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Option

Control of reproductive factors in herbivores

Title

SEROPREVALENCE AND RISK FACTORS OF THE MAIN ABORTIVE INFECTIOUS AGENTS OF CATTLE IN BATNA

Doctoral thesis presented by
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ABSTRACT

Infectious diseases are of increasing concern on dairy farms because of their potential impact on animal and human health, milk and meat production, food safety, and economics. The present PhD Thesis aimed to determine, at individual and herd level, the presence and risk factors of the main reproductive infectious agents of cattle in two regions from North Algeria: Batna, Khenchela, Setif (North-eastern) and Tiaret (North-western) Algeria respectively. The present Thesis is divided in three studies: Study-I analysed the prevalence and risk factors of the intracellular parasites *Neospora caninum* and *Toxoplasma gondii*; Study-II studied the prevalence and risk factors of the bacteria *Chlamydia abortus, Coxiella burnetii* and *Brucella abortus*; and Study-III analysed the prevalence and risk factors the Bovine Viral Diarrhoea Virus (Genus *Pestivirus*) and characterised molecularly the circulating strains.

In Study-I, we conducted a cross-sectional serological study on dairy farms from North-eastern Algeria (Batna, Khenchela, Setif). Blood samples from 344 dairy cattle from 22 herds were collected. The presence of antibodies against *N. caninum* and *T. gondii* was assessed by two commercial indirect ELISA tests, while only 151 out of 344 sera belonging to 10 farms were tested for *T. gondii* antibodies. Also, the presence of *N. caninum* and *T. gondii* DNA in aborted foetuses from the same dairy farms was analysed by qPCR. In addition, the risk factors of neosporosis and toxoplasmosis were analysed. Prevalence of antibodies against *T. gondii* and *N. caninum* was 9.9 (15/151; 95 % CI=5.9-15.5) and 5.5 % (19/344; 95 % CI=3.3-8.4), respectively. At the herd level, the prevalence of antibodies against *T. gondii* and *N. caninum* was 70.0 (7/10; 95 % CI=34.7-93.3), and 59.0% (13/ 22; 95 % CI=36.3-79.2) respectively. *Neospora caninum* and *Toxoplasma gondii* DNA were detected in 30% and 0% of aborted foetuses, respectively. The analysis of risk factors showed that the number of calving (>=6 vs <=2) (OR=6.3; 95% CI=1.7-23.4), presence of horses (yes vs no) (OR=5.9; 95% CI=1.0- 35.9) and the use of artificial insemination in the last mating (yes vs no) (OR=4.8, 95% CI=1.17-19.90) were significantly associated with *N. caninum* prevalence in the studied cattle herds. Conversely, the presence of standing water in the pasture (yes vs no) (OR=0.2; 95% CI=0.05-0.8) was considered as a protective factor. Retention of foetal membranes (*P*=0.081), increased inter-calving period (*P*=0.096) and the clinical reproductive disorder experience (CRDE) (*P*=0.077) were the most frequently reported clinical reproductive disorders among *T. gondii* seropositive cattle.

Study-II evaluated the seroprevalence and risk factors of the bacteria *Chlamydia abortus, Coxiella burnetii* and *Brucella spp* in dairy cattle from North-Eastern Algeria. Prevalence of antibodies against *Brucella spp, Coxiella burnetii* and *Chlamydia abortus* was 28.6% (127/437; 95% CI, 24.2-34.6%), 13.3% (46/344; 95% CI, 9.8-17.8%) and 1.45% (4/344, 95 % CI, 0.6-3.2), respectively. At a herd level *Coxiella burnetii* and *Chlamydia abortus* were observed in 11/22 (50.0%; 95% CI, 25.0-89.5%) and 4/22 (18, 1%; 95% CI, 5.0-46.6%) respectively. The following variables were identified as risk factors

for *Brucella spp* prevalence by the final multivariable logistic regression analysis: age (all categories) for cows over 60 months of age (OR=7.39; 95% CI=2.1-25.5%), artificial insemination (OR=1.46; 95% CI=0.4-4.3%), and rivers in the pasture (yes vs no) (OR=25.9; 95% CI=8.2-81.7%). Herd size (as a numeric variable) was found as a protective factor, with an increase of one animal in the herd resulting in a decrease of a 4% (1-0.96=0.04) in the odds. Visitors entering farms without personal protective equipment (yes vs no) was the main variable that was identified as a risk factor for *Coxiella burnetii* prevalence by the final multivariable logistic regression analysis (OR=5.70; 95% CI=1.70-19.10). On the other hand, season (Autumn vs Winter vs Spring) and water source (tap water vs well) were identified as protective factors, with an odds ratio of 0.09 (95% CI=0.02-0.49) and 0.09 (95% CI=0.02-0.44), respectively. The variables age (numeric), stray dogs (yes vs no), and presence of wild animals (mainly wolf, rodent, jackal, and Boar) in the building were identified as risk factors for *Chlamydia abortus* prevalence by the final multivariable logistic regression, with odds ratio of 1.03 (95% CI=1.00-1.05), 0.05 (95% CI=0.00-0.85) and 13.75 (95% CI=1.57-120.64), respectively.

In Study-III, we performed a cross-sectional seroprevalence study and evaluated the risk factors associated with BVDV on dairy farms from North-western Algeria. Blood samples from 234 dairy cattle from 31 herds were collected. Sera were analysed for the presence of antibodies against BVDV (Genus *Pestivirus*) using a commercial iELISA. The presence of *Pestivirus* RNA in sera was also analysed using a Reverse Transcription-qPCR and positive samples were sequenced. Additionally, we conducted a literature review of the presence of BVDV in ruminants in North Africa using a systematic search and compilation methodology to identify gaps of knowledge for future research. The prevalence of antibodies against *Pestivirus* at population (59.9%; 95% CI=49.0-70.7%) and farm (93.5%, 95% CI=78.6%-99.2%) level concur with epidemiological data reported in neighbouring countries. Risk factors associated with BVDV seroprevalence in cattle were mixed herd (presence of sheep in the farm), herd size (n>20), and parity (cow vs heifer). Furthermore, we confirmed the presence of BVDV-1a in Algeria. This study represents the first report of BVDV in cattle in Algeria (Tiaret province), on account of the fact that ruminant *Pestivirus* have historically been neglected pathogens in North Africa.

Our results confirmed the presence of *Neospora caninum, Toxoplasma gondii*, *Chlamydia abortus, Coxiella burnetii* and *Brucella spp*, and BVDV among dairy cattle in Algeria and highlighted their negative impacts on animal health and production and their public health implications.

Brucella spp and *Toxoplasma gondii* were found to cause reproductive disorders in the study area. A strong significant association was found between *Brucella spp* seroprevalence and abortion (P<0.001) in dairy cattle. The high BVDV prevalence and the presence of a potential Persistently Infected (PI) individual in the study area suggest an endemic epidemiological scenario of *Pestivirus*. Although *Chlamydia abortus* and *Coxiella burnetii* were found to be prevalent in the study area, with no

association with reproductive disorders. The lack of epidemiological and molecular transboundary studies of abortive pathogens in ruminants in North Africa is of concern for human and animal health as well as for wildlife conservation and further research is warranted.

Key words: Infectious abortion, Algeria, cattle, seroprevalence, risk factors, *Neospora caninum, Toxoplasma gondii, Brucella spp, Chlamyia abortus, Coxiella burnetiid,* BVDV, BD, detection and/or molecular characterization.

ARABIC SUMMARY (الملخص)

تشكل الأمراض المعدية مصدر قلق متزايد في مزارع الألبان بسبب تأثيرها المحتمل على صحة الإنسان والحيوان، إنتاج الألبان واللحوم، والأمن الغذائي والاقتصاد. هدفت أطروحة الدكتوراه الحالية إلى تحديد، على مستوى الفرد والقطيع، وجود عوامل الخطر للعوامل المعدية الرئيسية التي تسبب ضررًا تناسليًا للماشية في منطقتين بشمال الجزائر: باتنة خنشلة سطيف (الشمال- شرق) وتيارت(شمال غرب) الجزائر.

تم تقسيم الأطروحة الحالية إلى ثلاث دراسات: الدراسة الأولى حللت انتشار وعوامل الخطر من الطفيليات داخل الخلايا نيوسبورا كانينوم وتوكسوبلازما قوندي. درست الدراسة الثانية انتشار وعوامل الخطر لبكتيريا الكلاميديا المجهضة، وكوكسيلا بورنيتي، والبروسيلا. وحللت الدراسة الثالثة انتشار وعوامل الخطر لفيروس الإسهال الفيروسي البقري (جنس طاعون الحشرات) وتحديد السلالات المنتشرة على المستوى الجزيئي في الجزائر.

في الدراسة الأولى، أجرينا دراسة مصلية مقطعية على مزارع الألبان في شمال شرق الجزائر (باتنة، خنشلة، سطيف). تم أخذ عينات دم من 344 بقرة حلوب تنتمي إلى 22 قطيعاً. تم تقييم وجود الأجسام المضادة الموجهة ضد نيوسبورا كانينوم و توكسوبلازما قوندي من خلال استعمال اختبارIndirect ELISAالتجارية بالإضافة إلى ذلك، تم تحليل وجود الحمض النووي لنيوسبورا كانينوم و توكسوبلازما قوندي في الأجنة المجهضة من مزارع الألبان نفسها بواسطة qPCR. بالإضافة إلى ذلك، تم تحليل عوامل الخطر للإصابة بهذه الامراض.

كان انتشار الأجسام المضادة ضد توكسوبلازما قوندي ونيوسبورا كانينوم 9.9 % (151/15 95% CI=5.9-15.5) و5.5% (344/19؛ 95٪ CI= 3.3-8.4) على التوالي. على مستوى القطيع، كان معدل انتشار الأجسام المضادة ضد توكسوبلازما قوندي و نيوسبورا كانينوم 70.0% (10/7 95% CI= 34.7-93.3) و 59.0٪ (22/13؛ 95% CI=36.3-79.295) على التوالي. تم اكتشاف الحمض النووي نيوسبورا كانينوم وتوكسوبلازما قوندي في 30 ٪ و0 ٪ من الأجنة المجهضة، على التوالي. وأظهر تحليل عوامل الخطر أن عدد المواليد (<= 2 مقابل> = 6) (OR = 6.3؛ 95٪ CI 1.7-23.4=)، وجود الخيول (نعم مقابل لا) (OR = 5.9؛ 95 CI% =1.0-35.9)واستخدام التلقيح الاصطناعي أثناء التزاوج الأخير (نعم مقابل لا) (OR = 4.8، 95%CI = 1.17-19.90) ارتبطت بشكل كبير بانتشار نيوسبورا كانينوم في قطعان الماشية المدروسة. على العكس من ذلك، فإن وجود المياه الراكدة في المراعي (نعم مقابل لا) (OR = 0.2؛ 95%CI= 0.05-0.8) يعتبر عاملاً وقائيًا ضد العدوى. كان احتباس المشيمة (P = 0.081)، وزيادة الوقت الفاصل بين ولادتين (P = 0.096) والتجربة في الاضطرابات التناسلية السريرية (P = 0.077) من الاضطرابات التناسلية السريرية التي تم الإبلاغ عنها بشكل متكرر في الماشية التي أصيبت. بتوكسوبلازما قوندي.

قيمت الدراسة الثانية الانتشار المصلي وعوامل الخطر لكل من بكتيريا الكلاميديا المجهضة، كوكسيلا بورنيتي وبروسيلا في الأبقار الحلوب من شمال شرق الجزائر. وأخذت عينات دم من 437 بقرة حلوب من 30 قطيعاً. تم تحليل جميع الأمصال باستخدام Elisa Indirect. كان معدل انتشار الأجسام المضادة ضد البروسيلا، كوكسيلا بورنيتي، والكلاميديا المجهضة 28.6٪ (437/127؛ 95٪ CI=24.2-34.6٪)، 13.3٪ (344/46؛ 95٪CI=، 9.8-17.8٪) و1.45٪ (344/4، 95٪ CI=،0.6-3.2) على التوالي. على مستوى القطيع، لوحظت كلاميديا المجهضة وكوكسيلا بورنيتي في 22/11 (50.0٪؛ 95٪ CI=،25.0-89.5) و22/4 (18.1٪؛ 95٪ CI= 5.0-46.6) على التوالي،. تم تحديد المتغيرات التالية كعوامل خطر للإيجابية المصلية ضد البروسيلا من خلال تحليل الانحدار اللوجستي النهائي متعدد المتغيرات: العمر (جميع الفئات) للأبقار التي تزيد عن 60 شهرًا (OR = 7.39؛ 95٪CI=2.1-25.5)، التلقيح الاصطناعي (OR = 1.46؛ 95٪ CI= 0.4-4.3) الأنهار والجداول في المراعي (نعم مقابل لا) (OR = 25.9؛ 95٪ CI= 8.2-81.7). اعتبر حجم القطيع (كمتغير رقمي) عاملاً وقائياً، مع زيادة حيوان واحد فقط في القطيع مما أدى إلى انخفاض بنسبة 4٪ (1-

0.96 = 0.04) في الجانب الآخر. كان الزوار الذين يدخلون المزارع بدون معدات الحماية الشخصية (نعم أو لا) هو المتغير الرئيسي الذي تم تحديده كعامل خطر لانتشار كوكسيلا بورنيتي من خلال تحليل الانحدار اللوجستي النهائي متعدد المتغيرات (OR = 5.70؛ 95٪CI =1.70-19.10). في المقابل، تم تحديد الموسم (الخريف مقابل الشتاء مقابل الربيع) ومصدر المياه (مياه الصنبور مقابل البئر) كعوامل وقائية، مع نسبة أرجحية OR=0.09 (95٪CI= 0.02-0.49) وOR=0.09 (95٪CI=0.02-0.44)، على التوالي. تم تحديد متغيرات العمر (العددية)، والكلاب الضالة (نعم مقابل لا) ووجود الحيوانات البرية (بشكل رئيسي الذئب والقوارض وابن آوى والخنازير البرية) في المبنى على أنها عوامل خطر لانتشار الكلاميديا المجهضة عن طريق الانحدار اللوجستي النهائي متعدد المتغيرات، بنسبة أرجحية OR=1.03 (95٪CI =1.00-1.05)، OR=0.05 (95%CI=0.00-0.85%) وOR=13.75 (95٪CI = 1.57-120.64)، على التوالي.

في الدراسة الثالثة، أجرينا دراسة مقطعية للانتشار المصلي وقمنا بتقييم عوامل الخطر المرتبطة بـ BVDV في مزارع الألبان في شمال غرب الجزائر. تم أخذ عينات دم من 234 بقرة حلوب من 31 قطيع. تم تحليل المصل لوجود أجسام مضادة ضد BVDV (جنس *Pestivirus*) باستخدام iELISA تجاري. تم أيضًا تحليل وجود RNA لفيروس BVDV في الأمصال باستخدام النسخ العكسي - qPCR وتم تسلسل العينات الإيجابية.

بالإضافة إلى ذلك، أجرينا مراجعة الأدبيات حول وجود *Pestivirus* في المجترات في شمال إفريقيا باستخدام منهجية بحث وتجميع منهجية لتحديد الفجوات المعرفية لتحفيز إجراء أبحاث في المستقبل. انتشار الأجسام المضادة ضد BVDV على قطيع الماشية (59.9٪؛ 95%CI= 49.0-70.7) وفي المزرعة (93.5٪، 95٪ CI= 78.6 -99.2) يتوافق مع البيانات الوبائية المبلغ عنها في البلدان المجاورة. كانت عوامل الخطر المرتبطة بالانتشار المصلي لـ BVDV في الماشية مختلطة القطيع (وجود الأغنام في المزرعة)، حجم القطيع (ن> 20) وفئة الحيوان (البقر مقابل بقرة). بالإضافة إلى ذلك، أكدنا وجود BVDV-1a في الجزائر. تمثل هذه الدراسة أول تقرير عن الإصابة بفيروس BVDV في الأبقار في الجزائر (ولاية تيارت)، وذلك لأن فيروسات آفات المجترات كانت تاريخياً من مسببات الأمراض المهملة في شمال إفريقيا.

أكدت نتائجنا وجود نيوسبورا كانينوم, توكسوبلازما قوندي, البروسيلا ، كوكسيلا بورنيتي، الكلاميديا المجهضة و BVDV في أبقار الألبان في الجزائر وسلطت الضوء على آثارها السلبية على صحة الحيوان والإنتاج وآثارها على الصحة العامة.

تم العثور على ارتباط قوي بين الانتشار المصلي للبروسيلا والإجهاض (P <0.001) في الأبقار الحلوب. يشير الانتشار المرتفع لـ BVDV ووجود حيوان يحتمل أن يكون مصابًا بشكل دائم (PI) في منطقة الدراسة إلى سيناريو وبائي مستوطن لفيروس *Pestivirus*. على الرغم من انتشار الكلاميديا أبورتس وكوكسيلا بورنيتي في منطقة الدراسة، لا علاقة له بالاضطرابات الإنجابية. يعد الافتقار إلى الدراسات الوبائية والجزيئية العابرة للحدود حول مسببات الأمراض المجهضة في المجترات في شمال إفريقيا مصدر قلق لصحة الإنسان والحيوان وكذلك للحفاظ على الحياة البرية.

الكلمات المفتاحية: الإجهاض المعدي، الجزائر، الماشية، الانتشار المصلي، عوامل الخطر، *Neospora caninum*، *Toxoplasma gondii*، *Brucella spp*، *Chlammydia abortud, Coxiella burnetii*، BVDV، BD، الكشف و / أو التوصيف الجزيئي

RÉSUMÉ

Les maladies infectieuses sont de plus en plus préoccupantes dans les exploitations laitières en raison de leur impact potentiel sur la santé animale et humaine, la production de lait et de viande, la sécurité alimentaire et l'économie. La présente thèse de doctorat visait à déterminer, au niveau de l'individu et du troupeau, la présence et les facteurs de risque des principaux agents infectieux causant des troubles de la reproduction chez les bovins dans deux régions du nord de l'Algérie : Batna Khenchela Sétif (Nord-est) et Tiaret (Nord-ouest) de l'Algérie. La présente thèse est divisée en trois études : L'étude-I a analysé la prévalence et les facteurs de risque des parasites intracellulaires *Neospora caninum* et *Toxoplasma gondii*. L'étude II a étudié la prévalence et les facteurs de risque des bactéries *Chlamydia abortus*, *Coxiella burnetii* et *Brucella spp*; et l'étude III a analysé la prévalence et les facteurs de risque du virus de la diarrhée virale bovine (genre *Pestivirus*) et a caractérisé au niveau moléculaire les souches en circulation.

Dans l'étude-I, nous avons mené une étude sérologique transversale sur des fermes laitières du nord-est de l'Algérie (Batna, Khenchela, Sétif). Des échantillons de sang de 344 bovins laitiers appartenant à 30 troupeaux ont été prélevés. La présence d'anticorps dirigés contre *N. caninum* et *T. gondii* ont été évaluée par deux tests ELISA indirects commerciaux. De plus, la présence d'ADN de *N. caninum* et de *T. gondii* chez les fœtus avortés des mêmes fermes laitières a été analysée par qPCR. De plus, les facteurs de risque de néosporose et de toxoplasmose ont été analysés. La prévalence des anticorps contre *T. gondii* et *N. caninum* était respectivement de 9,9 (15/151 ; IC à 95% = 5,9-15,5) et 5,5% (19/344 ; IC à 95% = 3,3-8,4). Au niveau du troupeau, la prévalence des anticorps contre *T. gondii* et *N. caninum* était de 70,0 (7/10 ; IC à 95% = 34,7-93,3) et de 59,0% (13/22 ; IC à 95% = 36,3-79,2) respectivement. L'ADN de *Neospora caninum* et de *Toxoplasma gondii* a été détecté dans 30% et 0% des fœtus avortés, respectivement. L'analyse des facteurs de risque a montré que le nombre de vêlages (<= 2 vs > = 6) (OR = 6,3 ; IC à 95% = 1,7-23,4), la présence de chevaux (oui vs non) (OR = 5,9 ; IC à 95% = 1,0-35,9) et l'utilisation de l'insémination artificielle lors du dernier accouplement (oui vs non) (OR = 4,8, IC à 95% = 1,17-19,90) étaient significativement associées à la prévalence de *N. caninum* dans les troupeaux bovins étudiés. À l'inverse, la présence d'eau stagnante dans le pâturage (oui vs non) (OR = 0,2 ; IC à 95% = 0,05-0,8) a été considérée comme un facteur de protection contre l'infection. La rétention des membranes fœtales (*P* = 0,081), l'augmentation de l'intervalle vêlages- vêlages (*P* = 0,096) et l'expérience des troubles cliniques de la reproduction (CRDE) (*P* = 0,077) étaient les troubles de la reproduction clinique les plus fréquemment rapportés chez les bovins séropositifs à *T. gondii.*

L'étude II a évalué la séroprévalence et les facteurs de risque des bactéries *Chlamydia abortus*, *Coxiella burnetii* et *Brucella spp* chez les bovins laitiers du nord-est de l'Algérie. Des échantillons de sang de 437 bovins laitiers de 30 troupeaux ont été prélevés. Tous les sérums ont été analysés en utilisant Elisa Indirect. La prévalence des anticorps contre *Brucella spp, Coxiella burnetii* et *Chlamydia abortus* était de 28,6% (127/437 ; IC à 95%, 24,2-34,6%), 13,3% (46/344 ; IC à 95%, 9,8-17,8%) et 1,45% (4/344, IC à 95%, 0,6-3,2), respectivement. Au niveau du troupeau, *Coxiella burnetii* et *Chlamydia abortus* ont été observés respectivement dans 11/22 (50,0% ; IC 95%, 25,0-89,5%) et 4/22 (18, 1% ; IC 95%, 5,0-46,6%). Les variables suivantes ont été identifiées comme facteurs de risque de séropositivité contre la *Brucella spp* par l'analyse finale de régression logistique multivariée : âge (toutes catégories) pour les vaches de plus de 60 mois (OR = 7,39 ; IC à 95% = 2,1-25,5%), insémination artificielle (OR = 1,46 ; IC à 95% = 0,4-4,3%), et rivières et vapeur dans le pâturage (oui vs non) (OR = 25,9 ; IC à 95% = 8,2-81,7%). La taille du troupeau (en tant que variable numérique) a été considérée comme un facteur de protection, avec une augmentation d'un seul animal dans le troupeau entraînant une diminution de 4% (1-0,96 = 0,04) de la cote. Les visiteurs entrant dans les fermes sans équipement de protection individuelle (oui ou non) était la principale variable identifiée comme facteur de risque de prévalence de *Coxiella burnetii* par l'analyse finale de régression logistique multivariée (OR = 5,70 ; IC à 95% = 1,70-19,10). En revanche, la saison (automne vs hiver vs printemps) et la source d'eau (eau du robinet vs puits) ont été identifiées comme des facteurs de protection, avec un rapport de cotes de 0,09 (IC à 95% = 0,02-0,49) et de 0,09 (IC à 95% = 0,02-0,44), respectivement. Les variables âge (numérique), chiens errants (oui vs non) et présence d'animaux sauvages (principalement loup, rongeur, chacal et sanglier) dans le bâtiment ont été identifiées comme des facteurs de risque de prévalence de *C. abortus* par la régression logistique multivariée finale, avec un odds ratio de 1,03 (IC à 95% = 1,00-1,05), 0,05 (IC à 95% = 0,00-0,85) et 13,75 (IC à 95% = 1,57-120,64), respectivement.

Dans l'étude III, nous avons réalisé une étude transversale de séroprévalence et nous avons évalué les facteurs de risque associés au BVDV dans les fermes laitières du nord-ouest de l'Algérie. Des échantillons de sang de 234 bovins laitiers de 31 troupeaux ont été prélevés. Les sérums ont été analysés pour la présence d'anticorps contre le BVDV (Genus *Pestivirus*) en utilisant un iELISA commercial. La présence d'ARN de *Pestivirus* dans les sérums a également été analysée en utilisant une Reverse Transcription -qPCR et des échantillons positifs ont été séquencés.

En outre, nous avons mené une revue de littérature sur la présence du *Pestivirus* chez les ruminants en Afrique du Nord en utilisant une méthodologie de recherche et de compilation systématique pour identifier les lacunes dans les connaissances pour de possible futures recherches. La prévalence des anticorps contre le BVDV au niveau de du cheptel bovine (59,9% ; IC à 95% = 49.0-70.7%) et à la

ferme (93,5%, IC à 95% = 78.6%-99.2%) concorde avec les données épidémiologiques rapportées dans les pays voisins. Les facteurs de risque associés à la séroprévalence du BVDV chez les bovins étaient le troupeau mixte (présence de moutons dans la ferme), la taille du troupeau (n> 20) et la catégorie animale (vache vs génisse). De plus, nous avons confirmé la présence du BVDV-1a en Algérie. Cette étude représente le premier signalement de BVDV chez les bovins en Algérie (province de Tiaret), en raison du fait que les *Pestivirus* ruminants ont été historiquement des agents pathogènes négligés en Afrique du Nord.

Nos résultats ont confirmé la présence de *Neospora caninum, Toxoplasma gondii, Chlamydia abortus, Coxiella burnetii, Brucella spp,* et BVDV chez les bovins laitiers en Algérie et mettent en évidence leurs impacts négatifs sur la santé et la production animales et leurs implications pour la santé publique.

Nous avous constaté que *Brucella spp,* et *Toxoplasma gondii* causaient des troubles de la reproduction dans la zone d'étude. Une forte association significative a été trouvée entre la séroprévalence de *Brucella spp* et l'avortement (P <0,001) chez les bovins laitiers. La prévalence élevée du BVDV et la présence d'un individu potentiellement infecté de manière persistante (IP) dans la zone d'étude suggèrent un scénario épidémiologique endémique du *Pestivirus*. Bien que *C. abortus* et *C. burnetii* soient répandus dans la zone d'étude, sans aucun lien avec des troubles de la reproduction. Le manque d'études épidémiologiques et moléculaires transfrontières sur les agents pathogènes abortifs chez les ruminants en Afrique du Nord est préoccupant pour la santé humaine et animale ainsi que pour la conservation de la faune et des recherches supplémentaires sont justifiées.

Mots clés : Avortement infectieux, Algérie, bovins, séroprévalence, facteurs de risque, *Neospora caninum, Toxoplasma gondii, Brucella spp, Chlammydia abortud, Coxiella burnetii*, BVDV, BD, détection et/ou caractérisation moléculaire

LIST OF ABBREVIATIONS

AI: Artificial Insemination

B. abortus : *Brucella abortus*

BTV: Blue Tongue Virus

BVDV: Bovine Viral Diarrhoea Virus

CCD: Camera Couple Device

CFT: Complement Fixation Test

CI: Confidence Interval

C. abortus: *Chlamydia abortus*

C. burnetii: *Coxiella burnetiid*

Cp: Cytopathic

DNA: Deoxyribonucleic Acid

dsDNA: double strain Deoxyribo-Nucleic Acid

ELISA: Enzyme Linked Immunosorbent Assay

ICTV: International Committee for the Taxonomy of Viruses

IOFC: Income Over Feed Cost

IPC: Imported Cattle

CReSA: Institut de Recerca i Tecnologia Agroalimentàries Centre de Recerca en Sanitat Animal

IVV: Interval Calving-Calving

LB: Local Breeds

LIC: Local Improved Cattle

LH: Luteinizing Hormone

LLU: Large Livestock Unit

LPS: Lipo Poly Saccharide

MADR: Ministry of Agriculture and Rural Development

MAT: modified agglutination test

MOMP: Major Outer Membrane Protein

Neospora caninum : *N. gondii*

Ncp: Non-cytopathic

OR: Odd Ratio

PCR: Polymerase Chain Reaction

PI: Persistently Infected

RNA: Ribonucleic Acid

RT-PCR: Real Time-PCR

T. gondii: *Toxoplasma gondii*

USD: United States Dollars

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I. Introduction

Abortions and/or embryonic mortalities in cattle are encient and known pathologies but which still persist today in dairy cattle farms in Algeria

According to the Russian dictating "Better a goat which gives milk than a sterile cow", for this the production of milk is intimately linked to the calving of dairy cows. The objective of any breeder is to obtain a maximum lactation period with the optimum daily quantities of milk to hope to be the owner of economically profitable breeding while preserving animal welfare. Although, successful gestation to term is subject to many risks, mainly abortions or embryonic mortalities, whether early or late. These latter are of major concerns for farmers, given the economic and health impact they may have on farms.

Abortions can be idiopathic or result from metabolic or hormonal abnormalities, nutritional deficiencies, trauma, toxicities, or infectious agents. The latter represents the main aetiology of reproductive disorders overall (Ortega-Mora, 2007; Givens, 2006). The causes of abortions are numerous and varied, the infectious origins are in turn diverse. It is also important to note that some pathogens causing abortions in ruminants can be transmitted to humans and be dangerous; especially for pregnant women. An increase in the number of spontaneous abortions in a herd is a dramatic event for the farmer involved, and a range of epizootic and/or zoonotic diseases, or even emerging diseases, maybe the cause.

Controlling abortion and preventing this huge amount of economic loss are vital for breeders in Algeria. Many studies suggested that more than half of fertilizations result in embryo loss before pregnancy is detected (Borel et al., 2014; Reichel and Hill, 2018; Wolf-Jäckel et al., 2020). In such situation’s farmers, along with their veterinary practitioners, and potentially veterinarians state, expect rapid reliable results from diagnostic veterinary laboratories, a process that is not always easily achieved (Borel et al., 2014). Diagnostic rates in ruminant abortions are low worldwide, reaching approximately 50% of the cases (John Matthews, 2016). Nevertheless, diagnosis of the etiological agent has improved with time, from about 33–37% in the 1990s (Jamaluddin et al., 2016), to 44% in the 2000s (Anderson, 2007), to 58%(Clothier and Anderson, 2016) in 2014, but only if a full range of samples were collected. Conversely, in

Algeria the rate of diagnosis in ruminants remains very low, moreover, little scientific data in this field are available, suggesting the need for other additional investigations. Algeria suffers from a huge deficit to meet the national milk production needs, abortion and reproductive disorders probably represent one of the main causes. Establishing an aetiological diagnosis remains challenging awing to the large variety of bacteria, protozoa, viruses and fungi that have been in relation with abortion in cattle (Ghalmi et al., 2012; Achour et al., 2012; Abdelhadi et al., 2015; Hireche et al., 2016; Derdour et al., 2017; Rahal et al., 2018).

The ultimate goal is to try to obtain a satisfactory answer concerning some of the real obstacles facing the increase in milk production in Algeria which currently meets a third of national needs (MADR, 2013), besides, try to identify the probable direct and/or indirect causes of abortions due to infections causes. In the absence of studies and investigations or at least still insufficient responding to this problem and whose aim is to study the prevalence, the occurrence as well as the risks of certain abortive agents, in the first intention so that in second intention to try to list the appropriate recommendations in the Algerian context.

What is the proportion of the involvement of certain abortion agents in the phenomena of infertility and abortions? what are the risk factors for infections with abortive agents, namely *Neospora caninum, Toxoplasma gondii, Chlamydia abortus, Coxiella burnetii, Brucella spp* and Bovine Viral Diarrhea Virus (BVDV)? What are the consequences of these latter on reproduction parameters in cattle? Will the identification of the local strains help a good understanding of the infections that plague Algerian herds? Do wild animals which probably share common grounds with production animals, play a role in the achievement and spread of the abortive agents included in our study?

Serology is considered as one of the most widely used means in the diagnosis of infectious agents. In order to respond to this problem, a cross-sectional study was carried out, based on the collection of blood samples from dairy cattle farms in the north of Algeria (the north-easteren and the north-western). Epidemiological data from the different herds were collected based on a detailed questionnaire including a section on herd and breeder identification, a section on livestock, and another section on reproductive performance of the herd.

The detection of anti-abortive agent antibodies was performed by the iELISA technique, Antibody iELISA was used because it is largely used and also recommended by OIE for screening of brucellosis (OIE, 2008; IDvet innovative diagnostic, 2018). Most iELISAs use purified smooth LPS (Lipopolysaccharide) as antigen but a good deal of variation exists in the anti-bovine immunoglobulin conjugate used (Saegerman et al., 2004)

DNA and / or RNA extraction was conducted on organs from aborted foetuses, subsequently, the identification of the various abortive agents was carried out mainly by the PCR technique (RT [real time and reverse transcription]). In the end, the sequencing technique will surely provide a more comprehensive understanding of the source of the pathogen as well as a possible development of an effective means of prevention.

This thesis has two main chapters:

The first chapter is a review of the literature on the situation of dairy cattle breeding in Algeria, the current situation of milk production as well as the major constraints for dairy cattle breeding in Algeria, a synthesis of the main abortive agents in cattle in the countries of the Mediterranean basin with an emphasis on the abortion processes due to infections by *Neospora caninum, Toxoplasma gondii, Chlamydia abortus, Coxiella burnetii, Brucella spp* and BVDV.
Finally, a global approach to the means of diagnosis approach of abortion in a herd or flock around the world and in particular in countries belonging to the Mediterranean basin.

The second chapter is an experimental study bringing together three studies in two regions of northern Algeria:

1- Study I: Seroprevalence, risk factors and molecular detection of *Neospora caninum* and *Toxoplasma gondii* in cattle in North-Eastern Algeria.

2- Study II: Seroprevalence, risk factors and molecular detection of *Brucella abortus, Chlamydia abortus,* and *Coxiella burnetii* in cattle in north-eastern Algeria.

3- Study III: Seroprevalence, risk factors and molecular characterization of BVDV in North-Western region of Algeria

I. 1. BOVINE BREEDING IN ALGERIA

I. 1. 1. Bovine population in Algeria

The Mediterranean Maghreb constitutes a unique ecological area with close relations and exchanges at all levels of its economies. Livestock and particularly small ruminants play a major socioeconomical role in this region. In the past 50 years, the cattle population has increased from 865,700 heads to 1,895,126 (Yves Leforban et al., 1999; MADR, 2017a), and in the past 20 years the total livestock, including sheep, goat, cattle, camels and horses, has increased by 37%.

During the 2010-2017 period, sheep numbers represented 78% (26.4 million heads) of the total livestock in Algeria, followed by goats (14%, 4.8 million heads) and bovines (6%, 1.9 million heads), from which 52% were dairy cows (MADR, 2017b) (Figure 1).

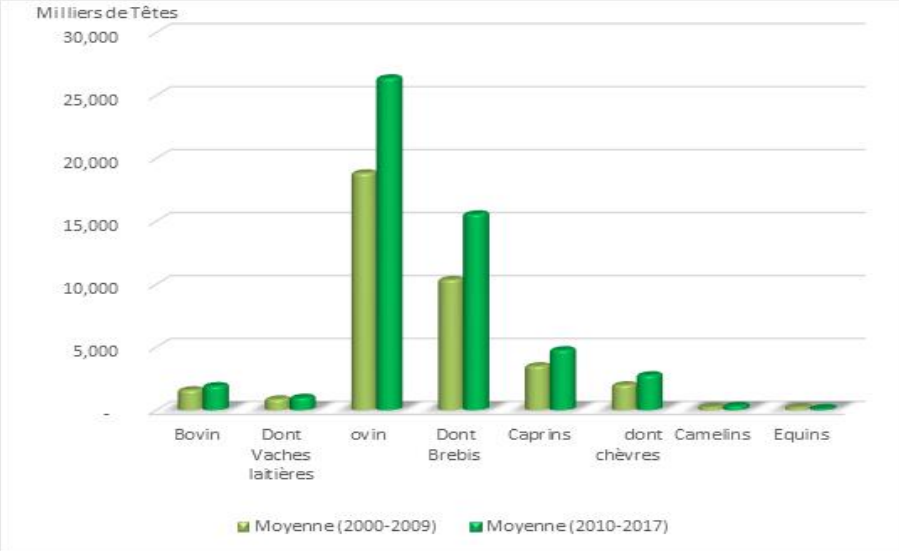


Figure 1. Evolution of the Algerian livestock from 2000 to 2017(MADR, 2017a)

The number of cattle varies considerably between the Maghreb countries: 2.8, 1.6 and 0.6 million in Morocco, Algeria and Tunisia, respectively. These figures have shown very slight changes in recent years (Figure 2) (Sraïri et al., 2013).

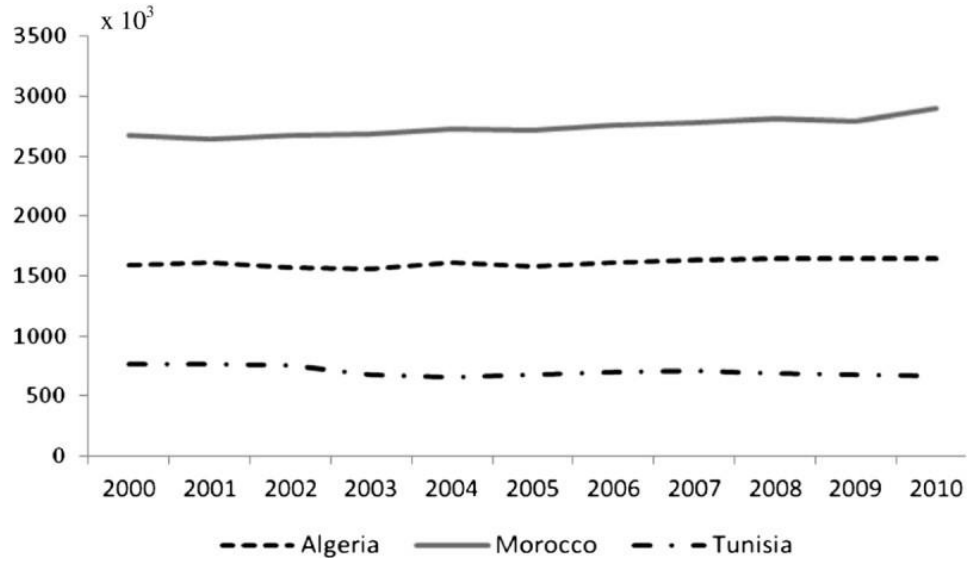


Figure 2. Evolution of cattle numbers in the Maghreb countries from 2000 to 2010 (Sraïri et al., 2013)

The cattle population in Algeria approaches two million heads, of which 70% are dairy cattle (30% of imported cattle "IPC" and 40% of local improved cattle "LIC") representing an important source of food for the inhabitants (Kali et al., 2011; Kardjadj, 2016). IPC cattle breeds are highly-productive breeds that have been mainly introduced from Europe. They are intensively or semi-intensively farmed in lowland and irrigated areas where fodder production is relatively high. The primary IPC breeds in Algeria are Holstein (either black and white or red and white coat colour) and Monbéliarde. There is also a hybrid breed generated by cross-breeding of IPC breeds and the local breed "*Brune de l'Atlas*". LIC breeds are located in mountain and forest areas. In 2012, LIC represented 38% of the national workforce and provided around 30% of total cow's milk production. Average milk yields of these local breeds (LB) range from 3,000 to 3,500 litres / dairy cow / year. Given the low milk production of LIC, milk products are mainly intended for self-consumption (i.e. feeding of young animals) and they are mostly used for meat production (Kali et al., 2011). Finally, the production from this category of LB is not counted because it is not the subject of dairy transactions.

The distribution of farms in Algeria is largely related to the richness of the pastures. About 80% of cattle farming is located in the northern regions of the country and 59% in eastern Algeria, which is the area with the highest rainfall in the country. Conversely, only 22% and 19% of

the farms are located in central and South-western areas, respectively, where sheep and goats are preferred due to the predominance of semi-arid areas (Kirat, 2007).

I. 1. 2. Current situation of milk production in Algeria

Algeria is the first consumer of milk in the Maghreb, with nearly 120L/inhabitant/year compared to 83L/inhabitant/year in Tunisia and 64L/inhabitant/year in Morocco (Sraïri et al., 2013; Hassani, 2013). In 2015, Algeria recorded an average estimated to 150L of milk per capita (Chemma, 2017) while the World Health Organization set an international standard of 90L/capita/year (Chemma, 2017; Boukhechem et al., 2019). Algeria is one of the largest importers of milk worldwide. Indeed, the dairy industry operates mainly on the basis of imported milk, which is becoming more and more expensive.

Before the 1970s, the cattle population in Algeria was almost entirely composed by local breeds, adapted to the agro-climatic conditions but achieving low productivity (between 600 and 1800kg of milk/ cow / lactation) (Yekhlef, 1989). The intensification program of the milk production, initiated in 1970 with the agrarian revolution, gradually introduced breeds with high genetic potential, mainly from Europe. The appearance of these breeds, primarily Montbéliarde, Frisonne Pie Noire, Pie breeds Eastern Red, Tarentaise and Holstein, reduced the presence of LB in the population structure (Djermoun et al., 2017). Since the 1980s, a succession of state policies has been approved, aimed at intensifying local milk production and aligning with modern cattle farming. This has resulted in significant changes in cattle production, including the modification of the genetic structure of the population (mentioned above), the progress of the zootechnical practices, the use of artificial insemination (AI), and the development of processing and marketing of raw milk.

The Maghreb countries have implemented programs of artificial insemination, using semen of high genetic merit dairy cattle. The official figures reveal that the number of AIs in 2011 reached 204,600, 320,000, and 305,000 in Algeria, Morocco and Tunisia respectively. The number of AIs is steadily increasing, yet their efficiency could be significantly improved as the conception rate in herds is frequently superior to the double by herd (Sraïri and Farit, 2001). Despite these undeniable efforts, the integration rate of local production, which can only satisfy

33% (MADR, 2013), remains low and a deficit still persists in milk production. In this context, a global vision of the structure and conditions of cattle production is necessary.

I. 2. MAJOR CONSTRAINS FOR DAIRY CATTLE FARMING IN ALGERIA

The dairy cattle population in Algeria is located in the North of country, particularly in the coast and the interior plains in humid and subhumid climates. This area holds most dairy cows (60%), fodder areas (60.9%) and national raw milk production (63%) (MADR, 2017b). The extension of cattle breeding remains limited towards the South of the country, due to the climatic conditions (5% of cattle farming). The low productivity of dairy cattle in Algeria is the result of several ecological, technical, and socio-economic obstacles, which limit the profitability of farms (Herbut and Walczak, 2018). The slow growth recorded in the cattle population in Algeria can be attributed to several factors (Kherzat Bahidja, 2006; Ghozlane, et al., 2010):

- Insufficient support policies for livestock and development of fodder crops.
- Insufficient water resources and development of irrigated areas.
- Shortcomings in the milk price policy, inducing farmers to lose interest in milk production.
- Shortcomings in mastering the technical management of farms in an integrated manner.
- Long cycles of droughts recorded in recent years.
- The appearance of several cases of infectious diseases (tuberculosis, brucellosis, foot and mouth diseases. etc.), which sometimes led to forced slaughter.
- Weak agricultural extension.
- Absence of farmer associations.

Bouras, (2015) concluded that the intensive agriculture is facing several obstacles that have slowed its development, which can be classified into four types: technical (non-mastery of the conduct of livestock dairy), environmental (difficulty in adapting animals), health (non-compliance with health standards) and economic constraints (confidence in food supplie). Ghozlane et al., (2010) argued that the poor reproductive management is also behind the poor performance; it is clearly highlighted by an insufficient policy of reform, reproduction, gestation control and heat detection. Additionally, analysis of reproductive criteria has shown

that the calving – fertilization/insemination interval is far above accepted standards, resulting in calving-calving intervals exceeding a year. However, environmental factors (climate and type of housing) and especially hygiene and milking equipment should also be considered. Bouzebda, (2007) reported that the conditions of animal husbandry at a farm level in six provinces (Guelma, Annaba, Souk ahras, El-Taref, Skikda, Tebessa) indicated an inadequate compliance of the animal husbandry regulations. Overall, the evaluation of production systems shows that milk outputs still lag way behind the true potential of the dairy cattle breeds.

I. 2. 1. Algerian policies

Algeria is still far from guaranteeing acceptable coverage of dairy demands by national production. In order to secure the milk supply, specific policies have been implemented in the Maghreb countries. These strategies consisted in the establishment of a dairy industry, based on the processing of either raw milk produced locally (in Morocco and Tunisia) or imported milk powder (in Algeria) (Sraïri et al., 2013). In Algeria, imports of powdered milk have been a major obstacle to the local development of the production and processing of raw milk. Nevertheless, the import bill for milk (including raw materials) fell to 849.2 million dollars (USD) in 2016 against one billion USD in 2015, a decrease of 18.66%, according to the Ministry of Agriculture and Trade (MADR, 2017b; Ministère du Commerce International, 2019). Official policies in the Maghreb countries encourage an improvement in the average milk yield per cow rather than an increase in the number of cattle. To achieve such an increase in milk production, one of the most important measures adopted has been a plan for crossing LB and breeds with high genetic value, such as Holstein, Montbeliard or Swiss Brown (Sraïri et al., 2013). Similarly, Algerian development policy for dairy production is based on a massive introduction of cattle breeds with high genetic milk yield potential from Europe (Madani et al., 2008).

According to Ferrah, (2000) , the cost of production of a litre of milk has increased from 22.4 DA in 2000 to 27 DA in 2004, which can be explained by the rising costs of food and cereals in the global market (Djebbara, 2008). Sraïri et al., (2013) identified remuneration as an important challenge for the dairy sector of Maghreb. In this sense, the current subsidies for dairy farming remain insufficient for the profitability of the sector (Senoussi, 2008).

2. 2. Breeder's qualification and feeding practices

The lack of technicality in the workforce is at the origin of the technical mismanagement of farms and consequently of the low yield. These inappropriate technical practices are translated into a low milk output. A recent publication by Boukhechem et al., (2019) on feeding practices of dairy cows in Algeria concluded that:

- Production values do not deviate from the national average, reflecting technical management problems in farms.
- Food wastage was observed in 50.2% of farms (coverage rates of nutrient requirements were greater than 110%), in addition to production costs.
- A food strategy based on covering the nutrient requirements of cows and according to scientific guidelines was lacking. This was mainly conditioned by forage availability and food price, resulting in an excessive use of concentrate to cover the needs of cows in spite of its negative impact on health status, profitability, and production cost.

Although these conclusions were also supported by several authors in Algeria (Kadi et al.,. 2007; Ghozlane et al., 2009). Ghozlane et al., (2009) argued that the production conditions in the study region (Constantine- North-eastern Algeria) are favourable for improving the level of milk production.

I. 2. 3. Climate

Several studies strongly suggest that warming of the Earth's climate will increase in next decades (Roth, 2017; Boni, 2019). Global temperatures are expected to rise by 1.4–3.0°C by the end of XXI century, and by 5.0°C in certain temperate areas of the planet. An increase in the frequency and intensity of extreme heat waves is also expected in the upcoming years. These climatic changes will undoubtedly be a significant problem for cattle breeders throughout the world. Different theories have attempted to explain the effects of high ambient temperatures on livestock production. The impact of high temperatures was once thought to be limited to tropical areas. However, it has extended into northern latitudes in response to the increasing global temperature. Heat stress has become an important challenge facing the global dairy industry due to climate change (Schär et al., 2004), as well as the increase in the number of livestock

and the intensification of agriculture (Renaudeau et al., 2012; Von Keyserlingk, and Hötzel, 2015).

Numerous studies have focused on the productivity related effects of heat stress in lactating cows (Kadzere et al., 2002), dry cows and calves (Tao and Dahl, 2013). In the European Union alone, estimated losses in dairy production in 2015 relative to the earlier years were between 70 and 550 kg of milk/day/100 cows. In 2014, economic losses were estimated at 670 million USD (using present-day milk prices), and this will probably rise to 2.2 billion USD/year by the end of the century (Mauger et al., 2015). Heat stress can also contribute to an increase in the number of cases of calving difficulties, postpartum paralysis, stillbirths, metritis and on other fertility problems (Roth, 2017). Sraïri at al., (2013) reviewed the dairy chains in North Africa (Algeria, Morocco and Tunisia) and concluded that, given the climate constraints, dairy intensification may not be possible without the production of high-quality irrigated fodder. Recently, interest is also moving into the study of the impacts of heat stress on animal welfare (Polsky and von Keyserlingk, 2017; Roth, 2017).

I. 2. 4. Water sources

Drinking water can be considered an essential nutrient for dairy cattle because it is used in various ways in milk production. The dairy industry consumes great amounts of water and generates large quantities of wastewater (Andrade et al., 2014). Regrettably, many areas of the Maghreb countries are characterized by structural aridity and unpredictable rainfall, resulting in an unsustainable use of groundwater resources to intensify agricultural activities (Wada, Beek and Bierkens, 2012). In a context of scarcity, water is even a more important resource and it is linked to an increase of milk volumes. In fact, climate constraints and lack of high-quality irrigated fodder can prevent the intensification of the dairy industry. Under these circumstances, water resource management agendas are in need of a holistic approach to environmental management (Hermanowicz, 2008).

Sraïri et al., (2009) reported that almost 1.8 and 10.6 cubic meters of water were necessarily to get a single kg of milk and of live weight gain, respectively. The same author (Sraïri et al., 2016) demonstrated a mean water footprint of 1.62±0.81 and 8.44±1.09 m^3/kg of milk and of live weight gain, respectively. Groundwater represented only 13.1% and 2.2% of the total water

used to produce milk and live weight gain, respectively, while rainfall represented 53.0% and 48.1% of the total water for milk and live weight gain. The remaining water volumes used came from surface irrigation water (7.4% for milk and 4.0% for live weight gain) and from virtual water (26.5% for milk and 44.7% for live weight gain) (Sraïri et al., 2016). Benyettou and Bouklikha, (2017) evaluated the variations and trends in temperatures and daily rainfall in Algeria over a period of 34 years (1982-2016). Their principal component analysis revealed four major regions in Algeria. The coastal region is characterized by a slight decrease in annual precipitation. The eastern littoral region and the eastern highlands show a stable rainfall regime (area of study of the present PhD Thesis). The western littoral region underwent extensive annual rainfall deficit from 1982 to 2004. The region of West high plateaus and central Algeria are characterized by rainfall deficits (area of our study). Finally, the climate is dry in the southern region since the Sahara is a very windy and arid area (Benyettou and Bouklikha, 2017).

I. 3. THE IMPORTANCE OF REPRODUCTIVE MANAGEMENT IN DAIRY CATTLE

Productivity and profitability are significantly impacted by the reproductive performance of a dairy herd. Improved reproductive performance has many beneficial effects: increased efficiency of milk production by shifting the milking herd to a more productive phase of the lactation (Ferguson and Galligan, 1999); improved Income Over Feed Cost (IOFC)[1] and milk yield per day of calving interval (Ribeiro et al., 2012); reduced reproductive culls (Pinedo, De Vries and Webb, 2010); reduced need for replacement animals; increased percentage of the lactating herd that is multiparous (Santos et al., 2010; Galvão et al., 2013); improved genetic gain because of more selective culling of lactating cows and more stringent selection of replacement animals (Kent Weigel, 2006; Santos et al., 2010); and reduced costs of reproductive interventions (Giordano et al., 2012; Galvão et al., 2013). However, significant improvement in reproductive performance results in a greater proportion of dry cows in the adult herd (Galvão et al., 2013), demanding proper planning to accommodate these animals and maternity needs.

[1] ***Income Over Feed Cost Calculations.*** The IOFC for each cow state is calculated by subtracting the cost of feeding from the milk production value at each cow state.

According to Sakaguchi et al., (2011), to achieve a sustainable development in the dairy industry, it is important that cows become pregnant at a biologically optimal time and at an economically profitable interval after calving. A coordinated series of physiological events are the key to a successful reproduction, including the resumption of ovarian cyclicity postpartum, the development and ovulation of a viable oocyte, fertilization, restoration of the uterus, embryo development and implantation, and maintenance of pregnancy until foetal maturation (Butler, 2003; Garnsworthy and Webb, 2008).

Fertility is related to the parity of cows, the number of times that an animal has given birth. Fertility issues affect reproductive performance of the herd, thereby negatively influencing productivity and return on investment of the farming business. According to international standards, an interval calving-calving (IVV) of 12-13 months is considered an economically optimal goal. The level of oestrus detection and the conception rate are essential components affecting the IVV. An inaccurate detection of oestrus is associated to loss of profit due to extended IVV and milk loss (Roelofs et al., 2010). Galvão et al., (2013) concluded that the accuracy of ED and the compliance with injections for timed artificial insemination affected reproductive performance, with compliance having a greater impact.

The challenges for optimizing fertility in dairy cattle (Roche and Diskin, 2001; Wiltbank, Gümen and Sartori, 2002; Robinson et al., 2006) involve two heterogeneous factors: biological changes in dairy cattle, and changes in the management and the economic environment of the dairy industry (Rotz Zartman and Crandall, 2005; De Vries, 2006). The biological factor includes certain characteristics shared worldwide regarding the genetic improvement of modern high-yielding dairy cattle, as well as metabolic profiles and reproductive function (Jorritsma et al., 2003). However, the human and economic factor differs across nations, areas and individual herds, and management decisions have a significant impact on fertility based on the evolution of biological and economic demands (Gröhn and Rajala-Schultz, 2000; Evans et al., 2006; Roche, 2006).

There are a number of key areas for improving fertility management in dairy cattle, including: i) managing large volumes of data, ii) genetic selection (including improved phenotypes for use in breeding programs), iii) nutritional management (including transition cow management), iv) infectious disease control, v) reproductive management (and automated systems to improve

reproductive management), vi) ovulation / estrogen synchronization, vii) rapid diagnosis of the reproductive status, and viii) management of male fertility. However, the negative association between infertility and production/profitability is not universal (Bello Stevenson, and Tempelman, 2012). For example, Cummins et al., (2012a; 2012b; 2012c) reported divergent fertility phenotypes with similar milk production, and there is evidence of fertility improving following the inclusion of a fertility sub index (includes calving interval and survival to the subsequent lactation) in multiple countries' national breeding objectives. Increases in milk yield observed in dairying over the past 50 years has been escorted by a decline in cow fertility in multiple regions of the world and diverse production systems (VanRaden et al., 2004; Walsh Williams and Evans, 2011). Nevertheless, there is increasing evidence that this decline has ceased and a phenotypic improvement in cow fertility is now being observed (Diskin et al., 2016).

Reproductive management of dairy animals has experienced extensive progress in the past 50 years, from the creation of prostaglandin drugs (Prostaglandin F2 Alpha [PGF2a]) for the synchronization of oestrus in the 1970s to the implementation of on-farm in vitro embryo production programs and the use of genomic selection to aid in breeding strategies. In addition, the intensive use of sophisticated protocols to synchronise ovulation coupled with timed artificial insemination, has dramatically improved fertility in recent years (Carvalho et al., 2018). However, whether such protocols ultimately mask primary fertility issues which would be apparent in the absence of such protocols is unclear. This progress has been possible because reproductive efficiency has long been identified as critical for the profitability of dairy herds. Herds with efficient reproductive programs benefit from having a large proportion of cows in the most productive phase of lactation (Ferguson and Galligan, 1999), greater availability of replacement animals, greater genetic progress (Giordano et al., 2012; Norman et al., 2009), reduced proportion of reproduction culls(Pinedo De Vries and Webb, 2010; Galvão et al., 2013) reduced cost of reproductive programs (Giordano et al., 2012; Galvão et al., 2013), and improved health.

Pregnancy begins with the fertilization of an oocyte with a sperm cell. In many dairy systems, the first insemination is undertaken using artificial insemination (AI). Although there are differences in male fertility (Berry Evans and Mc Parland, 2011), the use of AI programs

focuses on the female and the events leading up to first ovulation, subsequent cyclicity, the capacity for fertilization and pregnancy establishment. The fact that cows are undergoing homeorhetic mechanisms to support an increase in milk production in early postpartum and are typically at peak lactation during the breeding period, has led to a large volume of research linking the physiological events controlling milk production with those that control the interval to first ovulation, cyclicity, and overall fertility.

The traditional view is that the postpartum interval to first ovulation is an important metric for reproduction(Petersson et al., 2007). This is certainly true and, therefore, it is reasonable to be concerned about non-cycling cows. Non-cycling cows that ovulate for the first time during the breeding period (either in response to synchronization or spontaneously) have compromised fertility (Thatcher and Wilcox, 1973). During the early postpartum period, the ovary is primarily dependent on luteinizing hormone (LH). LH is released from the pituitary gland in pulses, and the frequency of these pulses is a major determinant of ovarian function postpartum (Butler, 2000); greater frequency, on average, results in ovulation. Follicle-stimulating hormone (FSH), also released from the pituitary, is generally viewed as nonlimiting for ovarian follicular growth and ovulation in the early postpartum (Lamming Wathes and Peters, 1981; Crowe Diskin and Williams, 2014). Cows that are not cycling generally have elevated FSH concentrations because the primary hormonal negative-feedback mechanism involving the dominant follicle is suppressed (Crowe Diskin and Williams, 2014). Extended intervals between postpartum and first ovulation are normal even in healthy, well-fed cattle because of the suckling effect (prolactine negative feedback on ovulation) (Wright et al., 1990). In dairy cows, a prolonged anovulatory period is not a normal event; rather, it indicates a blockage (perhaps metabolic) in the restoration of the hypothalamic-pituitary-ovarian axis. In the past, negative energy balance was considered a key risk factor of the anovulatory syndrome (Butler Everett and Coppock, 1981). Although the role of negative energy balance is well accepted, recently the extent to which negative energy balance explains the variation in the interval to first ovulation has been questioned.

3. 1. Reproductive efficiency and dairy herd profitability

Economic efficiency of dairy farms is the main goal of farmers and reproduction continues to be a critical component to maintain a dairy farm economically viable. In fact, the competitiveness of dairy farms often depends more on the improvement of technology and efficiency than on the size of the farm (Cabrera et al., 2010). Reproduction can have a multitude of impacts on a farm, from altering culling policies, increasing retention of better replacements, moving primiparous cows into a more productive second lactation, and improving milk production. The income of dairy farms is mainly originated from milk sales (88% of gross income), cows for dairy purposes, culled animals and calves (Santos et al., 2010). There are four ways to increase the volume of milk produced by a cow per day in a dairy herd:

1) By carrying out a genetic selection, based mainly on selection of individuals and artificial insemination
2) By improving nutrition
3) By better controlling diseases and management factors that reduce the yield (i.e. mastitis, metritis, heat stress)
4) By increasing the reproductive efficiency

Reproduction affects about 10% of gross farm income, the gross margin per cow is maximized when the herd's gestation rate (gestation efficiency) is greater than 30%. Gestation efficiency of a herd is established according to the gestation rate, which is calculated by multiplying the heat detection rate by the conception rate (Ferguson, 2003). Santos JEP, (2008) reported that four main factors affected the reproductive efficiency in dairy herds and were commonly monitored to evaluate reproduction: the voluntary waiting period, insemination rate, pregnancy per AI, and pregnancy loss. Shortening the IVV reduces the average days in milk of the herd and, consequently, a greater proportion of cows would be in earlier stages of lactation, when peak of milk production and greater IOFC occurs, whereas a smaller proportion of cows would be in later stages of lactation producing low amounts of milk with low IOFC (Ribeiro, et al., 2012).

Low oestrous detection rates result in low pregnancy rates and a large variation in age at first pregnancy and age at first calving, which are economically undesired (Santos JEP, 2008).

Diseases are also known to deeply reduce the income of a dairy cattle herd, for example subclinical ketosis in dairy cows reduces the productivity and therefore the efficiency of milk production and the profitability of the dairy farm (Mostert et al., 2018). Moreover, SCK is associated with an increased risk of displaced abomasum, metritis, mastitis, lameness and clinical ketosis among others (Suthar et al., 2013). $289 per case of SCK in relation only with abomasum displacement, metritis and ovarian dysfunction (McArt Nydam and Oetzel, 2012). According to the study of Mostert et al., (2018), the total cost of subclinical ketosis in dairy cows was 130€ per case per year, and varied from 83€ in parity one to 175€ in parity three. Costs were derived from a prolonged IVV (36%), from reduced milk production (24%), from treatment (19%), from discarded milk (14%) and from removal (6%). Estimating the economic impact of diseases may make farmers more aware of these problems, and can improve their decision-making regarding interventions to reduce illnesses. In Bejaia (North centre Algeria), Bellil and Boukrif, (2015) identified four systems of production according to a set of discriminative factors, two specialized in milk and two producers of both meat and milk. They report that the average cost of milk production by system was of 46.09, 50.80, 50.28 and 55.72 DA for systems 1, 2, 3 and 4 respectively, and was often greater than the sale price of milk (47 DA/litre). However, the sale of milk doesn't always constitute the only source of product and income.

I. 3. 2. Control of infectious diseases

Veterinarians managing fertility in dairy herds should regularly evaluate the herd health status for pathogens known to compromise reproductive efficiency. Infectious diseases are of increasing concern on dairy farms because of their potential impact on animal and human health, milk and meat production, food safety, and economics. Moreover, dairy farms are recognized as important reservoirs of foodborne pathogens.

Dairy cows are susceptible to production disorders and diseases during the peripartum period and early lactation (Roche et al., 2013; Bouamra Ghozlane and Ghozlane, 2016). In cattle, bacterial contamination of the uterus is ubiquitous at parturition. However, this does not automatically imply the establishment of uterine disease and subsequent fertility problems. It is generally a suppression in uterine immune function in addition to pathogen presence that

allows a shift in bacterial populations and establishment of disease in up to 20% of animals (Crowe and Williams, 2012).

Some pathogens are known to reduce conception rates while others may cause foetal losses and abortions. These diseases are known to have a significant effect on dairy production due to their effects on fertility (Walz et al., 2015; Bouamra Ghozlane and Ghozlane, 2016), milk production (McAloon et al., 2016), and, subsequently, culling (Smith et al., 2010). To implement appropriate and effective disease control programs at the national level, up-to-date and unbiased information on disease frequency is needed in Algeria. Control programs should be accompanied by continuous monitoring of herd status to assess the effectiveness of the program and progress towards goals. This can be achieved through serological testing for different infectious agents at the herd level (Houe Lindberg and Moennig, 2006). Testing of bulk milk samples is a particularly cost-effective strategy and has become part of surveillance and disease-control programs for several endemic infectious diseases of dairy cattle (Booth Cranwell and Brownlie, 2013). The application of a suitable disease control or elimination programs and monitoring at a national or a regional level should be based on knowledge of the baseline frequency and distribution of the disease in the population (Sayers et al., 2015).

I. 4. MAIN ABORTIVE INFECTIOUS AGENTS IN CATTLE IN THE MEDITERRAENAN BASIN

Abortion among dairy cows is one of the major causes of economic losses in the cattle industry (El-Tarabany, 2015). In many studies, only 30% or less of high-producing lactating cows calve following a single AI service. However, this rate can vary substantially based on environmental, genetic, and management conditions. The worldwide reported rate of abortion in dairy cows varies from 12% to 16% depending on the stage of gestation when pregnancies are diagnosed (Schlafer Fisher and Davies, 2000; Thurmond et al., 2005). Abortions may be idiopathic or the result of metabolic or hormonal abnormalities, nutritional deficiencies, trauma, toxicities, or infectious agents. The latter represents the leading aetiology of reproductive disorders (Givens, 2006; Ortega-Mora, 2007). Diagnostic rates in ruminant abortions are low worldwide, reaching approximately 50% of the cases (John Matthews, 2016). Nevertheless, diagnosis of the aetiological agent has improved over time, from about 33–37% in the 1990s (Jamaluddin et al., 2016), to 44% in the 2000s (Anderson, 2007) and 58% (Clothier and Anderson, 2016) in 2019, but only if a full range of samples are collected. An accurate and prompt diagnosis of abortive infectious agents in a herd requires cooperation between the herd veterinarian and a veterinary diagnostic laboratory. Combined efforts, good communication and appropriate sampling and testing approaches, greatly improves the chance of obtaining an aetiologic diagnosis (Anderson, 2007).

A significant proportion of embryonic loss in dairy cows occurs quite early after conception. Wiltbank et al. (2016) described four pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows, each corresponding to key physiological changes in the embryo, uterine environment, and ovary. These are: (i) during the first week after calving due to fertilization failure or death of the early embryo (20%-50%); (ii) from day 8 to 27, encompassing embryo elongation and maternal recognition of pregnancy with losses averaging 30%, but ranging from 25%-41%; (iii) from day 28 to 60, with losses of approximately 12%; and (iv) during the third month of pregnancy (∼2%). Pregnancy loss per day generally decreases as pregnancy progresses and is much lower after day 60 of pregnancy (Santos Rutigliano and Sá Filho, 2009; Diskin et al., 2016).

Analogous to many countries, abortion is a major problem for dairy producers in Algeria. Abortion, decreased calving percentage, stillbirths (i.e. expulsion of the foetus after day 260 of gestation), birth of weak calves and decreased milk production often leads to high economic losses for the farmer. Beyond to loss of foetus, abortion increases the number of AI required for obtaining a calve, imposes rebreeding costs, medical treatment costs and replacement costs to farmers (Peter, 2000; Weigel Palmer and Caraviello, 2003). Abortion in cattle was defined as foetal death between days 42 and 260 of pregnancy by Peter et al. (Peter, 2000). Similarly, Thurmond and Picanso, (1990) defined abortion as foetal death between 52 and 260 days in pregnancy and reported an abortion rate of 11% with losses of about 640 US$ per abortion. Norman et al., (2012) considered abortion cases only for cows with more than 150 days into pregnancy and reported an abortion rate of 1.3%. According to Eicker and Fetrow, (2003), the main factors affecting the value of a pregnancy are: cow parity, milk production level, persistence of lactation, breeding and replacement systems; which resulted in an average value of 200 USD. Gädicke Vidal and Monti, (2010) estimated that total net revenue for a lactation with abortion showed a mean loss of -143.32 USD in Chile.

Effects of abortions on profit may be greater in natural service since the open cow may not be identified until months after the abortion. The reproductive potential of these cows is lost for the year, resulting in early culling and associated replacement costs (BonDurant, 2005). Costs to the producer can be as high 1,900 USD per abortion based on stage of pregnancy, cow performance, current prices, and producer decisions (De Vries, 2006; Norman et al., 2012). Iran these costs have been found to vary significantly, ranging from 82 USD to 1,302 USD (Kalantari et al.,2008). Finally, late-term abortions have been estimated to cost between 500 USD and 900 USD per case (Hovingh, 2002; Kirk, 2003) and often result in early culling of productive cows for an additional loss of up to 1,000 USD (Kirk, 2003).

Many factors influence the viability of a bovine foetus during gestation, including hormonal fluctuations, genetics, compromised blood, nutrient or oxygen supply to the foetus, and exposure to pharmacologic, environmental, toxic, or infectious agents at critical times of gestation (Cabell, 2007; Evans, 2011; 2012). Causes of abortion may be either infectious or non-infections. Although non-infectious causes have gained more attention during recent years, e.g. the identification of lethal haplotypes (Charlier et al., 2016; Adams et al., 2016), infections

are generally thought to have a greater abortive potential and, thus, are considered more important.. Furthermore, infections agents are traditionally more readily diagnosed than non-infectious causes (Clothier and Anderson, 2016; Reichel Wahl and Hill, 2018). Borel et al., (2014) reviewed the most common and relevant abortive pathogens of cattle in Europe highlighting their epizootic and zoonotic potential (Table 1). Similarly, Reichel Wahl and Hill, (2018) evaluated the most important abortive pathogens of cattle in Australia and New Zealand (Figure 4).

Table 1. Overview of infectious causes of abortion in cattle in Europe (Borel et al., 2014)

Infectious agent	Agent name	Epidemiology	Time of abortion
Viruses	Bovine herpesvirus type-1	++, dt, epi.	
	Pestiviruses	++ a, dt, epi and vt	Early embryo loss
	Bluetongue virus	+, vb, enz	Second to third trimester
	Schmallenberg virus	++, vb, enz	
Bacteria	*Brucella* spp.	++, dt, epi, zoo	Second to third trimester
	Chlamydia abortus	+, dt, epi, zoo	Third trimester
	Coxiella burnetii	++, dt, epi, zoo	Second to third trimester
	Salmonella Abortusovis	-	Third trimester
	Miscellaneous bacteria	+	Second to third trimester
Parasites	*Neospora caninum*	++, ih and vt	3–8 months, usually 5 months
	Toxoplasma gondii	-	
	Tritrichomonas foetus	+	Early embryonic losses
Fungi	*Aspergillus fumigatus*	+	4 months to term

++, important in this species; +, occasional cause in this species; -, of unknown significance in this species. **epi**, epizootic; **enz**, enzootic; **zoo**, zoonotic; **vb**, vector borne; **dt**, direct transmission; **ih**, intermediate host; **vt**, vertical transmission

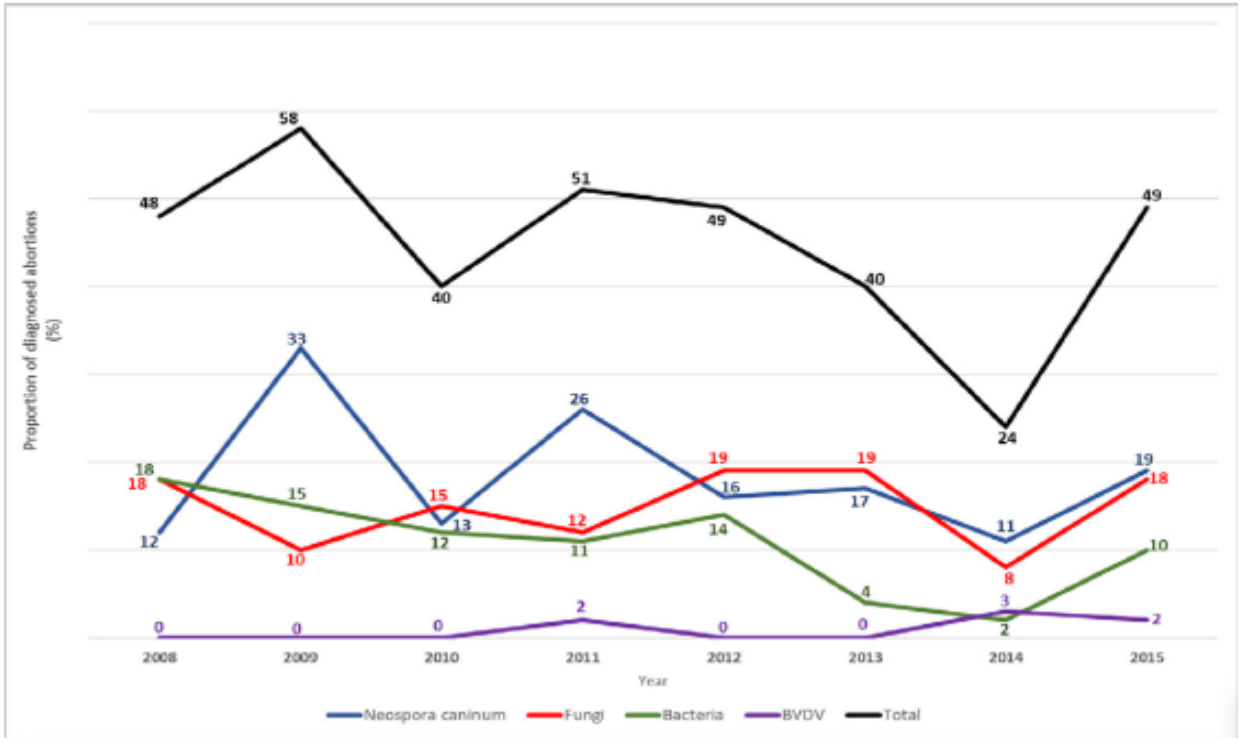


Figure 3. Percentage of aetiological diagnoses made per year, in 544 bovine abortion cases investigated at one veterinary diagnostic laboratory in New Zealand (Reitchel et al. 2018)

Abortion caused by infectious agents in ruminants is a major cause of economic losses worldwide. Effective management and control of outbreaks of abortive disease is essential in limiting their spread, and in preventing zoonotic infections. An increase in the number of spontaneous abortions in a herd is a dramatic event for the farmer involved. A range of enzootic, epizootic, emerging diseases and/or zoonotic diseases may cause abortions in cattle. Regarding breeding efficiency, abortion causes a larger IVV, hampering the achievement of the full genetic potential. Moreover, late abortion increases premature culling, generating increased replacement costs.

The mechanism of transplacental transmission has not been completely defined for many pathogens. However, there is evidence to suggest that placental macrophages may contribute to transmission of bacteria and fungi (Schlafer Fisher and Davies, 2000). Foetal response to infection depends on the stage of gestation when infection first occurs. In the first trimester, when the foetus has no effective immune system, infectious agents can directly kill foetal cells (Maley et al., 2006). At this stage, if the foetus continues its development, the calf may be born immunotolerant for the infectious agent, as in the case of BVDV. As the foetus develops, the immune system response becomes more complete. For example, from day 98 of gestation onwards, the foetus is capable of mounting an IgG immune response against *N. caninum* (Bartley et al., 2013). Within a few more days, developed bovine foetal lymphocytes are capable of mitogenic stimulation and the production of IL-2 (Bartley et al., 2013). Once the immune system has matured, infection may be controlled and cleared, or, conversely, the products of inflammation may negatively affect the foetus and even lead to foetal death (Srinivas et al., 2006; Kraus et al., 2012).

In order to prevent the allogeneic rejection of the embryo, the maternal immune system is diminished during pregnancy. Meanwhile, the foetal immune system only begins to develop during the second trimester. This maternal immunosuppression, combined with the initial state of foetal immune status, offers pathogens the ability to infect and grow uncontrollably. Infection triggers the release of prostaglandins during the inflammatory response, resulting in luteolysis and a cascade of events that lead to foetal expulsion (Neuvians et al., 2004; Skarzynski, Jaroszewski and Okuda, 2005). Once the foetus dies, the placental circulation collapses and becomes obliterated, characterized by intra-placental coagulation and endothelial disturbances

(Ornoy Crone and Altshuler, 1976). The separation of cotyledons from caruncles results in the termination of pregnancy. The foetus and foetal membranes are expelled, which is manifested by an abortion (Roescher et al., 2014).

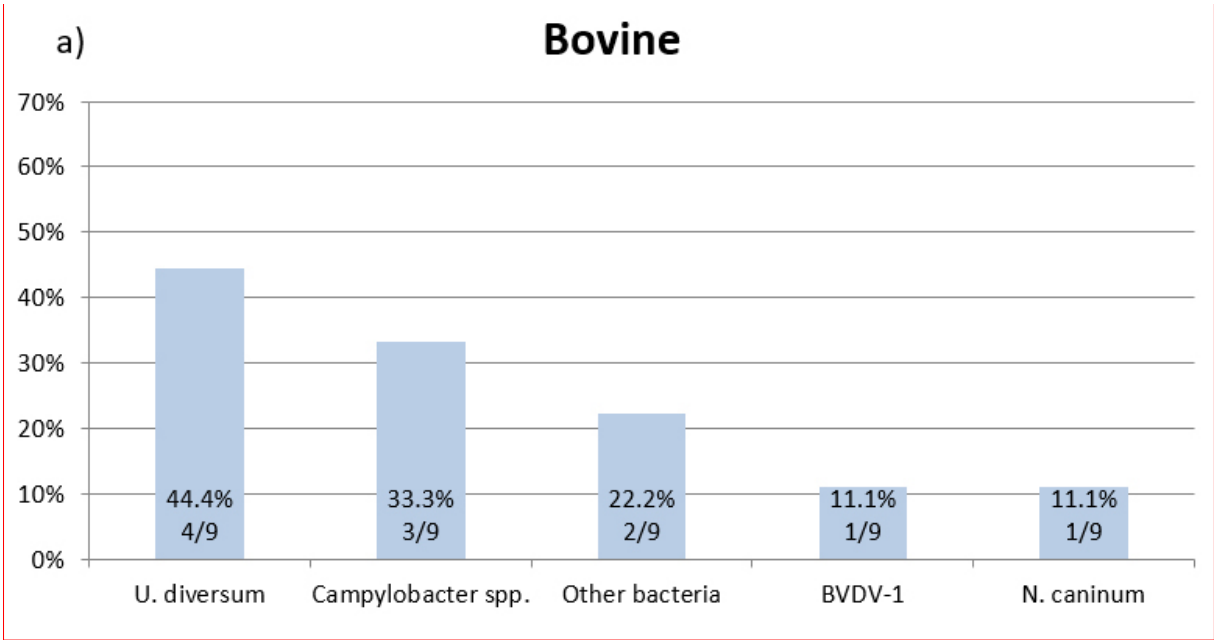


Figure 4. Frequency of diagnosed pathogens in cattle in relation to the total diagnosed cases, Ureaplasma diversum, Campylobacter spp, Bovine Viral Diarrhea Virus and *Neospora caninum*. Some cases had a mixed diagnosis (Jose Díaz-Cao et al., 2018).

The Mediterranean basin has an heterogeneneous scenario regarding the circulation of abortive pathogens in domestic ruminants. In France, monitoring bovine abortions is required for the surveillance of diseases such as Rift Valley Fever, Q fever or neosporosis (Anderson, 2007), especially when they are zoonotic. In the same country, the current bovine abortion surveillance system is designed to detect as early as possible any resurgence of bovine brucellosis (Fediaevsky and Garin-bastuji, 2011). It relies on the mandatory notification and testing of each and every aborting cow. Moreover, when at least three cows have aborted, the farmer can benefit from differential abortion diagnosis protocols developed by the animal health groupings (*Groupements de Défense Sanitaire*, GDS) to help identify whether the abortions could be linked to an enzootic disease.

In Turkey, circulation of several abortive pathogens has been detected in cattle.Yağcı Yücel et al., (2014) reported seropositiviy against *Toxoplasma gondii* (56.06%; n=132), *Listeria monocytogenes* (40,09%; n=132), and *Brucella abortus* (3.03%; n=132) in the region of Adana,

by the standard Sabin-Feldman Dye Test (SFDT), Osebold method and Microtube Agglutination Test (MAT) respectively (Yağcı Yücel et al., 2014). Also, Ozbek et al., (2008), detected a seroprevalence of 21.7% (n=23) against *Chlamydia trachomatis* using three diagnostic techniques (i.e. giemsa, immunoperoxidase and lugol stainings). Epidemic abortions caused by *Neospora caninum* were also reported by Kul et al., (2009).

In Greece, Lefkaditis et al., (2020) detected a 21.03% (n=875) of seropositivity against *N. caninum* in Holstein-Friesian dairy cows using the indirect fluorescent antibody technique. Positive farms were associated to previous history of infertility problems, such as abortions, increased number of AI services needed for conception, increased rate of returning to oestrus and retention of foetal membranes. In 2005, Billinis et al., (2005) estimated the prevalence of Bovine Viral Diahrroea Virus (BVDV) in Greece, using an antigen ELISA. Mean prevalences, adjusted for the test's accuracy and the herd-clustering effect, were 14% (95%CI: 11–18%) and 1.3% (0.8–1.8%), respectively. Herd size was not associated with the prevalence of antigen-positive or persistentily infected (PI) animals.

In Egypt, antibodies against *Pestivirus* were detected in goats with a prevalence between 33-72% (Løken Krogsrud and Bjerkås, 1991). One of nine cows aborted a *Pestivirus*-infected foetus, and all were antibody-positive. Selim et al., (2019) reported a seroprevalence of *Brucella* spp. of 16.7% and 16.25% in cattle and sheep, respectively. There was a significant association ($P < 0.05$) between the seroprevalence of brucellosis and sex in cattle and age in sheep level, where seroprevalence was 18.7% in female cattle and 22% in sheep over 2 years. In Egypt (Ahmed et al. 2019), 41.4% (94/127) of serum samples from cattle tested by a BTV ELISA were positive for bluetongue virus (BTV) antibodies. Of these 94 ELISA-positive cattle, only 83 EDTA-blood samples were available and were tested for BTV and epizootic haemorrhagic disease virus (EHDV) genome detection by RT-PCR and sequencing. In Egypt, Fereig et al., (2016) established a comprehensive record of the seroprevalence of *T. gondii*-specific antibodies using several animal hosts at different locations. The prevalence of antibodies was 38.7% in sheep, 28.7% in goats, 23.6% in cattle, and 22.6% in donkeys. The authors, also identified the risk factors associated with toxoplasmosis, using a cross-sectional epidemiological study.

In Tunisia, Amdouni et al., (2019) estimated the molecular prevalence of *T. gondii* infection in meat from slaughtered sheep, goats and cattle in Northwest Tunisia (Béja district). The overall molecular prevalence of *T. gondii* in sheep, goats and cattle was 33.3% (50/150), 32.5% (39/120) and 19.3% (29/150), respectively. During 2015, the incidence of clinical human brucellosis was estimated to 30.8 per 100,000 inhabitants affecting mainly males aged between 30 and 39 years. The overall animal seropositivity to *Brucella*, was 21 and 1.9% in case and control farms, respectively ($p < 0.0001$). Only five risk factors were found to be significant: overall animal seropositivity (OR = 65.2; 95%CI: 13.3–318.7); handling aborted females (OR = 43.1; 95%CI: 8.3–222.7); presence of male ruminants in the herds (OR = 18.5; 95%CI: 5.18–66) (Khamassi Khbou et al., 2017). Selmi et al., (2018) tested sera of healthy camels to detect antibodies againts *Coxiella burnetti* using an indirect ELISA and reported an overall prevalence of 44% (n=534). A meaningful high seropositivity was observed in female camels with a previous history of abortion (70%) (OR = 4.186, 95%CI: 2.05–8.51). *Listeria spp.* prevalence was also studied in 1134 samples from 378 Tunisian ruminants using PCR and it was detected in 5.7% of cattle and 10.2% of sheep (Barkallah et al., 2016). In addition, the true herd-level prevalence was 50.1% in cattle and 26.7% in sheep. At the herd level, risk factors for *Listeria spp.* test-positivity were abortion, herd composition and silage storage for cattle.

In Morocco, (Lucchese et al., 2016) 221 cattle sera from 25 farms were examined for the presence of *Brucella spp.* antibodies, 176 for *N. caninum*, 88 for *Leptospira spp.*, and 42 for BVDV, Bovine Herpesvirus 1 (BHV-1), and Bovine Herpesvirus 4 (BHV-4) (at least 1 sample per herd). Antibodies against the investigated pathogens were detected in all samples tested, with an overall seroprevalence of 33.48% for *Brucella spp.*, 8.52% for *N. caninum*, 9.09% for *Leptospira spp.*, 37.71% for BVDV, 50% for BHV-1 and 9.52% for BHV-4. Antibodies against *Leptospira spp.* serovars Hardjo, Pomona, and Tarassovi were identified and mixed infections were common. Additionally, abortions were reported in 23 (10.4%) of the 221 tested cattle. In Sidi Kacem Province in Morocco, cattle and small ruminant sera were tested for *Brucella spp.* antibodies using the standard Rose Bengal Test (sRBT) and the modified Rose Bengal Test (mRBT). The prevalence in cattle at individual and herd level was 1.9% and 9%, respectively. Bacteriology was also performed on 21 milk samples obtained from *Brucella*-seropositive cattle. for isolation and phenotyping of circulating *Brucella* species. Culture was positive for

three milk samples and *B. abortus* biovar 1was identified using Bruceladder® multiplex PCR and classical phenotyping.

In Israel, 58,048 pregnancies from 111 herds were studied in 1995 and the abortion density, the proportion of aborted cows, and the abortions per confirmed pregnancy were 4.2%, 5.9%, and 10.2%, respectively (Markusfeld-Nir, 1997). Seroprevalence of *N. caninum* antibodies has been reported at 35.5% in a sample of 1,078 pregnant cows (Mazuz et al., 2014). The percentage of abortions in seropositive cows was 3 times higher than in their seronegative counterparts (21.6 and 7.3%, respectively). No statistically significant association was found between the antibody level of positive cows during pregnancy and the proportion of aborting cows. However, 41.2% of the cows with antibody titers of 1:12,800 aborted. The risk of abortion for such animals was 2.7 times higher than for other seropositive cows which had lower titers of antibodies ($p=0.0072$). In the follow-up examinations of the seropositive cows during several pregnancies, the overall percent of abortions observed was significantly higher than in seronegative individuals (49.3 and 16.9%, respectively; $p<0.0001$). Moreover, the proportion of repetitive abortion observed was 5 to 1 (17.4 and 3.5%) in seropositive and seronegative dams, respectively ($p<0.001$). The rate of vertical transmission in positive dams was 61.0% and it appeared to be directly associated to antibody levels: the higher the titter in the cows during pregnancy, the higher the percentage of sero-positivity in their calves. Increased proportion of abortions was observed in seropositive cows both in summer and winter in comparison with spring and autumn. It was found that in seropositive cows, an increased number of pregnancies, which was directly related to the age of the dam, has been associated with an increased number of abortions.

I. 4. 1. Neospora caninum

Neospora caninum is an apicomplexan protozoan parasite that can cause neosporosis in cattle after consuming food or water contaminated with oocysts shed in canine faeces (Dubey et al., 2007; Taylor Coop et Wall, 2013). In many countries, *N. caninum* is the most frequently diagnosed cause of bovine abortion (Thilsted and Dubey, 1989; Dubey and Lindsay, 1996) and has been associated with epidemic and endemic patterns of abortion and congenitally infected calves with malformations (Dubey et al., 2017).

The biological cycle of *Neospora caninum* is heteroxenous. Dog (*Canis lupus familiaris*), coyote (*Canis latrans*), dingo (*Canis lupus dingo*) and grey wolf (*Canis lupus*) are the only species recognized as definitive hosts of *N. caninum*, in which the sexual phase of the cycle occurs, resulting in the shedding of oocysts in faeces (McAllister et al., 1998; Gondim et al., 2004; Dubey et al., 2011).

Neospora caninum can be transmitted postnatally (horizontally, laterally) by ingestion of tissues infected with tachyzoites or tissue cysts or by ingestion of food or drinking water contaminated by sporulated oocysts. The infective form for intermediate hosts is the sporulated oocyst, which is released in the faeces of definitive hosts (Figure 5). Transplacental (vertical, congenital) transmission, from an infected dam to the foetus, can also occur during pregnancy (Figure 5) (Dubey Schares and Ortega-Mora, 2007). In fact, vertical transmission accounts for 50–95% of infections and is the main route of transmission in intermediate hosts (Cardoso et al., 2012; Almería and López-Gatius, 2015), playing an important role in the continuation of the pathology in cattle herds (Santolaria et al., 2011).

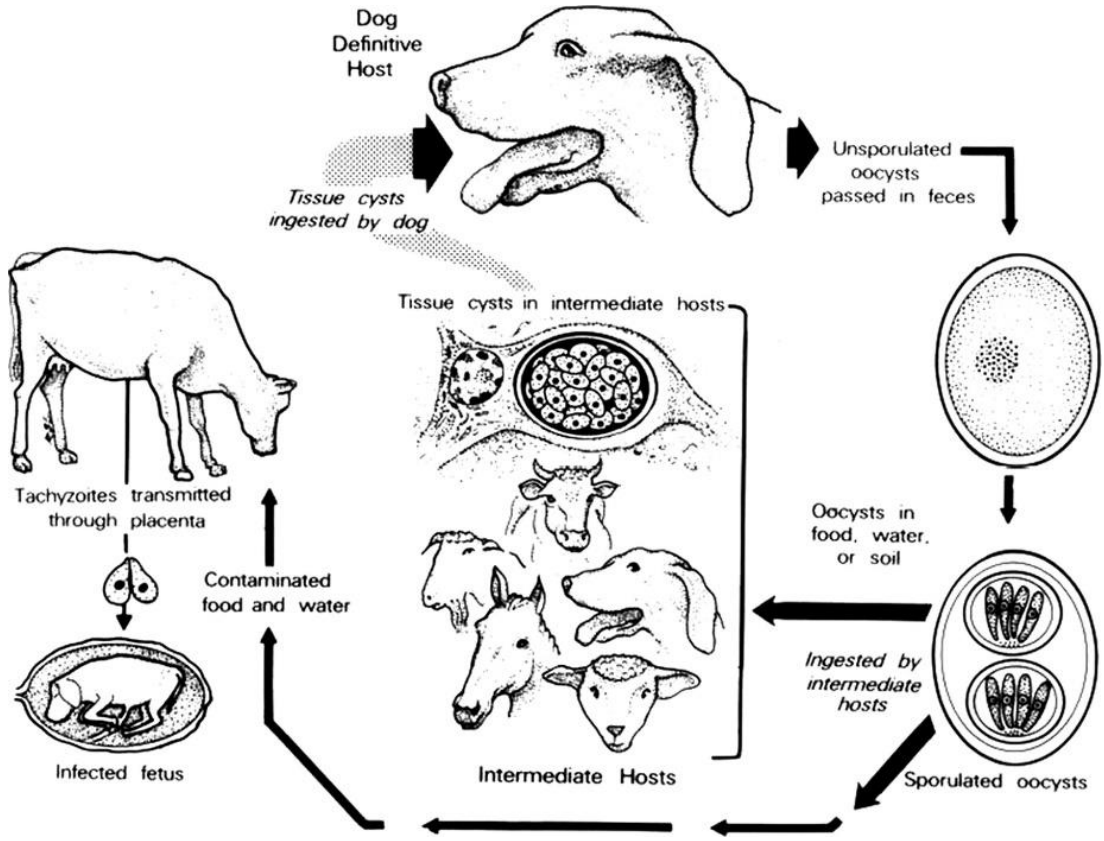


Figure 5. Life cycle of *Neospora caninum* (Dubey 1999)

The routine diagnosis of bovine neosporosis is based on detection of *Neospora caninum* specific antibodies in blood samples and milk. Although the detection of antibodies only indicates exposure to *N. caninum* (Dubey and Schares, 2006), since the first isolation of the parasite, a range of serological assays have been developed in dogs, cattle, and a variety of other potential host species.

At present, the strategies to control the presence of *N. caninum* in cattle are based on herd management and diagnosis (Dubey Schares and Ortega-Mora, 2007). The best control strategy for neosporosis at farm level is the serology in order to minimize vertical transmission by selective breeding and limiting horizontal transmission (to intermediate and definitive hosts) through application of hygienic disposal procedures for elimination of aborted foetal and maternal tissues.

Serological techniques are primarily employed to detect specific antibodies against *N. caninum* to differentiate exposed from non-exposed dams. Tachyzoites are the active form of the *N. caninum* parasite, representing the acute phase of the disease (Goodswen Kennedy and Ellis, 2013). Tachyzoites differentiate into bradyzoites, which characterize the chronic phase of the disease (Jiménez-Ruiz et al., 2012). All the serological assays are based on tachyzoite antigens (Dubey and Schares, 2006). These techniques include the several enzyme-linked immunosorbent assays (ELISAs), the indirect fluorescent antibody test (IFAT), *N. caninum* agglutination test (NAT), and western immunoblotting which is often used to confirm uncertain results in valuable samples (Álvarez-García et al., 2002). Although IFAT using whole fixed tachyzoites is the most reliable serological test for the detection of *N. caninum* antibodies, high cost and the need for specialized equipment and expertise have limited its use (Guido et al., 2016). The iELISA against recombinant antigens is a common serological test for the detection of *N. caninum* antibodies.

Polymerase chain reaction (PCR) is also used for the detection of DNA of the parasite. It has the advantage of being a quick, sensitive, and specific technique, but it still has high cost (Tramuta et al., 2011). The target samples to detect *N. caninum* DNA are brain or tissues from aborted animals (Wilkowsky et al., 2011). PCR can be applied for the diagnosis, protozoan DNA quantification, and identification of new hosts for the parasite (Dubey and Schares, 2011).

Various PCR formats have been developed, including real-time PCR (Pereira et al., 2014), nested PCR (J et al., 2014), and multiplex PCR (Tramuta et al., 2011), and can be used on aborted foetal tissue, amniotic or cerebrospinal fluid, blood, faeces, milk, semen, etc (Pereira et al., 2014).

Since the protozoan is closely associated with domestic dogs, humans do occasionally suffer from exposure; however, the disease is not considered a zoonosis (Dubey and Schares, 2011). Immunocompromised patients may become opportunistic hosts for the pathogen; thus, the disease emerges in this population (Oshiro et al., 2015).

Although neosporosis has been diagnosed in the main dairy and beef cattle producing countries, few data have been published in the literature about the disease in the Maghreb (Lucchese et al., 2016). In Algeria, the scarce reports available on its occurrence indicate that seroprevalence in cattle ranges from 12.37% to 19.64% (Ghalmi et al., 2012; Achour et al., 2012; Derdour et al., 2017).

Neospora caninum abortion process

Abortions caused by *N. caninum* can occur during all the gestation (Dubey Schares and Ortega-Mora, 2007; Dubey et al., 2017). *Neospora caninum* multiplication in the placenta induces cell death and causes abortion through the production of cytokines that are harmful for the maintenance of pregnancy. Cytokines are secreted locally and allow the producing cell to exert a powerful local effect on other cells of lymphoid and non-lymphoid origin, and hormonal regulation. It has also been suggested that placental infection and inflammation may trigger prostaglandin-induced luteolysis causing premature uterine contraction and foetal expulsion (Dubey et al., 2017). Different clinical consequences of bovine neosporosis can be observed depending on whether the infection occurs prior to conception or post-conception to birth or post-natally. Infection of dams during gestation results in either abortion or persistently infected (PI) calves (caused by exogenous transplacental transmission) (Figure 6). However, these infected dams only rarely transmit *N. caninum* to future progenies in successive gestations. Cows infected with *N. caninum* prior to gestation may give birth to seronegative calves without evidence of *N. caninum* infection (Dubey et al., 2017). Therefore, these non-pregnant infected animals can clear the infection and develop immunity that protects against abortion or

transmission to successive generations. On the other hand, endogenous transplacental transmission occurs as the result of reactivation of an existing persistent infection within a cow during pregnancy (Figure 6) and can cause abortion or transmission of the infection to successive progeny (Almería Serrano-Pérez and López-Gatius, 2017). Thus, cattle infected during gestation and cattle PI do not easily develop effective immunity to the parasite (Almeria et al., 2003).

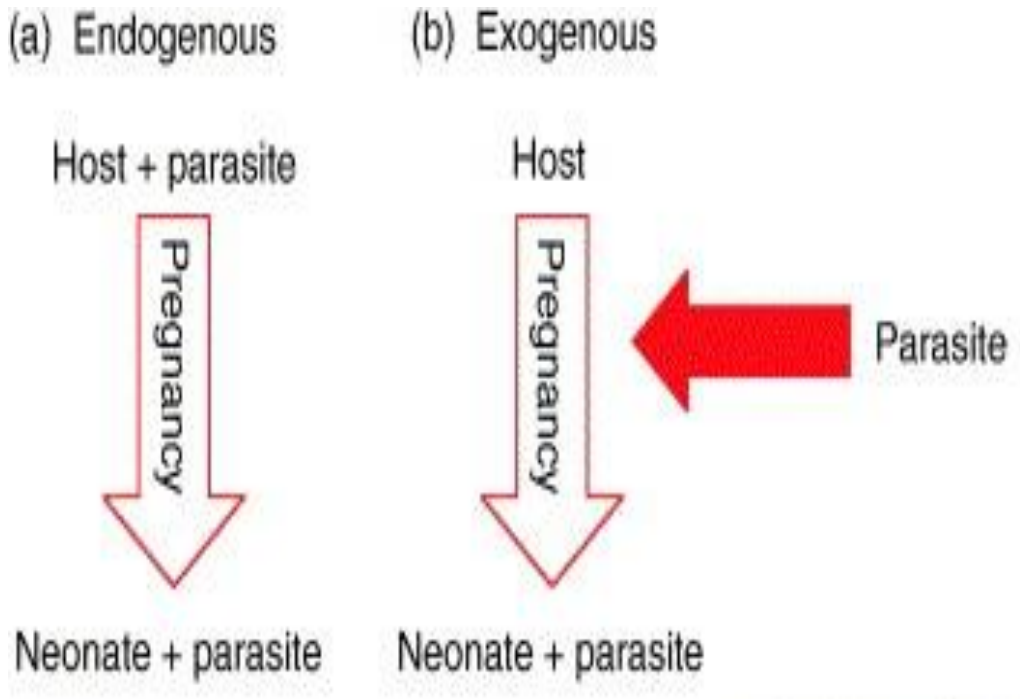


Figure 6. Host–parasite relationship and pregnancy. Image shows the difference between (a) endogenous and (b) exogenous transplacental infection, as defined in the main text (Trees and Williams, 2005).

I. 4. 2. Toxoplasma gondii

Toxoplasma gondii is a zoonotic intracellular protozoan parasite of worldwide distribution. Wild and domestic felids are the definitive hosts and, therefore, are the only known hosts that excrete oocysts in faeces (Tenter Heckeroth and Weiss, 2000). Humans and virtually all warm-blooded species, including birds, can be intermediate hosts and become infected by the ingestion of food and water contaminated with sporulated *T. gondii* oocysts, by consumption of tissue cysts in infected animal tissues, or congenitally (De Marez et al., 1999; Hill and Dubey, 2002; Tenter Heckeroth and Weiss, 2000),. Transmission can also occur via tachyzoites present in blood products, organ transplants, or unpasteurized milk able to infect all warm-blooded animals, including humans (Figure 7).

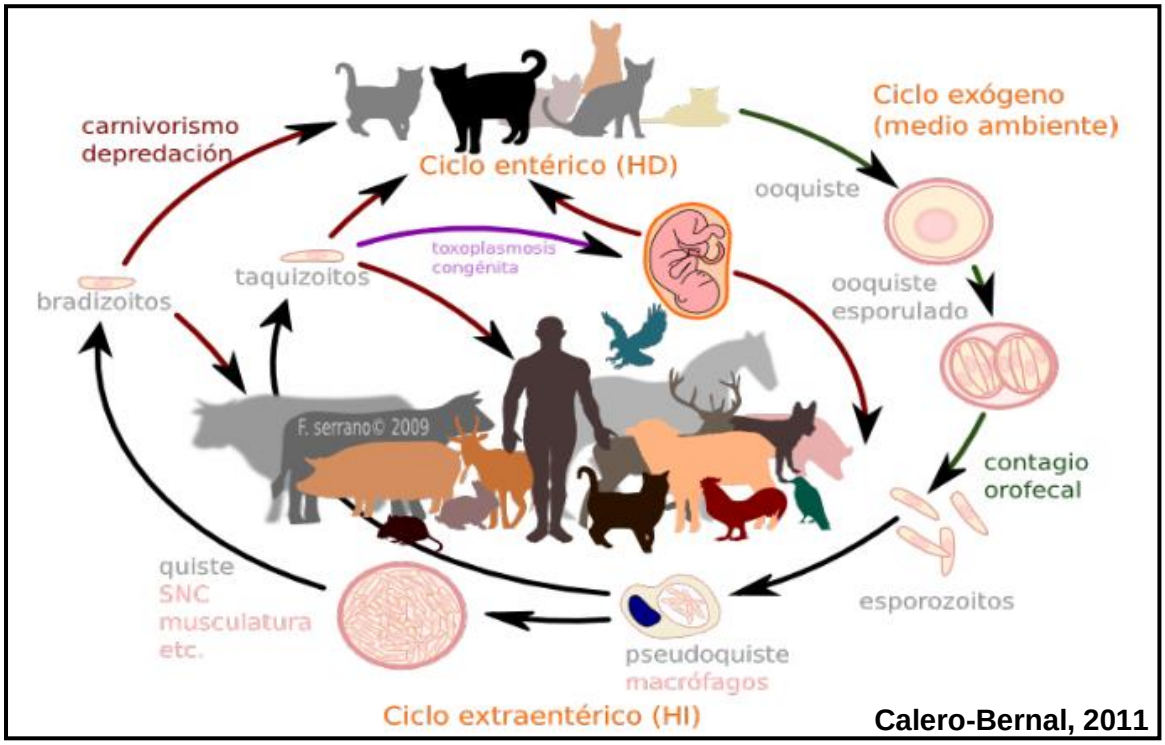


Figure 7. The life cycle of *Toxoplasma gondii* [Calero-Bernal, 2011].

Infection with *T. gondii* can induce embryonic resorption, mummification, abortions, neonatal deaths or birth of weak and non-viable new-borns.

Toxoplasma gondii distribution varies widely according to species, farms and countries. Sheep, goats and pigs are the most sensitive species, recording the highest seroprevalences and constituting a potential risk to humans (Tenter Heckeroth and Weiss, 2000; Dumetre et al., 2006; Opsteegh et al., 2011). Although the detection of *T. gondii* in bovine tissues is rare, consumption of raw or undercooked beef might be an important source of human infection (Said et al., 2017). In addition, based on quantitative risk assessment, beef was predicted to be the most important source of meat borne infections in the Netherlands and Italy (Belluco Patuzzi and Ricci, 2018).

A variety of serological tests, such as dye test (DT), modified agglutination test (MAT), ELISA, immunosorbent agglutination assay (ISAGA), IFAT and indirect hemagglutination assays

(IHA), have been developed to detect different antibody classes or antigens. The dye test proved highly sensitive and accurate, but may be unreliable in cattle and avian species (Dubey et al., 1993). The major disadvantage of the DT is the requirement of live tachyzoites and the high potential risk to laboratory staff conducting the test (Reiter-Owona et al., 1999). The gold standard method for isolating the parasite is the mouse bioassay, which may increase the sensitivity of *T. gondii* detection in infected cattle (Burrells et al., 2018).

Several immunofluorescence and ELISA tests have been developed. The ELISA method usually includes the solid phase antigen or antibody, enzyme labelled antigen or antibody, and the substrate of the enzyme reaction, which can be modified to test both antibodies and antigens. Different types of ELISA have been developed to detect *T. gondii* antibodies or antigens, such as indirect ELISA and sandwich ELISA (Figure 8).

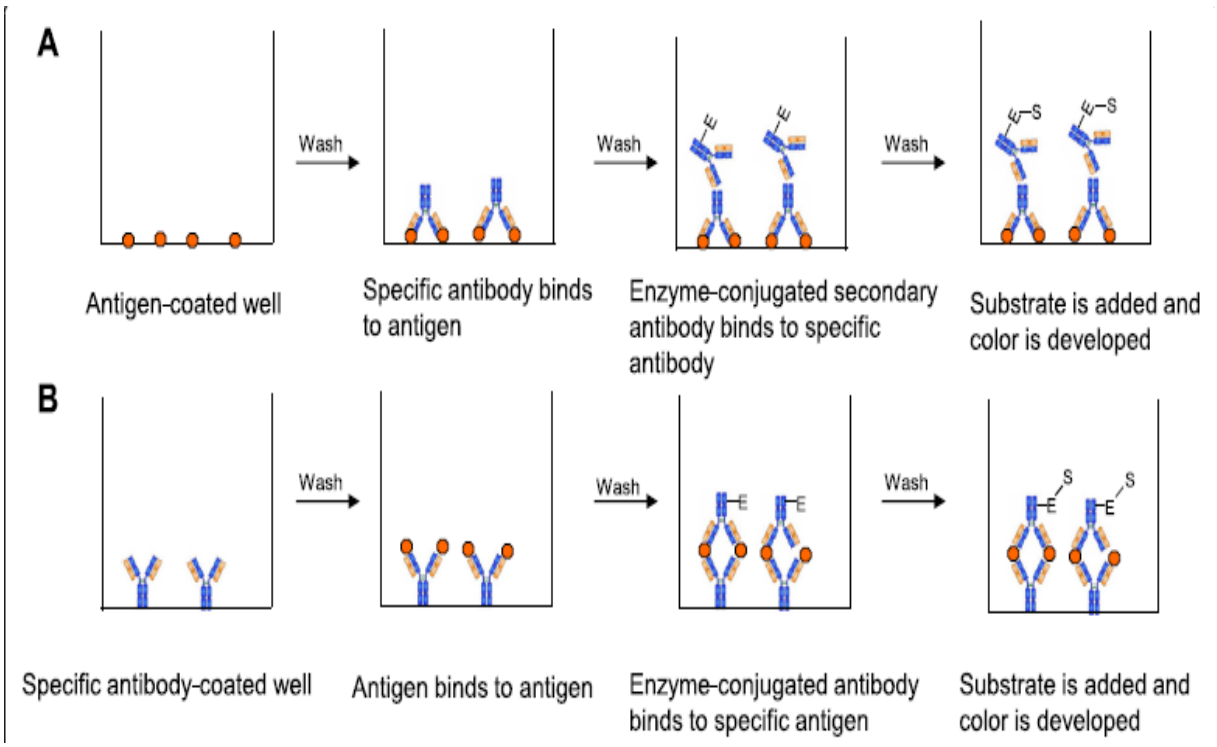


Figure 8. Schematic diagram of ELISA (Liu et al., 2015). A. Indirect ELISA: primarily used for detection of *T. gondii* antibodies rather than antigen; involves the specific antigens coated onto the solid phase, enzyme conjugated secondary antibody and substrate. B. Sandwich ELISA: used for the detection of *T. gondii* antigens; involves the specific antibody coated onto the solid phase, enzyme conjugated antibody and substrate.

Diagnosis of toxoplasmosis has been improved by the emergence of molecular technologies to amplify parasite nucleic acids. Among these, PCR-based molecular techniques have been useful for the genetic characterization of *T. gondii*. Several PCRs have been developed for the detection of toxoplasmosis. Among these techniques, nested PCR is sensitive and reliable but it is time consuming and not practical for high-throughput screening. The advent of innovative qualitative and quantitative real time PCR techniques has proven useful in various applications, including pathogen detection and gene expression investigations.

Approximately 30% of human population worldwide is chronically infected with *T. gondii*. People can become infected with *T. gondii* via ingestion of undercooked or raw meat containing tissue cysts or ingestion of oocyst-contaminated food or water (Moncada and Montoya, 2012). Human infections are generally asymptomatic, but a severe form might occur in cases of congenital toxoplasmosis and in immunocompromised individuals (Tenter Heckeroth, and Weiss, 2000). Although *T. gondii* has been reported in the main dairy and beef cattle producing countries, there is scarce data about the presence of this parasite in livestock farming in North-African countries, including Algeria (Khatima and Abdellah, 2015; Derdour et al., 2017; Khames et al., 2018), Morocco (Benkirane et al., 2015; Lucchese et al., 2016; Meriem Essayagh et al., 2017), Sudan (Elfahal et al. 2013a), Egypt (Fereig et al., 2016) and Tunisia (Lachkhem et al., 2015).

Toxoplasma gondii abortion process

T*oxoplasma gondii* infection affects all warm-blooded animals with a wide species variation in the disease it causes. Previous studies have uncovered the fact that the asexual cycle of *T. gondii*, especially in the intermediate hosts, is closely associated with its virulence. The asexual cycle was artificially divided into five critical phases including glide, attachment, invasion, intracellular proliferation and egress(Pittman and Knoll, 2015; White and Suvorova, 2018). Additionally, the parasites egressed from the infected host cells can re-invade other vacant cells through motility and re-attachment (Kato, 2018). *Toxoplasma gondii* often parasitizes its host without any clinical manifestations under normal conditions. However, it can cause severe infection depending on the strain of parasite and the route of transmission (Yu et al., 2007). Infections in naïve pregnant sheep may result in abortion or neonatal infection, while in cattle the parasite is eliminated quickly from the tissues and clinical abortion has not been reported

(Esteban-Redondo and Innes, 1997). Pregnant cattle inoculated with *T. gondii* oocysts or tissue cysts developed transient fever and anorexia and gave birth to healthy calves (Munday, 1978). Other studies (Koestner and Cole, 1961; Dubey, 1986) have also shown that toxoplasmosis does not appear to cause abortion or neonatal mortality in cattle under natural conditions. However, Costa et al., (2011) concluded that congenital infection of *T. gondii* in cattle, while infrequent, does occur naturally (Canada et al., 2002). The pathogenicity of the strain of *T. gondii* may influence the likelihood of this route of transmission.

Few studies describe the abortion mechanism of *T. gondii* in cattle, in contrast to sheep and goats. Bari Yeasmin and Alam, (1993) reported that the pathological changes were more common and severe in the placenta than in foetus and placental damage was probably the primary cause of death in black Bengal goats. If established for the first time during pregnancy, a progressive infection may develop in the gravid uterus (Buxton and Innes, 1995). In these circumstances, tachyzoites invade the caruncular septa, the maternal tissue of the placentome, and then invade the placental villi and the foetus (Buxton and Finlayson, 1986). The ability of the foetal immune system to respond to *T. gondii* develops progressively after 70 days of gestation. Infection before this age results in rapid foetal death with resorption, mummification, maceration or abortion of the foetus. Infection later in pregnancy may be less damaging and result in stillborn or weak lambs, or even clinically normal lambs that are infected and immune to natural challenge (Buxton and Finlayson, 1986). Stillborn and weak lambs usually have brain damage such as focal leukomalacia and a characteristic non-suppurative meningo-encephalitis (Buxton et al., 1982). Lambs that survive the first few days of life generally grow normally to adulthood without neurological defects (Buxton and Innes, 1995). Moreover, a previous study confirmed that reactivation of *T. gondii* cysts in chronically infected sheep and goats poses an important risk for endogenous transplacental transmission in sheep and goats during pregnancy (Williams et al., 2005; Hide, 2016). Despite the vertical route of transmission has traditionally been thought to be rare, the current evidence in sheep is ambivalent and controversial (Hide, 2016).

I. 4. 3. Brucella

To date, the genus *Brucella* consists of eleven species (Smirnova et al., 2013). Among the different species of genus *Brucella*, *B. abortus* is the most common species infecting cattle

worldwide while *B. melitensis* mainly affects sheep and goats (Alton, 1990), and occasionally affects cattle (Reisberg Selim and Gaede, 2013; OIE, 2009). Other relevant species include *B. ovis* in sheep, *B. suis* in pigs and *B. canis* in dogs (Smirnova et al., 2013).

In livestock farming, brucellosis causes abortion and infertility in both male and female animals and reduced milk yields. *Brucella* spp. are excreted in vaginal secretions of infected females and are at their highest level immediately after abortion or birth. Therefore, products of abortion and birthing materials are the main source of contagion, although vertical and sexual transmission and transmission through lactation also occurs. Venereal transmission is not a major route of infection under natural conditions, but artificial insemination with contaminated semen is a potential source of infection (McDermott and Arimi, 2002; Neta et al., 2010). Extensive production systems exhibit low rates of disease transmission and lower disease burden, while intensification promotes transmission due to increased stocking densities, animal contacts and a higher birth index (McDermott Grace and Zinsstag, 2013; Ducrotoy et al., 2014; Grace et al., 2012; Jones et al., 2013; Racloz et al., 2013).

A battery of serological tests including Milk Ring Test (MRT), Fluorescence Polarization Assay (FPA), intradermal test, Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), Coombs test and ELISA are extensively used for diagnosis of bovine *Brucella* infection (Nielsen, 2002; Abernethy et al., 2012). Currently, there is no diagnostic test sufficiently sensitive and specific to detect all stages of infection in live animals (McGiven et al., 2003; Poester et al., 2010). Serological tests are reliable but sometimes false positivity due to cross-reacting antibodies against *Yersinia enterolitica* and some other zoonotic pathogens may reduce the specificity (See et al., 2012).

The introduction of new animals in the herd has been identified as the main risk factor for seropositivity (Musallam et al., 2015), besides, abortion in animals, age of the animal and awareness about brucellosis(Chand and Chhabra, 2013). The disease, eradicated in many developed countries, is a re-emerging neglected zoonosis endemic in several zones, especially in the Mediterranean region (McDermott Grace and Zinsstag, 2013). In later stages of control programs, in which eradication is the goal, a strict monitoring program using highly discriminatory methods for strain characterization is crucial. Characterization of circulating

strains allows the determination of the source of infection and the transmission routes (Robinson, 2003; Almendra et al., 2009). Genotyping and identification of *Brucella* species based on molecular approaches have proved to be powerful tools to confirm the presence of the pathogen and to assess the genetic relationship among field isolates (Dorneles et al., 2014; Mick et al., 2014; Allen et al., 2015). The great advantage of these methodologies are the reduced risk of laboratory-acquired infections, short time-consuming and accessibility (Scholz and Vergnaud, 2013). Among the molecular typing methodologies, the Multiple Locus Variable Number Tandem Repeats VNTR Analysis (MLVA) has proved to be a valuable tool in molecular epidemiology studies, allowing source tracking and characterization of new *Brucella* species (Dorneles et al., 2014; Mick et al., 2014; Allen et al., 2015; Whatmore et al., 2014; Xiao et al., 2015). Isolation and molecular description of the prevalent *Brucella spp.* are useful to determine the origin of the infection and to establish appropriate measures to control brucellosis (Godfroid et al., 2013).

Brucellosis stands first in the list of zoonotic bacterial diseases, and 500,000 cases are reported annually in disease-endemic regions (Johansen et al., 2017). The World Health Organisation (WHO) estimated that in 2010 there were 0.83 million cases of human brucellosis globally (47% of these were identified as foodborne in origin). Nevertheless, the actual figure is likely to be much higher than this, due to widespread under-reporting and misdiagnosis (WHO, 2015; Kirk et al., 2015; Jennings et al., 2007). Nearly every case of human brucellosis has an animal origin and, therefore, control is primarily a veterinary problem (Paul Nicoletti, 2002). Brucellosis gains public health importance when the bacteria are transmitted to human via unpasteurized milk, meat, and animal by-products from infected animals (Garcell et al., 2016). Brucellosis in humans is characterized by undulant fever, general malaise, and arthritis. The name ‘Malta fever’ is occasionally used for typical fever conditions caused by *Brucella spp.* (Figure 9).

Brucellosis is an endemic infectious disease not only in animals but also in humans in Algeria and in Maghreb (Reviriego and Domínguez, 2000; Aggad and Boukraa ,2006; Calistri et al., 2013; Lounes et al., 2014; Ammam Grele and Belmamoun, 2018; Yahyaoui Azami et al., 2018; Abdelbaset et al., 2018). Many studies have investigated the animal and herd seroprevalence, histopathological identification, risk factors and molecular characterisation of *Brucella spp.* in

cattle in Mediterranean countries (Selim et al., 2019; Khamassi Khbou et al., 2017; Kaaboub et al., 2019; Aggad and Boukraa, 2006; Moustafa Kardjadj, 2017; 2018; Yahyaoui Azami et al., 2018; Barkallah et al., 2016; Şahin et al., 2008).

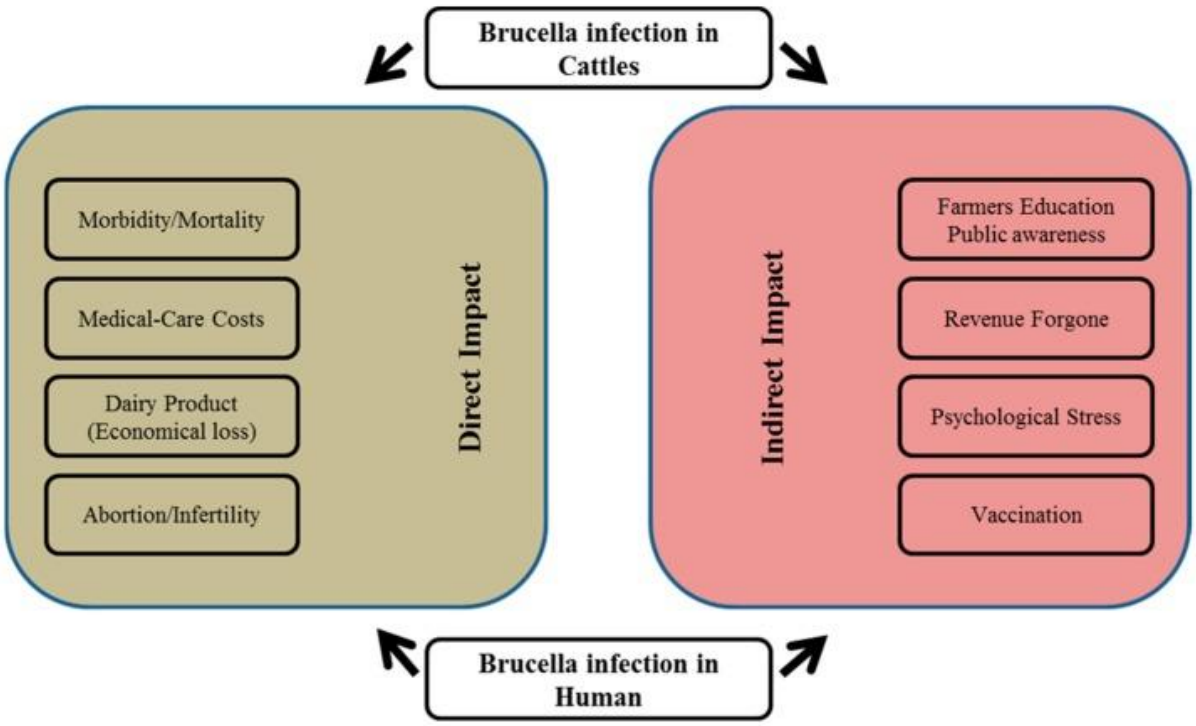


Figure 9. Summary of the impact of *Brucella* infection in humans and cattle (Khan and Zahoor, 2018).

Brucella abortion process

Brucellosis is the second most important zoonotic disease in the world after rabies according to the World Organisation for Animal Health (Wareth et al., 2014). *Brucella abortus* is the primary agent of brucellosis globally. *Brucella melitensis* is emerging as an important pathogen of cattle worldwide (Wareth et al., 2014). Cattle erythritol, a four-carbon polyol, is a sugar abundant in bovine placental tissues. The ability to catabolise erythritol preferentially over other sugars by bacteria of the genus *Brucella* has been largely recognized and has been associated to the capability to induce abortions in infected ruminants. This ability appears to be the cause of the localization of *B. abortus* in the placenta of pregnant cows and has been linked to its virulence (Smith H, et al., 1962). The pathway and the genes involved in the catabolism of erythritol in *Brucella spp.* have been previously identied (Sperry and Robertson, 1975; Rodríguez et al., 2012). The main consequence of bovine brucellosis is abortion, due to a series of biochemical events that, if exacerbated, may lead to foetal expulsion or embryonic death. Under pathological

conditions, there is an overproduction of free radicals, causing cell and tissue damage, which requires the activation of the antioxidant system (Tonin et al., 2014). Perin et al., (2017) concluded that: 1) pregnant cows seropositive for *Brucella spp.* suffer oxidative stress, which may enhance the occurrence of abortion; and 2) the reduction in the activity of deaminase adenosine in seropositive cows is a compensatory mechanism to decrease the inflammatory process triggered by the disease and, consequently, the tissue damage that can lead to abortion.

The ability of the pathogen to survive and replicate within different host cells explains its pathogenicity (Muflihanah et al., 2013; Ray et al., 2009; Mohammad and Esmaeil, 2012). Pathogenesis depends upon various factors such as species, dose of the inoculum, route of transmission and host immune status (Djønne, 2007). Extensive replication in placental trophoblasts is associated with abortion (Djønne, 2007; Rodríguez et al., 2012; Corbel and Organization, 2006), while persistence in macrophages and other cell types leads to chronic infections (Gilbert et al., 1991; Pappas et al., 2006). Protective immunity to the host is conferred by T-cell mediated macrophage activation by the antigenic protein of *Brucella spp.* and the production of specific antibodies. Moreover, other elements of the immune response such as tumour necrosis factor (TNF), interferons and complement activation are also involved. Following infection, the immunoglobulin M (IgM) titer increases initially followed by the immunoglobulin G (IgG) titer. Thus, the detection of IgM indicates an early immune response and IgG correspondingly indicates chronic infection or relapse (McDonald et al., 2006). Rodríguez et al., (2012) results corroborate that erythritol is used preferentially over other compounds and provide a neat explanation for the stimulation of *B. abortus* growth induced by erythritol (Rodríguez et al., 2012).

I. 4. 4. Chlamydia abortus

Chlamydia spp. are Gram-negative, obligate intracellular bacteria that can infect a wide range of animal hosts including humans, causing reproductive and respiratory diseases in many countries around the world (Aitken and Longbottom, 2008) (Figure 10). Chlamydial infections in cattle can cause abortion (Borel et al., 2006), vaginitis and endometritis (Wittenbrink et al., 1993), infertility (Wehrend et al., 2005) and chronic mastitis (Biesenkamp-Uhe et al., 2007). Several other disease syndromes can also be caused by *Chlamydia* infection, including urogenital tract pathology, pneumonia, conjunctivitis, enteritis, polyarthritis and

encephalomyelitis, as well as subclinical infections (Holliman et al., 1994). *Chlamydia abortus* (formerly *Chlamydophila abortus*), *Chlamydia pecorum* (formerly *Chlamydophila pecorum*) and *Waddlia chondrophila* are recognised causes of reproductive disease in cattle (Guest editorial, 2006). There is also increasing interest in the role of another *Chlamydia*-like species, *Parachlamydia acanthamoeba* in abortion in both cattle and humans (Wheelhouse et al., 2010; Blumer et al., 2011).

Definitive diagnosis of *Chlamydia* species requires either identification of the bacteria by microscopic examination of stained smears, detection of bacterial antigen by micro-immunofluorescence (MIF) or by ELISA, or detection of bacterial DNA by PCR or by microarray (Rodolakis and Yousef Mohamad, 2010). DNA amplification techniques have allowed several reclassifications of the order of Chlamydiales in recent decades. The most recent revision has led to the reunification of all known species of the family Chlamydiaceae into one genus: *Chlamydia* (Sachse et al., 2015). Currently, 11 species are recognized: *C. abortus*, *C. pecorum*, *C. psittaci*, *C. pneumoniae*, *C. felis*, *C. caviae*, *C. trachomatis*, *C. suis*, *C. muridarum* and the novel *C. avium* and *C. gallinacea*. Complement fixation test is considered the standard serological test for detection of chlamydial antibodies by the Organisation Internationale des Epizooties (OIE) (OIE, 2020). However, the test lacks specificity in ruminants, mainly due to the heat-resistant LPS antigen which is common to all Chlamydiaceae species (Salinas et al., 2009). The MIF test is still regarded as the standard serological assay for species-specific detection of chlamydial antibodies. Nevertheless, poor sensitivity and cross-reactivity with MIF have been reported (Maass et al., 1998). Several ELISAs using purified whole elementary bodies, LPS, or *C. abortus* major outer membrane protein (MOMP) show improved sensitivity and specificity to detect antibodies against *C. abortus* (Rodolakis and Yousef Mohamad, 2010).

Infection with *C. abortus* in pregnant women after contact with aborting/lambing sheep and goats may also lead to abortion and, if untreated, to life-threatening illnesses. In humans, different serovars of *C. trachomatis* cause eye and urogenital infections, being the leading cause of infectious blindness worldwide (blinding trachoma) and the most common bacterial sexually transmitted infection (O'Connell and Ferone, 2016). Respiratory infections in humans are caused by *C. pneumoniae*, a chlamydial species also infecting horses, frogs, reptiles and

marsupials (Roulis Polkinghorne and Timms, 2013). *Chlamydia psittaci* has the best-known zoonotic potential and is associated with severe respiratory disease in humans, while birds are commonly infected without clinical manifestations. Numerous other animal pathogenic chlamydial species, including *C. felis, C. caviae, and C. suis*, are recognized or suspected to cause infrequent human infections with various clinical presentations.

Although abortion and reproductive disorders such as infertility are of major economic importance in both dairy and beef cattle, there is little information on the prevalence and epidemiology of bovine chlamydial infection in North-Africa (Maghreb). The chlamydial infection status of Algeria's herds and flocks has been previously investigated (Derdour et al., 2017; Merdja et al., 2015; Hireche et al., 2016), even in the Maghreb's country (Benkirane et al., 2015; Abdessalem Rekiki et al., 2005; Djellata et al., 2019), Egypt (Osman et al., 2011) and Turkey (Halil Ibrahim Gokce et al., 2007). However, the lack of comprehensive understanding of *Chlamydia spp.* dynamics limits the potential identification of a significant causal link. Therefore, studies and surveys in this direction are needed to fill and complete the epidemiological gaps.

Chlamydia abortus abortion process

The bacterium is transmitted through faeco-oral and/or venereal routes. *Chlamydia abortus* can establish subclinical infections until pregnancy, when it can invade the placenta and induce an inflammatory cascade leading to placentitis and abortion. It has been experimentally demonstrated that *Chlamydia spp.* multiply primary in the cotyledons, where they cause severe inflammation and necrosis. Since the bacteria affect placental function, abortion or peri-natal deaths are well-described consequences of infection (Cavirani et al., 2001; Wang Shieh and Liao, 2001). Abortions usually occur after the seventh month of gestation but have been reported as early as the fifth month (Parkinson, 2019). Most cows show no clinical signs before abortion. However, experimental infections showed an intermittent, mucoid, vulvar discharge, together with transient diarrhoea, pyrexia and lymphopenia (Kaltenboeck Hehnen and Vaglenov, 2005). Retention of the foetal membranes after abortion is common and infertility that is not associated with abortion can also occur (Kaltenboeck Hehnen and Vaglenov, 2005). Infection in the last trimester of pregnancy may also result in the birth of live, weak calves. Early host–pathogen interactions could explain differential pathogenesis and subsequent

disease outcome in ruminant species (Animal and Plant Health Agency (APHA), 2013). Vaccines are available for use in sheep but none has yet been developed for use in cattle.

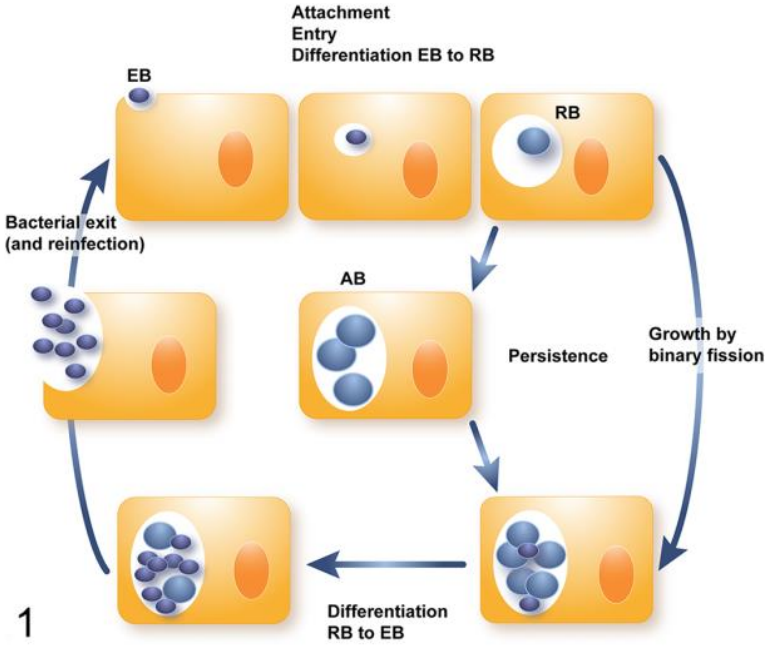


Figure 10. *Chlamydia* developmental cycle(Borel, Polkinghorne, and Pospischil, 2018). The elementary body (EB) attaches to a host cell and differentiates into a reticulate body (RB) after entry, enclosed in a membrane-bound vacuole. The RB grows by binary fission and later differentiates into EBs, which are released and infect a new host cell. The persistent state is characterized by the formation of the aberrant body (AB), which may be induced by stressful conditions.

I. 4. 5. Coxiella burnetii

Coxiella burnetii, the causative agent of query (Q) fever or coxiellosis, is an obligate intracellular bacterium that can infect a wide variety of hosts including arthropods (particularly ticks), fish, reptiles, birds, mammals and humans (Cutler Bouzid and Cutler, 2007). *Coxiella burnetii* can cause sporadic abortion in cattle (Jensen et al., 2007; Rády Glávits and Nagy, 1985). However, infection with *C. burnetii* without associated placental pathology has been recently reported and the bacteria has also been detected in the vagina of healthy cattle (Guatteo et al., 2006; Hansen et al., 2011).

Inhalation of aerosols containing *C. burnetii* is the main source of infection for cows and humans (Isken et al., 2013). Moreover, this agent can be shed by infectious animals in milk, urine and faeces and in high numbers in the amniotic fluid, vaginal discharges, aborted tissues, placenta and semen (Plummer et al., 2018), (Scientific report of EFSA and ECDC, 2012), (Guatteo et al., 2007). A high contamination rate has been reported in dairy products, especially

if unpasteurized (Eldin et al., 2013). Noteworthy is the fact that asymptomatic individuals and intermittent cattle shedders may remain negative in serological tests, unnoticeably shedding the pathogen into the environment for several months or years (De Cremoux et al., 2012). Sporadic cases of *C. burnetii* transmission by sexual contact, blood transfusion and transplantation have also been reported (Petty Te and Pursell, 2017). The importance of ticks in the transmission of *C. burnetii* remains unclear (Psaroulaki et al., 2006; Pluta et al., 2010; Knobel et al., 2013).

Intra-herd infection dynamics of a dairy herd are mainly influenced by this heterogeneity of the shedding routes (Courcoul et al., 2011). *Coxiella burnetii* transmission and spread dynamics among ruminants are influenced by local environmental conditions such as vegetation, soil moisture and sewage water (Nusinovici et al., 2015; Pandit et al., 2016). Different farm-level factors, such as farm location, density and proximity to other infected ruminant farms or contacts with farm visitors, were found to be associated with higher *C. burnetii* seroprevalence (Schimmer et al., 2014). Domestic ruminants (i.e. goats, sheep, cattle) are considered to be a major reservoir of *C. burnetii* (Alvarez et al., 2012). Moreover, wildlife may also be an important source of the pathogen (Nicole Borel Polkinghorne and Pospischil, 2018). As mentioned above, *C. burnetii* is highly resistant and the environment itself can serve as a reservoir (De Bruin et al., 2013).

Reproductive disorders such as abortions, stillbirths and delivery of weak and unviable new borns have been reported in livestock infected with *C. burnetii* (Bildfell et al., 2000). In ruminants, *C. burnetii* mainly causes reproductive disorders (spontaneous abortion, premature delivery, stillbirth and weak offspring) in pregnant ewes, goats and cattle (Agerholm, 2013) as well as metritis and infertility in cows (Scientific report of EFSA and ECDC, 2012). Other reproductive conditions in cattle have also been associated with *C. burnetii*. There are biological indications of species differences in relation to the impact on reproduction. Recent molecular studies have shown that different strains of *C. burnetii* exist and those strains are associated with different ruminant hosts, although cross infection does occur.

Diagnosis of *C. burnetii* in animals is based on detection of bacteria, bacterial DNA, or antibodies (Rodolakis, 2009). Isolation of *C. burnetii* is hazardous, difficult and it requires Biosafety Level 3 laboratories, due to the zoonotic nature of the microorganism (Masala et al.,

2004). Diagnosis can also be based on PCR detection of *C. burnetii* DNA in different biological samples, including placenta, vaginal mucus, milk, colostrum, faeces and tissues from aborted foetuses (Rousset et al., 2010). Unfortunately, PCR is not reliable to determine the infection status because of the variability in shedding between animals (different shedding routes, potentially intermittent shedding). Exposure to *C. burnetii* can be screened indirectly by serological tests. The complement fixation test (CFT) (OIE recommended test), indirect immunofluorescence assay (IFA) or ELISA (EU recommended test) may be used, but the latter is thought to be the most robust and has good specificity and high sensitivity (Emery et al., 2014). ELISA is reported to be highly sensitive and specific for the detection of antibodies against *C. burnetii*, and can be used in individual serum samples as well as in bulk milk. On the other hand, CFT protocol is complex and fails to detect antibodies in sheep or goats (Kovácová Kazár and Simková, 1998). Taking into account that the clinical signs of coxiellosis in animals are non-specific and infection may be asymptomatic, especially in cattle, laboratory tests are crucial for the diagnosis of *C. burnetii*. (Guatteo et al., 2007). As a general principle, the methods for the diagnosis of *C. burnetii* allow only an interpretation at the population level and are not reliable at the individual level.

Coxiella burnetii infection in humans has been reported worldwide, with the exception of New Zealand (Cutler Bouzid and Cutler, 2007), and in all continent except Antarctica (Dean et al., 2013), Europe (Van den Brom et al., 2013), Oceania (Tozer et al., 2011), North America (Anderson et al., 2009) and South America (Costa et al., 2005). The seroprevalence of *C. burnetii* in humans ranges from less than 1% in Canada (Messier et al., 2012) to 52.7% in Cyprus (Psaroulaki et al., 2006). However, the prevalence reaches 65.1% when evaluated in high risk groups such as veterinarians from the Netherlands after the outbreak that occurred between 2007 and 2009 (Van den Brom et al., 2013). Despite most of the recent human outbreaks are known to originate from small ruminants, intensive cattle farming with high prevalence could become a concern for public health (Guatteo, 2011). Lacheheb and Raoult, (2009) reported a high seroprevalence of *C. burnetii* among the human inhabitants of Setif region (Algeria), reaching 15.5% and being significantly higher among inhabitants of rural areas (20%). However, to date, no epidemiological survey has targeted the livestock from Setif region in Algeria. The disease has been described in humans in Cyprus (Cantas et al., 2011), Syria (Bottieau et al., 2000) and Iraq (Faix et al., 2008).

Coxiella burnetii circulation has been reported in several Middle-East countries. Prevalence in Eastern Turkey was 5.8% in cattle and 10.5% in sheep (Cetinkaya et al., 2000). In Iran the prevalences reported were 27.5% in sheep, 54% in goats and 0.83% in cattle (Abbasi-Doulatshahi et al., 2015). A study investigating animals with history of abortion in Jordan revealed a prevalence of 12.1% in sheep and 10.7% in goats (Aldomy Wilsmore and Safi, 1998). In a recent serological survey of 2,699 animals across Egypt, significant interspecies and regional variations were observed (Klemmer et al., 2018). Camels exhibited the highest rates of seropositivity (40.7%), followed by cattle (19.3%), buffalo (11.2%), sheep (8.9%) and goats (6.8%). Regarding regional differences, the highest rates of seropositive animals were observed in the Eastern desert (27%), compared to the Nile Delta (16.4%) or Western Desert (17%). Pasture-based production systems also had significant lower levels of seropositive animals (9.9%) compared with either nomadic (19.4%) or stationary husbandry. Moreover, *C. burnetii* DNA was identified in the placenta and vaginal swab from an aborted goat in a study of 109 abortions from Egyptian dairy goats, sheep and cattle (Abdel-Moein and Hamza, 2017). In Italy, seroprevalence surveys in animals are scarce, and reports have mainly focused on reproductive disorders and, particularly, on abortion as the major clinical problems (Vicari et al., 2013). The only extensive investigations conducted to date were carried out in Sardinia, revealing a seroprevalence of 38% and 47% on sheep and goat farms, respectively (Masala et al., 2004); and in Piedmont, revealing a seroprevalence of 38,7% for sheep and 19,5% for goats (Rizzo et al., 2016). Although comparisons among groups belonging to different productive orientations and geographic areas revealed some critical differences, in most cases the real drivers of *C. burnetii* infection in flocks and herds were intrinsic farm factors, such as production system and management (Nokhodian et al., 2016; Rizzo et al., 2016).

Coxiella burnetii abortion process

Two microscopic forms of *C. burnetii* are known based on their pathogenicity (large-cell variant and small-cell variant). The large-cell variant is the vegetative form in infected cells. The small-cell variant is the extracellular form, which is shed in milk, urine, faeces, placenta, and amniotic fluid (Maurin and Raoult, 1999). The small-cell variant is resistant to high temperatures and desiccation, conferring the capacity for airborne transmission and long-term environmental persistence to this form of *C. burnetii* (Van Schaik et al., 2013). Differences in

the clinical presentations of coxiellosis in cattle could be the result of differences in bacterial genotype. Although the role of *C. burnetii* in bovine abortion is clear, its association with other reproductive disorders of cattle, such as infertility, premature delivery, endometritis, metritis, and mastitis is controversial (De Biase et al., 2018; Agerholm, 2013). Additionally, *C. burnetii* DNA has frequently been detected in cases of endocarditis in cattle at slaughter (Agerholm et al., 2017); however, the clinical significance of this finding remains undetermined. It is generally accepted that chronic infection with *C. burnetii* may cause abortion, premature birth, dead or weak offspring in cattle, sheep and goats. Bacterial DNA and antigen have also been detected in endometrial biopsies of cows with repeated breeding failure (De Biase et al., 2018). Although these findings suggest an association between *C. burnetii* and reproductive disorders in cattle, they have not been compared to those of healthy cows and reliable conclusions cannot be drawn (De Biase et al., 2018). Agerholm, (2013) reported that the outcomes of infection during pregnancy can involve a range of conditions, including abortion, delivery of premature offspring, stillbirth and weak offspring (APSW complex) as well as production of clinically normal progeny that may or may not be infected (Figure 11).

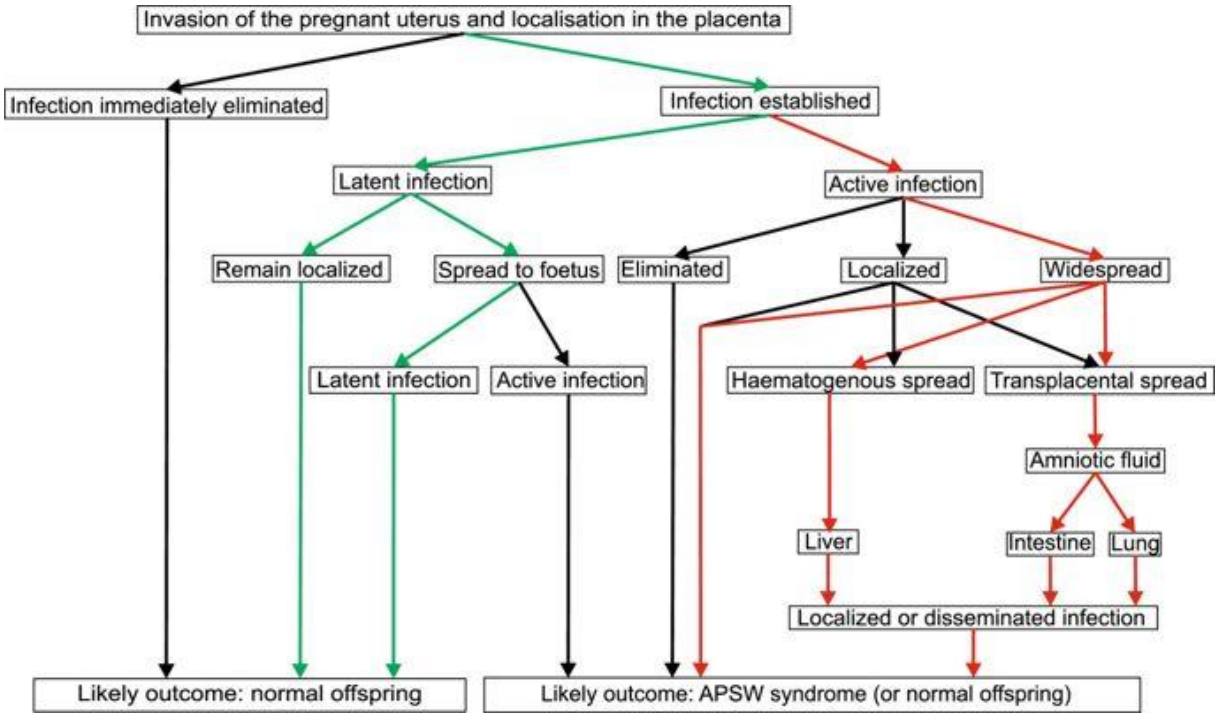


Figure 11. Review of *Coxiella burnetii* associated reproductive disorders in domestic animals. APSW: Abortion, Premature Offspring, Stillbirth and Weak Offspring *(Agerholm, 2013)*.

I. 4. 6. Bovine Viral Diarrhoea Virus

The Family Flaviviridae comprises four genera: genus *Pestivirus*, genus Hepacivirus, genus Pegivirus and genus Flavivirus (Lefkowitz et al., 2018; ICTV, 2021). The genus *Pestivirus* includes eleven recognised species formally named *Pestivirus* A to K. However, these *Pestivirus* are most commonly known by their classical nomenclature. Traditionally, *Pestiviruses* have been classified and named according to the affected species and the diseases they cause. However, *Pestiviruses* have the ability to cross species barriers and to infect a wide range of *Cetartiodactyla* species.

The three classical *Pestivirus* affecting cattle are Bovine Viral Diarrhoea Virus type 1 (BVDV-1; *Pestivirus* A), BVDV-2 (*Pestivirus* B) and Hobi-like virus (*Pestivirus* H). Bovine Viral Diarrhoea Virus is enveloped, spherical, approximately 50 nm in diameter and its genome is a single-stranded positive-sense RNA molecule, of 12.5 kb long. The genome of BVDV contains a single open reading frame flanked by a 5' and a 3' untranslated regions (UTR). The OFR encodes a polyprotein of 3,900 amino acids, approximately, which will be cleaved into twelve structural (S) and non-structural (NS) proteins. The S proteins are the capsid protein C and the envelope glycoproteins Erns, E1 and E2 (Thiel et al., 1993). The NS proteins are p20 (Npro) that is an autoprotease, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. In addition, uncleaved E2-p7 and NS2-3 polypeptides are produced. As a consequence of their RNA genome, *Pestivirus* display high mutation rates, which, in some cases, may lead to the emergence of new virus lineages. Bovine Viral Diarrhoea Virus -1 and -2 are divided into two biotypes, cytopathic (cp) and non-cytopathic (ncp), based on their effects on cultured cells.

Bovine Viral Diarrhoea Virus causes disease in cattle and it is distributed worldwide, including in many Mediterranean countries (Derdour et al., 2017; Feknous et al., 2018; Mahin Wellemans and Shimi, 1985; Mahin et al., 1982; H. Yilmaz et al., 2012; Kadir et al., 2008; Beaudeau et al., 2005; Thabti et al., 2002; Decaro et al., 2012; Aslan Azkur and Gazyagci, 2015; 2015; Lanave et al., 2017; Arias et al., 2003; Billinis et al., 2005; Mainar-Jaime et al., 2001). The virus was first described in 1957 as the causative agent for bovine viral diarrhoea (BVD) (Lee and Gillespie, 1957). The infection with BVDV is responsible for massive economic losses in cattle industry worldwide through reduced milk production, abortions, and a shorter lifespan of the infected animals (Richter et al., 2017).

Bovine Viral Diarrhoea Virus can be transmitted horizontally and/or vertically. Horizontal transmission causes acute infections characterized by a short period of viraemia. The virus spreads among ruminants by the oro-nasal route and can be detected in serum between day 4 and 11 post-infection. This acute form of BVD is characterized by a post-natal infection of an immunocompetent host and courses with a mild transient viraemia followed by the production of neutralizing antibodies (OIE, 2021). Clinically, acute ncp-BVDV infection courses with enteric disease consisting of diarrhoea, pyrexia and mild depression, with high morbidity and low mortality. However, the acute fatal haemorrhagic syndrome has been associated to ncp-BVDV-2 (Carman et al., 1998).

The success of BVDV has been the vertical or congenital transmission to foetus. The infection of cattle during pregnancy originates different clinical situations. While the course of infection in the pregnant female is clinically mild or unapparent and similar to the acute horizontal infection described above, the consequences for the foetus are of importance. The ability to cross the placenta and the infection of the foetus causes different consequences depending on the phase of the gestation when the infection occurs. Although the death of the foetus/embryo can occur at any stage of gestation after BVDV infection, it is at the first stages when there is a higher probability of death. After death, the embryo is typically reabsorbed, which usually goes undetected. If the infection occurs before foetal immunocompetence, BVDV can replicate in the foetal tissues without control. Approximately 50% of these infected foetuses die; if they survive and are born alive, calves remain PI. Individuals PI are characterized by the continuous replication and excretion of the virus, as the immune system recognises the virus as a self-antigen. The PI new born will have colostral antibodies against the virus (OIE, 2021). In the epidemiology of BVDV, these PI animals represent the major source of transmission of the virus within and among cattle herds, causing significant losses in cattle farming worldwide (Brownlie et al., 1987) (Figure 12). Persistent infected animals shed large quantities of virus during their lives, whereas cattle with transient infections excrete small quantities of virus over a short period of time (14 days in average) (Brownlie et al., 1987) and are less important for the spread of infection (Niskanen Lindberg and Tråvén, 2002; Sarrazin et al., 2014).

Mucosal disease (MD) is another enteric syndrome associated to BVDV infection, which can lead to the death of the infected animal. This syndrome is associated to cp BVDV biotypes. The presence of the cp biotype in an animal can be the consequence of: 1) superinfection of a cp biotype in a PI animal (Bolin, 1995), 2) recombination between ncp biotypes, or 3) mutation of an already existent biotype (Loehr et al., 1998). Mucosal disease presents low morbidity and high mortality and is characterized by diarrhoea, profound depression and death. At necropsy, erosions in the mucosa at various sites along the gastrointestinal tract are observed. Histological examination shows destruction of the gut-associated lymphoid tissue, which is replaced by inflammatory cells.

The impact of BVDV on the health status of the herd depends on the time and duration of infection, the virulence of the virus strain, herd immunity (Rodning et al., 2012), disease prevalence, herd production level and concomitant infections (Stott Humphry and Gunn, 2010). Induced costs are mainly due to production losses, derived from the immunosuppressive and abortive actions of the etiological agent, and to the biosecurity and immunization measures often implemented for its control or eradication (Richter et al., 2017; Thomann et al., 2017).

The most commonly used tests to detect the presence of a PI animal include virus isolation, reverse transcription PCR (RT-PCR), IHC (Immunohistochemistry), antigen-capture ELISA (Table 2). Studies investigating the molecular epidemiology of BVDV can provide invaluable information about the variability of viral strains existing in a population and, in turn, inform the development of control programs, vaccine choice and determine likely infection sources. Several epidemiological surveys have proven that BVDV-1 is the predominant *Pestivirus* circulating in European cattle population, although very recently BVDV-2 outbreaks have been reported. The main subtypes detected in Europe are BVDV-1b and BVDV-2a (Lanave et al., 2017). However, in North Africa there is a single study that identified BVDV-2a and BVDV-1b from cattle with clinical history in Tunisia (Thabti et al., 2002). Control programmes, particularly vaccination, have not been implemented in Algeria.

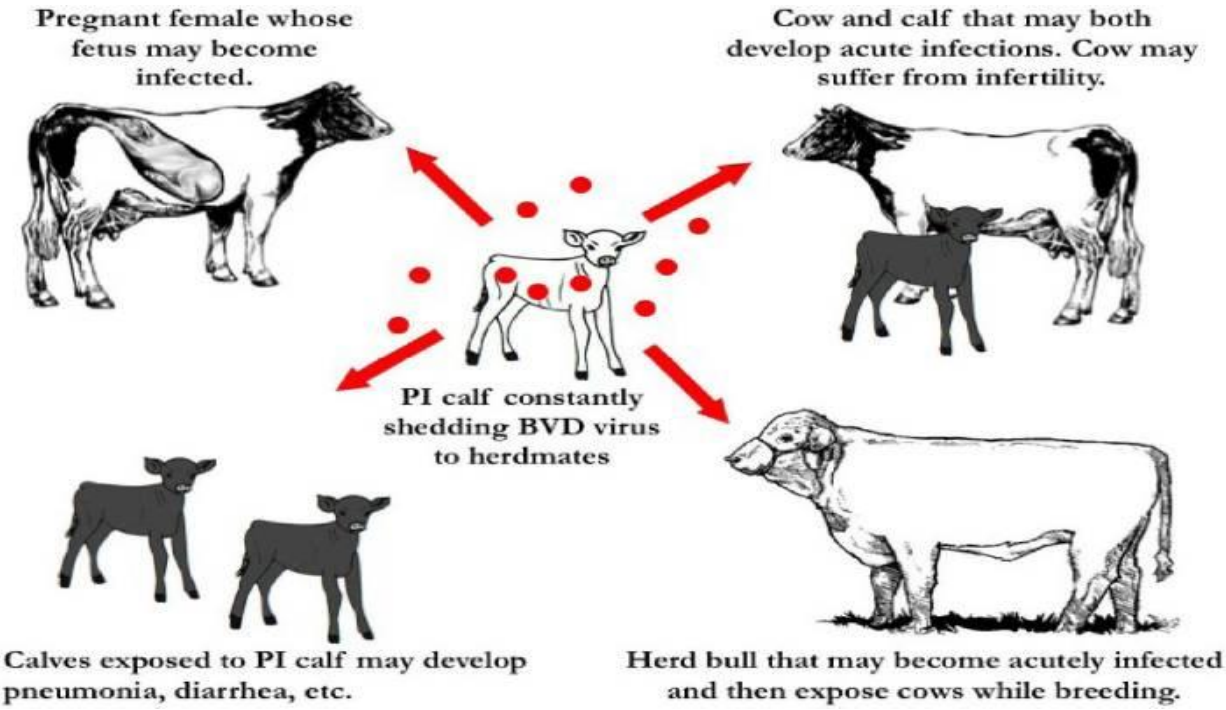


Figure 12. Shedding of Bovine Viral Diarrhoea Virus by Persistently Infected (PI) individuals (Khodakaram-Tafti and Farjanikish, 2017)

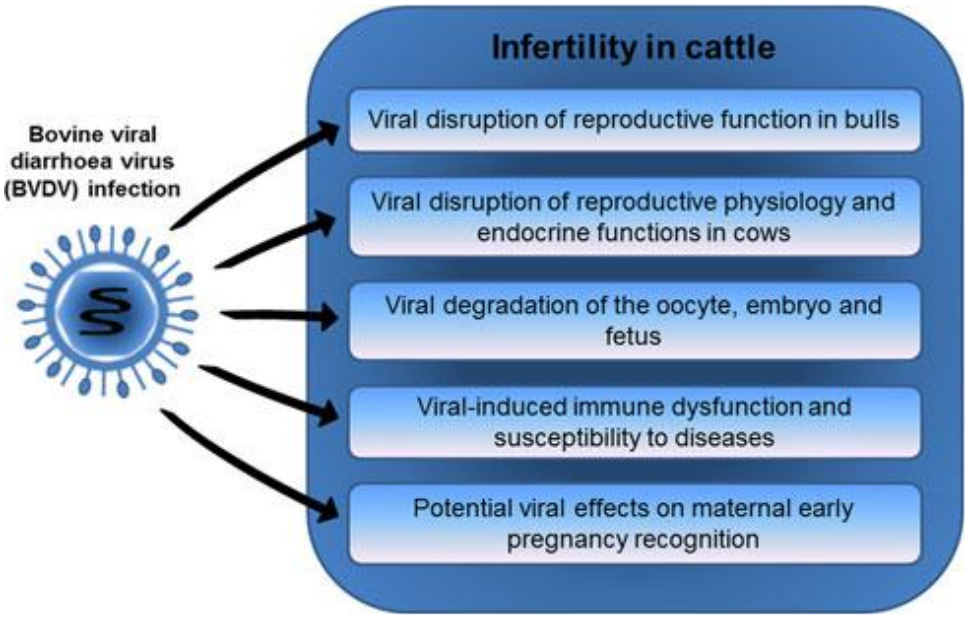


Figure 13. Mechanisms linking Bovine Viral Diarrhoea Virus infection with infertility in cattle (Oguejiofor et al. 2019).

Table 2. Suggested diagnostic laboratory tests for the detection of Bovine Viral Diarrhoea Virus - persistently infected (PI) animals (OIE, 2021).

Test	Cost	Advantages	Disadvantages	Specimens/shipping
Virus isolation	Moderate to high cost	-Gold standard -High specificity -Virus available for future studies	-Slow procedure -Potential false negatives: interference with maternal antibodies -Retest positives in 3 weeks to confirm PI	-Whole blood (10 ml) or serum (2-3 ml) and tissue samples -Send in container with cold packs -Do not freeze the samples
Immunohistochemistry	Low cost	-High sensitivity	-Labour-intensive -Slow procedure -Formalin usage	-Skin samples-ear notch and tissue samples -Send fresh on wet ice or stored in formalin
Antigen-Capture ELISA in serum samples	Low cost	-High sensitivity -Easy to carry out	-Potential false negatives -Retest positives in 3 weeks toconfirm PI	-Serum at 4-8ºC
Antigen-Capture ELISA in skin samples	Low cost	-High sensitivity -Usually identifies only PI animals	-Will generally not identify PI animals	-Skin samples-ear notches -Send in insulated container with cold packs -Do not allow to dry out
Antigen-Capture ELISA in tissue/leukocytes	Low cost	-High sensitivity	-Labor-intensive to prepare buffy coat	-Whole blood (EDTA) or tissues at 4-8ºC
Polymerase chain reaction (PCR)	Moderate to high cost	-High sensitivity	-Retest positives in 3 weeks to confirm PI	-Blood/serum, ear notches, milk, semen and tissues at 4-8ºC

I. 4. 6. 1. Molecular characterization

Rapid and accurate detection of emerging viruses is essential for rapid response, optimized clinical care and to limit the spread of these viruses. Ideally, a diagnostic test needs to be rapid, cheap, accurate, and applicable in remote settings (Powers and Waterman, 2017). In the past, diagnosis of pathogenic viruses was based on virus isolation and serology. However, these methods have some drawbacks. Virus isolation is expensive and laborious. Serology tests lack the sensitivity and specificity required for detection of viruses at a low level, although it is useful for large population screenings.

Dramatic advances in molecular methods have revolutionized the detection and characterization of emerging viruses. Molecular methods are methods which are commonly employed in molecular biology studies and other disciplines of biological sciences dealing with manipulation and analysis of nucleic acids and proteins. Currently, molecular methods find

wide applications in the diagnosis and research of pathogenic viruses. One of the molecular methods widely used in the detection and identification of pathogenic viruses is the polymerase chain reaction (PCR).

PCR

Globally, the most widely used molecular tests today are based on the amplification of the target genome (DNA or RNA) and are based on the PCR technique (Mullis et al., 1986). A virus is a small infectious agent that can only multiply inside living organisms by directing the host cell's machinery to generate more virus. The genetic material of a virus is either RNA or DNA. The involvement and contribution of PCR in the field of diagnostics is considerable from the point of view of performance in terms of sensitivity and specificity. PCR is an in vitro enzymatic process used to amplify a single, or a few copies, of DNA/RNA across several orders of magnitude, resulting in thousands to millions of copies of a specific DNA/RNA fragment. The principle of amplification of a genomic region makes it possible to multiply a DNA or RNA molecule, initially present in very small quantities, in order to be detected or for other applications requiring a lot of material like isolation and culture. The specificity of PCR is linked to the use of oligonucleotides complementary to the targeted nucleotide sequence. The design of oligonucleotides is decisive in the specificity of the reaction. The targeted area of the genome needs to slightly variable and highly conserved to ensure the hybridization of the primers within the same viral family. Good primer design and optimized PCR conditions are essential for a successful reaction.

PCR types and probes

Conventional PCR provides a qualitative or even semi-quantitative result and it is now being replaced in favour of quantitative real-time PCR (qPCR). The qPCR provides absolute or relative quantitative information. This allows the quantification of the genome of a microorganism or the expression of a gene (RT-qPCR) from the host involved, for example, in response to infection. qPCR is today implemented around two large fluorescence formats: SYBR Green, which uses the properties of a specific fluorophore of double stranded DNA, and a second type based on the use of hydrolysis or Taqman probes.

SYBR Green chemistry is based on the use of DNA intercalating molecules which emit fluorescence when incorporated into a double strand of DNA, even for small amounts of DNA. This technology has increased sensitivity, potentially allowing the detection of only 20 pg of genome DNA initially present in a sample (Karlsen Steen and Nesland, 1995). The specificity of the reaction is also ensured by the interpretation of the fusion curve, which provides information relating to the nitrogenous base composition of the amplified DNA fragment.The use of a Taqman probe considerably increases the specificity of the reaction since it is a nucleotide sequence complementary to the region of DNA to be amplified. In addition to the primers, hybridization of the probe will be necessary for the detection of the targeted agent. In addition, fluorescence will only be emitted if the DNA polymerase synthesizes the DNA strand complementary to the DNA sought.

The choice between SYBR Green chemistry and Taqman chemistry is made according to the specificity desired. A Taqman PCR is preferred when the aim is to reduce the risk of variability. SYBR Green chemistry has the advantage of being able to amplify a potentially variable zone, provided that the sites of hybridization of the primers are stable. The analysis of the fusion curve offered by the SYBR Green technology provides, for example, the possibility of identifying the presence of mutants or of relevant motifs, in particular thanks to High Resolution Melting technology.

PCR is the technology of choice for finding a specific pathogen. However, the field of diagnosis sometimes requires, depending on the clinical context, the search for several agents in the same sample and in a single reaction. The objective of the maneuver is not only economical, but it also saves time and preserves the samples. This is called multiplex PCR. The Multiplex PCR is now widely marketed in the form of kits using Taqman technology. Indeed, a reaction dedicated to the search for several pathogens will be done by labeling different probes with fluorophores whose emission wavelengths are in distinct spectra. The limiting factor of the multiplexing capacity is determined by the availability and the diversity of the fluorochromes as well as by the capacity of the thermocycler to be able to discriminate the different fluorescence signals in real time.

High throughput PCR

PCR remains a flagship tool in view of its qualities of specificity and sensitivity. However, the challenge lies in the possibility of being able to search for a large panel of microorganisms in a large number of samples, simultaneously. The development of innovative diagnostic methods is accompanied by a decrease in reaction volumes, microfluidic systems coupled with signal detection devices based on nanotechnologies, which are now part of the landscape of molecular analysis (Coelho et al., 2017). This reduction in volumes therefore allows a multiplication of reactions. The conventional 96-well plate format has been supplemented by a 384-well format to increase screening capacity; however, qPCR still shows limited multiplexing capacity. The analysis proposed by Biomark (Fluidigm) is qualified as a high-throughput test because it allows the screening of 48 or 96 samples against 48 or 96 pairs of primers in a single qPCR reaction. Nevertheless, this is still targeted research requiring prior knowledge of the agents sought and their genomes, all provided that a genetic modification does not hamper the analysis.

The ingredients needed for PCR assay include template DNA, primers, nucleotides, and thermostable DNA polymerase. The DNA polymerase is the key enzyme responsible for linking individual nucleotides together to form the PCR product. The nucleotides constitute four bases, adenine (A), thymine (T), cytosine (C), and guanine (G) that act as the building blocks used by the DNA polymerase to synthesize the PCR product. The primer is a short piece of single-stranded DNA (generally about 18–22 bases) with a defined sequence complementary to the target DNA that is to be detected and amplified. A number of factors need to be considered in designing primers. The size of the primers is optimized to be long enough for adequate specificity and short enough for the primer to bind easily to the template at annealing temperature. During the PCR process, the primers function as starting points for DNA synthesis. They are required because the DNA polymerases can only add new nucleotides to an existing strand of DNA. The pairing of primers in the reaction (forward and reverse primers) specify the exact DNA fragment to be amplified.

Bovine Viral Diarrhoea Virus molecular detection

The RNA genome of BVDV is one of the largest (12500 bases=12.5 kb) among members of the Flaviviridae family (Colett et al., 1988). The virus genome is single stranded RNA and it consists of a long 5′ untranslated region which contains an internal ribosomal entry site (IRES) for translation of viral proteins (Chon et al., 1998). It is composed of a single ORF flanked by 5´ and 3´ untranslated regions (UTR) and encodes for a long polyprotein (NH2-Npro-CErns-E1-E2-p7-NS2-3-NS4A-NS4B-NS5A-NS5B-COOH) that is processed by viral and cellular proteases, thus generating structural and non-structural proteins (Figure 14).

Reverse transcription-polymerase chain reaction (RT-PCR) is a rapid and sensitive method for detection of viral RNA that has the advantage of being insensitive to toxic substances in the specimen. A general RT-PCR protocol includes four different steps: extraction of RNA prior to the PCR, reverse transcription to complementary DNA, primer-directed amplification, and detection of amplified products. Characterization of the BVDV virus genome has classically been performed by sequence analysis in any of three regions of the BVDV genome. These regions are the 5'UTR (Vilcek et al., 2001)(Table 3), non-structural N-terminal protein (Npro) region and the E2 region (Flores et al., 2000; Tajima et al., 2001). The Npro and the E2 regions are in the ORF and both are highly conserved within the BVDV genome. However, the 5'UTR is considered the most highly conserved region of *Pestiviruses* (Deng and Brock, 1993). There is good agreement in genotypic classification when using any of these regions and all have been used to characterize the BVDV virus at the subgenotype level (Kim et al., 2009; Vilcek et al., 2001).

Table 3 Primer information and sensitivity of the 5'-UTR–based RT-PCR assays used for detection of *Pestiviruses* in bovine sera (Monteiro et al., 2019).

Primer	Position (nt)	Target (bp)	Sequence (5'–3')	Sensitivity§		
				BVDV-1	BVDV-2	HoBiPeV
324-326	108–395*	288	ATGCCCWATTAGTAGGACTAGCA TCAACTCCATGTGCCATGTAC	10	10	$>10^3$
HCV90-368	107–389*	283	CATGCCCATAGTAGGAC CCATGTGCCATGTACAG	10	10^2	10^3
BP189-389	190–390*	201	AGTCGTCARAGTGGTTCGAC TCCATGTGCCATGTACA	1	1	1
BVDV-2 2F-2R	143–365†	223	GCGGTAGCAGTGAGTTTATTGG TTTACTAGCGGGATAGCAGGTC	ND	10	ND
N2-R5	183–332‡	150	TCGACGCATCAAGGAATGCCT TAGCAGGTCTCTGCAACACCCTAT	ND	ND	1

ND= not detected until 10^4 $TCID_{50}$/reaction;
RT-PCR = reverse transcription
PCR, UTR= untranslated region
*Position based on BVDV_1 sequence NADL (M31182.1)
†Position based on BVDV_2 sequence 890 (U18059)
‡Position based on HoBiPeV sequence D32/00 HoBi (AB871953.1)
§Sensitivity tests were performed using Senger (BVDV_1), 890 (BVDV_2) and SV757/15 (HoBiPeV) strains
The value is presented in $TCID_{50}$/reaction

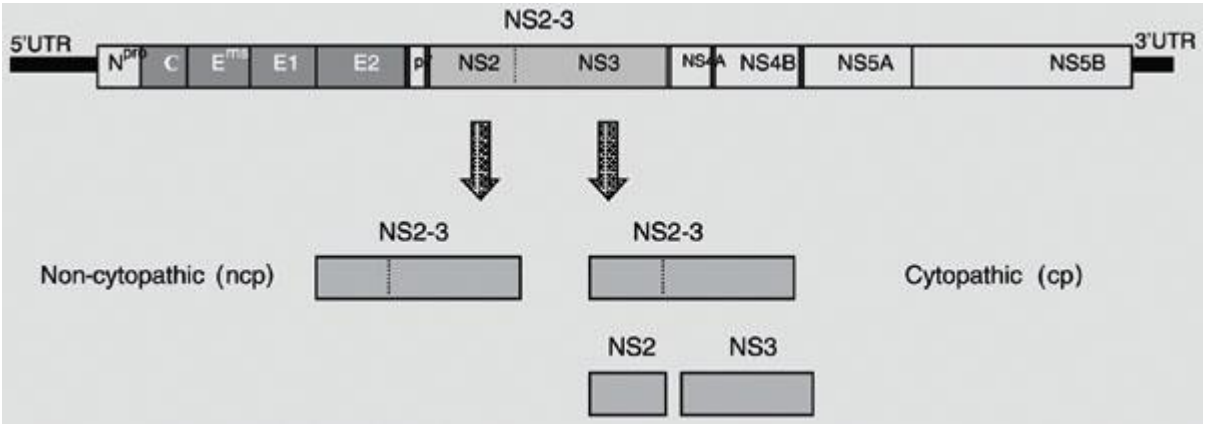


Figure 14. Organization of the Bovine Viral Diarrhoea virus genome and processing of the NS2-3 polypeptide in cytopathic (cp) and non-cytopathic (ncp) isolates. In ncp isolates, NS2-3 is expressed as a single-long polypeptide; in cp isolates both the entire NS2-3 a single-long polypeptide; in cp isolates both the entire NS2-3 and NS3 polypeptides are found. In cp viruses, NS3 expression may result from NS2-3 cleavage or translation of a duplicated gene. UTR = untranslated region.

I. 4. 6. 2. Sequencing

The ability of pathogenic viruses to adapt to new environments, cross species barriers, develop resistance to antiviral drugs, and to evade existing vaccines is associated with the accumulation of mutations throughout their genomes. DNA sequencing technologies allow determination of the exact nucleotide sequence of each viral genome, in order to better understand viruses especially in terms of genetic diversity, evolution, pathogenesis, ecology, and vaccine design. Together with PCR screening can be used to track the ancestral relationships between viruses and infer their possible origins. Sanger sequencing is the technique of choice for a punctual, rapid and targeted need when there is no need to use high-speed sequencing. DNA sequencing by the Sanger technique (Sanger Nicklen and Coulson, 1992) is a method of reading a series of nitrogenous bases within a DNA molecule, it is a sequencing technique by termination of reaction. The method of Maxam, Gilbert (Maxam and Gilbert, 1977) has been much less utilized.

Manual method

The most used method is Sanger sequencing. The principle of this technique is based on the use of DNA polymerase having the capacity to synthesize a complementary strand from a template strand. The synthesis of the complementary strand is initiated following the fixing of a specific primer for the PCR product to be analyzed. This primer, located upstream of the DNA to be sequenced, allows the elongation of a new complementary strand following the random incorporation of deoxyribonucleotides triphosphate (dNTP) in excess, and dideoxynucleotides (ddNTP) in limiting quantities, which are incorporated very rarely and at random. The ddNTP differ from dNTP by their 3 ′ end where the group -OH is replaced by - H. This modification prevents the phosphodiester bond with the following nucleotide and interrupts the chain extension. At the end of the reaction, the medium is composed of fragments of all sizes which are then separated by electrophoretic migration on polyacrylamide gel, according to their molecular masses.

Automated methods

Adaptation of the Sanger method to fluorescence

The ddNTPs (ddATP, ddTTP, ddCTP, ddGTP) are each marked by a fluorophore of different color with a specific emission spectrum. As a result, each DNA fragment synthesized carries a terminal fluorophore called the elongation terminator (BigDye terminator). The advantage of this technique is that it is carried out in a single sequencing reaction with a reaction mixture composed of ADN matrice, enzyme, dNTP, ddATP, ddCTP, ddGTP and ddTTP. The mixture is then injected into an automatic sequencer. The migration of PCR products is carried out by capillary electrophoresis: the DNA fragments are separated according to their length by an intense electric field. Through a resonance energy transfer system, the donor fluorochrome is excited by an argon laser beam whose emission takes place at two distinct wavelengths (488 nm and 514.5 nm). The emitted fluorescence is picked up by the acceptor fluorochrome which will re-emit according to a unique fluorescence spectrum for each ddNTP. This fluorescence emission is picked up by a cell Charge Couple Divice camera (CCD) and processed by computer to associate the corresponding base and, thus, define the nucleotide sequence of the initial DNA strand (Figures 15 and 16) (Mayer, 2011). The chromatograms obtained are then analyzed using software such as BIOEDIT v7.0.1 and then compared to the sequences listed in the database (GenBank), National Center for Biotechnology Information (NCBI), via the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990).

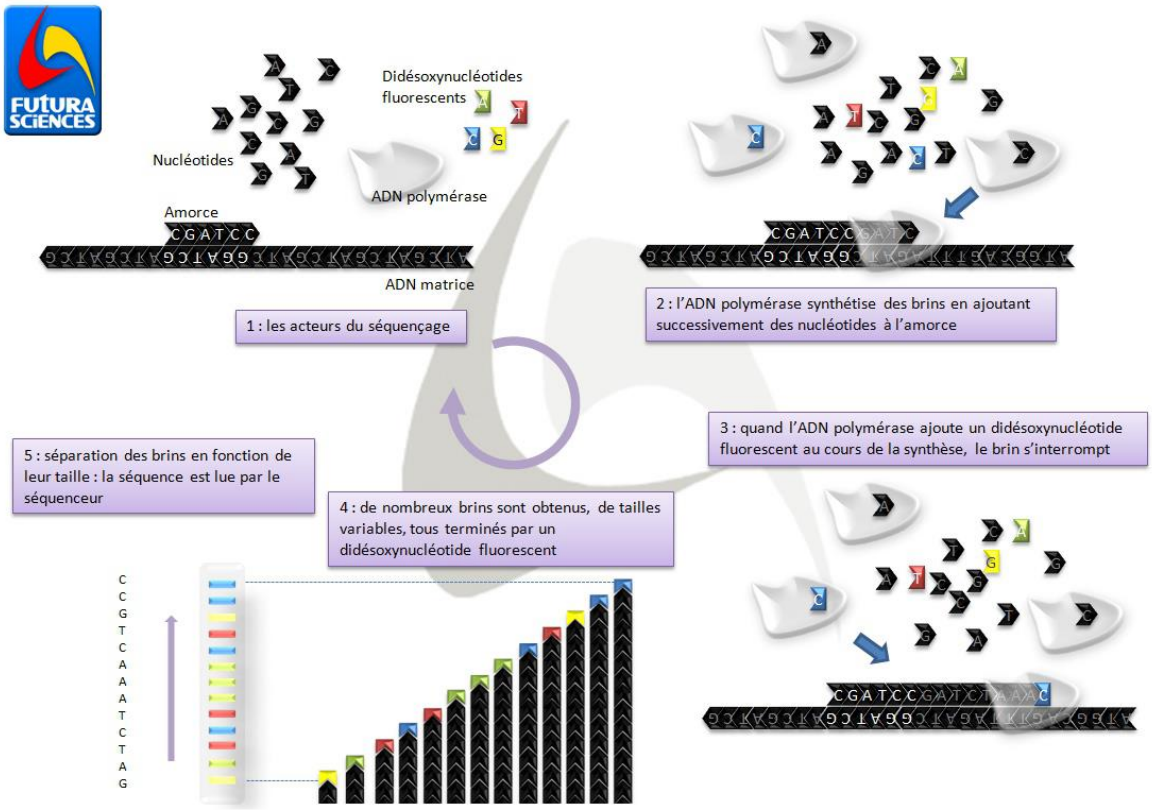


Figure 15. Sanger sequencing method adapted to fluorescence (Mayer, 2011)

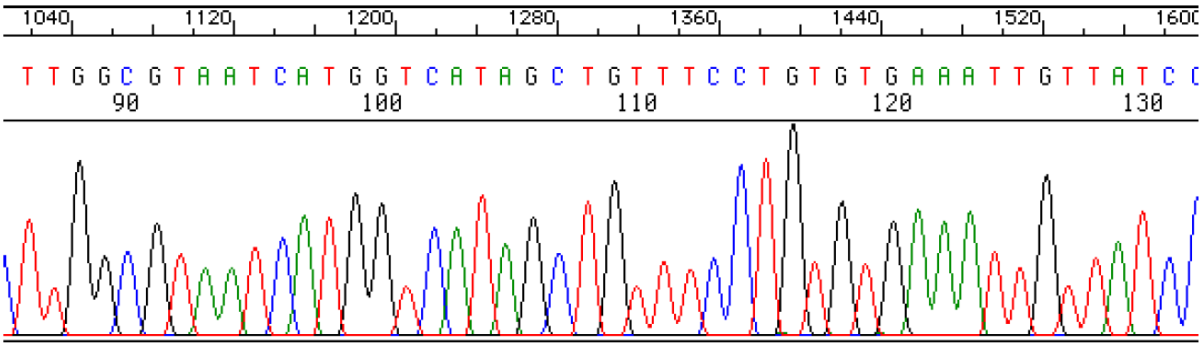


Figure 16. General scheme of sequencing according to the technique Big Dye.

Pyrosequencing

Pyrosequencing allows rapid sequencing at a lower cost than sequencing by the Sanger method. The dNTP is added one after the other, not all together as in the Sanger method. If the added nucleotide is complementary to the nucleotide of the template strand, it is incorporated into the strand being synthesized and an inorganic pyrophosphate (PPi) is released. The light signal is picked up by a CCD sensor and translated by a peak on the pyrogram™ (Figure 17). The drawback of this method is the size limit of the analyzable fragments (up to only 100 nucleotides).

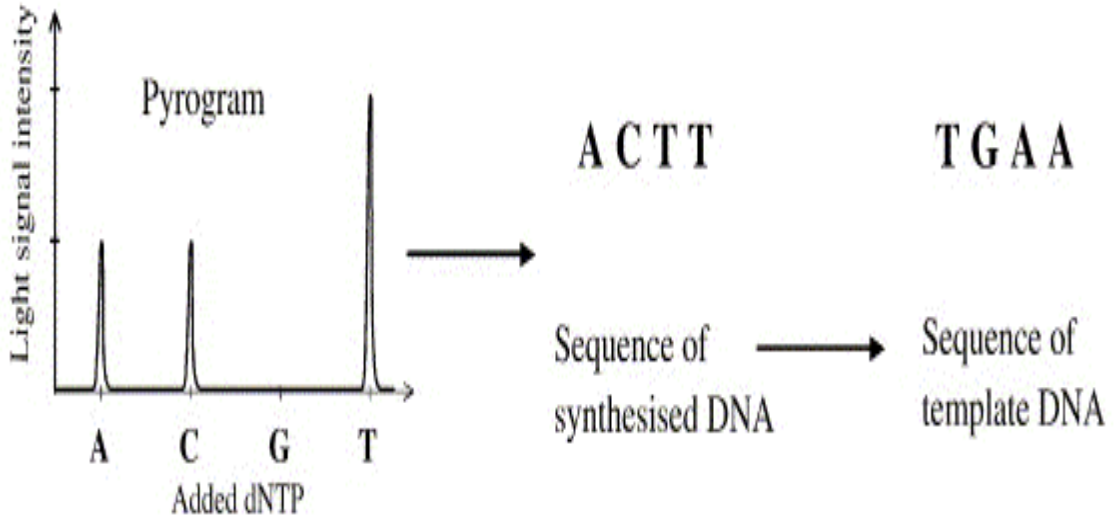


Figure 17. Representation of a pyrogramme, from *Ahmadian Ehn and Hober, (2006)*.

I. 5. Diagnosis approach of abortion in a herd

Controlling abortion and preventing the high economic losses derived from it are vital for cattle breeders in Algeria. Previous studies suggested that over half fertilizations result in embryo loss before pregnancy is detected in Algeria. In such situation, farmers, along with their veterinary practitioners and potentially state veterinarians, expect rapid reliable results from veterinary diagnostic laboratories, a process that is not always easily achieved (Nicole Borel et al., 2014). Establishing an aetiological diagnosis remains challenging owing to the large variety of bacteria, protozoa, viruses and fungi that have been associated with abortion in cattle. Economic constraints limit and reduce considerably the range of diagnostic methods available for routine diagnostics, and decomposition of the conceptus or lack of proper foetal and/or maternal samples further restrict the diagnostic success (Wolf-Jäckel et al., 2020). Given that rapid and accurate laboratory diagnosis is central to controlling abortion outbreaks, the submission of

tissue samples to laboratories offering the most appropriate tests is essential. Direct antigen and/or DNA/RNA detection methods are the currently preferred methods of reaching an aetiological diagnosis. Ideally, these results are confirmed by the demonstration of corresponding macroscopic and/or histopathological lesions in the foetus and/or the placenta (Nicole Borel et al., 2014). Even under optimal conditions, the percentage of aetiological diagnoses of abortion reached in ruminants can be relatively low (John Matthews, 2016; Moeller, 2011). Wolf-Jäckel et al., (2020) categorized the diagnostic findings of aborted and stillbirth bovine foetus or foetal tissue, foetal placenta and maternal blood samples into four main groups:

1. Bacterial infection was diagnosed by the isolation of bacteria and the presence of consistent lesions.
2. Mycoses were diagnosed by the presence of hyphae or yeast cells in tissue sections associated with inflammation.
3. Protozoal infection was diagnosed by findings of non-suppurative inflammation in foetal organs as follows: cases were considered positive if focal to multifocal non suppurative necrotizing encephalitis was found together with non-suppurative interstitial myocarditis and/or non-suppurative hepatitis. In the absence of brain lesions or exclusion of the brain due to extensive decomposition, the presence of non-suppurative interstitial myocarditis together with non-suppurative hepatitis was regarded as being diagnostic of protozoal abortion.
4. Infection with BVDV was diagnosed by demonstration of the BVDV antigen within foetal tissues.

In the case of dual infection (i.e. bacterial and protozoal infections), the case was diagnosed as protozoal abortion because the protozoa-associated inflammatory lesions were more severe than the bacteria-associated lesions. Overall, this allowed the identification of the likely cause of abortion associated with the main infectious agents.

However, Nicole Borel et al., (2014) reported that globally and particularly in Europe, diagnostic laboratories usually focus on the most likely aetiologies, and those with zoonotic potential. In New Zealand, Reichel et al., (2018) reported that *N. caninum* ranks highly as an

important cause of reproductive loss along with fungal and bacterial infections and concluded that effective disease control strategies require rapid diagnoses at diagnostic laboratories.

Abortion in dairy cows brings about breeding, productive and economic damages. The cost of abortion varies according to factors such as time of gestation, milk production, days in milk, time of insemination after parturition, cost of nutrition, sperm costs and laboratory costs, which differ from country to country. The costs of laboratory examinations may be considerable and, even under optimal conditions, the percentage of aetiological diagnoses reached can be relatively low. In Algeria, abortion is not a notifiable disease. Therefore, official data on the incidence of cases are not available from the Algerian Ministry of agriculture and rural development. Nevertheless, many unpublished studies have been performed in different regions and cities, most of them in the form of master's and PhD thesis. In opposite, many investigations on the seroprevalence of abortive agents have been published in several ruminant species from different regions of Algeria(Kardjadj, 2016; Ghalmi et al., 2012; Achour et al., 2012; Derdour et al., 2017; Khames Yekkour Fernández-Rubio, et al., 2018; Hireche et al., 2016; Merdja et al., 2015; Djellata et al., 2019; Feknous et al., 2018; Saidi et al., 2018) and neighbouring countries (Amdouni et al., 2019 ; Yahyaoui Azami et al., 2018 ; Mahin et al., 1982 ; Benkirane et al., 2015 ; Lucchese et al., 2016 ; Fassi Fihri et al., 2019 ; Meriem Essayagh et al., 2017 ; Wareth et al., 2014 ; Ahmed et al., 2019 ; Fereig et al., 2016 ; Jennings et al., 2007 ; Klemmer et al., 2018 ; Abdel-Moein and Hamza, 2017). Although abortion of infectious origin is considered a significant problem in dairy cattle farming because of its economic loss, the declaration and investigation of cases is not mandatory in Algeria.

II. Hypothesis and Objectives

The hypothesis of the present Thesis is that the abortive diseases of infectious origin are widespread in domestic ruminants in Algeria. Therefore, the main objective of the different studies that compose the Thesis is to determine the presence of the main abortive pathogens and their risk factors in cattle in Algeria. In Study-I, a cross-sectional serological study for the detection of antibodies against *N. caninum* and *T. gondii* was conducted on dairy farms from North-eastern Algeria. Also, the presence of *Neospora caninum* and *Toxoplasma gondii* DNA in aborted foetuses from the same dairy farms was analysed by qPCR. In addition, the risk factors of neosporosis and toxoplasmosis were analysed. The Study-II evaluated the seroprevalence and risk factors of the bacteria *Chlamydia abortus*, *Coxiella burnetii* and *Brucella spp* in dairy cattle from North-estern Algeria. In the Study-III, we performed a cross-sectional serosurvey and evaluated the risk factors associated with BVDV on dairy farms from North-western Algeria. The presence of *Pestivirus* RNA in sera was also analysed using a Reverse Transcription-qPCR and positive samples were sequenced. Additionally, we conducted a literature review of the presence of *Pestivirus* in ruminants in North Africa using a systematic search and compilation methodology to identify gaps of knowledge for future research.

III. Studies

III. 1. Study I. Seroprevalence, risk factors and molecular detection of *Neospora caninum* and *Toxoplasma gondii* in cattle in north-eastern Algeria

III. 1. 1. Introduction

Toxoplasma gondii and *Neospora caninum* are two closely-related, intra-cellular apicomplexan protozoan parasites of worldwide distribution that have been implicated in abortion and reproductive disorders, mainly in ruminants (Dubey Schares and Ortega-Mora, 2007; Dubey, 2009). Toxoplasmosis, caused by *T. gondii*, affects most species of warm-blooded animals, including birds, and is zoonotic (Dubey, 2009). Cats which are the only hosts of *T. gondii* that can excrete environmentally resistant oocysts, are most frequently infected with *T. gondii* via predation on infected birds and rodents. *Neospora caninum* is considered one of the most important causes of abortion in cattle worldwide (Quintanilla-Gozalo et al., 1999; Dubey, Schares and Ortega-Mora, 2007).

Accordingly, the study aimed:

(i) To determine the individual and herd seroprevalence of protozoans like *Neospora caninum* and *Toxoplasma gondii*, in dairy cattle in North-eastern of Algeria.

(ii) To investigate potential risk factors related to seropositivity of dairy cattle herds.

(iii) To identify the occurrence of reproductive disorders in relation with infectious diseases.

(iv) To confirm the presence of these two pathogens by using molecular detection.

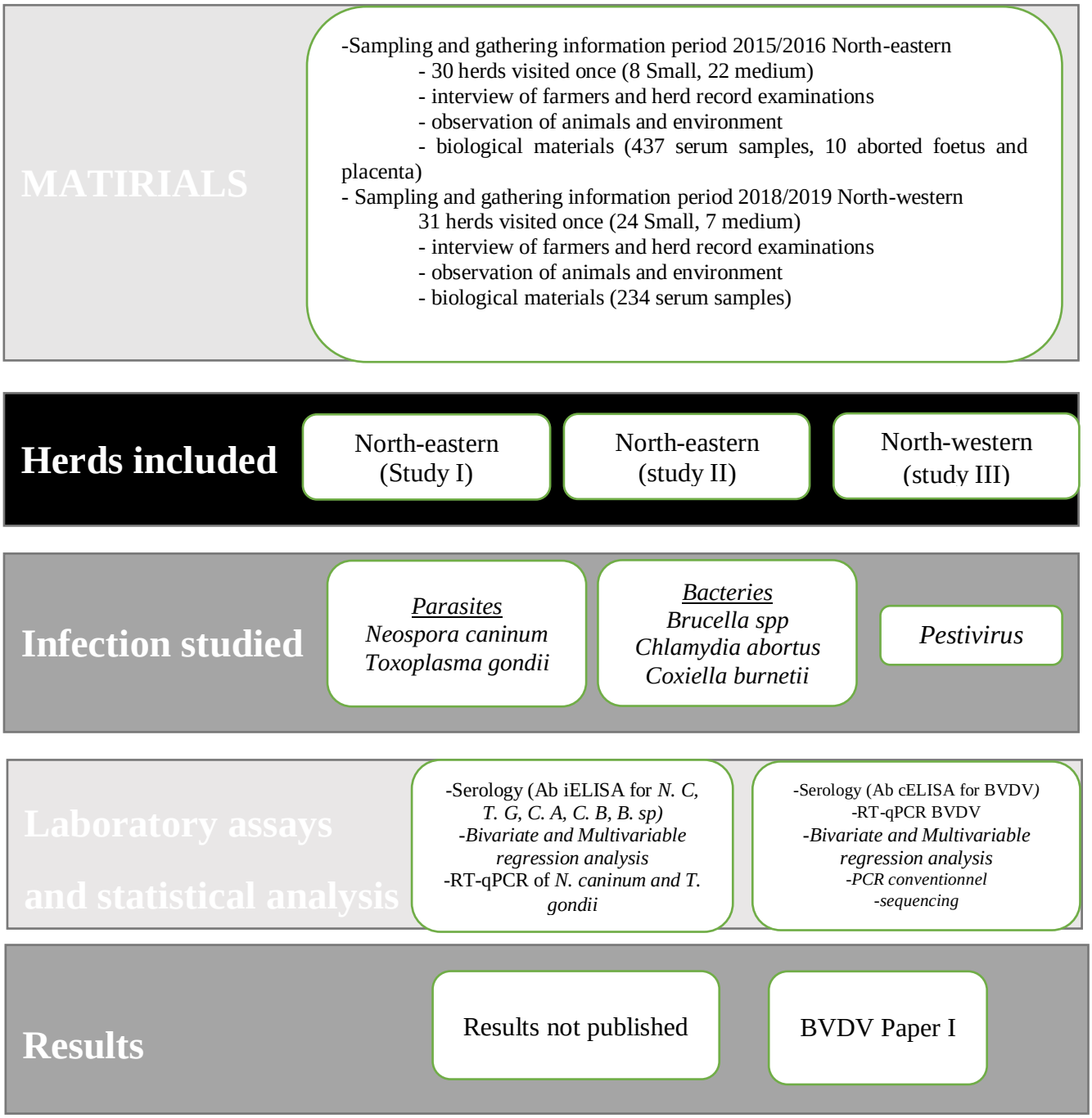


Figure 18. Overview of the study; data and materials collected, infections studied, laboratory and statistical analysis performed and resulting publication

III. 1. 2. Materials and methods

III. 1. 2. 1. Area of study and target population:

The study area included Batna (Region-I; 35°.55"N 6°.15"E), Khenchela (Region-II; 35.43"N, 7.14"E) and Setif (Region-III; 36°.0"N 5°.3"E), these three regions are in the North-eastern Algeria (Figure 19). Batna region is located in the Aurès region, at 1,037 m above sea level with an area of 12,192 km^2 and is known to have many different climates (semi-arid cold, cold desert, warm, summery Mediterranean, hot desert and finally warm-summer Mediterranean climate) with an annual rainfall of about 329 mm with a rainy season from January to April, average annual temperature is 14.2 °C (6.2ºC to 25.58°C) (Climat Batna, 2020). This region is located approximately 214 Km from the Mediterranean coasts. Batna region is delimited to the north, by the province of Mila; to the north-east by the province of Oum-El-Bouaghi; to the east by the province of Khenchela, to the south by the province of Biskra; and to the north-west by the province of Setif (Figure 19).

Setif region (North-Eastern Algeria; north-west border of the Batna region) has a warm and temperate climate. In winter, the rains are much more important in Sétif than they are in summer. The Köppen-Geiger classification is of the Csa (Mediterranean climate) type (hot dry-summer). The average annual temperature is 13.3°C (4.5ºC to 24.0ºC) in Setif. Average annual precipitation reached 469 mm (Climat Sétif 2020). As stated by Mouffok Charef-Eddine, (2014), cattle farming in Setif and Bordj Bou Arréridj (North-Eastern Algeria) is generally structured in small, medium-sized workshops of 13 Large Livestock Unit (LLU), including generally eight dairy cows, three heifers and two bull calves. Cattle in the region are often associated with sheep (42%) or operated alone (47%). Commercial strategies are based on total (45%) or partial sales (50%) milk according to the farming system used. In addition, calves are often sold at a late age (64%) as lean or finished on the farm, thus helping to improve the farm's cash flow. The results of the typology highlighted the presence of five types of cattle workshops expressing an increasing gradient of specialization.

1. The balanced mixed system is poorly represented (4%).
2. The dairy system characterizes farms specializing in milk production (15%) made up of a reduced herd (9 LLU) dominated by dairy cows (7 heads).

3. The mixed meat-oriented system is the dominant system in the study area with more than 50% of the cases. Over 60% of farmers in this group associate cattle with a relatively large herd of meat sheep (> 30 heads).
4. The suckler cattle (meat) system represents only 5% of farms with relatively large herd size.
5. The mixed dairy-oriented system (20%) whose income is made up more from the sale of milk (Mouffok 2018).

Khenchela's climate is classified as warm and temperate. In Khenchela, the rains are less intense than they are in winter. According to Köppen and Geiger (Climat Batna, 2020), the climate is classified as Csb (supra-Mediterranean climate). The average temperature in Khenchela is 12.6°C (4.2ºC to 21.6ºC). The rainfall here averages 446 mm being July the driest month. Essentially, the north-eastern region of Algeria is known to include the most important number of dairy herd cattle in comparison with other Algerian region as previously cited (bovine breeding in Algeria chapter) about 80% of cattle farming is in the northern regions of the country, 59% in the east, which is the wettest area of the country, against 14% in the west, where sheep and goats are preferred, and 22% in the centre and only 5% in the south of the country (Kirat, 2007).

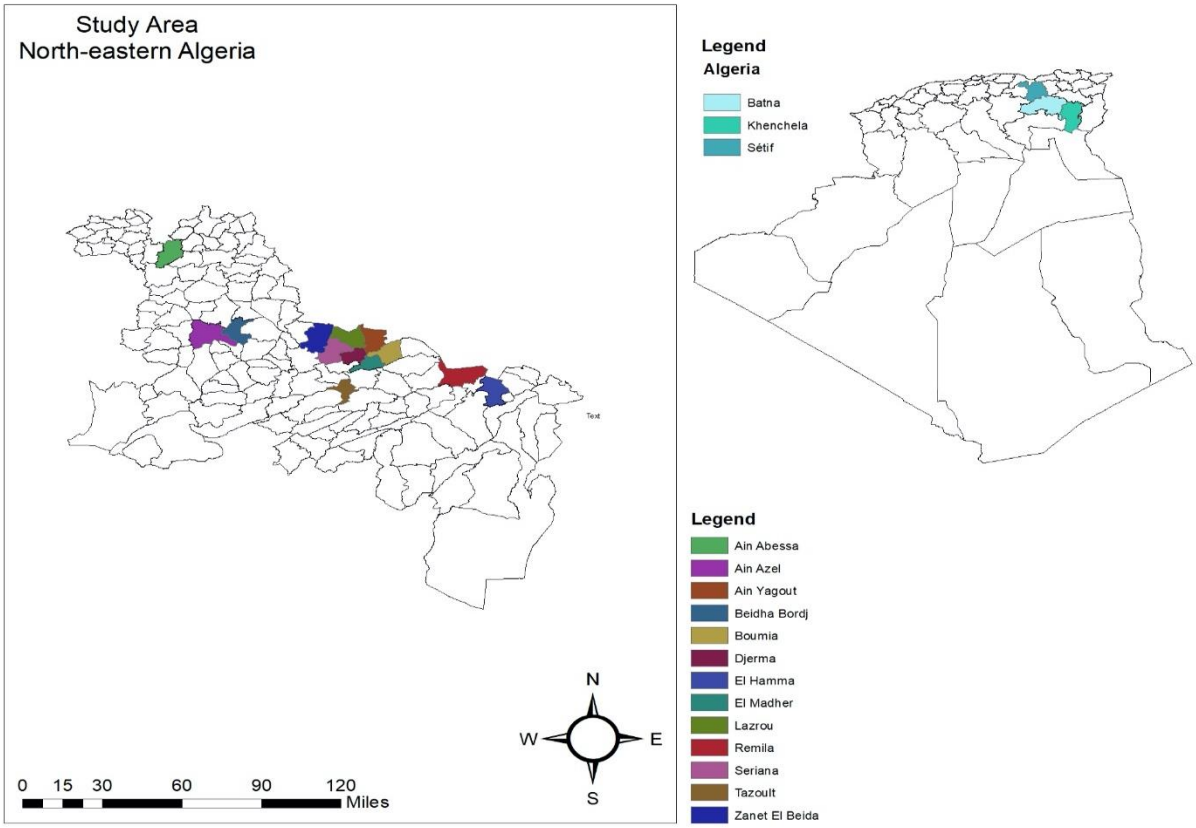


Figure 19. Representative map of north-eastern Algeria sampling.

III. 1. 2. 1. Calculation of the sample size.

A representative sample of female cattle between 6 and 182 months of age was drawn by random sampling in two steps. A two-stage sampling survey was carried out in north-western Algeria. For the first stage of sampling (sampling of herds), the sample size for disease detection was calculated based on the following formula (Dohoo et al., 2003).

$$n_1 = \left(1 - (1 - \alpha_1)^{\frac{1}{D_1}}\right) \times \left(N_1 - \frac{D_1 - 1}{2}\right)$$

where α_1 was the confidence level (set at 95%), D_1 was the minimum number of infected herds (estimated as $D_1 = Prev_1 \times N_1$), where $Prev_1$ was the minimum herd prevalence to be detected (set at 10%), and N_1 was the population of herds (which in our case were 292 dairy herds). The estimate of n_1 was 30 herds. The Official Veterinarian in Batna, Khanchela (Hamma and Roknia municipalities) and Setif (Ain Abbas, Ain Azel and Beidha Bordj municipalities) Veterinary Office provided a list of all cattle herds registered in the province, which included information of the herd owner, the address or number of animals. The sampling frame included

292 dairy cattle herds. No formal random process was used for the selection of herds. Instead, from the list, a herd was randomly selected, and the herd owner was contacted, and asked, first a) whether they complied with the inclusion criteria, and then b) whether they were willing to participate. The process was repeated until the number of herds needed for the first stage was completed. The inclusion criteria comprised that the herd had at least one female animal above six months, and that the milk was not only for own consumption (i.e. some of the milk was sold). This age category was selected to avoid interference as much as possible the detection of maternal antibodies in the seroprevalence studies (Chase Hurley and Reber, 2008).

For the second stage (sampling of animals within herds), the sample sizes for disease detection were also calculated based on the formula by Dohoo et al., (2003):

$$n_{2i} = \left(1 - (1 - \alpha_2)^{\frac{1}{D_{2i}}}\right) \times \left(N_{2i} - \frac{D_{2i} - 1}{2}\right)$$

where α_2 was the confidence level (set at 95%), D_{2i} was the minimum number of infected animals in herd i (estimated as $D_{2i} = Prev_2 \times N_i$), where $Prev_2$ was the minimum within-herd prevalence to be detected (set at 30%), and N_{2i} was the population size of herd i (size of herds selected in stage 1 varied between 7 and 62). The estimate of n_2 varied between 4 and 8. The sampling of animals within herds (second stage) was also random, although because of the lack of proper sampling frames, no formal random process was used either. Random animals in the herd were selected until the number of animals needed for the second stage was completed. However, because of logistics problems, the number of samples per herd could not always be completed, and therefore some extra samples were collected in some of the remaining herds, and also a few extra herds were sampled. Within herds, animals were randomly selected to allow the detection of infected individuals if infection was present in at least 30% of animals with a 95% confidence (i.e. up to 10 animals depending on the size of the herd). Sample sizes for the two stages were calculated using Epitools (Sergeant, 2018). Holstein/Friesian, Monbeliard and crossbreed were the most common breeds.

In total, 30 herds were visited and 437 animals were sampled. Eigth herds were small scale dairy herds (1-10 cattle) and 22 medium scale herds (10-100 cattle) (Table 4). Holstein/Friesian, Monbeliard and crossbreed were the most common breeds in the two regions. The crossbred cow is the principal component of herds in the smallholder farming, it is resulting of crossing between local breed and imported dairy cow, commonly, the frisonne Holstein and

Monbeliarde. Blood samples from 437 dairy cattle (344 from 22 dairy farms, from Batna and Khanchela; 93 dairy cattle from Setif) were collected by a simple random sampling method from animals aged between 9 and 180 months (Table 4, 5, 6, 7) (Figure 19).

Table 4. Size, number of herds, municipalities and animal categories sampled from the two study locations (regions).

Characterises	**Regions**	**North-East region (2015-2016)**
Herd size		
Herds		**30**
Small (1-10 cattle)		8
Medium (10-100 cattle)		22
Municipalities		14
Parity		
Heifer		42
Cow		395
Total		**437**

Table 5. Region, Municipalities, Number of animals and Number of herds.

Province	***Municipality***	***Number of animals***	***Number of herds***
Batna	Djermaa	47	1
	Ain assafir	25	2
	Maadher	24	2
	Lazrou	14	2
	Seriana	25	1
	Ain yagout	41	5
	Zana Baidha	45	3
	Boumia	31	2
	Total	**252**	**18**
Khanechela	Hamma	52	3
	Roknia	40	1
	Total	**92**	**4**
Setif	Ain abbas	24	2
	Ain Hdjar	21	2
	Douar Ajail	38	3
	Ain Azel	10	1
	Total	**93**	**8**
Total		437	30

Table 6. Summarize of animals and herds per region.

	Batna	**Khanchela**	**Setif**	**Total**
Animals	252	92	93	437
Herds	18	4	8	30

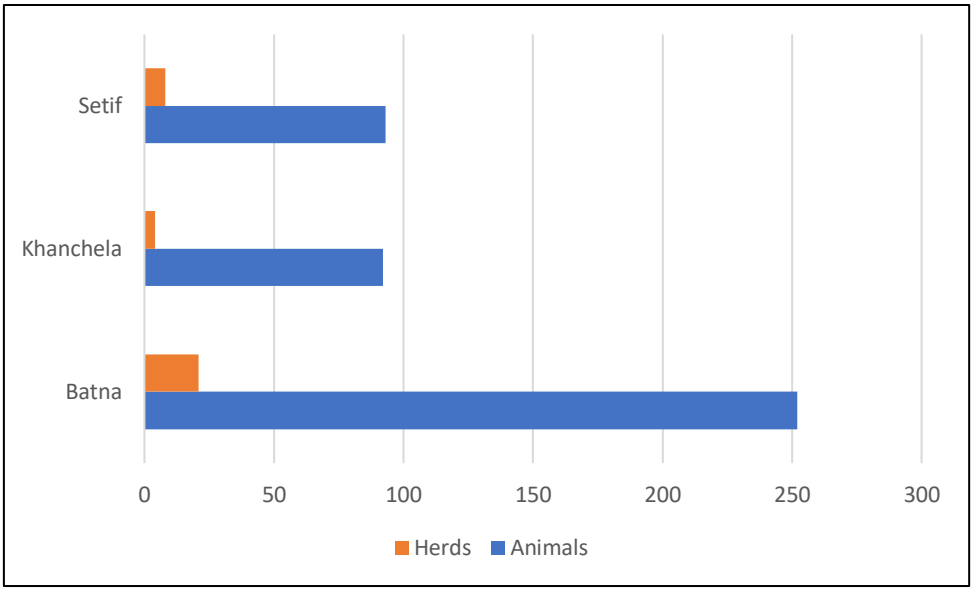


Figure 20. Design of sampling in each region. Blue: number of animals sampled. Orange: number of herds sampled.

Table 7. Distribution of animals sampled by age in north-eastern Algeria.

Age category	Frequency	Percentage %
≤24 months	41	9.4
>24 months ≤48	133	30.4
>48 months ≤60	113	25.9
> 60 months	150	34.3
Total	437	100

Samples were collected during field trips conducted between September 2015 and May 2016, where each herd was visited once. Blood sampling (5 ml) were taken from the coccygian vein of the animals on sterile dry vacutainer tubes, using disposable needles. The samples were immediately sent on ice to laboratory. The sera were extracted by centrifugation at 1000g for 10 minutes, aliquoted in labeled Eppendorf tubes and then serum was removed and stored at -20°C until further testing. In case where centrifuge was not available, the blood sample were left in the fridge (+8°C) for maximum of 72 hours for serum separation. Serum samples were then pipette into sterile tubes, transported on ice to a local laboratory and immediately frozen at approximately -20°C. Information from all regions (north-Eastern Algeria) was gathered through direct observation at farms, interviews of farmers, and collection of biological material from animals. In addition, epidemiological data of each farm/breeder was recorded (Appendix 1) with the collaboration of a qualified veterinary.

III. 1. 2. 2. Serology

The search for antibodies against *N. caninum* and *T. gondii* was carried out by indirect ELISA techniques IDSCREEN® *Neospora caninum* indirect (Innovative diagnostic, Grabels, France) and IDSCREEN® Toxoplasmosis indirect multispecies (Innovative diagnostic, Grabels, France), respectively. These tests were performed at the laboratory of serology of '*The refuge*' private in Batna according to manufacturers' instructions and cut-off recommendations (Table 10). These kits are based on an indirect ELISA technique using a purified antigenic extract of *Neospora caninum*, and the P30 antigen specific to *Toxoplasma gondii*. The Sensitivity and specificity of these iELISAs test figure out in the below table.

Table 8. Sensitivity and specificity of Indirect ELISA used for the antibody detection of each pathogen agent according to the manufacturers.

	Sensitivity	Interval confidence	Specificity	Interval confidence
Neospora caninum	99,6%	(CI95%: 98.9–100)	98.9%	(CI95%: 97.4–100)
Toxoplasma gondii	98,36%	(CI 95%: 95.29%-99.44%)	99,42 %	(CI 95%: 98.8%-100%),

The wells are sensitized with the antigens. The samples to be tested and the controls are distributed in the wells. Anti-pathogen antibodies, if present, form an antigen-antibody complex that masks the epitopes of the pathogen. A conjugate anti-pathogen labeled with peroxidase (HRP) is distributed in the wells. It attaches to the epitopes of the pathogen, which remain free, forming an antigen-antibody-conjugate-HRP complex. After removal of the excess conjugate by washing, the reaction is revealed by a developer solution (TMB). The resulting coloration is linked to the quantity of specific antibodies present in the test sample:

- in the presence of antibodies in the sample, a blue color appears which becomes yellow after addition of the stop solution.
- in the absence of antibodies in the sample, wells remained clear

The color of each well is proportional to the level of anti-pathogen antibodies present in the diluted sample. After stopping the reaction, the results (optical density) were read by an ELISA plate reader. (DIALAB ELX800 G, Autriche), set at a wavelength of 450 nm. Positive and negative control sera of the five diseases are supplied with the kit. (Figure 21).

Protocol:

Almost the same protocol was performed for both pathogens.

All reagents were bringing to room temperature (21°C ± 5°C) before use and were homogenized by Vortex.

1. 90µl of Dilution Buffer 2 was distributed in each well.
   10µl of negative control was distributed in wells A1 and B1.
   10µl of positive control was distributed in wells C1 and D1.
   10µl of each sample to be tested in the remaining wells.
2. Cover the microplate and incubate 45 min ± 4 min at 21°C (±5°C).
3. The wells were emptied by Washing each well 3 times with at least 300µl of washing solution （Note: Avoid drying out of the wells between washes) (**ORGANO TEKNIKA washer).**
4. The 1X Conjugate was prepared by diluting the concentrated conjugate 10X to 1/10th in Dilution Buffer 3.
5. 100µl of 1X Conjugate was distributed in each well.
6. The plate was covered and incubated for 30 min ± 3 min at 21°C (± 5°C).
7. The wells were emptied again and each well was washed 3 times with at least 300µl of washing solution (**ORGANO TEKNIKA washer).**
8. 100µl of revelation solution was distributed in each well.
9. The plate was covered and incubated for 15 min ± 2 min at 21°C (±5°C) in the dark.
10. 100µl of Stop Solution was then dispensed into each well to stop the reaction.
11. Optical densities were measured and recorded at 450 nm (Microwell system).

Validity criteria and interpretation

The Tables 9 and 10 below summarize the validity criteria and interpretation of each ELISA.

Table 9. Validity criteria for each disease using the Indirect ELISA in cattle sera from north-eastern Algeria.

Validity	Antigen used	DO_{PC}	DO_{CP}/DO_{NC}	S/P calculation
N. caninum	purified antigenic extract of *Neospora caninum*	> 0.350	>3	$(OD_{sample} - OD_{NC})/ (OD_{PC} - OD_{NC})$ x 100
T. gondii	P30 antigen specific to *Toxoplasma gondii*	> 0.350	>3.5	OD_{sample}/OD_{PC} X 100

Table 10. Interpretation for each disease using the ELISA in cattle sera from north-eastern Algeria.

Interpretation	Negative	Doubtful	Positive	Acute infection (Strongly positive)
Neosppora caninum	S/P ≤40%	40<S/P≤50%	S/P ≥50%	-
Toxoplasma gondii	S/P ≤40%	40<S/P<50%	50≤S/P<200%	S/P ≥200%

Figure 21. ELISA plates' washer (ORGANO TEKNIKA; Microwell system); sera plate; reagents; ELISA reader (Personal photographs)

III. 1. 2. 3. Molecular detection of N. caninum and T. gondii

The analyses were conducted in different research and diagnostic laboratories. Veterinarian laboratory of biotechnology research center Constantine in Algeria as well as in the Animal Health Research Centre (IRTA-CReSA), Campus of the Autonomous University of Barcelona, Bellaterra, Spain.

Between 2016 and 2017, ten aborted foetuses of dairy cows (aged 1-8 months of gestation) were obtained from seven dairy farms located in the study area (Northeastern Algeria) (Figure 19, 20). Aborted, mummified, stillborn foetuses and bovine placentas could be collected from breeders willing to participate in the study and for whom previous serological studies were carried out, in purpose confirm that the abortion is not caused by Brucellosis. A blood sample was taken from the jugular vein or the coccygeal vein of cows that had abortion and from which aborted foetuses could be removed.

Figure 22. Cattle abortions mummified foetus (personal photographs).

After identification, the foetus samples were wrapped in plastic and placed in the portable cooler with cold storage block, then immediately sent to the laboratory. In total, 10 abortions, stillbirths, placentas, and mummified foetus were collected (Figures 22). In the laboratory, the foetus samples were placed on a clean work surface. After macroscopic examination of the placenta and foetus, these latter were autopsied. A total of 53 samples from different foetal tissues (brain, kidney, eye, spleen, liver, lung, heart, placentas) and mummified foetus were collected. For each tissue two to three samples were separately placed in sterile plastic jars (Figure 23) and stored at -20°C while waiting for DNA extraction. Blood serum of the mother was collected after centrifugation or sedimentation of the whole blood to be tested for *Brucella* spp antibodies by the rapid hemagglutination test (Rose Bengal).

III. 1. 2. 3. a. DNA extraction from animal tissue

Tissue samples were homogenized mechanically and DNA was extracted using the commercial kit QIAamp DNA Mini Kit® (QIAGEN, Hilden, Germany) from 25 mg (10 mg spleen) of each tissue. After the extraction, DNA samples were stored at -20ºC until the execution of the RT-qPCR reactions.

i. Principal

The QIAamp DNA Mini Kit uses a fast spin column or vacuum operation to simplify the purification of DNA from animal tissue samples. DNA specifically binds to the QIAamp silica gel membrane and contaminants flow away. PCR inhibitors, such as divalent cations and proteins, can be removed in two effective washing steps. the kit is based on the principle of ionic interactions and uses columns containing silica membranes capable of retaining DNA in a specific way by adjusting the pH and the salt conditions.

The preparation passes through a filtration column, thus proteins, lipids and polysaccharides are not retained by the membrane, after washing the membrane which makes it possible to rid the sample of contaminants, the DNA is then eluted with the elution buffer AE supplied with the kit (aqueous solution containing very little salt). The pure DNA bound to the spin column can be eluted with water or buffer in the kit. Genomic, mitochondrial, bacterial, parasitic or viral DNA purified from animal tissue samples by QIAamp DNA technology can be used in PCR and blotting experiments.

ii. Things to do before starting

Two water baths were heat

- One to 56°C.
- One to 70°C

Buffer AE or distilled water was equilibrated to room temperature for elution in step 11.

The Buffers AW1 and AW2 have been prepared according to the following instructions:

AW1 and AW2: the appropriate amount of ethanol (96-100%) was added as indicated on the bottle for each one.

In case where a precipitate has formed in Buffer ATL or Buffer AL, it was dissolved by incubating at 56°C.

iii. Procedure

- The tissues samples were removed from storage. The amount of tissue was determined. no more than 25 mg (10 mg spleen).
- According to the manufacture's instruction, the tissue sample can be cut up, grind, or mechanically disrupt.

Time will be reduced if the sample is mechanically homogenized in advance. Giving to our available means we opted to use the mechanic homogenization by the TissuesLyser II (QIAGEN)

Up to 25 mg of tissue (10 mg spleen) was added to a 1.5 ml microcentrifuge tube containing 80 µl PBS and one small streel balls. The sample was Homogenized using the TissueLyser II and small steel ball (Figure 23).

- 20 µl proteinase K was added and was mixed by vertexing, and incubated at 56°C until the tissue was completely lysed. To ensure efficient lysis, samples were placed in a shaking water bath or on a rocking platform and overnight lysis was opted.
- Brief centrifugation of the 1.5 ml microcentrifuge tube was done to remove drops from the inside of the lid
- 4 µl RNase A (100 mg/ml) was added, mixed by pulse-vortexing for 15 s, and incubated for 2 min at room temperature (15–25°C). Briefly the 1.5 ml microcentrifuge tube was centrifuged to remove drops from inside the lid before adding 200 µl Buffer AL to the samples were mixed again by pulse-vortexing for 15 s, and incubated at 70°C for 10 min.

The 1.5 ml microcentrifuge tube were centrifuged, briefly, to remove drops from inside the lid. In the case where white precipitate was formed when Buffer AL was added and according to the manufacturer's instructions, in most cases, it was dissolved during incubation at 70°C and the precipitate did not interfere with the QIAamp procedure or with any subsequent application.

- 200 µl ethanol (96–100%) was added to the sample, and mixed by pulse-vortex for 15s. After mixing, the 1.5 ml microcentrifuge tube was centrifugated to remove drops from inside the lid. It was essential that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution.

Carefully the mixture was applied from the previous step (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The cap was closed, and centrifuged at 6000 x *g* (8000 rpm) for 1 min.

The QIAamp Mini spin column was placed in a clean 2 ml collection tube (provided), and the tube containing the filtrate was discarded.

Each spin column was closed to avoid aerosol formation during centrifugation. It was essential to apply all of the precipitate to the QIAamp Mini spin column.

Centrifugation is performed at 6000 x *g* (8000 rpm) to reduce noise.

According to the manufacturer's instructions, centrifugation at full speed will not affect the yield or purity of the DNA. In the event that the solution has not completely passed through the membrane, second centrifugation at a higher speed until all the solution has passed through was needed.

- Carefully the QIAamp Mini spin column was opened and 500 µl Buffer AW1 was added without wetting the rim. the cap was closed, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

The QIAamp Mini spin column was placed in a clean 2 ml collection tube (provided), and the collection tube containing the filtrate was discarded.

- Carefully the QIAamp Mini spin column was opened and 500 µl Buffer AW2 was added without wetting the rim. The cap was closed and centrifugated at full speed (20,000*g*; 14,000 rpm) for 3 min.
- Manufacturer's recommended: the QIAamp Mini spin column was placed in a new 2 ml collection tube (not provided) and the old collection tube with the filtrate was discarded. Centrifugated at full speed for 1 min (This step helps to eliminate the chance of possible Buffer AW2 carryover)
- The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube and the collection tube containing the filtrate was discarded. Carefully the QIAamp Mini spin column was opened and 200 µl Buffer AE or distilled water was added. Incubated at room temperature for 1 min, and then centrifugated at 6000 x *g* (8000 rpm) for 1 min.

The previous step was repeated to increase DNA yield (5 min incubation of the QIAamp Mini spin column loaded with Buffer AE or water, before centrifugation.

For long-term storage of DNA, eluting in Buffer AE and placing at –15°C to -30°C was done, because DNA stored in water is subject to acid hydrolysis.

According to the manufacturer's information, 25 mg of tissue will yield approximately 10–30 µg of DNA in 400 µl of water (25–75 ng/µl), with an A260/A280 ratio of 1.7–1.9.

III. 1. 2. 3. b. BioDrop Microvolume Quantitation of Nucleic Acids (DNA)

In order to have an appropriate idea about the DNA concentration and purification, the BioDrop Microvolume Quantitation of Nucleic Acids (DNA) was performed in the molecular biology laboratory in the research centre of Biotechnology in Constantine Algeria.

i. Principal

Micro-volume measurement of DNA is a routine application in many life science laboratories. Quantification and purity measurement of DNA is a key first step before performing experiments such as PCR, qPCR, Next Generation Sequencing. The success of these experiments demands accurate and precise quantification of the DNA starting material. These experiments typically require highly concentrated solutions which are available only in small volumes (2ng/µl for simple and 200ng/100µ for qPCR). In addition, the high cost of the reagents makes accurate initial quantification even more crucial.

ii. Procedure

In the end of the DNA extraction procedure, the quantification of the DNA and purity was performed using a Biodrop^{TM} µLITE (Resolution Life Science Software, Montreal Biotech, Abs 260/280mm ratio) spectrophotomer. Ratio above 1.8 were considered pure and samples below this threshold were discarded. DNA samples concentration ranging between 5-130 *ng/µl*. The integrated sampling port was used and sampling volumes as low as 0.5 µl dsDNA volumes were pipetted and measured accurately. After each measurement, cleaning with distilled water was carried out to prevent any transfer of samples.

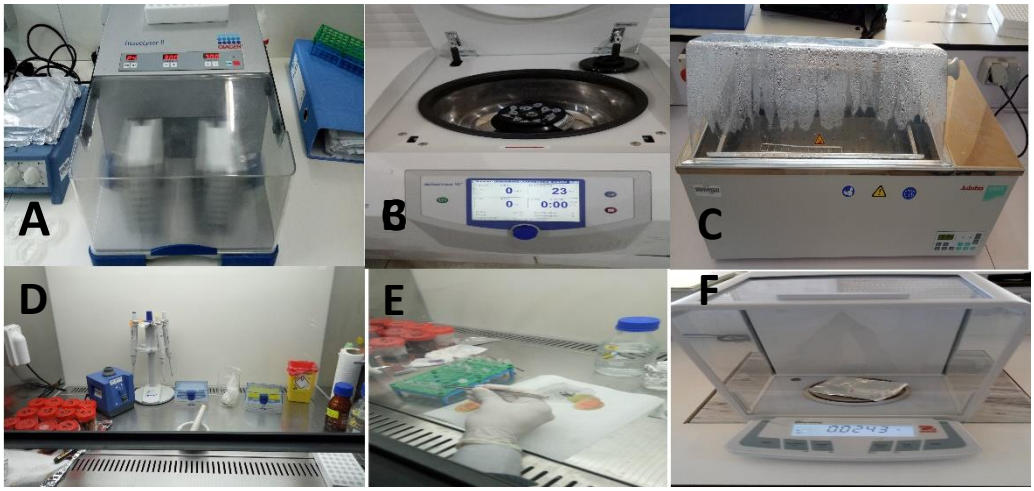


Figure 23. Materials used for DNA extraction (A-Tissue Lyser, B-refrigerated centrifuge, C-tidal bath, D-Laminar flow hood, micro pipettes, Vortex, samples, PBS, mortar, absorbent paper, bins, E-steel balls, F- precise balance) (Personal photographs).

Figure 24. kit QIAamp DNA Mini Kit® (QIAGEN, Hilden, Germany) (Personal photographs).

Figure 25. Biodrop™ µLITE (Resolution Life Science Software, Montreal Biotech, Abs 260/280mm ratio) Spectrophotometer (personal photographs).

III. 1. 2. 3. c. RTqPCR Neospora caninum and Toxoplasma gondii

The Nc-5 gene, a repeated DNA sequence in the *N. caninum* genome, has been shown to be an effective target. *N. caninum* Real-time PCR (qPCR) was performed using the pair of primers Np6+/Np21+, amplifying and based on the 337 bp-DNA fragments (Müller et al., 1996). DNA from *N. caninum* NC-1 isolate was used as positive control and DNA from VERO cells as negative control. In another hand, *Toxoplasma gondii* Real Time qPCR was based on the 529 bp-DNA fragment (Homan et al., 2000). A positive control (*T. gondii* TS-4, ATCC 40050) and negative control were included in all experiments. Both qPCR was performed using the 7500 Fast Real Time PCR system thermocycler (Applied Biosystem).

i. Primers Neospora caninum and Toxoplasma gondii

The primers used to detect *Neosppora caninum* were Np21+ (5'-CCCAGTGCGTCCAATCCTGTAAC-3') and Np6+ (5'-CTCGCCAGTCAACCTACGTCTTCT-3') and the NC-probe (5' 6FAM-CATCGGAG GACATCGCTCACTGACTG-TAMRA 3') (Table 11).

The primers used for detecting *Toxoplasma gondii* were Toxo-SE (900 nM, 5'-AGGCGAGGGTGAGGATGA) and Toxo-AS (900 nM, 5'-TCGTCTCGTCTGGATCGCAT) and the probe Toxotaqman (300 nM, 5'- 6FAM-CGACGAGAGTCGGAGAGGGAGAAGA TGT--BHQ1 -3') (Table 11).

Table 11 primers, probes of *Neospora caninum* and *Toxoplasma gondii* used for the RT qPCR amplification.

Target gene	Primers	Sequence 5'-3'	Probe 5'-3'
NC5	Np21+	CCCAGTGCGTCCAATCCTGTAAC	6FAM-CATCGGAG GACATCGCTCACTGACTG-TAMRA
	Np6+	CTCGCCAGTCAACCTACGTCTTCT	
TOX	ToxoSE	AGGCGAGGGTGAGGATG	6FAM-CGACGAGAGTCGGAGAGGGAGAAGATGT-BHQ1
	ToxoAS	TCGTCTCGTCTGGATCGCAT	

ii. Composition reaction mix

Prepare the reaction mix for each sample PCR reactions were performed in a 25 µl reaction mixture containing:

1. 2 µl of the sample,
2. 0.45 µl of each primer,
3. 0.15 µl of the probe,
4. 12.5 µl of TaqMan®2x Universal PCR MasterMix (Applied Biosystem, Warrington, UK)
5. 9.45 µl of sterile water.

The TaqMan® Universal PCR Master Mix no AmpErase® UNG, used in the reaction, was a convenient mix of components (except primers, probes, template, and water) necessary to perform a Real-Time Polymerase Chain Reaction (PCR).

iii. The TaqMan® Universal PCR Master Mix no AmpErase® UNG contains

• AmpliTaq Gold® DNA Polymerase, UP (Ultra-Pure)
• dNTPs with dUTP
• ROX™ Passive Reference
• Optimized buffer components

iv. Protocol

RT-qPCR were performed using a 7500 Fast Real Time PCR system thermocycler (Applied Biosystem) at the following conditions (Table 12).

1. The reaction mix was prepared for each sample using the components listed above.

• The volume of each component of the PCR reaction mix was calculated by multiplying the volume of each component by the number of replicates for each sample.

• we performed two technical replicates of each reaction according to the manufacture's recommendation.

2. The tube(s) was capped and vortexed briefly to mix the solutions.

3. The tube(s) was centrifugated briefly to spin down the contents and eliminated any air bubbles from the solutions.

4. The appropriate volume of each reaction mixture was transferred to each well of an optical reaction plate.

5. The plate was covered with a MicroAmp® Optical Adhesive Film. For standard 96-well plates, we may use MicroAmp® Optical Caps (Figure 26).

6. The plate was centrifugated briefly to spin down the contents and eliminate air bubbles from the solutions.

7. A compression pad was applied to the plate.

8. In the system software, the plate document or experiment was opened that corresponds to the reaction plate.

9. The reaction plate was loaded into the real-time PCR system.

10. The run was Started.

Table 12. Thermal Cycling Parameters for *Neospora caninum* and *Toxoplasma gondii*

Parameter	**UNG Incubation***	**Polymerase activation****	**PCR (40 cycles)**	
	Hold	Hold	Denature	Anneal/extend
Temperature	50°C	95°C	95°C	60°C
Time (mm:ss)	2:00	10:00	00:15	1:00

* Required for optimal UNG activity. If using TaqMan® Universal PCR Master Mix, no AmpErase® UNG, this step is not necessary.

** Required to activate the DNA Polymerase.

Figure 26. Cover the plate with a MicroAmp® Optical Adhesive Film, MasterMix (Applied Biosystem, Warrington, UK), 7500 Fast Real Time PCR system thermocycler (Applied Biosystem) and work plan (Personal photographs).

III. 1. 2. 4. Statistical analysis

The variables age, breed, number of calving, number pregnancy, herd size, standing water, presence of rivers and streams, use of disinfectant, municipality, artificial insemination and region were selected and ($P \leq 0.20$) for multivariate analysis of *N. caninum* in cows (Table 17).

The variables age, breed, stage of gestation, the number of calving, number of pregnancy, region, municipality, artificial insemination practice, presence of stagnant water and/or rivers and the use of disinfectant were selected ($P \leq 0.25$) for multivariate analysis (Table 17) for *T. gondii* in cattle. No variables were identified as risk factors by the final multivariable logistic regression.

For numeric variables, we used the Student's t-test. For categorical variables, we used the Chi-squared Test, except when the sample size for any of the categories was small (i.e. lower than 5), in which case we used the Fisher's exact test.

III. 1. 3. Results

Animal-level and herd-level seroprevalence for *N. caninum* were 5.5% (19/344; 95% CI, 3.3%–8.4%) and 59.0% (13/22; 95% CI, 36.3%-79.2%) respectively, with specific seroprevalence at farm level ranged from 0.0% (0 out 25;95% CI: 0-13.7%) to 23.0% (3 out of 13; 95% CI:

0.50%-53.8%). Positive cattle were found in all three age groups, and the seroprevalence ranged from 3.4% to 12.2 %.

Animal-level and herd-level seroprevalence for *T. gondii* were 9.9 % (15/151; 95 % CI, 5.9%-15.5%) and 70.00 % (7/10; 95 % CI, 34.75%-93.33%) respectively, and with specific seroprevalence at farm level ranged from 0% (0 out of 11: 95%CI: 0.00-28.49%) to 20.00% (5 out of 20: 95%CI: 6.83%-40.70%). Positive animals and herds were also found in all districts. *Toxoplasma gondii* prevalence in different ages of cattle ranged from 7.8% to 10.9 %. The serological results are presented in the Table 13.

Table 13. Results of serological screening for abortive diseases on sera from cows from the wilayas of the study areas.

Abortive agent	Number animals examined	No. of Positive	No. of Negative	Seroprevalence (%)
Toxoplasma gondii	151	15	136	9.9
Neospora caninum	344	19	325	5.5

i. Distribution of N. caninum and T. gondii according to farms

A herd is considered to be seropositive when it contains at least one female who is seropositive for an abortive infection. The percentage of herds infected with *N. caninum* and *T. gondii* was 59.0% (13/22) and 70.0% (7/10) respectively. It is necessary highlight the presence of two seropositive herds with five abortive diseases (*N. caninum*, *T. gondii*, *Chlamydia abortus*, *Coxiella burnetii*, *Brucella mellitensis*; data exposed in Study II), a frequency of 9.09% (2/22; 95% CI, 1.1%-32.8%).

Table 14. Serological results of farms with regard to abortive agents.

Abortive agent	Number of seropositive herds (%)
Toxoplasma gondii	7/10 (70.0%)
Neospora caninum	13/22 (59,0%)

Table 15 Distribution of seropositive cattle herds with a single abortion agent.

Abortive agent	Number of herds seropositive (%)
Toxoplasma gondii	0/10 (0%)
Neospora caninum	0/22 (0%)

Univariate analysis by Pearson's Chi-square test revealed a significant difference ($p < 0.05$) between *Neospora caninum* seropositivity and *Toxoplasma gondii*. Among the 15 *Toxoplasma gondi* seropositive sera, 4 of them (33.3%) also contain anti-*Nesopora caninum* antibodies. (Table 16).

Table 16. Distribution of *Neospora caninum* and *Toxoplasma gondii* seropositive and seronegative cattle.

		Toxoplasma gondii		
		seronegative	seropositive	total
Neospora caninum	Seronegative	143 (92.9%)	11 (7.1%)	154
	Seropositive	8 (66.7%)	4 (33.3%)	12
Total		151 (91.0%)	15 (9.0%)	166

P-value=0.014

ii. Risk factors associated to Neospora caninum and Toxoplasma gondii infection

The following variables were identified as risk factors for *N. caninum* infection by the final multivariable logistic regression Table 18: number of calving (>=6 vs <=2), presence of horses (yes vs no), Standing water (Yes vs no) and the use of artificial insemination in the last mating (yes vs no) with odds ratios 6.3, 5.9, 0.2 and 4.8 respectively. No variables were found to be risk factors in seropositive cattle to *Toxoplasma gondii*

Table 17. Animal level putative *risk* factors in relation to *Neospora caninum* and *Toxoplasma gondii* (ELISA) serostatus in dairy cattle of *n*orth-*e*a*stern* Algeria region established using the Chi-square test or Fisher's test.

Variables	Category	*N. caninum*			*T. gondii*		
		No. animals examined	Proportion of seroreactors (95% CI)	P-value	Number of animals examined	Proportion of seroreactors (95% CI)	P-value
Age (years)	≤2	149	3.4 (1.3-7.2)	0.061*	77	7.8 (3.3-15.4)	0.872
	2-6	146	5.5 (2.6-10.1)		64	10.9 (5.0-20.3)	
	>6	49	12.2 (5.3-23.5)		25	8 (1.7-23.3)	
Breed	Crossbreed	91	3.3 (0.9-8.5)		41	4.9 (1.0-14.7)	0.225*
	Montbéliarde	120	4.2 (1.6-8.9)	0.062*	19	15.8 (4.7-36.4)	
	Holstein	100	11.0 (6.0-18.2)		80	12.5 (6.6-21.0)	
	Brune des alpes	25	0.0 (0.0-9.5)		20	0.0 (0.0-11.7)	
	Fluck	8	8 (0.0–26.2)		6	0.0 (0.0-33.0)	
Gestation	No	168	6.0 (3.1-10.3)		82	11.0 (5.6-19.1)	0.555
	Yes	176	5.1 (2.6-9.1)	0.917	84	7.1 (3.0-14.1)	
Stage of gestation	1-3 months	57	1.8 (0.2-7.9)	0.285	31	6.5 (1.4-19.1)	0.117*
	4-6 months	57	6.8 (2.3-15.3)		27	14.8 (5.2-31.5)	
	7-9 months	61	8.2 (3.2-17.0)		25	0.0 (0.0-9.5)	
Number of calving	<=2	155	4.4 (2.0-8.5)	0.008*	74	9.2 (4.2-17.2)	0.189*
	3 - 5	128	4.3 (1.6-9.1)		54	4.4 (0.9-13.5)	
	>=6	22	16.7 (6.7-32.7)		12	10.5 (2.3-29.7)	
Number pregnancy	<=2	97	3.7 (1.4-7.9)	0.042*	42	8.8 (3.8-17.3)	0.675
	3 - 5	176	5.1 (2.6-9.1)		79	8.9 (4.1-16.6)	
	>=6	32	15.6 (6.2-30.9)		19	10.5 (2.3-29.7)	
Herd size	Small <20	72	8.3 (3.6-16.4)	0.337	39	5.1 (1.1-15.4)	0.525
	Large >20	272	4.8 (2.7-7.8)		127	10.2 (5.9-16.4)	
Mixing	No	89	3.4 (1.0-8.7)	0.422	8	12.5 (1.4-45.4)	0.539
	Yes	255	6.3 (3.8-9.8)		158	8.9 (5.2-14.0)	
Presence of sheep	No	81	2.5 (0.5-7.7)	0.264	-		-
	Yes	263	6.5 (4.0-9.9)		166	9.0 (5.4-14.1)	
Presence of cats	No	28	3.6 (0.4-15.5)	0.968	-	-	-
	Yes	316	5.7 (3.5-8.7)		166	9.0 (5.4-14.1)	
Presence of dogs	No	11	0.0 (0.0-20.0)	0.885	11	0.0 (0.0-20.0)	0.591
	Yes	333	5.7 (3.6-8.6)		155	9.7 (5.8-15.1)	
Visit of other farmers	No	128	4.7 (2.0-9.4)	0.780	128	9.4 (5.2-15.3)	0.829
	Yes	216	6.0 (3.4-9.8)		38	7.9 (2.3-19.6)	
Standing water	No	102	9.8 (5.2-16.7)	0.047*	86	8.1 (3.7-15.3)	0.883
	Yes	242	3.7 (1.9-6.7)		80	10.0 (4.8-18.0)	
Rivers and streams	No	189	7.4 (4.3-11.8)	0.102*	92	8.7 (4.2-15.7)	0.999
	Yes	155	3.2 (1.2-6.9)		74	9.5 (4.3-17.7)	
Use of disinfectant	No	207	3.4 (1.5-6.5)	0.058*	45	4.4 (0.9-13.5)	0.360
	Yes	137	8.8 (4.9-14.4)		121	10.7 (6.2-17.2)	
Region	Batna	252	6.7 (4.1-10.3)	0.116*	166	9.0 (5.4-14.1)	-
	Khenchela	92	2.2 (0.5-6.8)		-	-	
Municipality	EL-MADHER	24	4.2 (0.5-17.9)	0.137*	24	4.2 (0.5-17.9)	0.392
	DJARMA	47	8.5 (2.9-19.0)		47	10.6 (4.2-21.8)	
	SERIANA	25	0.0 (0.0-9.5)		25	20.0 (8.1-38.4)	
	LAZROU	14	21.4 (6.4-46.9)		14	7.1 (0.8-28.8)	
	BOUMIA	31	3.2 (0.4-14.1)		31	3.2 (0.4-14.1)	
	AY_A	25	12.0 (3.5-28.7)		25	8.0 (1.7-23.3)	
	AIN_YAGOUT	41	4.9 (1.0-14.7)		-	-	
	ZANA EL_BAIDA	45	6.7 (1.9-16.7)		-	-	
	HAMMA	52	1.9 (0.2-8.6)		-	-	
	ROKNIA	40	2.5 (0.3-11.1)		-	-	
IA in five last year				0.046*			0.791
	No	198	3.0 (1.3-6.1)		61	9.8 (4.2-19.2)	
	Yes	47	8.5 (2.9-19)		47	10.6 (6.9-27.0)	
	Both	99	9.1 (4.6-15.9)		58	6.9 (2.0-13.4)	
IA in last mating				0.018*			0.999
No	No Yes	198146	3.0 (1.3-6.1)		61	9.8 (4.2-19.2)	
Yes			8.9 (5.1-14.3)		105	8.6 (4.3-15.1)	

* P-value≤0.25

Table 18. The final multivariable logistic regression model for factors associated with *Neospora caninum* infection in dairy cattle at the individual level in north-eastern Algeria.

Factor	Odds Ratio	Confidence interval	p-value
Number of calving (2 vs 1)	0.77	0.25 – 2.40	0.654
Number of calving (3 vs 1)	6.36	1.72 – 23.43	0.005
Presence of horses (yes vs no)	5.99	1.00 – 35.97	0.050
Standing water (yes vs no)	0.21	0.05-0.83	0.026
IA last mating (yes vs no)	4.83	1.17-19.90	0.029

Table 19. Occurrence of reproductive disorders in relation to *Neospora caninum* and *Toxoplasma gondii* serostatus. **CRDE: Clinical reproductive disorder experience.*

Variable	Cattle	*N. caninum*			Cattle	*T. gondii*		
	No. animals examined	No. of positive	Proportion of seropositive (95% CI)	P-value	No. animals examined	No. of positive	Proportion of seropositive (95% CI)	P-value
Abortion								
No	283	15	5.3 (3.1-8.4)	-	110	7	6.36 (2.60, 12.67)	-
Yes	61	4	6.6 (2.3-14.8)	0.697	30	4	13.33 (3.76, 30.72)	0.382
Endometritis								
No	273	16	5.86 (3.39-9.34)	-	124	10	8.06 (3.94, 14.33)	-
Yes	32	1	3.12 (0.17-16.22)	0.817	16	1	6.25 (0.16, 30.23)	1.000
Weak calf								
No	290	16	5.52 (3.19-8.81)		132	11	8.33 (4.23, 14.42)	
Yes	15	1	6.67 (0.17-31.95)	1.000	8	0	0.00 (0.00, 36.94)	0.862
Retained fetal membrane	283	15	5.30 (3.00-8.59)	-	128	8	6.25 (2.74, 11.94)	-
No	22	2	9.09 (1.12-29.16)	0.792	12	3	25.00 (5.49, 57.19)	**0.081**
Yes								
Repeat breeding								
No	215	10	4.65 (2.25-8.39)	-	95	6	6.32 (2.35, 13.24)	
YES	90	7	7.78 (3.18-15.37)	0.417	45	5	11.11 (3.71, 24.05)	0.517
Anoestrus								
No	244	16	6.56 (3.79-10.43)	-	107	6	5.61 (2.09, 11.81)	-
Yes	61	1	1.64 (0.04-8.80)	0.236	33	5	15.15 (5.11, 31.90)	0.158
Increased inter calving period	185	9	4.86 (2.25-9.03)	-	78	3	3.85 (0.80, 10.83)	-
No	120	8	6.67 (2.92-12.71)	0.678	62	8	12.90 (5.74, 23.85)	**0.096**
Yes								
CRDE*								
No	144	8	5.56 (2.43, 10.65)	-	54	1	1.85 (0.05, 9.89)	-
Yes	161	9	5.59 (2.59, 10.35)	1.000	86	10	11.63 (5.72, 20.35)	**0.077**

iii. Molecular detection of Neospora caninum and Toxoplasma gondii

Globally, three aborted foetuses and one mummified foetus out of 10 aborted foetuses (30%) collected and tested by qPCR were found positive for *N. caninum*, with Ct values ranging from 35 to 39 (Figure 27). Nine of the 53 tissue samples from the analysed foetuses were positive for *N. caninum* DNA, for an overall positivity rate by qPCR of 15.09% Table 20. Target DNA was amplified from the brain, eye, lung, liver, placenta, stomach contents and mummified body. Among the four *N. caninum*-positive foetuses, DNA was detected in 33.3% of mummified body samples and 25% of eye samples. All foetuses' samples were negatives for the presence of *T. gondii* DNA (Table 20).

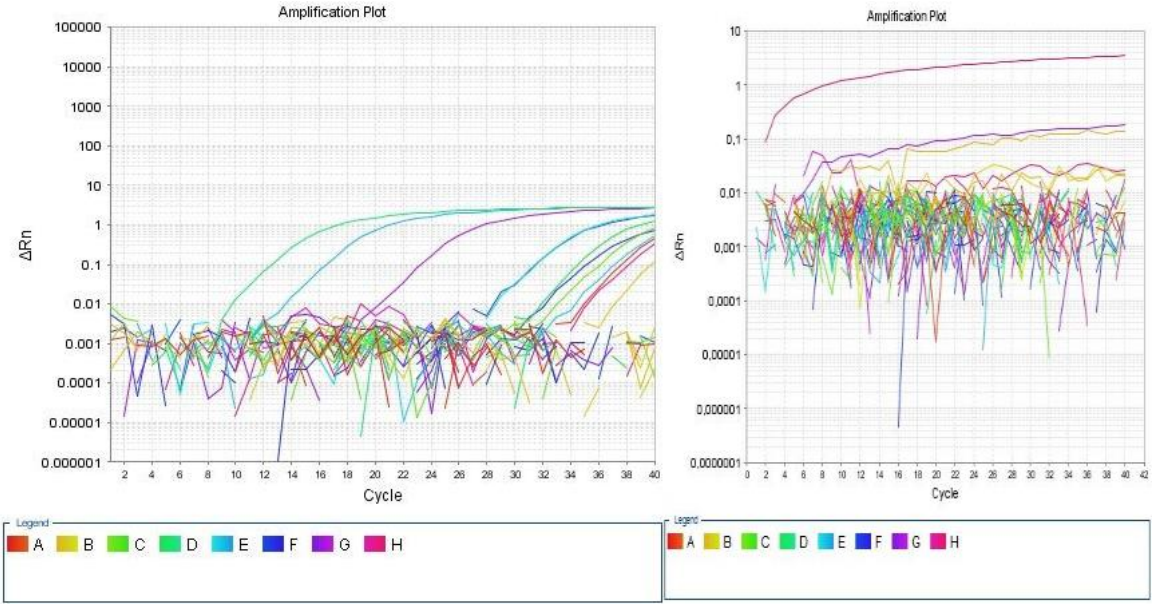


Figure 27 Amplification and fusion curves obtained with primers N21 + and N6 +, amplification curves. melting curves and specific melting temperature of the amplified product (95 ° C). The DNA of the *N. caninum* NC-1 isolate was used as a positive control and the DNA of VERO cells was used as a negative control and as a positive control (left) and the DNA of *Toxoplasma gondii* from the RH a strain. was used, and DNAse-free water was used as a negative control. included in each series (right). A sample is positive when it has an amplification curve and a melting temperature identical to that of the positive control.

Table 20. Distribution of qPCR positive and negative samples according to specimen's type.

Specimen	Number tested	qPCR result			foetus			
		N°+ve (%)	N°−ve (%)		1	2	3	4
Brain	4	1 (25%)	3 (75%)		+	-	-	-
Eye	4	1 (25%)	3 (75%)		-	-	-	+
Lung	4	1 (25%)	3 (75%)		-	+	+	-
Liver	11	2 (18.18%)	9 (81.81%)		-	-	+	-
Kidney	4	0 (0%)	4 (100%)		-	-	-	-
Spleen	6	0 (0%)	6 (100%)		-	-	-	-
Heart	4	0 (0%)	4 (100%)		-	-	-	-
Stomach content	2	1 (50%)	1 (50%)		NT	-	+	NT
Placenta	7	1 (14.28%)	6 (85.71%)		-	-	+	-
Mummified body	3	1 (33.33%)	2 (66.66%)		NT	NT	+	NT
Total	53	8 (15.09%)	41 (83.67%)					

III. 1. 4. Discussion

The aim of our study was the investigation of individual-level seroprevalence of *T. gondii* and *N. caninum* in dairy cattle in north-eastern Algeria and clarify factors associated with individual-level seroprevalence of these pathogen infections. To our knowledge, this is the first report of herd-level seroprevalence of *T. gondii* and *N. caninum* in cattle in the region.

Cross-sectional study design associated with serological investigations, as used in the study, is widely used in veterinary epidemiology and used to assess the burden of a particular disease in a defined population (Dohoo Martin and Stryhn, 2009). The advantage of cross-sectional design is that it is unambiguous, straightforward, inexpensive, and needs only one sampling occasion. It provides descriptive characteristics of a population at a particular point in time and includes both old and new cases (Dohoo Martin and Stryhn, 2009). Nevertheless, it is less suitable for determining when the disease occurred or for how long it has lasted. The finding of antibodies in a single serum sample only indicates that infection has occurred sometime in the past which make its diagnostic value as indicator of present active infection limited (Levin, 2006). It is impossible to determine the sequence of events, namely whether exposure occurred before, during, or after the onset of disease outcome (Levin, 2006). In some type of ELISA's against some diseases, a high level of antibodies could indicate a possible acute phase of the diseases. One disadvantage of cross-sectional design compared to, for example, longitudinal study design is consequently the weakness in determining cause-effect relationships (Dohoo Martin and Stryhn, 2009). The association between seropositivity and reproductive disorders found in the present study is therefore not necessarily causal even though it is statistically significant. Other

study designs, such as longitudinal study or case-control study, would have been stronger field visits.

Sample-size determination is often an important step in planning an epidemiological study. There are several approaches to determining sample size. It depends on the type of the study. Descriptive, observational and randomized controlled studies have different formulas to calculate sample size. An adequate sample size helps guarantee that the study will yield reliable information, regardless of whether the ultimate data suggest a clinically important difference between the treatment being studied, or the study is intended to measure the accuracy of a diagnostic test or the incidence of a disease. Ideally, to get a true prevalence estimate of a given infection in a population with good precision, all animals should be included (census). Due to the insufficiency of resources, only a fraction (sample) of the population is used to represent the whole population. This fraction needs to be optimal and representative to allow inferences to be made about the target population (Dohoo Martin and Stryhn, 2009), which necessitates a random sampling strategy.

The calculation of sample size needed was complicated by several factors: unknown prevalence and heterogeneity in management systems. Pertinent literature on seroprevalence in Algeria is scarce and concerns studies conducted in other parts of the country with different management systems, study design, and laboratory techniques, limiting its relevance. The minimum sample size was increased to take into account the mentioned challenges. It is therefore likely that the sample size in the present study allows implications to be made about the target population; furthermore, the herds included were generally typical and are very likely representative of other herds in the study areas.

Most of the information on reproductive disorders and risk factors was collected using an interview-based questionnaire, which is susceptible to communication challenges. To minimize the risk of information bias/misinformation, for this, on each excursion, the purpose of which was collect samples and information, a qualified veterinary accompanies the breeders to avoid any possible ambiguities in the responses collected. This method was resource-demanding but advantageous compared to online or paper-based data collection from veterinarian practices. However, the information provided by the farmers depends largely on their knowledge, record keeping, and capacity to remember what happened up to, in some cases, more than 5 years

before interview. Because of the lack in records and farmers have a poor understanding of reproductive disorders, the frequency of reproductive disorders in the area might have been underestimating. For a dairy herd, the only record important to most farmers is number of calving and milk yield in which this latter information could not be provided even for veterinary inspection. Information on reproductive performance indicators in general (age at first service, age at first pregnancy, conception rate, and calving interval) was sought by the owners. Early embryonic loss, such as fertilization/conception failure and early embryonic mortality, was not possible to assess in all breeders, which might cause further underestimation of the occurrence of reproductive disorders.

Neospora caninum and *Toxoplasma gondii* have global epidemiological distribution (Khan and Zahoor, 2018; Lanave et al., 2017; Barati et al., 2017; Almería and López-Gatius, 2013; Hemphill and Gottstein, 2000; Dubey and Webster, 2010; Knobel et al., 2013) and were selected in accordance with their recognized impact on the ability to cause reproductive disorders in cattle, public health and economic importance, and likely local importance (Khan and Zahoor, 2018; Lanave et al., 2017; Barati et al., 2017; Almería and López-Gatius, 2013; Hemphill and Gottstein, 2000; Dubey and Webster, 2010; Knobel et al., 2013). The incidences of reproductive disorders in bovine are increasing over years. This scenario is further aggravating due to more emphasis on selection and rearing of animal for specific commercial purposes which compromises livestock reproduction.

Bovine neosporosis control programs are currently based on herd management and serodiagnosis because at present, there is no effective treatment or vaccine for *N. caninum* infection, and control measures are based on herd management and diagnosis over the world. Although a wide variety of serological tools have been developed, enzyme-linked immunosorbent assays (ELISAs) are the most commonly commercialized and used tests. Although IFAT using whole fixed tachyzoites is the most reliable serological test for detection of *Neospora* antibodies, high cost and the need for specialized equipment and expertise have limited its use. Serological techniques are primarily employed to detect specific antibodies against *N. caninum* to differentiate infected from non-infected animals. These techniques include a wide variety of enzyme-linked immunosorbent assays (ELISAs). In general, most of

the available tests for the diagnosis of bovine *N. caninum* infection have shown strong performances (Dubey and Schares, 2006b).

In the present study, Antibody iELISA used a purified antigenic extract of *Neospora caninum* and have a sensitivity of 99.6 (CI95%: 98.9–100) and specificity of 98.9 (CI95%: 97.4–100). According to Alvarez-García et al., (2013) iELISA used in the study is one of the best-adjusted ELISAs that showed excellent Sensitivity and Specificity values (>95%). Another important issue is the study of cross-reactions with closely related apicomplexan parasites with relevance to cattle, such as *Sarcocystis spp* and *Besnoitia besnoiti*. It is well known that 100% of cattle are infected with *Sarcocystis spp* (Dubey et al., 1989). As satteled by Alvarez-García et al., (2013) the iELISA used in the study it would be desirable to discard cross-reactions by employing a wide panel of appropriate sera.

Toxoplasma gondii is not the main causative agent of abortion in cattle, and the contribution of milk and meat from infected cattle to the prevalence of *Toxoplasma* in humans is unknown in north-Africa (Dubey, 1986). In fact, serological methods appear to lack sensitivity and specificity, even though the qualitative detection of antibodies remains a standard tool. At the same time, there are differences within the serological techniques. Moreover, Dubey et al., (1995) found that the diagnostic performance of a MAT was higher than that of ELISA.

Toxoplasma gondii, *Neospora* spp., *Sarcocystis* spp., *Hammondia* spp. and *Besnoitia besnoiti* are genetically related cyst-forming coccidia. Serology is frequently used for the identification of *T. gondii*, *Neospora* spp. and *B. besnoiti*-exposed individuals. Serologic cross-reactions occur in different tests among animals infected with *T. gondii* and *H. hammondi*, as well as among animals infected by *T. gondii* and *N. caninum*.

In the present study antibody iELISA was used with *P30* antigen specific *to Toxoplasma gondii* and have a sensitivity and specificity of 98.36 % (CI 95%: 95.29%-99.44%) and 99.42 % (CI 95%: 98.8%-100%), respectively. The surface protein (P30) was designated SAG1, which is the product of the *SAG1* gene. However, as it was observed more recently that *SAG1* genes belong to a superfamily of related genes, named *SRS* (SAG1-related sequences), which encode a superfamily of structurally related surface proteins from *T. gondii*, the name of SAG1 (P30)

has been changed to SRS29B (Wasmuth et al., 2012). This iELISA test does not discriminate between natural infection and vaccination, but, importantly, there is no history of vaccination against toxoplasmosis in all over the word. Consequently, the presence of antibodies due to vaccination can be excluded, which simplifies the interpretation of serological results.

The present study found that the associations between the number of calving and *N. caninum* infection in dairy cattle were significant. While many studies demonstrated that age, breed, and number of pregnancies were risk factors (Asmare et al., 2013). The seroconversion risk can increase with time or gestation number (Rinaldi et al., 2005), suggesting that horizontal transmission is important in some herds(Dubey et al., 2007). According to the literature, Guimarães et al., (2004) indicated that older cows showed higher seropositivity for *N. caninum*, indicating a greater possibility of horizontal transmission of the disease possibly owing to the increased risk of infection by horizontal transmission. This fact suggests the existence of sporulated *N. caninum* oocysts in the environment, which characterizes horizontal transmission, as observed by (Dijkstra et al., 2001). In opposite, some studies regarding neosporosis have shown that foetal infection decreases with the rise of the number of gestations or lactation, and consequently with the animal's age, mainly because animals acquire immunity against the parasite (Almería et al., 2010). The risk of being seropositive may increase with age or gestation number in beef and dairy cattle. Sanderson et al., (2000); Rinaldi et al., (2005) suggested that horizontal transmission of *N. caninum* is of particular importance in some herds.

In the present study, the presence of standing water in the pasture was identified as a protective factor associated with the animal-level prevalence of *N. caninum* infection. In opposite direction, a possible way of infection could be the consumption of water contaminated with *N. caninum* oocysts from feces of infected wild or domestic felines and canids because they may come to a standing water region to drink and at the same time defecate in or near the water source region. Studies have reported that abortion epidemics may be correlated with the ingestion of food or water contaminated with oocysts (Sun et al., 2015). Moreover, flooding may also be a risk factor because it can spread *N. caninum* oocysts (Justo et al., 2013).

Contrary to Justo et al. (2013), the results of (Gindri et al., 2018) showed that flooding was actually associated with a lower *N. caninum* seroprevalence (OR = 0.5).

The finding of this study indicates that the presence of horses in the same farm with cattle in north-eastern Algeria are at risk factor of neosporosis ($p<0.05$, OR=5.9). Many studies made similar conclusion (Hobson et al., 2005; Zhou et al., 2017).

Horses can be infected by *Neospora caninum* or *Neospora hughesi*, this latter is newly recognized parasite that closely related to *Neospora caninum* and is a cause of equine protozoal myeloencephalitis. The presence of shared antigens was demonstrated by the cross reactivity in the *Neospora* agglutination test as well as in the indirect fluorescent antibody test between *Neospora caninum* and *Neospora hughesi* in horses (Ae et al., 1996; Dubey et al., 2001). Horses are known to be intermediate host of *Neospora hughesi* (Dubey et al., 2007a) mostly in the United States of American areas. In contrast, Dubey and Lindsay, (1996b) concluded that although *Neospora caninum* have a wide range of host, neosporosis is rare in animals other than cattle and dogs.

Studies in Southern Brazil reported relatively higher *Neospora* prevalences on farms using natural breeding (Martins et al., 2013; Ferre et al., 2005), demonstrated the presence of the parasite in 15% semen samples from naturally infected bulls. Besides, intrauterine infection of cows with tachyzoites led to seroconversion and detection of DNA of the parasite in 66% of the animals studied (Serrano et al., 2006). In opposite, previous studies have shown that the use of beef semen significantly reduces the risk of *N. caninum* abortions in seropositive dairy cows (Lopez-Gatius et al., 2005; Almería and López-Gatius, 2013). Sala et al., (2018) hypothesized that long-term systematic use of beef semen in seropositive breeders, may reduce *N. caninum* incidence and prevalence, due to seropositive descendant exclusion from remount insemination with beef-breed semen halves the abortion rate (Lopez-Gatius et al., 2005; Almería and López-Gatius, 2013). In study of Ortega-Mora *N. caninum DNA* was detected in non-extended fresh semen samples and frozen extended semen straws by nested-PCR (Ortega-Mora et al., 2003). Doosti et al., (2015) reported that frozen semen samples, which used for artificial insemination in Iranian Insemination Centres, plays an important role in the spread of bovine neosporosis. The findings of the study showed the high presence of *N. caninum* infection ($P<0.05$) in fresh and frozen bull's semen samples that were used for artificial insemination in Iranian Insemination Centers and animal husbandries (Sharifzadeh Doosti, and Dehkordi, 2012).

Until now, insufficient data are available on cattle toxoplasmosis in the world, and there have been limited number of reports on cattle toxoplasmosis from Algeria. The overall seroprevalence of toxoplasmosis reported in the present study in north-eastern Algeria using ELISA (9.03%) is higher than that reported in cattle from Medea (north of Algeria) (4.4%) using the MAT (Khames et al., 2018), Djelfa province in Algeria (3.92%) based on the IFAT (Khatima and Abdellah, 2015), Tanzania (3.6%) (Schoonman, Wilsmore and Swai, 2010) using the LAT, France (7.8%) using MAT, Malaysia 7.9% in local cattle and 4% in yellow cattle, (Chandrawathani et al., 2008) using IFAT, and Brazil (2.68%) using IFAT. The current literature report seroprevalences of antibodies against *T. gondii* in cattle ranging from 3.3% in Mexico to 90.9% in the Netherlands (Webster, 2010). Lower prevalence of infection in cattle also reported in some countries sunch as Ethiopia (10.7%) using iELISA (Tilahun et al., 2018), Sudan (13.3%) using iELISA (Elfahal et al., 2013b), and in female cattle from south-west from Iran (15.77%) using MAT (Hamidinejat et al., 2010). Thailand reported a prevalence of 22.3% (Jittapalapong et al., 2008) using the LAT. The difference in the prevalence between studies could be attributed to the different techniques used in estimating these prevalence's.

Cattle can be readily infected with *T. gondii*, nevertheless, they are considered poor hosts. This resistance to clinical toxoplasmosis could be explained by a more effective immune response to *T. gondii* infection (Esteban-Redondo and Innes, 1997).

III. 2. Study II. Seroprevalence and risk factors of *Brucella abortus*, *Chlamydia abortus*, and *Coxiella burnetii* in cattle in north-eastern Algeria

III. 2. 1. Introduction

Productivity and profitability have a huge impact on reproductive performance of a dairy herd. Fertility, in turn, is related to the parity of cows. To establish sustainability development in the dairy industry, it is important that cows are pregnant at a biologically optimal time and at an economically profitable interval after calving. Veterinarians managing fertility in dairy herds should regularly evaluate the herd health status for pathogens known to compromise reproductive efficiency. Infectious diseases are of increasing concern on dairy farms because of their potential impact on animal and human health, milk and meat production, food safety, and economics.

Dairy farms are recognized as important reservoirs of foodborne pathogens. Some infections pathogens are known to reduce conception rates while others may cause foetal losses and abortions. To implement appropriate and effective disease control programs at the national level, up-to-date and unbiased information on disease frequency is needed in Algeria. It is important that they are accompanied by continuous monitoring of herd status against abortive pathogens, including *Brucella abortus*, *Chlamydia abortus* and *Coxiella burnetii* to assess the effectiveness of the program and progress toward goals; this can be achieved through serological testing at the herd level.

Accordingly, the study aimed:

(v) To determine the individual and herd seroprevalence of *Brucella abortus, Chlamydia abortus and Coxiella burnetii* in non-vaccinated dairy cattle in north-eastern of Algeria.

(vi) To investigate potential risk factors related to seropositivity of antibodies against these bacteria in dairy cattle herds.

(vii) To identify the occurrence of reproductive disorders in relation with these infectious diseases.

III. 2. 2. Materials and methods

III. 2. 2. a. Area of study and target population

The study area is described in Section III. 1. 2. (Study I). Briefly, the study area included Batna, Khanchela and Setif regions from north-eastern Algeria (Figure 19). Batna region (12,192 km^2), located in the Aurès region approximately 214 Km from the Mediterranean coasts, has many different climates (semi-arid cold, cold desert, warm, summery Mediterranean, hot desert and finally warm-summer Mediterranean climate) with an annual rainfall of about 329 mm (Figure 19). Setif region has a warm and temperate climate with an average annual precipitation of 56mm (Climat Sétif, 2020). Khenchela's climate is classified as warm and temperate supra-Mediterranean climate with an annual average rainfall of 446 mm. Essentially, the north-eastern region of Algeria is known to include the most important number of cattle herds, and about 80% of cattle farming is located in the northern regions of the country (Kirat, 2007).

III. 2. 2. b. Calculation of the sample size

Calculation of the sample size was previously described in Study-I of the present Thesis. Briefly, a two-stage sampling survey was carried out in north-western Algeria. For the first stage of sampling (sampling of herds), the sample size for disease detection was calculated based on the following formula (Dohoo et al., 2003) (III. 1. 2. b. section of the present Thesis).

$$n_1 = \left(1 - (1 - \alpha_1)^{\frac{1}{D_1}}\right) \times \left(N_1 - \frac{D_1 - 1}{2}\right)$$

For the second stage (sampling of animals within herds), the sample sizes for disease detection were also calculated based on the formula by Dohoo et al (2003) (III. 1. 2. b. sction of the present Thesis):

$$n_{2i} = \left(1 - (1 - \alpha_2)^{\frac{1}{D_{2i}}}\right) \times \left(N_{2i} - \frac{D_{2i} - 1}{2}\right)$$

Within herds, animals were randomly selected to allow the detection of infected individuals if infection was present in at least 30% of animals with a 95% confidence. Sample sizes for the two stages were calculated using Epitools (Sergeant, 2018). Holstein/Friesian, Monbeliard and crossbreed were the most common breeds.

III. 2. 2. c. Herd animals and management

In total, 30 herds were visited and 437 animals included, 8 herds were small scale dairy herds (1-10 cattle) and 22 medium scale herds (10-100). Tables 4 to 7 (Study – I) shows the distribution of herds of different sizes and samples in the north-eastern regions.

III. 2. 2. d. Study design

A graphical overview of the study design of all the thesis, the materials collected, and the analysis performed is provided in the Figure 19. Study I and II *Neospora caninum*, *Toxoplasma gondii*, *Brucella spp*, *Coxiella burnetii*, and *Chlamydia abortus* as primary aetiology of abortion was based on serological investigations of sera from the North-eastern region in Algeria as well as molecular identification including RT-PCT from aborted bovine foetuses and foetal membranes were used in this study.

III. 2. 2. e. Study period and epidemiological data collection

All material for the project was collected during field trips conducted between September 2015 and May 2016, where each herd was visited once. Information from all regions (north-eastern Algeria) was gathered through direct observation at farms, interviews of farmers, and collection of biological material from animals. Table 21 summarizes the most of the information on animals and farms management which was collected using an interview-based questionnaire (Appendix 1). To minimize the risk of information bias/misinformation, for this, on each excursion, the purpose of which was collect samples and information, a qualified veterinary accompanies the breeders to avoid any possible ambiguities in the responses collected. Because of the lack in records and farmers have a poor understanding of reproductive disorders, the frequency of reproductive disorders in the area might have been underestimating (Appendix 1).

Table 21. Epidemiological information's collected during cattle sampling.

Characteristic	Variables
Animals and farm	breed (Holstein, Monbeliarde, Brune des Alpes, Fleckvieh and crossbreed), age (≤2 years, between 2-6 years and >6years), animal categories (calves, heifers, milking cows, dry cows), herd size (small <20, Large >20)
Reproductive Performance	Reproduction technique (natural breeding (NB), artificial insemination (AI), NB+AI), reproductive disorders (repeat breeding***(Repeat or normal), anoestrus (yes/no), still birth (yes/no), birth of weak calf (yes/no), calving interval** (Expected" or "Prolonged), diagnoses of reproductive diseases (brucellosis) (yes/no), gestation status (pregnancy, no pregnancy, stage of pregnancy), abortion, number of calving and pregnancy*, endometritis (yes/no), retained fetal membrane ((yes/no). Clinical reproductive disorder experience CRDE (yes/no): abortion and/or stillbirth and/or retained fetal membrane and/or dystocia and/or prolonged uterine discharge (metritis) and/or the birth of defective and/or weak calf.
Bio-Security	Veterinarian assistance (yes, no), Presence of other animals (sheep, horses, rats, cats, dogs), standing water, sanitary management

* Number was defined as the number of abortion and calving experienced by a cow
** Expected refers to calving every 12–18 months while prolonged refers to over 18 months.
*** Cows reported requiring 3 or more services per pregnancy were categorized as repeat breeders.
**** abortion was defined as loss of the foetus between 42 and 260 days of gestation, and stillbirth was defined as a calf that was born dead between 260 days and full-term or died within 24 h following birth.

III. 2. 2. f. Serology

Blood samples were collected and stored as previously reported (III. 1. 2. g.; Study I).

The search for antibodies against the three targeted abortive agents was carried out by indirect ELISA techniques. These tests were performed at the laboratory level of serology of the refuge practice in Batna. All ELISAs were conducted in accordance with the manufacturer's instructions and cut-off recommendations (Table 24). Antibodies against all three pathogens were tested by indirect ELISA technique using the Indirect Multi-species kit (innovative diagnostic, Grabels, France). All ELISA kits have almost the same principal and protocol. These kits are based on an indirect ELISA technique using a purified antigenic extract of *Coxiella burnetii* phase 1 and 2 strains (isolated in France from the placenta of a bovine abortion), *Brucella abortus* lipopolysaccharide and a synthetic peptide antigen from a MOMP specific to *Chlamydia. abortus*. The Sensitivity and specificity of these iELISAs test figure out in the below table.

Table 22. sensitivity and specificity of Indirect ELISA used for the antibody detection of each pathogen agent according to the manufacturers.

	Sensitivity	Interval confidence	Specificity	Interval confidence
C. abortus	70%	(CI95%: 53.5 - 83.4%)	100%	(CI95%: 90.5 - 100%)
C. burnetii	100 %	(CI95%: 89.28%- 100%) *****	100 %	(CI95%: 97.75%-100%) ******
B. abortus	100%	(CI95%: 89.57% - 100%)	99.74%	(CI95%: 99.24% - 99.91%)

****Performed on 32 samples.***
*****specificity performed on 167 samples.***

Principal of the serologic technic and protocol followed in the case of bacterial abortive agents were similar to the *N. caninum* and *T. gondii* and were summarized in the Study I- III. 1. 2. 2. Serology.

i. Validity criteria and interpretation

The Tables 23 and 24 below summarize the validity criteria and interpretation of each ELISA test.

Table 23. Validity criteria for each disease using the Indirect ELISA in cattle sera from north-eastern Algeria.

Validity	Antigen used	DO_{PC}	DO_{CP}/DO_{NC}	S/P calculation
Chlamydia abortus	synthetic peptide antigen from a MOMP specific to *Chlamydia abortus*	> 0.350	>3	OD_{sample}/OD_{PC} X 100
Coxiella burnetii	*Coxiella burnetii* phase 1 and 2 strain	> 0.350	>3	OD_{sample}/OD_{PC} X 100
Brucella spp	*Brucella abortus* lipopolysaccharide	> 0.350	>3	$(OD_{sample} - OD_{NC})/(OD_{PC} - OD_{NC})$ x 100

Table 24. interpretation for each disease using the Enzyme-linked immunosorbent assays (ELISA) in cattle sera from north-eastern Algeria.

Interpretation	Negative	Doubtful	Positive	Acute infection (Strongly positive)
Chlamydia abortus	S/P ≤50%	40<S/P<60%	S/P ≥60%	-
Coxiella burnetiid	S/P ≤40%	40<S/P≤50%	50<S/P≤80%	S/P >80
Brucella spp	S/P ≤110	110<S/P<120%	S/P ≥120%	-

III. 2. 2. 3. Statistical analysis

The variables age, breed, number of calving, stage of gestation, use of disinfectant, cleaning method, mixing species, sheep, quarantine practice, herd size, visit of other farmers, standing water, presence of rivers and streams in the pasture, municipality, region and artificial insemination were selected ($P \leq 0.20$) for multivariate analysis of *Brucella abortus* in cows (Table 30).

The variables age, breed, mixing species, sheep, presence of rivers and streams in the pasture, visit of other farmers, municipality, region and artificial insemination were selected ($P \leq 0.20$) for multivariate analysis of *Coxiella burnetii* in cows (Table 30).

The variables age, breed, mixing species, sheep, visit of other farmers, municipality, region and artificial insemination were selected ($P \leq 0.20$) for multivariate analysis of *Chlamydia abortus* in cows (Table 34).

III. 2. 3. Results

On an individual scale, the results obtained showed a predominance of brucellosis with an animal seropositivity rate of 28.6% (127/437; 95% CI, 24.2%-34.6%), followed by Q fever with A low prevalence of *Chlamydia* (1.45%, 95 % CI, 0.6%-3.2%) was also observed (5/344).

i. Distribution of bacterial abortive diseases according to farms

A herd is considered to be seropositive when it contains at least one female who is seropositive for an abortive infection. Consequently, the distribution of herds shows that 22/30 (73.3%; 95% CI, 46.0-86.5%) have tested positive for brucellosis alone or in combination with other abortifacients. The percentage of herds infected with *Toxoplasma gondii* is 70.0% (7/10) herds. concerning Neosporosis the percentage of seropositive herds was 13/22 (59.0%; 95% CI, 31.5-77.5%). The other abortive diseases encountered *Coxiella burneti* and *Chlamydia abortus* had a prevalence of 11/22 (50.0%; 95% CI, 25.0-89.5%) and 4 / 22 (18,1%; 95% CI, 5.0-46.6%) respectively (Table 25-27).

ii. Multiple serological response (Study–I and Study-II pathogens)

Of the 22 farms surveyed, 3 (13.63%) herds showed positive serological tests for Brucellosis and Neosporosis (Study I). In contrast, 15 (68.1%) herds showed positive serologies for 2, 3 or 4 abortion agents. Table 25 shows the different types of associated infections. The association “Brucellosis, Neosporosis and Toxoplasmosis” as well as “Neosporosis, Brucellosis, Q fever, Chlamydiosis, Toxoplasmosis” are the most frequent, both represented by 20.0% of farms. The other associations are represented by: Neosporosis, Brucellosis, Q fever and Neosporosis-Brucellosis with a similar prevalence respectively (13.6%), Brucellosis, Toxoplasmosis and Neosporosis, Q fever, Toxoplasmosis with a similar prevalence of 10.0% respectively. Finally, with low within-herd prevalence, the associations Neosporosis, Q fever and Brucellosis, Q Fever, Chlamydiosis through a similar prevalence of 4.5% respectively.

Table 25. Distribution of farms with multiple immune status against several abortive agents.

Multiple serological response	**No. seropositive (%)**	**Municipality**
Neosporosis – Brucellose	3/22 (13.6%)	AIN_YAGOUT
		AIN_YAGOUT
		ZANA EL_BEIDA
Neosporosis - FQ	1/22 (4.5%)	ROKNIA
Brucellosis – Toxoplasmosis	1/10 (10.0%)	SERIANA
Neosporosis - FQ - toxoplasmosis	1/10 (10.0%)	DJERMA
Neosporosis – Brucellosis - FQ	3/22 (13.6%)	ZANA EL_BEIDA
		ZANA EL_BEIDA
		HAMMA
Brucellosis - FQ - *Chlamydia*	1/22 (4.5%)	EL_MADHER
Brucellosis – Neosporosis - Toxoplasmosis	2/10 (20.0%)	EL_MADHER
		BOUMIA
Néosporosis - FQ – *Chlamydia* - Toxoplasmosis	1/10 (10.0%)	AYOUN ASSAFIR
Néosporosis- Brucellosis- FQ-*Chlamydia*-Toxoplasmosis	2/10 (20.0%)	LAZROU
		AYOUN ASSAFIR
Total	**15/22 (68.1)**	**10**

On an individual scale, 23 female cattle showed antibodies to two to three abortifacients at a time (Table 27). 7 cattle showed a positive serological association for brucellosis and toxoplasmosis. On the other hand, only 4 cattle were simultaneously seropositive with *Chlamydia*, Q fever and neosporosis, brucellosis. 3 cattle were seropositive for neosporosis, Q fever and neosporosis, toxoplasmosis. However, no bovine was seropositive with the five abortifacient agents at the same time but on the other hand, we detected a bovine seropositive with four pathologies (neosporosis, toxoplasmosis, Q fever and chlamydiosis).

Table 26. Distribution of seropositive cows by herd and by municipality.

Number of Herd	Municipality	Region	Neosporosis	Toxoplasmosis	Q Fever	Chlamydiosis	Brucellosis
1	DJERMA	BATNA	4	5	8	0	0
2	SERIANA		0	5	0	0	16
3	EL_MADHER		1	1	0	0	11
4	EL_MADHER		0	0	2	1	5
5	LAZROU		3	1	2	1	2
6	LAZROU		0	0	0	0	0
7	BOUMIA		1	1	0	0	8
8	BOUMIA		0	0	0	0	0
9	AYOUN ASSAFIR		2	1	2	2	0
10	AYOUN ASSAFIR		1	1	1	1	1
11	AIN_YAGOUT		0	ND	0	0	6
12	AIN_YAGOUT		1	ND	0	0	12
13	AIN_YAGOUT		0	ND	0	0	5
14	AIN_YAGOUT		0	ND	0	0	3
15	AIN_YAGOUT		1	ND	0	0	9
16	ZANA EL_BEIDA		1	ND	8	0	5
17	ZANA EL_BEIDA		1	ND	2	0	3
18	ZANA EL_BEIDA		1	ND	0	0	3
19	HAMMA	KHANCHELA	1	ND	2	0	3
20	HAMMA		0	ND	4	0	0
21	HAMMA		0	ND	5	0	0
22	ROKNIA		1	ND	10	0	0
23	AINABASSA	SETIF	ND	ND	ND	ND	1
24	AINABASSA		ND	ND	ND	ND	1
25	AINHDJAR		ND	ND	ND	ND	4
26	AINHDJAR		ND	ND	ND	ND	5
27	DOUARADJAIL		ND	ND	ND	ND	8
28	DOUARADJAIL		ND	ND	ND	ND	7
29	DOUARADJAIL		ND	ND	ND	ND	9
30	AIN_AZEL		ND	ND	ND	ND	0

Seronegative to all diseases

Seropositive to one pathogen

Seropositive to two pathogens

Seropositive to three pathogens

Seropositive to four pathogens

Seropositive to five pathogens

Table 27. Animals' seroprevalence.

Bruc+Toxo	FQ+Chl	Neo+Bruc	Neo+Toxo	Neo+FQ	Bruc+FQ	Neo+FQ+CHL+Toxo	Number of diseases	Herds	Municipality	Region	Age (months)
			1				2	1	DJERMAA	BATNA	9
			2	1			3	1	DJERMAA	BATNA	72
1							2	2	SERIANA	BATNA	24
2							2	2	SERIANA	BATNA	24
3							2	2	SERIANA	BATNA	60
4							2	2	SERIANA	BATNA	60
5							2	3	MADHER	BATNA	48
	1						2	4	MADHER	BATNA	48
					1		2	4	MADHER	BATNA	48
				2			2	5	LAZROU	BATNA	48
						1	4	5	LAZROU	BATNA	96
6							2	7	BOUMIA	BATNA	60
		1					2	7	BOUMIA	BATNA	48
	2						3	9	AYOUN ASSAFIR	BATNA	96
	3						2	9	AYOUN ASSAFIR	BATNA	60
7		2	3				3	10	AYOUN ASSAFIR	BATNA	36
	4						2	10	AYOUN ASSAFIR	BATNA	72
		3					2	15	AIN YAGOUT	BATNA	84
					2		2	16	ZANA EL BAIDA	BATNA	72
				3			2	16	ZANA EL BAIDA	BATNA	60
		4					2	17	ZANA EL BAIDA	BATNA	84

Table 28. Distribution of seropositive and seronegative females for *Brucella spp.* and *Coxiella burnetii*

		Coxiella burnetii		
		seronegative	seropositive	total
Brucella spp	Seronegative	208 (82.5%)	44 (17.5%)	252
	Seropositive	90 (97.8%)	2 (2.2%)	92
Total		298 (86.6)	46 (13.4%)	344

P-value<0.0001

Table 29. Distribution of seropositive and seronegative females for *Brucella spp* and *Toxoplasma gondii*

		Toxoplasma gondi		
		seronegative	seropositive	total
Brucella spp	Seronegative	115 (93.5%)	8 (6.5%)	123
	Seropositive	36 (83.7%)	7 (16.3%)	43
Total		151 (91.0%)	15 (9.0%)	166

P-value=0.054 fisher =0.067

Table 30. Distribution of seropositive and seronegative females for *Coxiella burnetii* and *Chlamydia abortus*

		Chlamydia abortus		
		seronegative	seropositive	total
Coxiella burnetii	Seronegative	298 (100%)	0 (0.0%)	298
	Seropositive	41 (89.1%)	5 (10.9%)	46
Total		339 (98.5%)	5 (1.5%)	344

P-value<0.0001

Table 31. Distribution of seropositive and seronegative females for *Chlamydia abortus* and *Toxoplasma gondii*

		Toxoplasma gondii		
		seronegative	seropositive	total
Chlamydia abortus	Seronegative	148 (91.9%)	13 (8.1%)	161
	Seropositive	3 60.0%)	2 (40.0%)	5
Total		151 (91.0%)	15 (9.0%)	166

P-value=0.014 fisher=0.065

ii. Risk factors associated with the seropositivity of abortion bacterial agents.

Risk factors significantly associated with seropositivity towards *Brucella spp, Coxiella burnetii and Chlamydia abortus.* The univariate analysis revealed seven factors significantly associated with seropositivity towards of *Coxiella burnetii*. For numeric variables, we used the Student's t-test. For categorical variables, we used the Chi-squared Test, except when the sample size for any of the categories was small (i.e. lower than 5), in which case we used the Fisher's exact test.

Associated with Brucella abortus

The following variables were identified as risk factors by the final multivariable logistic regression Table 32: age (all categories) for cows more than 60 months OR=7.39 (95% CI, 2.1%-25.5%) artificial insemination OR=1.46 (95% CI, 0.4%-4.3%), and finally, rivers and steaming in the pasture (Yes vs No) OR=25.9 (95% CI, 8.2%-81.7%) However, Herd size (as numeric variable) was found as a protective factor, consequently, an increase of one animal in the herd (namely, an increase of one unit in the size of the herd) translates as a decrease of a 4% (1-0.96 = 0.04) in the odds of the herd (Table 32).

Table 32. The final multivariable logistic regression model for factors associated with *Brucella* infection.

Factor	Odds Ratio	Confidence interval	P-value
Age (24, 48 months)	5.58	1.59-19.57	0.007
Age (48, 60 months)	5.66	1.55-20.68	0.009
Age (> 60 months)	7.39	2.14-25.53	0.002
Herd size (numerical)	0.96	0.93-0.96	0.002
Rivers and Streams (yes vs no)	25.95	8.23-81.76	<0.000
IA in five last year			
Naturel	0.17	0.03-1.01	0.051
Artificiel	1.46	0.48-4.39	0.505

Association with Coxiella burnetii

Visit of other farmers with no specific protection (yes vs no) was the main variable that was identified as risk factor by the final multivariable logistic regression with odds of ratios 5.70, in another hand, the season (Autumn vs Winter vs Sprint) and water source (tap water vs well) were identified as protective factors (decrease seropositivity) with odd ratio of 0.09 and 0.09, respectively (Table 33).

Table 33. The final multivariable logistic regression model for factors associated with *Coxiella burnetii*

Factor	Odds Ratio	Confidence interval	P-value
Season			
Winter	0.29	0.09-0.89	0.031
Sprint	0.09	0.02-0.49	0.005
Visit of other farmers (yes vs no)	5.70	1.70-19.10	0.005
Water source (tap water vs well)	0.09	0.02-0.44	0.003

Table 34. Risk factors in relation with *B. abortus, C. abortus* and *C. burnetii*.

	B. abortus, melitensis, suis			***Chlamydia abortus***			***C. burnetii***		
characteristics	n	No. of positive (%)	*P*	n		*P*	n	% of positive	*P*
≤2 years6-48*	41	10 (24.4)	**0.001**	38	0 (0)	0.240	38	2 (5.3)	0.112
2-6 years49-72	318	81 (25.5)	*	257	3 (1.2)		257	40 (15.6)	
>6years73-182	78	36 (46.2)		49	2 (4.1)		49	4 (8.2)	
Breed			**0.000**			0.032			0.000
Crossedbreeds	113	59 (52.2)	*	91	0 (0)	*	91	2 (2.2)	*
Montbéliarde	159	35 (22)		120	1 (0.8)		120	26 (21.7)	
Holstein	126	26 (20.6)		100	3 (3)		100	17 (17)	
Brune des alpes	28	5 (17.9)		25	0 (0)		25	0 (0)	
Fleckveih	11	2 (18.2)		8	1 (12.5)		8	1 (12.5)	
number of calving			0.997			0.033			1.000
<=2	234	65 (27.8)		194	1 (0.5)	*	194	26 (13.4)	
3 - 4	153	47 (30.7)		116	2 (1.7)		116	16 (13.8)	
>=5	50	15 (30)		34	2 (5.9)		34	4 (11.8)	
Number of pregnancies			**0214**			0.090			0.819
<=2	213	59 (27.7)		194	1 (0.5)		194	26 (13.4)	
3-5	157	42 (26.8)		116	2 (1.7)		116	16 (13.8)	
>=5	67	26 (38.8)		34	2 (5.9)		34	4 (11.8)	
Gestation			0.280			0.372			0.533
No	204	55 (27)		168	1 (0.6)		168	20 (11.9)	
Yes	233	72 (30.9		176	4 (2.3)		176	26 (14.8)	
Stage of gestation (month)			0.211			0.205			0.462
1-3	75	22 (29.3)		57	1 (1.8)		57	6 (10.5)	
4-6	76	27 (35.5)		57	2 (3.5)		57	11 (19.3)	
7-9	81	23 (28.4)		61	1 (1.6)		61	9 (14.8)	
AI 5yr			**0.000**			0.037			0.009
No	222	45 (20.3)	*	198	1 (0.5)		198	33 (16.7)	*
Yes	78	9 (11.5)		47	0 (0)		47	8 (17)	
Both	137	73 (53.3)		99	4 (4)		99	5 (5.1)	
AI in last mating			**0.019**			0.167			0.053
No	222	45 (20.3)	*	198	1 (0.5)		198	33 (16.7)	
Yes	215	82 (38.1)		146	2.7 (5)		146	13 (8.9)	
use of disinfectant			0.126			0.021			0.790
Yes	262	73 (27.9)		207	0 (0)		207	29 (14)	
No	175	54 (30.9)		137	5 (3.6)		137	17 (12.4)	
Cleaning method			**0.000**			0.503			0.494
Balayage	108	49 (45.4)	*	77	0 (0)		77	8 (10.4)	
Balayage et toyauterie	329	78 (23.7)		267	5 (1.9)		267	38 (14.2)	
mixing with other spp			**0.000**			1.000			0.006
No	99	9 (9.1)	*	89	1 (1.1)		89	20 (22.5)	
Yes	338	118 (34.9)		255	4 (1.6)		255	26 (10.2)	
Visit of another farmer			0.184			0.654			0.030
No	159	49 (30.8)		128	1 (0.8)		128	10 (7.8)	
Yes	278	78 (28.1)		216	4 (1.9)		216	36 (16.7)	
Quarantine practice			**0.001**			0.545			0.660
No	365	119 (32.6)	*	272	5 (1.8)		272	38 (14)	
Yes	72	8 (11.1)		72	0 (0)		72	8 (11.1)	
presence of sheep			**0.000**			0.472			0.004
No	91	8 (8.8)	*	81	0 (0)		81	19 (23.5)	
Yes	346	119 (34.4)		263	5 (1.9)		263	27 (10.3)	
Presence of calving box			**0.013**			1.000	.		0.824
No	354	114 (32.2)	*	261	4 (1.5)		261	36 (13.8)	
Yes	83	13 (15.7)		83	1 (1.2)		83	10 (12)	
EL-MADHER DJARMA	47	0 (0)	**0.000**	47	0 (0)	0.001	47	(8 (17)	0.001
SERIANA	25	16 (64)	*	25	0 (0)		25	0 (0)	
LAZROU	24	16 (66.7)		24	1 (4.2)		24	2 (8.3)	
BOUMIA	14	2 (14.2)		14	1 (7.1)		14	2 (14.3)	
AY_A	31	8 (25.8)		31	0 (0)		31	0 (0)	
AIN_YAGOUT	25	1 (4)		25	3 (12)		25	3 (12)	
ZANA EL_BAIDA	41	35 (85.4)		41	0 (0)		41	0 (0)	
HAMMA	45	11 (24.4)		45	0 (0)		45	10 (22.2)	
ROKNIA	52	3 (5.8)		52	0 (0)		52	11 (21.2)	
AIN ABASSA	40	0 (0)		40	0 (0)		40	10 (25)	
DOUAR ADJAIL	24	2 (8.3)		-	-		-	-	
AIN AZAL	21	9 (42.9)		-	-		-	-	
AIN HEDJAR	38	24 (63.2)		-	-		-	-	
Batna	252	89 (35.3)							
Khenchela	92	3 (3.3)		252	5 (2)		252	25 (9.9)	
Setif	93	35 (37.6)		92	0 (0)-		92-	21 (22.8)-	
Small <20	130	52 (40)	**0.013**	72	3 (4.2)	0.063	72	7 (9.7)	0.407
Large >20	307	75 (24.4)	**6**	272	2 (0.7)		272	39 (14.3)	
River streaming			**0.000**			0.383			0.008
No	223	19 (8.5)	*	189	4 (2.1)		189	34 (18)	
Yes	214	108 (50.5)		155	1 (0.6)		155	12 (7.7)	
Standing water			**0.000**			0.156			0.765
No	136	16 (11.8)	*	102	3 (2.9)		102	15 (14.7)	
Yes	301	111 (36.9)		242	2 (0.8)		242	31 (12.8)	

Association with Chlamydia abortus

The following variables were identified as risk factors by the final multivariable logistic regression Table 35: age (numeric), stray dogs (yes vs no) and wild animal in the building with odds ratios 1.03, 0.05 and 13.75 respectively.

Table 35. The final multivariable logistic regression model for factors associated with *Chlamydia infection.*

Factor	Odds Ratio	Confidence interval	P-value
Age (numeric)	1.03	1.00-1.05	0.066
Stray dog (yes vs no)	0.05	0.00-0.85	0.038
Wild species in the building	13.75	1.57-120.64	0.018

Table 36. Occurrence of reproductive disorders in relation to *Chlamydia abortus*.

Variable	Cattle	*Chlamydia* seropositive cattle		
	Number of animals examined	No. of Positive	Proportion of seropositive (95% CI)	P-value
Abortion				
No	245	4	1.63 (0.45, 4.13)	
Yes	60	1	1.67 (0.04, 8.94)	>0.999
Endometritis				
No	273	5	1.83(0.60, 4.22)	
Yes	32	0	0.00 (0.00, 10.89)	0.971
Weak calf				
No	290	5	1.72 (0.56, 3.98)	
Yes	15	0	0.00 (0.00, 21.80)	>0.999
Retained foetal membrane				
No	283	5	1.77 (0.58, 4.07)	
Yes	22	0	0.00 (0.00, 15.44)	>0.999
Anoestrus				
No	244	4	1.64 (0.45, 4.14)	
Yes	61	1	1.64 (0.04, 8.80)	>0.999
Repeat breeding				
No	215	2	0.93 (0.11, 3.32)	
Yes	90	3	3.33 (0.69, 9.43)	0.311
CRDE				
No	149	1	0.67 (0.02, 3.68)	0.395
Yes	156	4	2.56 (0.70, 6.43)	

Table 37. Occurrence of reproductive disorders in relation to *Coxiella burnetii*.

Variable	Cattle	*Coxiella brunetii* seropositive cattle		
	Number of animals examined	No. of Positive	Proportion of seropositive (95% CI)	P-value
Abortion				
No	245	34	13.88 (9.81, 18.85)	
Yes	60	10	16.67 (8.29, 28.52)	0.729
Endometritis				
No	273	41	15.02 (11.00, 19.82)	
Yes	32	3	9.38 (1.98, 25.02)	0.553
Weak calf				
No	290	43	14.83 (10.94, 19.45)	
Yes	15	1	6.67 (0.17, 31.95)	0.617
Retained foetal membrane				
No	283	44	15.55 (11.53, 20.30)	
Yes	22	0	0.00 (0.00, 15.44)	0.092
Anoestrus				
No	244	37	15.16 (10.91, 20.29)	
Yes	61	7	11.48 (4.74, 22.22)	0.596
Repeat breeding				
No	215	26	12.09 (8.05, 17.22)	
Yes	90	18	20.00 (12.31, 29.75)	0.107
CRDE				
No	149	21	14.09 (8.94, 20.73)	>0.999
Yes	156	23	14.74 (9.58, 21.30)	

Table 38. Occurrence of reproductive disorders in relation to *Brucella abortus.*

Variable	Cattle	*B. abortus* seropositive cattle		
	Number of animals examined	No. of Positive	Proportion of seropositive (95% CI)	P-value
Abortion				
No	313	68	21.73 (17.28, 26.71)	
Yes	82	48	58.54 (47.12, 69.32)	<0.000
Endometritis				
No	357	101	28.29 (23.68, 33.27)	
Yes	38	15	39.47 (24.04, 56.61)	0.211
Weak calf				
No	380	111	29.21 (24.68, 34.06)	
Yes	15	5	33.33 (11.82, 61.62)	0.956
Retained foetal membrane				
No	364	103	28.30 (23.73, 33.23)	
Yes	31	13	41.94 (24.55, 60.92)	0.163
Anoestrus				
No	324	95	29.32 (24.42, 34.61)	
Yes	71	21	29.58 (19.33, 41.59)	>0.999
Repeat breeding				
No	277	74	26.71 (21.60, 32.34)	
Yes	118	42	35.59 (27.00, 44.93)	0.098
CRDE				
No	189	39	20.63 (15.10, 27.11)	
Yes	206	77	37.38 (30.75, 44.37)	<0.000

III. 2. 4. Discussion

The aim of our study was the investigation of individual-level seroprevalence of *Brucella abortus, Chlamydia abortus* and *Coxiella burnetii* in dairy cattle in eastern Algeria and clarify factors associated with individual-level seroprevalence of these pathogen infections.

Cross-sectional study design associated with serological investigations, as used in the study I, is widely used in veterinary epidemiology and used to assess the burden of a particular disease in a defined population (Dohoo et al., 2009). The advantage of cross-sectional design is that it is unambiguous, straightforward, inexpensive, and needs only one sampling occasion. It provides descriptive characteristics of a population at a particular point in time and includes both old and new cases (Dohoo et al., 2009). Nevertheless, it is less suitable for determining when the disease occurred or for how long it has lasted. The finding of antibodies in a single serum sample only indicates that infection has occurred sometime in the past which make its

diagnostic value as indicator of present active infection limited (Levin, 2006). It is impossible to determine the sequence of events, namely whether exposure occurred before, during, or after the onset of disease outcome (Levin, 2006). In some type of ELISA's against some diseases, a high level of antibodies could indicate a possible acute phase of the diseases. One disadvantage of cross-sectional design compared to, for example, longitudinal study design is consequently the weakness in determining cause-effect relationships (Dohoo et al., 2009). The association between seropositivity and reproductive disorders found in the present study is therefore not necessarily causal even though it is statistically significant. Other study designs, such as longitudinal study or case-control study, would have been stronger field visits.

Sample-size determination is often an important step in planning an epidemiological study. There are several approaches to determining sample size. It depends on the type of the study. Descriptive, observational and randomized controlled studies have different formulas to calculate sample size. An adequate sample size helps guarantee that the study will yield reliable information, regardless of whether the ultimate data suggest a clinically important difference between the treatment being studied, or the study is intended to measure the accuracy of a diagnostic test or the incidence of a disease. Ideally, to get a true prevalence estimate of a given infection in a population with good precision, all animals should be included (census). Due to the insufficiency of resources, only a fraction (sample) of the population is used to represent the whole population. This fraction needs to be optimal and representative to allow inferences to be made about the target population (Dohoo et al., 2009), which necessitates a random sampling strategy.

The calculation of sample size needed was complicated by several factors: unknown prevalence and heterogeneity in management systems. Pertinent literature on seroprevalence in Algeria is scarce and concerns studies conducted in other parts of the country with different management systems, study design, and laboratory techniques, limiting its relevance. The minimum sample size was increased to take into account the mentioned challenges. It is therefore likely that the sample size in the present study allows implications to be made about the target population; furthermore, the herds included were generally typical and are very likely representative of other herds in the study areas.

Most of the information on reproductive disorders and risk factors was collected using an interview-based questionnaire, which is susceptible to communication challenges. To minimize the risk of information bias/misinformation, for this, on each excursion, the purpose of which was collect samples and information, a qualified veterinary accompanies the breeders to avoid any possible ambiguities in the responses collected. However, the information farmers provide depends largely on their knowledge, record keeping, and capacity to remember what happened up to, in some cases, more than 5 years before interview. Because of the lack in records and farmers have a poor understanding of reproductive disorders, the frequency of reproductive disorders in the area might have been underestimating. For a dairy herd, the only record important to most farmers is number of calving and milk yield in which this latter information could not be provided even for veterinary inspection. Information on reproductive performance indicators in general (age at first service, age at first pregnancy, conception rate, and calving interval) was sought by the owners. Early embryonic loss, such as fertilization/conception failure and early embryonic mortality, was not possible to assess in all breeders, which might cause further underestimation of the occurrence of reproductive disorders. *Brucella* spp. typically results in abortion in late gestation that is relatively easily recognized by farmers.

All infectious agents studied have global epidemiological distribution (Khan and Zahoor, 2018; Lanave et al., 2017; Barati et al., 2017; Almería and López-Gatius, 2013; Hemphill and Gottstein, 2000; Dubey and Webster, 2010; Knobel et al., 2013). They were all selected in accordance with their recognized impact on the ability to cause reproductive disorders in cattle, public health and economic importance, and likely local importance (Khan and Zahoor, 2018; Lanave et al., 2017; Barati et al., 2017; Almería and López-Gatius, 2013; Hemphill and Gottstein, 2000; Dubey and Webster, 2010; Knobel et al., 2013) exceptionally in Algeria where vaccine does not practical against them. Practical and financial considerations made it necessary to focus on only a few. There are several other important infectious agents known to cause reproductive disorders in cattle such as *Leptospira* spp., *Campylobacter foetus, Listeria* spp., *Haemophylus somnus, Trichomonus foetus*, *Sarcocystis neuroni* and Bovine Herpes Virus -1 (BHV- 1) (Yoo, 2010). The incidences of reproductive disorders in bovine are increasing over years. This scenario is further aggravating due to more emphasis on selection and rearing of animal for specific commercial purposes which compromises livestock reproduction. Commonly, in Algeria, there is a paucity of data on endemic zoonosis other than *Brucella spp*

(Khan and Zahoor, 2018; Lanave et al., 2017; Barati et al., 2017; Almería and López-Gatius, 2013; Hemphill and Gottstein, 2000; Dubey and Webster, 2010; Knobel et al., 2013; Lounes et al., 2014). In Algeria, few studies have been done on humans and livestock (Lacheheb and Raoult, 2009; Rouatbi et al., 2019; Croxatto et al., 2014; Lounes et al., 2014), accentuating the need for more research on this infection. Additionally, molecular epidemiological information is still lacking.

In the current study, Antibody iELISA was used because it is widely used and also recommended by OIE for screening of brucellosis (OIE, 2008; IDvet innovative diagnostic, 2018). The iELISA has a sensitivity of 100% (CI95%: 89.57% - 100%), a specificity of 99.74% (CI95%: 99.24% - 99.91%), and performed with *Brucella abortus* lipopolysaccharide as antigen. ELISAs are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen but a good deal of variation exists in the anti-bovine Ig conjugate used (Saegerman et al., 2004). Most iELISAs detect mainly IgGs or IgG sub-classes. Their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions, notably those due to *Yersinia enterolitica* (YO9) infection. These cross-reactions seen in iELISAs motivated the development of cELISAs. These tests are more specific, but less sensitive, than iELISAs (Nielsen et al., 1995). The OIE considers these tests “prescribed tests for trade”(OIE, 2008).
The ELISA used in the study detects antibodies directed against *Brucella abortus, melitensis* and *suis* in serum and plasma. Khames et al., (2017) concluded that Bruce-ladder multiplex PCR and conventional bio typing showed that Algerian cattle are infected mostly by *B. abortus* biovar 3, and to less extent by *B. abortus* biovar 1 and *B. melitensis* biovar 3. The test is straightforward and versatile with short and overnight incubations for individual serum or plasma samples, or pools of up to 10 samples.

When testing bovine samples, cross-reactions due to *Yersinia enterolitica* may be observed with this test, especially for free range animals. The kit is easy-to-use, and results are obtained in 90 minutes. This test does not discriminate between natural infection and vaccination with *B. abortus* S19, but, importantly, there is no history of vaccination against brucellosis in Algeria. Consequently, the presence of antibodies due to vaccination can be excluded, which simplifies the interpretation of serological results.

Enzyme linked immunosorbent assay (ELISA) with blood is preferred for large-scale screening of the infection status livestock (OIE, 2019). In dairy cattle, seroconversion against *C. burnetii* tends to occur within the first ninety days of lactation, with young multiparous cattle being the most likely to seroconvert (Böttcher et al., 2011). As stated by Niemczuk et al., (2014); Sidi-Boumedine et al., (2010) the use of a combination of different laboratory methods, preferably ELISA for serology and PCR for the agent detection, is suggested to achieve the correct diagnosis of Q fever in cattle.

Antibody iELISA used in the current study is based on *Coxiella burnetii* phase 1 and 2 strains (isolated in France from the placenta of a bovine abortion). It can be used on serum, plasma and milk of ruminants. *C. burnetii* exists in two antigenic phases: phase I and phase II. This antigenic difference is important in diagnosis. Anti- phase II-antibodies are produced early after infection with virulent *C. burnetii*, whereas the increase of anti-phase I antibody titers is delayed. The two forms of the infection, acute and chronic, have different serological profiles:

- in acute cases of Q fever, antibody levels to phase II *C. burnetii* are usually much higher than to phase I *C. burnetii*, and are generally first detected during the second week of illness.
- in chronic Q fever, both phase I and phase II antibody titres are high.

ELISA is preferred over IFA and CFT, particularly for veterinary diagnosis, because it is convenient for large-scale screening. The ID Screen® Q Fever Indirect Multi-species ELISA used in the study uses phase I and II *Coxiella burnetti* as antigens. The manufacturer claims 100 % (CI95%: 89.28%- 100%) sensitivity performed on 32 samples and 100 % (CI95%: 97.75%-100%) specificity performed on 167 samples of animals with known infection status. Similarly, to other studied diseases there is no vaccination in Algeria against the Q fever. This test does not discriminate between natural infection and vaccination, but, importantly, there is no history of vaccination in Algeria, therefore, the presence of antibodies due to vaccination can be excluded, which simplifies the interpretation of serological results.

Chlamydia abortus

Serological diagnosis of *Chlamydia abortus* may be achieved by the complement fixation test (CFT) or by ELISA. These tests, which use LPS or whole bacteria as antigens, generally present low specificity and sensitivity levels, and cross reactions are often observed with the lipopolysaccharide of *Chlamydophila pecorum*. The IDvet Screen® *Chlamydophila abortus* Indirect Multi-species ELISA used in the present study aims to reduce these false positive reactions through the use of a synthetic peptide antigen from a MOMP specific to *Chlamydia abortus.*

The PCR and real-time PCR tests, although highly sensitive and used to detect *Chlamydia* in different animals in other countries have seldom been used on a large scale due to high cost. O'Neill et al., (2018) reported in his investigation that aimed to compare three commercial ELISA kits to detect *C. abortus* antibodies in ewes and to determine which of the kits had the highest sensitivity. The IDvet kit used a MOMP peptide antigen, the MVD-Enfer kit is based on a POMP90–3 antigen while the LSI kit plates are coated with chlamydial LPS. Moreover, the sensitivity was highest with the LSI test kit at 94.74%, followed by the MVD-Enfer and IDvet kits, at 78.95% and 73.68% respectively.

In the present study antibody iELISA was used and the manufacturers claims a specificity of 100% (CI95%: 90.5 - 100%) and sensitivity of 70% (CI95%:53.5%-83.4%). This test does not distinguish between natural infection and vaccination, but, prominently, there is no history of vaccination in Algeria against Chlamydiosis, subsequently, the presence of antibodies due to vaccination can be let off, which simplifies the interpretation of serological results.

The aim of this study was to determine the seroprevalence of *Brucella* spp. infections in unvaccinated cattle in selected districts of north-eastern region of Algeria and identify associated risk factors. From our study, there was evidence of previous cattle exposure to brucellosis in the study area.

In Algeria, control programs against brucellosis are applied based on various strategies: screening-slaughtering program against cattle brucellosis, and Rev-1 vaccination program against small ruminants' brucellosis (Kardjadj, 2016), whereas on the other hand, no vaccine is practice for cattle.

The overall animal level seroprevalence in our study (28.6%) was higher than previously reported in Algeria (7/280; 2.5%) using the Rose Bengal test (Kaaboub et al., 2019). Similarly, Yahia et al., (2018) stated a seroprevalence of 1.4% in their study carried out between 2004-2013 in Djelfa province (located in the high plateaus, 300 km south of Algiers capital of Algeria). In the same way. Ammam et al., (2018) reported 6.30% in human seroprevalence in contact with cattle from North-western Algeria (Sidi Bel Abbes). World Health Organisation (WHO) argued that human brucellosis incidence in Maghreb countries was 10 to 25 times underestimated (Berger, 2016). In opposite, the animal seroprevalence in the current study was lower when compared to the 31.5% in both southern and northern Tiaret province (Aggad and Boukraa, 2006) using the buffered plate antigen test, and also 40.10% in Angola (Franco C. Mufinda et al., 2015) using Rose Bengal test.

The reported herd level seroprevalence in our study (73.3%) was higher when compared to the 7/57 farms (12.28%) in media (north centre Algeria) (Kaaboub et al., 2019). Further, Moustafa Kardjadj, (2018) reported a within herd prevalence of 12% (95%; CI 4.65%–19.35%) using iELISA in their study carried out in five regions (north-central, north-western, north-eastern, steppe, and the Sahara region). The same observation was reported in Morocco at individual (1.9%; 95% CI 1.2%-2.8%) and herd 9% (95% CI 4.5%-1.5%) levesl by Yahyaoui Azami et al., (2018). In addition, based on serological (Rose Bengal test and iELISA) and molecular (real-time PCR) analyses, the true adjusted animal population level prevalence was 23.5 % in cattle in central-eastern Tunisia with a true adjusted herd level prevalence of brucellosis of 55.6 % (Barkallah et al. 2017). There, Khamassi Khbou et al., (2017) reported an overall animal seropositivity to *Brucella spp*., of 21% and 1.9% in case and control farms, respectively.

Despite a lack of epidemiological data, it is admitted the disease is endemic in Maghreb, with brucellosis prevalence in small ruminants ranging from 0.1% for Morocco to 6% and 7.5%, respectively for Algeria and Tunisia (Ayayi Justin Akakpo et al., 2009). Refai Mohamed, (2002) stated that, brucellosis infection was reported in almost all domestic animals, particularly cattle, sheep and goats in countries of the Near East region (Saudi Arabia, Kuwait, Israel, Oman, Iraq, Iran, United Arab Emirates, Jordan, Israel, Palestinian, Syria, Yemen Sudan, Egypt, Libya, Somalia Tunisia and Turkey).

In the study area, semi-extensive farming management is the most type used by breeders. It may be explaining the high level of animal and within herds seroprevalence of antibodies against *Brucella* spp. in agreement with multiple studies that reported that extensive production systems exhibit low rates of disease transmission and lower disease burden, while intensification promotes transmission due to increased stocking densities, animal contacts and a higher birth index (Grace et al., 2012; McDermott Grace and Zinsstag, 2013; Jones et al., 2013; Racloz et al., 2013; Ducrotoy et al., 2014).

In the present investigation, the potential risk factors included age (all categories: for cows more than 60 months (OR=7.39 [95% CI, 2.1%-25.5%]), presence of rivers and/or runoff (OR=25.9 [95% CI, 8.2%-81.7%]) in pastures and the use of artificial insemination (OR=1.46 (95% CI, 0.4%-4.3%)). In opposite, herd size (as numeric variable) was found as a protective factor; an increase of one animal in the herd (namely, an increase of one unit in the size of the herd) translates as a decrease of a 4% (1-0.96 = 0.04) in the odds of the herd.

Age is one of the possible factors associated with the occurrence of brucellosis. The prevalence was lower among the young animals screened in this study compared to the older ones. Usually, young animals are protected by maternal immunity until when the immunity disappears, thus susceptibility seems to be low among them. The high prevalence seen in the older animals is demonstrating the chronic nature of brucellosis. Brucellosis appears to be more associated with sexual maturity (Mukasa-Mugerwa and Africa, 1989). This is in accordance to previous studies, and its explanation lies in the fact that the older animal is, the longer is the potential exposure to the pathogen (Abutarbush, 2010; Megersa et al., 2011). Age was previously found to be significantly associated with seropositivity for brucellosis in cattle in Media (North center

Algeria) (Kaaboub et al., 2019), Niger (Mohammed et al., 2011; Boukary et al., 2013). While, Mohammed et al., (2011) observed that age, sex, location, and herd size played role in the epidemiology of brucellosis among cattle herds in Jigawa state, north western Nigeria.

The data generated in the present study agrees with Delafosse et al., (2002) Koutinhouin. B et al., (2003), Muma et al., (2006), Zubairu Ardo and Mai, (2014) Asmare K et al., (2010), Ibrahim et al., (2010), Hailu Degefu et al., (2011), B et al., (2011), Tialla et al., (2014) and Dirar Nasinyama and Gelalcha, (2015) that reported that the relationship between the increase of the risk of infection with age logically corresponds to a greater probability of exposure to risk in older animals to remain infected, and to be dangerous to other animals.

Farms which practiced artificial insemination (AI) had 1.46 (95% CI, 0.48-4.39) times more likely to have brucellosis infection compare to farms with natural mating. *Brucella* spp. antigen could be transmitted via Artificial insemination gun during non-aseptic AI process by inseminator. Two of the major goals of artificial insemination of domesticated animals are to achieve continuous genetic improvement and to prevent or eliminate venereal disease. According to McDermott and Arimi, (2002); Neta et al., (2010) venereal transmission is not a major route of infection under natural conditions, but artificial insemination with contaminated semen was reported as potential source of infection in Brazil and sub-Saharan Africa countries.

B. abortus is one of the pathogens that directly affect the testicular parenchyma where it could become cultured; genital tract cells produce erythritol promoting this pathogen's growth and are thus its preferred localization Givens and Marley, (2008). reported that brucellosis infection in bulls could lead to reduced libido and lower semen quality and infertility. The shedding of *B. abortus* in the semen of bulls has been reported and this may pose a risk of disease transmission by AI (Eaglesome M.D and Garcia M. M, 1993). Johanna Lindahl et al., (2019) stated that artificial insemination was also associated with increased risk of brucellosis infection in dairy herds. Aprizal Panus et al., (2018) indicated that farms which were not having disinfection before the artificial insemination seems 2.8 times more likely to have brucellosis infection in their farms compare to farms with having disinfection, assessing the importance of disinfection as protective factor to reduce brucellosis infection.

Nicoletti, (1980) (Nicoletti, 1980) suggested that although *Brucella abortus* survives under certain conditions in pasture and water, the absence of direct contact between susceptible and infected animals or infected biological material (carcasses, uterine secretions, aborted foetuses, and semen for artificial insemination) almost eliminate the risk of disease spread. In contrast, Shome, (2014) concluded in their investigation in India that animals bred with natural mating were more seropositive for *Brucella* infection than animals bred with artificial insemination. In the same way, Cárdenas et al., (2019) said that when herds with and without artificial insemination were compared, it was observed that farms that used natural breeding with bulls from non-certified herds had a higher risk than farms using artificial insemination (OR = 2.45, *p*-value = 0.037), but when the bulls came from brucellosis-free farms, farms with natural breeding were less affected (OR = 0.30, p-value = 0.004) than farms using artificial insemination, whether with frozen semen from certified brucellosis-free herds or fresh semen from uncontrolled Columbian herds.

Nevertheless, given that in Algeria semen comes from The National Center for Artificial Insemination and Genetic Improvement (CNIAAG), with quality control standards, artificial insemination is a consolidated and safe reproductive procedure (http://www.cniaag.dz/) (‘CNIAAG’ 2020). It should be worried that the exclusive use of artificial insemination for reproductive management might be a substitution variable for herds that have better production standards and therefore greater concern over health issues, given the quality of the genetic material in the herd.

In the present survey, larger herds were considered protective factors, more precisely, an increase of one animal in the herd (namely, an increase of one unit in the size of the herd) translates as a decrease of a 4% (1-0.96 = 0.04) in the odds of the herd to contract brucellosis infection. The explanation of that is probably, purchase of cows (introduction of new animals to the herd without any controlling) in small herds increased the prevalence and risk of brucellosis infection in comparison to middle and large herd sizes. One explanation could be that cattlemen with small herds are less careful, by economical constraints, in purchasing animals. McDermott and Arimi, (2002) stated that brucellosis risk in the extensive livestock production systems is more important regarding large herd sizes, extensive movement of cattle, and common mingling with other herds at common grazing and watering points from sub-

Saharan Africa. The same reflexion took place regarding large herd size in Ethiopia (Ibrahim et al., 2010; Terefe et al., 2017), in Tanzania (Sagamiko et al., 2018), in Uganda (Bugeza et al., 2019), (Oloffs, 1996), (Fred Unger et al., 2003), in Ghana (Tasiame et al., 2016), in Zambia (Muma et al., 2007), in Negeria (Ogugua et al., 2018), in Italy (Calistri et al., 2013), and in Brazil (De Alencar Mota et al., 2016).

It is reasonable that a larger flock size increases the risk of infection by increasing the contact rate between susceptible and infected animals, particularly, in the case of intensive breeding. Further, sanitary measures are poorer in larger herds compared to small herds. Unhygienic practices, cattle concentrations, and mixing encourage spread of the infection amongst the animals. Furthermore, Calistri et al., (2013) suggested that the association between the brucellosis infection and the number of animals in the herd is probably linked to the number of parturitions and abortion, and therefore probability of *Brucella* spread, and maybe to the number of contact among animals, through animal movements or in the pastures.

In the present survey, presence of rivers and steaming in the pasture (Yes vs no) were considered as risk factors to seropositivity of animals (*P*-value<0.001, OR=25.9 [95% CI, 8.2%-81.7%]). Our finding is in accordance with several studies (Calistri et al., 2013; Mazeri et al., 2013; Pandey et al., 1999; Hellmann, Staak and Baumann, 1984). Transmission is possible at watering points when cattle share water with wild animals, which could explain the higher risk of infection in herds that shared water with wild animals.

Animal-level and herd-level seroprevalence in unvaccinated cattle for *Coxiella burnetii* were 13.3% (46/344; 95% CI, 9.8%-17.8%) and 11/22 (50.0%; 95% CI, 25.0%-89.5%) respectively, with specific seroprevalence at farm level (at least one positive animal) ranged from 0.0% (0 out 47;95% CI: 0-5.2%) to 41.7% (5 out of 12; 95% CI: 18.0%-68.8%). The age of the cows ranged between 6 months and 15 years. Positive cattle were found in all three age groups, and the seroprevalence ranged from 5.3% to 15.6 %.

In Algeria, Q fever is considered an endemic infection. Interestingly, very few studies have documented the seroprevalence of Q fever in Algerian farm animals and most investigations have focused on sheep and goats (Khaled et al., 2016; Yahiaoui et al., 2013; Rahal K et al., 2011). Consequently, Q fever has been unexplored in Algeria. As a consequence of the lack of

published results, the value of ruminants as *C. burnetii* reservoirs and their role in diffusion of this pathogen are currently not totally known.

The overall animal level seroprevalence in our study (13.3%) was lower than that reported earlier in south-eastern Algeria by Benaissa et al., (2017) who reported a seroprevalence of *C. burnetii* at the animal level equal to 71.2% (95% CI: 65.2–78.3) and 85.3% (95% CI: 72.8–97.8) at the herd level in camel using ELISA. Obaidat and Kersh, (2017) in Jordan revealed that 62.9% (95% confidence interval: 55.1% to70.0%) of the tested ruminant farms were positive for *C. burnetii* antibodies using Ab ELISA in Bulk milk (Obaidat and Kersh 2017). These results are much lower than those observed in Europe, for example, ELISA testing showed 38.1% in cattle for individual for seropositivity in Hungary (Gyuranecz et al., 2012), in northern Spain, ELISA anti-*C burnetii* antibody prevalence was slightly higher in beef cattle (6.7±2.0%).

The seroprevalence observed in the studied area was also lower compared to the results from other African countries. The seroprevalence in cattle was estimated to be between 40% and 59.08% in Nigeria, Sudan and Zimbabwe however, higher than carried out in Chad about 4% (Guatteo, 2011). Alos, previous studies recorded the presence of infection in east Turkey at rates of 5.8% in animal level using IFAT (Çetinkaya et al., 2000).

The overall animal level seroprevalence in our investigation was higher than the previously reported in Iran, where the seroprevalence of Q fever in domestic cattle was 3.23%, whereas all IPC were negative (Ghasemi et al., 2018). Furthermore, the overall animal level seroprevalence in our investigation was lower than that reported recently in Lebanon 30.63% at herd level (Dabaja et al., 2019).
Finally, Guatteo, (2011) concluded that infected animals are detected in all the 5 continents (Africa, America, Asia, Europe, Oceania), New Zealand being the only country with a reported apparent prevalence of zero.

The inhalation of contaminated aerosols seems to be the main route and its low infectious dose (Jones et al., 2016). In relation with recent Q fever outbreaks, the presence of contaminated aerosols with *C. burnetii* or its presence in dust taken from animal premises confirmed potential transmission risks to humans. Infection of animal and human and contamination of environment

with *C. burnetii* requires transport through the atmosphere. It is assumed that *C. burnetii* is absorbed or fixed at the aerosol surface and becomes airborne. *C. burnetii* is resistant to heat and dryness and can survive for more 150 days in the environment.

The analysis of seroprevalence in the three age categories revealed that prevalence of antibodies increased with age, with very low prevalence in animals less than 2 years (5.3%) without be statistically significant (Table 34). This was in agreement with other studies and indicated horizontal transmission and maintenance of infection within adult populations (McCaughey et al., 2010; Ruiz-Fons et al., 2010; Taurel et al., 2011).

In the current study, the visit of other farmers was considered a risk factor (OR=5.70, *P*=0.005). In the same manner, Woldehiwet, (2004) and Paul et al., (2012) suggested in their investigations that farm personnel often act as mechanical transmitters of contaminated fomites from an infected herd to uninfected ones. Therefore, a lack of precautionary measures for visitors (such as washing hands and changing clothes and boots) before entering the farm including veterinarians, food factory staff, and professional hoof trimmers are considered as a potential risk to transmitted *C. burnetii*. Similarly, another investigation stated that the factors including animal contact with human visitors from outside the farm, artificial insemination by other people than artificial insemination technician, and herd health contract for routine health evaluation of the herd by the veterinarian were associated with increased antibody *C. burnetii* positivity with odd ratio 4.2, 7.7, 4.3 respectively (Agger et al., 2013). A study conducted in the United States of America by Whitney et al., (2009) in which the aims were to estimate seroprevalence and risk factors among veterinarian, concluded that antibodies against *C. burnetii* were detected in 113 (22.2%) of 508 veterinarians and risk factors associated with seropositivity included age ≥ 46 years, routine contact with ponds, and treatment of cattle, swine, or wildlife.

The season (Autumn vs Winter vs Sprint) and water source (tap water vs well) were identified as protective factors (decrease seropositivity) with odd ratio of 0.09 and 0.09, respectively. Little information is available about the probability of the influence of season and water source on *C. burnetii* seropositivity in cattle, however, in one study *C. burnetii* was reported that the higher risk of introduction and/or transmission of *C. burnetii* in larger herds is possibly due to

the higher number of calving or lambing females during the parturition season (Woldehiwet, 2004).

In the present study, no relationship was found between the size of the herd and the seroprevalence level. Nevertheless, other studies showed controversial results. Thus, whereas Taurel et al., (2011) found a significantly higher seroprevalence in herds with less than 46 animals, others McCaughey et al., (2010); Ryan et al., (2011) observed higher seroprevalence in larger dairy herds. The results obtained in the present study, assessed that no significant association between *C. burnetii* seropositivity and reproductive disorders. In a common direction, some researchers reported a lack of association between high seroprevalence of *C. burnetiid* and reproductive disorders in cattle (Raoult Marrie and Mege, 2005; Muskens et al., 2011; Astobiza et al., 2012; Muskens et al., 2012; Obaidat and Kersh, 2017). These findings suggest that infection can persist in cattle sometimes without producing significant clinical signs (Paiba et al., 1999).

In opposite, the results reported by Khalili Sakhaee et al., (2012) showed that 51.35% of dairy cattle with reproductive problems and 10.3% cattle without problems were *C. burnetii* seropositive, signifying a close association between a history of reproductive disorders and Q fever seropositivity ($P<0.05$). (Bildfell et al., (2000) reported that bovine placentitis was highly associated with the presence of *C. burnetti,* along the same lines McCaughey et al., (2010) (McCaughey et al. 2010) stated the same conclusion. Equally, in cattle, metritis is frequently the unique manifestation of the disease (Woldehiwet, 2004; To et al., 1998; Parisi et al., 2006).

In conclusion, this study confirms the widespread existence of *C. burnetii* antibodies in dairy cattle in the study area (north-eastern Algeria) and suggests that further studies on the public health consequences of *C. burnetii* shedding in ruminant milk would be beneficial. Questions emerged regarding the potential impact of *Coxiella burnetii* on the general population as well as persons at risk, such as pregnant women.

Although few studies have investigated the prevalence of chlamydial infections in cattle in North Africa, reported prevalence rates vary hugely all over the world. In the current study, an animal-level and herd-level seroprevalence for *Chlamydia abortus* were 1.45% (5/344; 95% CI, 0.6%-3.2%) and 4/22 (18.1***%;*** 95% CI, 25.0%-89.5%), respectively, in Batna and Khanchla

province (North-eastern region of Algeria) (Figure 19) with specific seroprevalence at farm level (at least one positive animal) ranged from 0.0% (0 out of 47;95% CI: 0%-5.2%) to 12.5% (1 out of 8; 95% CI: 1.4%-45.4%). The age of the cows ranged between 6 months and 15 years. Positive cattle were found in all three age groups, and the seroprevalence ranged from 3.4% (1.3%-7.2%) and 12.2% (5.3%-23.5%).

Detection of antibodies against *C. abortus* is due to natural infection, since vaccination is not practiced in Algeria. However, the seroprevalence rate in cows may be overestimated with the use of ID Screen® *Chlamydophila abortus* Indirect Multi-species ELISA kits due to antigenic cross-reactivity with *Chlamydophila pecorum* and other organisms that have some similarities to *C. abortus.* Further, the ELISA used in the study aims to reduce these false positive reactions through the use of a synthetic peptide antigen from a MOMP specific to *Chlamydia abortus* ('IDvet | Innovative Diagnostic Kits' n.d.) (see Diagnosis challenge). The rate of such cross-reactions depends on the incidence of these organisms in cow population and thus could differ with geographical area, animal age, and sex or with the property of origin (McCauley et al., 2010). There has been no report on the seroprevalence of organisms that cross-react with *C. abortus* in dairy herds in Algeria. Therefore, it was difficult to estimate how much of the seroprevalence rate was due to infection with these organisms.

Several studies have reported considerable variation in the seroprevalence of chlamydial infection in cattle. Research on the disease has been mainly carried out in small ruminants (sheep and goats) and dairy cattle. Our results were significantly lower than the prevalence observed in the same country with an animal level seroprevalence of 12.2% (45/368) for *C. abortus,* and a seroprevalence at herd level of 29.8% (37/124) using ELISA, concurring with previous studies from north-centre of Algeria (Djellata et al., 2019).

Our results were meaningfully lower than the prevalence observed in Jordan, animal and herd level 19.9% and 66.3%, respectively (Abdelsalam Talafha et al., 2012). Other investigations carried out in several region in turkey which seroprevalence ranged from 5% to 20% in cattle (Berri et al., 2004; Da Silva et al., 2006; Entrican, 2002). Other studies was carried out in Algeria in small ruminant (Merdja et al., 2015). Halil Ibrahim Gokce et al., (2007) reported that 8.33% of cattle were positive for antibodies specific to *C. abortus* and 26.92% (7/26) of herds

examined in Turkey. Globally, seroprevalence of *C. abortus* in cattle ranged from 4.76 to 12.67% in the north-eastern part of Turkey using iELISA.

Seroprevalence to *C. psittaci* in dairy herds in Italy has been estimated at 24%, although the prevalence was appreciably higher in cows with reproductive problems (Cavirani et al., 2001). In Germany, seropositivity was associated with infertility (Sting R, et al. 2003), while this value rose to 41.5% in cows from farms with an increased incidence of reproductive disorders (Wehrend et al., 2005). In contrast, a study in Sweden, reported a seroprevalence of 28%, but could find no correlation between the presence of chlamydial antibody and reproductive disease (Godin et al., 2008). Further studies of both dairy herds and breeding bulls also concluded that *C. abortus* infection is uncommon or absent in Sweden (Karlsson et al., 2010). The overall seropositivity was 11.8% in cattle in Reunion Island (Cardinale et al., 2014)
The complement fixation test (CFT) results showed that the seroprevalence of *Chlamydia spp*. infections in the asymptomatic cattle population was 4.15%, while in the cattle with reproductive disorders 7.20% in the polish dairy population (Szymańska-Czerwińska Niemczuk and Galińska, 2013). In China, fifty out of the 134 herds (37.31%) had at least one *C. abortus* seropositive animal, and 535 cattle were seropositive (11.92 %) for *C. abortus* by Hemagglutination assay (IHA) test at the cut-off dilution of 1:16 (Sun et al., 2015). However, 158 of 974 (16.22%) white yaks were seropositive for *C. abortus* antibodies at the cut-off of 1:16 in by HIA in yack in north-eastern China. Reinhold Sachse and Kaltenboeck, (2011) stated that data published in the last two decades suggest a high seroprevalence of chlamydial infection in herds worldwide (Austria, Germany, Switzerland, Italy, Sweden, Taiwan and United states of America), with seropositivity at a herd level ranging from 45% to 100% in cattle.

At the individual animal level, our results was higher than reported by Derdour et al., (2017)who reported an animal seroprevalence of 0.83 (CI95%, 0%–1.77%) using ELLISA exactly in Algiers (north-centre of Algeria) (Derdour et al., 2017).

The following variables were identified as risk factors by the final multivariable logistic regression to Chmlamydia abortus cattle seropositive (Table 35): age (numeric), wild animal in the building with odds ratios 1.03 and 13.75 respectively. In opposite, stray dogs (yes vs no) was considered as a protective factor (OR=0.05).

Age of cattle (years) as a continuous variable was analysed in the logistic regression model, and the results showed that the prevalence was different significantly with ages (OR=1.03, *P-value*=0.066) demonstrating that the age is a predisposing factor for *C. abortus* seroprevalence. Significantly, the seroprevalence of *C. abortus* infection was nearly gradually increased with increase of age in cattle (Table 35). As the growth of the age, the seroprevalence of *C. abortus* infection went up all the time, indicating that there may be a cumulative likelihood for exposure to *C. abortus* infection with age in these surveyed regions. which was consistent with previous studies in other aged animals susceptible to *C. abortus* in white yaks in China (Qin et al., 2015). The seroprevalence of *C. abortus* vary significantly across age groups. In the same sense to our finding, a study from Bosnia reported significant regional differences in addition to differences between age groups(Softic et al., 2018).

In our knowledge, a very limited information is available on the presence of Chlamydiaceae in wildlife, particularly in North Africa. In the present investigation, the presence of wildlife in the pasture or/and in the building (Fox (Li et al., 2018), wolf, , boar and maybe birds(Nicole Borel Polkinghorne and Pospischil, 2018; Burnard and Polkinghorne, 2016)) were considered as an important risk factor (OR=13.75, *P*=0.018). Chlamydiosis is common in livestock, poultry, companion, and wild animals which may serve as reservoir for this organism and play a role in the contamination of the environment and spread of the disease (Berri et al., 2004; Hotzel et al., 2004; Hoffmann et al., 2015). Susceptible animals are infected through ingestion or inhalation of *C. abortus*-infected material, as a result of contamination of calving pens or of pasture by foetal membranes and discharges. Further, evidence is slowly mounting for the zoonotic and/or cross-species transmission potential of other bacteria within the order *Chlamydiales* with an increasing number of reports suggesting contact with wildlife is a risk factor for these infections as well (Hoffmann et al., 2015). Highlighting of *Chlamydia* spp infection in wild animals in Algeria could clarify the understanding of this pathogen.

In non-pregnant animals, this bacteria can exist in a latent form, possibly in lymphoid tissue, where it remains until at least the onset of pregnancy (Da Silva J.C. De Freitas and Müller, 2006; Entrican, 2002). However, the infection cannot be diagnosed either serologically or by direct detection of the pathogen (*e.g.*, modified Ziehl-Neelsen staining, PCR) until the time of

abortion, when infectious organisms are excreted and maternal *C. abortus* antibody titres rapidly increase. Zhaocai et al (2018) (Li et al., 2018) reported the evidence for the existence of *C. abortus* in farmed fur animals for the first time (the fox is one of them). Other possibility is that, it has been reported that *Chlamydia psittaci, Chlamydophila pneumonia* and *C. abortus* can be found in the faecal material and respiratory exudates of infected birds which results in contamination of the environment and spread of the disease in cattle(Borel et al., 2006). In our study, since the stray dogs were present in the study area, they were considered as a protective factor against cattle *Chlamydia abortus* infection (OR=0.05, P-value=0.03), this may be explained by the fact that they help to keep wild animals away from the farms. Thus, reduce the bacterial load that can infected cattle.

Factors, including presence of regular veterinary services, farm workers visiting neighboring farms, source of feed and water, presence of calving pens, and abortion rate were not significantly associated with seropositivity to C. abortus in this study.

Using the IDvet iELISA, no difference in chlamydial seroprevalence was found between cows with reproductive problems (including abortion), compared to healthy control cows (Godin et al., 2008; Petit et al., 2008). Our results, using iELISA, also showed that chlamydial seropositivity was independent of reproductive disorders history including abortion. Although chlamydiae are not considered a threat to the livestock industry, since abortions are sporadic and abortion storms are rare events, they could affect 20% of pregnant cows (Reinhold Sachse and Kaltenboeck, 2011). On the other hand, one must take into account the subclinical effect of *Chlamydia* infections, which have significant economic repercussions (Reinhold et al., 2008).

It is recommended that seropositive animals should be eliminated from flocks and herds. An appropriate vaccine against *C. abortus* should also be applied for ewes and cows to reduce the incidences of infection.

This is among the first report confirming and detect the seroprevalence of *C. abortus* in cattle offering basic data for prevention and control of this latter in cattle. Limiting access of wild animals to the farms (building and pasture) are important measures to minimize the risk for infection and reinfection not only with *Chlamydophila* spp. but also other infections affecting human and animal health. More studies using additional sensitive and specific molecular and

serological tests, such as PCR, monoclonal antibodies and recombinant protein-based ELISA, and characterization of field isolates, will improve herd management, control, and treatment of chlamydial infections. This, in turn, will lead to an intensification in animal production, improvement of animal welfare, an increase in economic return, and also reduction in the likelihood of zoonotic risk to humans.

III. Study III. Seroprevalence, risk factors and molecular characterization of BVDV in north-western region of Algeria

III. 3. 1. Introduction

The livestock sector in Algeria is enormous in animal numbers but the production is disproportionally small. In Algeria, there are several constraints to reach an acceptable level of dairy production and consequently, developing the Algerian dairy industry. One of them and probably the most restrictive is the absence of controlling program against the main infectious reproductive diseases. Many studies were conducted and concluded that the appearance of several cases of contagious diseases (tuberculosis, brucellosis, foot and mouth disease, etc), which sometimes led to forced slaughter therefore it was until now an absolute obstacle to achieve self-sufficiency of milk production. In addition, analysis of the reproductive criteria has shown that the calving fertilization insemination interval is far above accepted standards; this resulted in a calving-calving interval exceeding three hundred and sixty days. Similarly, a poor reproductive management is also behind these poor performances; it is clearly highlighted by a poor policy of reform, reproduction, gestation control and heat detection.

Bovine viral diarrhoea virus (BVDV), belonging to the genus *Pestivirus*, the family Flaviviridae, has a markedly negative impact on the economy to the livestock industry worldwide, through reduced milk production, abortions, and a shorter lifespan of the infected animals. Mortality, morbidity, premature culling and clinical signs include upper respiratory disease, fever, transient immune suppression, death among young stock, reproductive losses, still birth and the generation of PI animals.

Even thought, the epidemiological surveillance and vaccine against BVDV are the main methods used over all the world to control and minimize the effect of the disease. Algeria is still quite late in the practice of these procedures. Serological screening and molecular characterization studies are needed to achieve an effective management of BVDV disease, particularly the fact to detect and eliminate PI animals from herds. To date, the nucleotide sequencing and phylogenetic analysis of BVDV infection has not been systematically assessed in cattle from Algeria. The aims of this study, conducted between 2018 and 2019, were i) to estimate the BVDV seroprevalence in Tiaret region (north-western Algeria) cattle herds, ii) to identify associated risk factors, to confirm the BVDV impact in Algeria through serological screening based on ELISA tests and Reverse Transcriptase RT PCR, iii) to analyse the genetic diversity of *Pestivirus* circulating in cattle herds of north-western Algeria. This epidemiological

data should may lead to improve the visibility of this neglected disease in Algeria, suggest detection and elimination of PI animals and to develop a monitoring plan for the country.

III. 3. 2. Materials and methods

III. 3. 2. 1. Area of study and target population

Tiaret region sits at about 1143 mm above sea level with average temperature about 14.7°C and a mean annual rainfall of about 529 mm. The study area covers an area of 20,399.10 km^2 and covers part of the Tell Atlas in the north and the highlands in the Center and South. Tiaret region is located approximately 160 Km from the Mediterranean coasts. It is limited by several provinces, namely: the province of Tissemsilet and Relizane in the north; Laghouat and El-Bayadh in the South; the province of Mascara and Saida and to the West; the province of Djelfa to the East.

The average number of head of cattle per farm in Tiaret increased from 15 cows in 2009 to between 50 and 100 heads in 2015. The wilaya of Tiaret currently has a herd made up of 42,600 dairy cows including 12,000 imported cows (WWW.APS.DZ, 2015). The target population of this study is dairy cattle where the sale of milk is the main source of income for the farmer. The husbandry system in our target population is semi-intensive for the entire dairy herds (Figure 28).

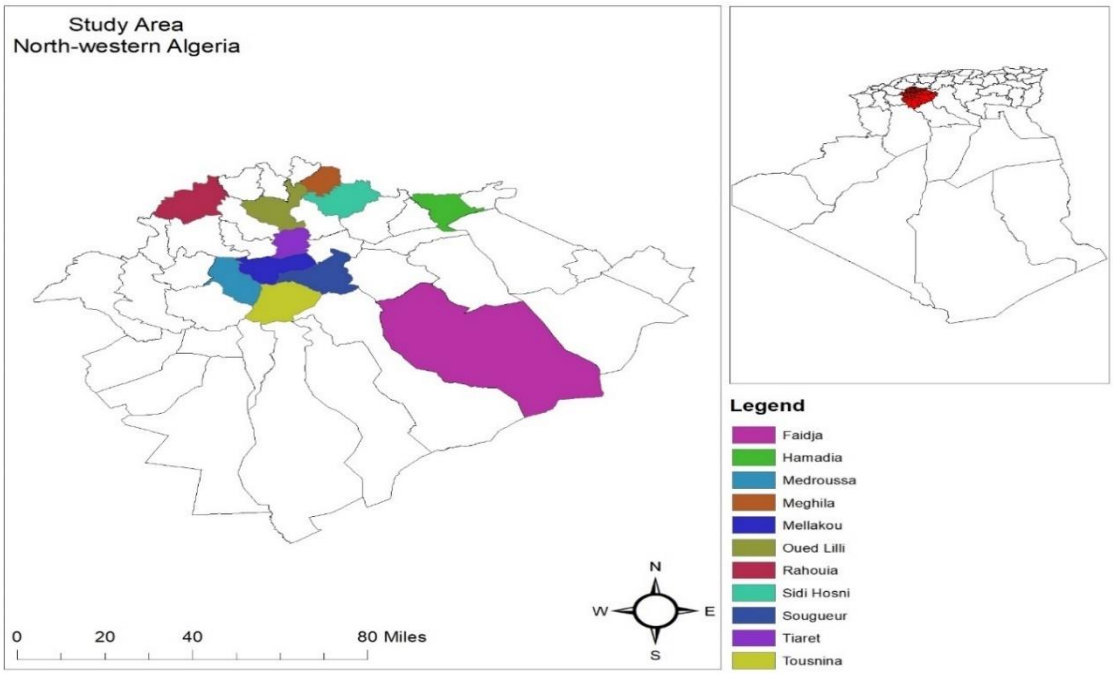


Figure 28. Representative map of the north-western Algeria sampling.

III. 3. 2. 2. Herd animals and management

In total, 31 herds were visited and 234 animals were included in the study. Twenty-four herds were small scale dairy herds (1-10 cattle) and seven were medium scale herds (10-100 cattle). Table 35 shows the distribution of herds of different sizes in the regions. Holstein/Friesian, Monbeliard and crossbreed were the most common breeds in the regions. The crossbred cow is the principal component of herds, it is resulting of crossing between local breed and imported dairy cow, commonly, the frisonne Holstein and Monbeliarde. Dairy cattle originating from 31 dairy farms, in the region of Tiaret from 17 municipalities (35°22'23.6"N 1°19'16.5"E) north-western Algeria (Figure 28). The herd size varied from farm to farm with a range of 10 to 70 cattle. Blood samples from 234 dairy cattle were collected by a simple random sampling method from animals aged between 9 and 180 months (Table 39, 40, 41).

Table 39. Size, number of herds, municipalities and animal categories sampled from the two study locations (regions).

	Tiaret (North-western Algeria) (2018-2019)
Herds	31
Small (1-10 cattle)	24
Medium (10-100 cattle)	7
Municipalities	17
parity	
Heifer	45
Cow	190
Total	235

Table 40. Distribution of animals sampled by age in north-western Algeria.

Age category	**Frequency**	**Percentage %**
≤24 months	38	16.2
>24 months ≤48	74	31.9
>48 months ≤60	29	12.3
> 60 months	93	39.6
Total	234	100

Table 41. Region, Municipalities, Number of animals and Number of herds.

Province	***Municipality***	***Number of animals***	***Number of herds***
Tiaret	Rahouia	7	1
	Tousnina	47	5
	Kebouba	18	1
	Mecharraf	12	2
	Mellakou	13	2
	Charra	3	1
	Medroussa	3	1
	Tiaret	19	2
	Rechigua	10	1
	Hammadia	8	1
	Oued Sousellem	14	3
	Harmela	8	1
	Souguer	12	1
	Sid labid	39	1
	Biben Mesbah	5	1
	Faija	16	4
	Sid Hosni	1	1

III. 3. 2. 3. Study design

Within the present study, which is a cross-sectional study, the selection of animals was done at two stages. A two-stage sampling survey was carried out in north-western Algeria (Tiaret province; 35°22'10.1"N 1°19'47.7"E) between June 2018 and August 2019. For the first stage of sampling (sampling of herds), the sample size for disease detection was calculated based on the following formula (Dohoo Ian, Stryhn HE, and Martin W, 2003).

$$n_1 = \left(1 - (1 - \alpha_1)^{\frac{1}{D_1}}\right) \times \left(N_1 - \frac{D_1 - 1}{2}\right)$$

where α_1 was the confidence level (set at 95%), D_1 was the minimum number of infected herds (estimated as $D_1 = Prev_1 \times N_1$), where $Prev_1$ was the minimum herd prevalence to be detected (set at 10%), and N_1 was the population of herds (which in our case were 289 dairy herds). The estimate of n_1 was 29 herds.

The Official Veterinarian in Tiaret Veterinary Office provided a list of all cattle herds registered in the province, which included information of the herd owner, the address or number of animals. The sampling frame included 289 dairy cattle herds. No formal random process was used for the selection of herds. Instead, from the list, a herd was randomly selected, and the herd owner was contacted, and asked, first a) whether they complied with the inclusion criteria, and then b) whether they were willing to participate. The process was repeated until the number of herds needed for the first stage was completed. The inclusion criteria comprised that the herd had at least one female animal above six months, and that the milk was not only for own consumption (i.e. some of the milk was sold). This age category was selected to avoid interference as much as possible the detection of maternal antibodies in the seroprevalence studies (Chase Hurley and Reber, 2008).

For the second stage (sampling of animals within herds), the sample sizes for disease detection were also calculated based on the formula by Dohoo et al., (2003):

$$n_{2i} = \left(1 - (1 - \alpha_2)^{\frac{1}{D_{2i}}}\right) \times \left(N_{2i} - \frac{D_{2i} - 1}{2}\right)$$

where α_2 was the confidence level (set at 95%), D_{2i} was the minimum number of infected animals in herd i (estimated as $D_{2i} = Prev_2 \times N_i$), where $Prev_2$ was the minimum within-herd prevalence to be detected (set at 30%), and N_{2i} was the population size of herd i (size of herds selected in stage 1 varied between 7 and 62). The estimate of n_2 varied between 4 and 8. The

sampling of animals within herds (second stage) was also random, although because of the lack of proper sampling frames, no formal random process was used either. Random animals in the herd were selected until the number of animals needed for the second stage was completed. However, because of logistics problems, the number of samples per herd could not always be completed, and therefore some extra samples were collected in some of the remaining herds, and also a few extra herds were sampled.

Within herds, animals were randomly selected to allow the detection of infected individuals if infection was present in at least 30% of animals with a 95% confidence (i.e. up to 10 animals depending on the size of the herd). Sample sizes for the two stages were calculated using Epitools (Sergeant, 2018). Holstein/Friesian, Monbeliarde and crossbreed were the most common breeds. Blood samples were collected from 234 dairy cattle aged between 9 and 180 months by a two-stage sampling design. Serum samples were obtained by centrifuging at 1200g for 10 min and stored at −20°C until tested. Figure 18 summarizes and gives an overview of the study design (previously reported, Study I), the materials collected, and the analysis performed. Papers 1 (*Pestivirus* in ruminant in North Africa) was based on serological investigations of sera from the north-western region in Algeria as well as molecular characterization including RT-PCT, Conventionnel PCR and Sequencing the *Pestivirus*.

Study period and Epidemiological data collection

All the biological samples were collected during field trips conducted from June 2018 and August 2019, where each herd was visited once. Information from both regions (north-eastern and north-western) was gathered through direct observation at farms, interviews of farmers, and collection of biological material from animals.

The same approach of the first study was applied to achieve the third investigation. As previously cited, Table 39 summarizes the information on animals and farms management which was collected using an interview-based questionnaire (appendix 1). To minimize the risk of information bias/misinformation, for this, on each excursion, the purpose of which was collect samples and information, a qualified veterinary accompanies the breeders to avoid any possible ambiguities in the responses collected. This method was resource-demanding but advantageous compared to online or paper-based data collection. However, the information

provided by the farmers depends largely on their knowledge, record keeping, and capacity to remember what happened up to, in some cases, more than 5 years before interview. Because of the lack in records and farmers have a poor understanding of reproductive disorders, the frequency of reproductive disorders in the area might have been underestimating.

III. 3. 2. 4. Serology.

The analysis of the sera was carried out by IDEXX BVDV p80 Ab (Montpelier, France) which is an enzyme linked immunoassay (ELISA) for the detection of antibodies directed against p80 protein for diagnostic of Bovine Viral Diarrhoea Virus (BVDV) and Mucosal Disease (MD) in individual serum, plasma and milk samples and in pools of serum (maximum 10) and tank milk samples from bovine origin.

Descriptions and Principles

The IDEXX BVDV p80 Ab is based on the principle of competition between the bovine antibodies and a Peroxidase coupled monoclonal anti-p80-antibody "WB112". Microplates are coated with p80 proteins attached to the wells via a specific WB103 antibody. Samples to be tested are diluted and incubated in the wells. Upon incubation of the test sample in the coated wells, p80 protein specific antibodies form immune-complexes with the p80 protein. After washing away unbound material, an anti-p80 protein antibody enzyme conjugate is added. In presence of the p80 protein-antibody immune-complex, the conjugate is blocked from binding to its corresponding epitopes on the microplate. Conversely, in the absence of p80 protein-antibodies in the test sample, the conjugate is free to bind to its corresponding epitopes on the microplate. Unbound conjugate is washed away and an enzyme Substrate (TMB) is added. In presence of the enzyme, the Substrate is oxidized and develops a blue compound becoming yellow after blocking. Subsequent color development is inversely proportional to the amount of anti-p80 protein antibodies in the test sample. The result is obtained by comparing the sample absorbance with the Negative Control mean absorbance

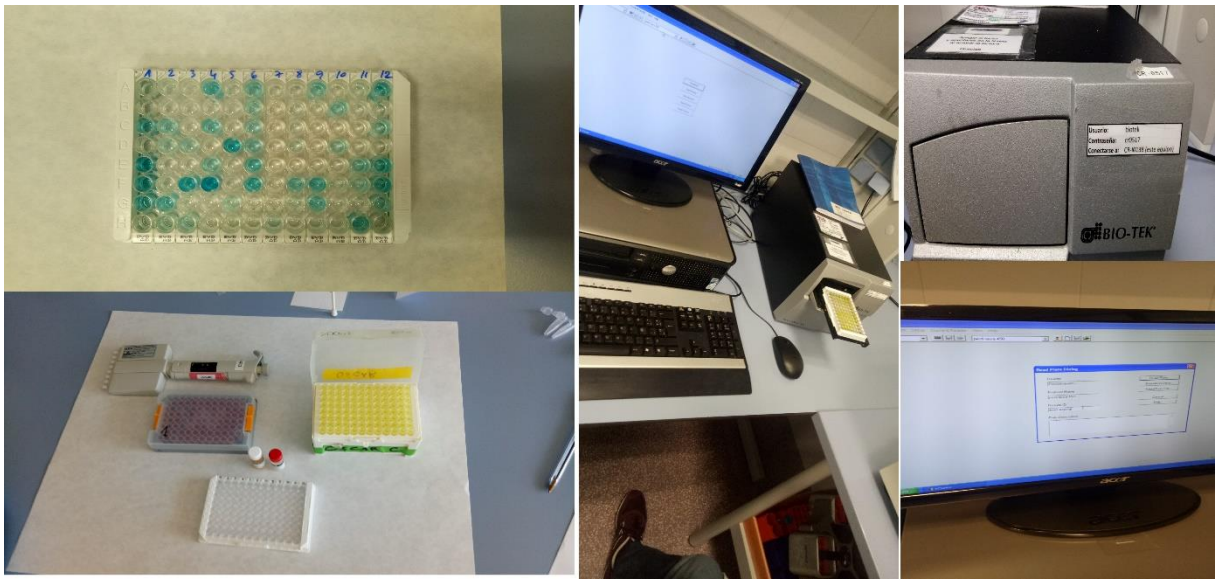


Figure 29 ELISA reader (BIO TEK), samples, plate and micropipette
(Personal photographs)

Protocol

All reagents were allowed to come to 18–26°C before use. Reagents were mixed by gentle inverting or swirling.

1. coated plates were obtained and the sample position was recorded
2. Dilution buffer N. 9, controls and samples were dispensed

90µL of Dilution Buffer N.9 was dispended in each well.

10µL of Negative Control was dispended into two appropriate wells.

10µL of Positive Control was dispensed into one appropriate well.

10 µL of sample was dispensed into remaining wells (1 well per sample)

contents of the wells were homogenized using a microplate shaker.

The microplate was covered and overnight incubate was performed for 16–24 hours at 2–8°C.

3. the solution was removed and each well was washed with approximately 300 µL of Wash Solution 3–5 times. Each plate was taped onto absorbent material after the final wash to remove any residual wash fluid.
4. 100µL of DILUTED Conjugate was dispensed into each well.
5. The microplate was covered and incubated for 30 minutes (±3 min.) at 18–26°C.

6. The solution was removed and each well was washed with approximately 300 µL of Wash Solution 3 times. Each plate was taped onto absorbent material after the final wash to remove any residual wash fluid.
7. 100µL of TMB Substrate N.9 was dispensed into each well.
8. Incubated 20 minutes (±3 min.) at 18–26°C away from direct light.
9. 100µL of Stop Solution N.3 was dispensed into each well.
10. Absorbance values of samples and Controls were measured and recorded at 450 nm.
11. Calculation:

Controls

$$NCx = \frac{NC1 + NC2}{2}$$

i.Validity criteria

Controls

$$NCx \geq 0.800 \qquad PC: NCx < 0.20$$

ii.*Interpretation*

Samples

$$S/N\% = (\frac{Sample\ (450)}{NCx}) \times 100$$

Table 42. BVD/MD diagnostic for bovine Individual Serum and Plasma samples

Negative	Suspect	Positive
S/N ≥ 50 %	40 % < S/N < 50 %	S/N ≤ 40 %

III. 3. 2. 5. Molecular detection

Reverse transcription-polymerase chain reaction (RT-PCR) is a rapid and sensitive method for detection of viral RNA that has the advantage of being insensitive to toxic substances in the specimen. A general RT-PCR protocol includes four different steps: a previous extraction of RNA, reverse transcription to complementary DNA (cDNA), primer-directed amplification, and detection of amplified products. Furthermore, by direct sequencing of the PCR products, phylogeny studies can be performed for rapid and exact identification of virus variants. These regions are the 5'UTR (Vilcek et al., 2001), non-structural N-terminal protein (Npro) region and the non-structural protein two and three (NS2/3) region (Flores et al. 2000; Tajima et al. 2001). However, the 5'UTR is considered the most highly conserved region of *Pestivirus* (Deng and Brock, 1993). Analysis of the 5'UTR, a highly conserved region of the genome, has shown to be a reliable and reproducible method for genetic characterization of BVDV isolates (Ridpath 2005). Additionally, it is the target region for most PCR-based diagnostics, and as such a suitable target for direct sequencing from the PCR product.

RNA extraction from pools samples

First step was the constitution of pool samples (n=4). Serum pools were compiled by removing 50ul from the individual serum. Total RNA was extracted and achieved using the commercial kit IndiMg ®pathogen kit (Indical Bioscience GmbH, Leipzig, Germany) and eluted in 100µl RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms (Buffer AVE, 30ng/µl) according to the manufacturer's instructions (descripted below). The 200 µL of the resulting pooled sera were used for BVDV detection by RT (Reverse transcription)-PCR.

Principal

The IndiMag Pathogen Kit uses MagAttract magnetic-particle technology for nucleic acid purification. This technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The workstation processes a sample containing magnetic particles.

- Step 1. A magnetic rod, protected by a rod cover, enters a well containing the sample and attracts the magnetic particles.

- Step 2 The magnetic rod cover is positioned above another well and the magnetic particles are released.

Steps 1 and 2 are repeated several times during sample processing. The purification procedure is designed to ensure convenient, reproducible handling of potentially infectious samples (Figure 30). DNA and RNA bound to the magnetic particles are then efficiently washed, followed by an air-drying step. High-quality nucleic acids are eluted in Buffer AVE (Figure 31).

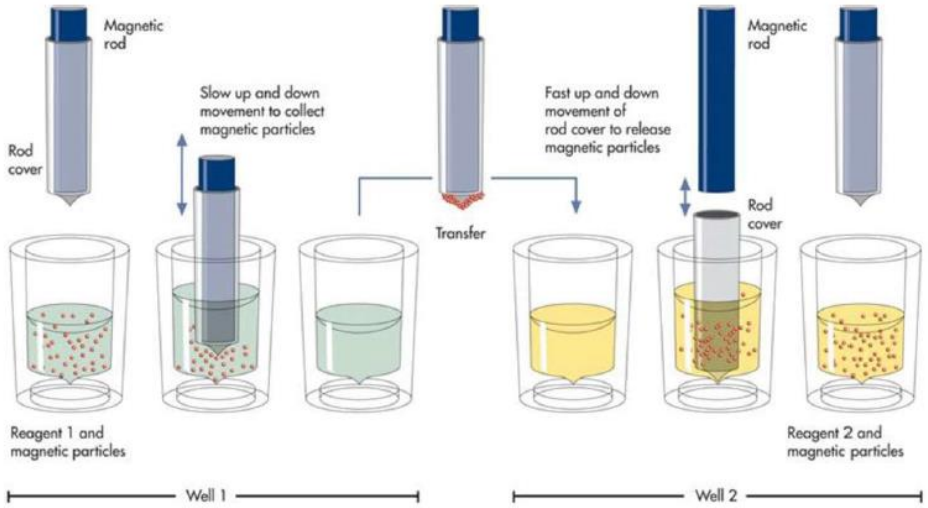


Figure 30 Schematic of the magnetic bead principle.

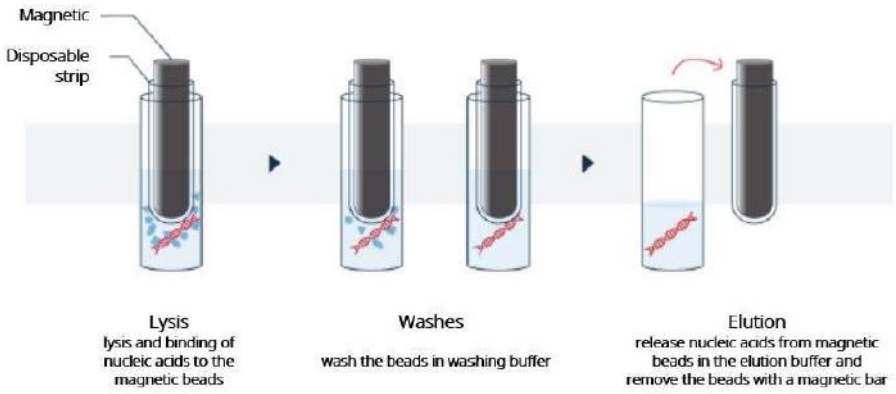


Figure 31 Schematic description of protocol steps

Things to do before starting

- 96 samples and were Buffers thawed at room temperature (15–25°C).
- Carrier RNA was Dissolved in Buffer AVE as indicated in the tube.
- Isopropanol (100%) was added to Buffer ACB and ethanol (96-100%) to Buffer AW1 AW2 before use until the bottle labels for volume.

Protocol

1. Five C-Blocks and one 96 well Microplate PM were labeled.
2. 20 µl Proteinase K was piped into the bottom of the S-Block well and 200 µl of sample was added [2]
3. Buffer VXL mixture was prepared and mixed thoroughly for 30 second (Table 43).

Table 43 Preparation of Buffer VXL mixture.

	Number of samples	
Reagent	1	96
Buffer VXL	100 µl	9.6 ml
Buffer ACB	400 µl	38.4 ml
MagAttract Suspension G	25 µl	2.4 ml
Carrier RNA (1 µg/µl)	1 µl	96 µl

4. 500 µl Buffer VXL mixture was added to each sample in the block.
5. Four S-Blocks (slots 2-6) and one 96 well Microplate MP according to (Table 44)
6. The BioSprint 96 was switched on at the power switch (Figure 32).
7. The front door of the protective cover was opened
8. The protocol "BS96 cador v2" was selected using the ↑ and ↓ keys (Figure 32)
9. "Start" was pressed and the messages was followed for loading the worktable as shown in Table 44.

Table 44 BioSprint 96 wortable setup and reagent volumes.

Slot	Loading message	Format	Item to add	Volume per well (µl)
6	Load rod cover	S-Block	Large 96-rod cover	-
5	Load elution	96-well microplate MP	Buffer AVE	100
4	Load wash 3	S-Block	Buffer (96- 100%)	750
3	Load wash 2	S-Block	Buffer AW2	700
2	Load wash 1	S-Block	Buffer AW1	700
1	Load lysate	S-Block	Lysate*	720

*Includes 20µl Proteinase K, 200 µl sample and 500 µl Buffer VXL mixture

[2] Note: in case where sample volume was less than 200 µl, PBS was added until 200 µl.

Figure 32. BioSprint 96 DNA Plant Kit, indimag reagents, S Block (Personal photographs).

III. 3. 2. 6. Phylogenetic analysis of BVDV

RNA extraction from individual samples

In order to get a hight amount of RNA to achieve the next step (sequencing), the twelve samples constituted the four positive pools were used to performing a second manual RNA extraction using NucleoSpin® RNA Plus kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). RNA was stored at – 70°C until phylogenetic analysis.

The basic Principle

The **NucleoSpin® RNA Plus** kit is designed to purify RNA from a variety of cell and tissue types. The NucleoSpin® RNA Plus kits allow purification of high-quality RNA. The **NucleoSpin® RNA Plus** kits allow purification of RNA with an A260/A280 ratio generally exceeding 1.9. This kit introduces the NucleoSpin® gDNA Removal Column, a spin column, which quickly and effectively removes genomic DNA contamination without the need of DNase digestion. One of the most important aspects during the isolation of RNA is to prevent degradation of the RNA. Cells and tissues are first lysed by incubation in a chaotropic ion lysis buffer solution, which immediately inactivates RNases. The lysate is added to the NucleoSpin®

gDNA Removal Column to clarify the lysate and to remove contaminating gDNA. After the addition of the Binding Solution to the flow-through, the RNA is bound to the NucleoSpin® RNA Plus Column. Two subsequent wash steps remove salts, metabolites, and macromolecular cellular components. High quality RNA is eluted with RNase-free H2O.

1	Homogenize sample and lyse sample			350 µL LBP
2	Remove gDNA and filtrate lysate			11,000 x *g*, 30 s
3	Adjust RNA binding conditions			100 µL BS Mix
4	Bind RNA			Load sample 11,000 x *g*, 15 s
5	Wash and dry silica membrane		1st wash 2nd wash 3rd wash 1st and 2nd 3rd	200 µL WB1 600 µL WB2 250 µL WB2 11,000 x *g*, 15 s 11,000 x *g*, 2 min
6	Elute RNA			30 µL RNase-free H_2O 11,000 x *g*, 1 min 30 µL RNase-free H_2O 11,000 x *g*, 1 min

Figure 33. Protocol-at-a-glance NucleoSpin® RNA Plus

Figure 34. Manual RNA extraction, reagents, work plan (Personal photographs).

The one-step RT-PCR

All sera were also analysed for the presence of *Pestivirus* RNA using a RT-PCR. The RT-PCR was performed using primers 324 and 326 (Vilček et al., 1994) and a commercial kit (One-Step PCR kit, Qiagen Inc., Hilden Germany). Each 50µl Reverse Transcription PCR (RT-PCR) reaction was performed in the 96 well plate format using 7500 Fast Real Time PCR system thermocycler (Applied Biosystem). Each 50µl RT-PCR reaction was prepared using 0.2 uM *Pestivirus* Forward 324 (5'-ATGCCCWTAGTAGGACTAGCA-3') and Reverse 326 (5' TCAACTCCATGTGCCATGTAC-3') primers, 100ng RNA template was added to each sample. RT-PCR reactions were performed in single RNA and it was reverse transcribed at 50°C for 30 minutes followed by deactivation of reverse transcriptase at 95°C. cDNA was amplified by 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 60 seconds a final hold for 10 minutes at 72°C was followed by an infinite hold at 4°C.

Gel electrophoresis

The RT-PCR-amplified products were examined by electrophoresis in a 2% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. In this study, A negative control (sterile water), and a positive control RNA from BVDV-1 (IRTA-CReSA, Spain), were included in each amplification run.

Phylogenetic analysis

Pestivirus positive amplicons were sequenced as described below and the 5' untranslated region (5'-UTR) was characterized. The phylogenetic tree was calculated by the neighbour-joining method using an automatic root location. To test the reliability of the branches in the tree, a bootstrap analysis of 1000 replicates was performed by creating a series of randomly selected bootstrap samples.

This procedure included routine precautions and safety measures to avoid cross contamination of the samples (Belák and Ballagi-Pordány, 1993). The segment of the BVDV genome used to characterize the virus was the highly conserved 5'UTR. The primers used were described according to the site of binding when using the BVDV-NADL strain of reference (GenBank accession number M31182). The primer sequences were designed to amplify a region of the 5'UTR common in BVDV viruses (Vilček et al., 1994).

III. 3. 2. 7. The review of the presence of ruminant Pestivirus in North Africa

The review of the presence of ruminant *Pestivirus* in North Africa was performed using a systematic search and compilation methodology of peer-reviewed literature available to identify gaps of knowledge for future research. North Africa is the UN subregion comprised by Algeria, Egypt, Libya, Morocco, Sudan, Tunisia and Western Sahara. We searched Web of Science: All Databases (WoS; Thomson Reuters) literature database using "topic" searcher. We used the words "(*Pestivirus* AND Algeria OR Egypt OR Libya OR Morocco OR Sudan OR Tunisia OR Western Sahara)" (44 articles) and then we discarded research papers on Classical Swine Fever Virus (44 *Pestivirus* articles - 21 CSFV articles = 23 Ruminant *Pestivirus* articles from North-African countries). Finally, we added any relevant literature that was not originally included in WoS (2 articles).

III. 3. 3. Results

The prevalence of antibodies against *Pestivirus* in cattle found in the present study, adjusted for the two-stage design was 59.9% with a 95% CI [49.0-70.7%]. The overall seroprevalence of infected herds was 93.5% (29 out of 31) with a 95% CI [78.6%-99.2%] and the within-herd seroprevalence ranged from 0.0% to 100.0%. Considering the sensitivity and specificity of the IDEXX p80 test, 60% and 97%, respectively (Hanon et al., 2017), and an estimated apparent prevalence of 59.9%, the true prevalence of disease would 99.8%. The model with all the factors that were significantly associated ($p<0.05$) with the presence of *Pestivirus* infection included: presence of sheep, size of herd, parity, breed, presence of standing water and number of calving. After studying the correlation, the best-fitting model included: presence of sheep (OR=5.64; 95% CI [2.0, 15.9]; p=0.001) and parity (OR=3.80; 95% CI [1.6, 8.9]; p=0.002) (Table.46) The variance of the random effect was 0.48, therefore evidencing the heterogeneity among herds. RT-PCR resulted positive in 3 out of the 234 analysed animals. Only one of these three positive samples (a heifer of 20 months) could be sequenced targeting the *Pestivirus* 5'UTR region, confirming the presence of BVDV-1a in cattle from Algeria (Tiaret_2019; GenBank Acc. No. MT157227) (Figure.35-36)

Review of ruminant pestiviruses in North Africa

Our literature review (25 research articles) confirmed the presence of ruminant *Pestivirus* in all North African countries, except in Libya and Western Sahara, where no data was available. A summary of the studies on *Pestivirus* in livestock in North Africa is presented in Table 48. The most studied and reported *Pestivirus* were *Pestivirus* A and B (BVDV-1, -2) in cattle, sheep and dromedary camels. However, the few studies on *Pestivirus* D (BDV) reported high seroprevalences in Algeria, Morocco and Tunisia, and an outbreak of severe clinical Border Disease in Tunisia in small ruminants. The review of the main risk factors for the presence of *Pestivirus* in livestock in North Africa found them to be heterogeneous (Table 48). On the other hand, no information about *Pestivirus* in wild ruminants in North African countries was recorded.

Table 45. Animal level putative factors in relation BVDV (iELISA) serostatus in dairy cattle of north-western Algeria region established using the Chi-square test or Fisher's exact test.

Variables	Category	BVDV		
		Number of animals examined	Proportion of seroreactors (95% CI)	P-value
Age (years)	≤2	42	40.5 (23.6-64.8)	0.006
	2-6	128	57.8 (45.4-72.6)	
	>6	64	71.9 (52.6-95.9)	
Breed	Crossbreed	47	42.6 (26.0-65.7)	0.030
	Montbéliarde	90	57.8 (43.2-75.8)	
	Holstein	95	68.4 (52.8-87.2)	
	Brune des alpes	1	100 (2.5-100)	
	Fluck	1	0 (0.0-85.3)	
parity	Heifers	45	42.2 (25.4-65.9)	0.022
	Cows	189	62.5 (51.7-74.8)	
Gestation	No	87	63.2 (47.6-82.3)	0.352
	Yes	147	56.1 (44.4-69.2)	
Stage of gestation	1-3 months	76	50.0 (35.4-68.6)	0.293
	4-6 months	47	66.6 (44.8-93.6)	
	7-9 months	25	56.0 (30.6-94.0)	
Number of calving	<=2	117	51.6 (39.1-66.0)	0.002
	3 - 5	56	60.6 (42.0-84.8)	
	>=6	61	70.4 (51.0-95.0)	
Number pregnancy	<=2	83	54.2 (43.5-64.6)	0.007
	3 - 5	79	62.0 (51.0-72.1)	
	>=6	52	71.2 (57.9-82.1)	
Herd size	Small <20	105	60.0 (50.5-69.0)	0.721
	Large >20	129	57.7 (49.1-65.9)	
Mixing	No	164	53.2 (43.0-66.1)	0.029
	Yes	70	69.9 (51.8-92.5)	
Food source	purchased	73	56.2 (44.7-67.1)	0.098
	mixed on the farm	47	72.3 (58.5-83.5)	
	both	114	54.8 (45.7-63.7)	
Water source	tap	8	25.0 (5.6-59.2)	0.065
	Well or drilling	216	59.0 (52.4-65.4)	
	River water	10	80.0 (49.7-95.6)	
Quarantine	No	176	57.2 (46.7-69.7)	0.542
	Yes	58	61.9 (44.2-86.4)	
Cleaning method	Scanning	94	61.5 (46.9-79.8)	0.024
	Piping	0	0	
	Both	110	50.8 (38.8-66.7)	
	Not practice	30	76.4 (63.3-91.1)	
Presence of sheep	No	46	28.3 (15.0-48.2)	0.000
	Yes	188	66.1 (55.3-79.1)	
Presence of cats	No	7	71.3 (49.1-69.4)	0.703
	Yes	227	58.1 (23.2-93.5)	
Presence of horses	No	101	43.6 (31.7-58.2)	0.000
	Yes	133	70.0 (57.1-86.4)	
Visit of other farmers	No	77	58.4 (38.5-60.9)	1.000
	Yes	157	58.7 (47.2-71.9)	
Calving box	No	222	58.3 (48.9-69.5)	0.765
	Yes	12	66.7 (28.8-87.5)	
Standing water	No	140	54.4 (43.4-68.7)	0.0182

	Yes	94	64.9 (49.6-83.4)	
Rivers and streams	No	168	59.6 (49.0-73.1)	0.623
	Yes	66	56.0 (39.5-77.2)	
Use of disinfectant	No	86	59.2 (44.2-78.0)	1.000
	Yes	148	58.2 (46.5-71.8)	
Municipality	Ainfrid sidhosni	1	100.0 (14.7-100.0)	0.001
	Bibenmisbah	5	60.0 (20.9-90.6)	
	Chara	3	100.0 (46.4-100.0)	
	Faija	16	56.3 (32.6-77.8)	
	Hammadia	8	25.0 (5.6-59.2)	
	Harmela	8	50.0 (19.9-80.1)	
	Kebouba	18	72.2 (49.4-88.5)	
	Mecharaf	12	75.5 (47.1-92.4)	
	Medroussa	3	100.0 (46.4-100.0)	
	Mellakou	13	46.2 (22.1-71.7)	
	Oued souseleme	14	64.3 (38.5-84.9)	
	Rahouia	7	42.9 (13.9-76.5)	
	Rechiga	10	90.0 (61.9-98.9)	
	Remelia	12	100.0 (81.5-100.0)	
	Sidelabid	39	56.4 (40.9-71.1)	
	Tiaret	19	68.4 (46.1-85.6)	
	Tousnina	47	36.2 (23.6-50.4)	
IA in five last year	No	198	58.6 (48.7-70.6)	0.854
	Yes	12	66.7 (28.8-87.5)	
	Both	25	56.0 (30.6-94.0)	
IA in last mating	No	200	59.1 (48.8-70.7)	0.886
	Yes	34	55.8 (33.6-87.3)	

Table 46. The final multivariable logistic regression model for factors associated with Bovine Viral Diarrhea Virus infection in dairy cattle at the individual level in north-western Algeria.

Factor	Odds Ratio	Confidence interval	p-value
Presence of sheep	10.7	4.3-29.8	<0.001
Presence of standing water (yes vs no)	0.2	0.1-0.6	0.003
Herd size (small vs large)	0.9	0.8-1.0	0.025
Animal categories (heifers vs cows)	2.5	1.0-6.5	0.048
Number of births (<=2, 3-4, >=5)	1.2	1.1-1.5	0.008

Table 47. Occurrence of reproductive disorders in relation to BVDV serostatus in dairy cattle in north-western Algeria.

Variable	Cattle	BVDV seropositive cattle		
	Number of animals examined	No. of Positive	Proportion of seropositive (95% CI)	P-value
Abortion				
No	176	109	61.9 (57.6-68.9)	
Yes	14	10	71.4 (45.5-89.5)	0.285
Endometritis				
No	183	114	62.3 (55.1-69.1)	
Yes	7	5	71.4 (35.2-93.5)	0.703
Weak calf				
No	188	117	62.2 (55.2-68.9)	
Yes	2	2	100 (33.3-100)	0.634
Retained foetal membrane				
No	183	114	62.3 (55.1-69.1)	
Yes	7	5	71.4 (35.2-93.5)	0.702
Anoestrus				
No	177	108	61.0 (53.7-68.0)	
Yes	13	11	84.6 (59.1-96.7)	0.105
Repeat breeding				
No	148	91	61.5 (53.5-69.0)	
Yes	42	28	66.7 (51.7-79.4)	0.251
Increased inter calving period				
No	129	77	59.7 (51.1-67.9)	
Yes	61	42	68.9 (56.6-73.4)	0.078
CRDE				
No	130	79	60.8 (52.2-68.9)	
Yes	60	40	66.7 (54.277.6)	0.164

Table 48. Characteristics of studies investigating seroprevalence BVDV in North Africa country in cattle.

Country	Year sampling	*Pestivirus*	Species	Diagnostic method	N	Prevalence	Risk factors	Ref.
Algeria	2011-2013	BVDV	Cattle	Ab-ELISA	360	1.4%	Abortions	(Derdour et al., 2017)
	2019	BVDV-1a	Cattle	Ab-ELISA RT-qPCR	234	58.9% 1.3%	Herd size Presence of sheep Increase of age	Present Study
	2015-2016	BDV/BVDV	Sheep	Ab-ELISA VNT Ag-ELISA RT-PCR	576 197 689 689	68.2% 68.2% 0% 0%	Climate: arid vs Mediterranean Landscape: mountain vs plateau Flock management: sedentary vs transhumant Presence of goat	(Feknous et al., 2018)
	2016-2017	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA	111	9.0% 41.4%	Sheep, goat, cattle in mixed herd	(Saidi et al., 2018)
Morocco	1984	BVDV	Cattle	IFF	524	48.5%	Extensive management system Local ruminants Ruminants without apparent respiratory symptoms	(Mahin et al., 1985)
	NA	BDV	Sheep	Ab-ELISA qPCR Ag-ELISA	760 543 150	28.9% 0% 0%	Intensive farming Presence of cattle	(Fassi et al., 2019)
	NA/1982?	BVDV	Cattle	Disease	1			(Mahin et al., 1982)
	NA	BVDV	Cattle	Ab-ELISA	42	37.7%		(Lucchese et al., 2016)
Tunisia	1995	BDV	Sheep	Disease Sequencing	NA 9	NA	Vaccine contamination	(Thabti et al., 2005a)
	1993	BDV	Sheep from 1 flock with BD clinical history	Abortion VNT	2,576 53 aborted sheep	17.7% 100%		(Zaeim et al., 1993)
	2001-2002	BVDV2a BVDV1b	Cattle from 2 farms (F) with BVD clinical history	Ab-ELISA PCR Sequencing	F1-188 F2-820 F1 F2	87% 82% 2.6% 0.2% BVDV2a BVDV1b	Importation of infected cattle/semen	(Thabti et al., 2005b)
Egypt	NA	BVDV	Cattle Buffalo Sheep Goats Dromedary camel	VNT (BVDV strains)	128 150 178 137 59	49.2% 52.0% 27.5% 31.4% 52.5%	Species: Cattle/Buffalo vs sheep/goat/dromedary	(Zaghawa, 1998)
	2011	BDV/BVDV	Sheep Goat	*IHC* *MAbs* RT-PCR MDBK Sequencing	5 4	0% 50% 0% 25% BVDV1b BVDV1a	-Neurological signs	(Abdel-Latif et al., 2013)
	2012-2013	BVDV	Cattle Buffalo	Ab-ELISA	480 260	40% 23%	Species: Cattle vs Buffalo	(Selim et al., 2018)
	2011	BVDV	Cattle Buffalo	Ab-ELISA Ag-ELISA MDBK IFAT IPMA	151 97 22 21 19 3	100% 62.2% 14.5% 13.9% 12.5% 1.9%	- NA	(El-Bagoury et al., 2012)
	2017	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA RT-PCR	80 80 10	11.2% 7.5% 0%	Camels from Sudan	(El Bahgy et al., 2018)
Libya		No Data						

Western Sahara		No Data						
Sudan	2017	BVDV	Dromedary camel-smuggler into Egypt	Ab-ELISA Ag-ELISA RT-PCR	120 120 7	47.5% 31.6% 42.8%		(El Bahgy, et al., 2018)
	2000-2006	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA RT-PCR	260 186 13	84.6% 7% 100%		(Intisar et al., 2010)
	2000-2012	BVDV	Dromedary camel	Ic-ELISA	474	9.0%	- Mixed virus infection - Pneumonia - Lacrimation	(Saeed et al., 2015)
	2005-2008	BVDV	Cattle	Ab-ELISA	688	25.7%	- Khartoum state - Rainy season (July to October) - Females - Old cattle - Abortions - Neonatal deaths	(Elhassan et al., 2011)

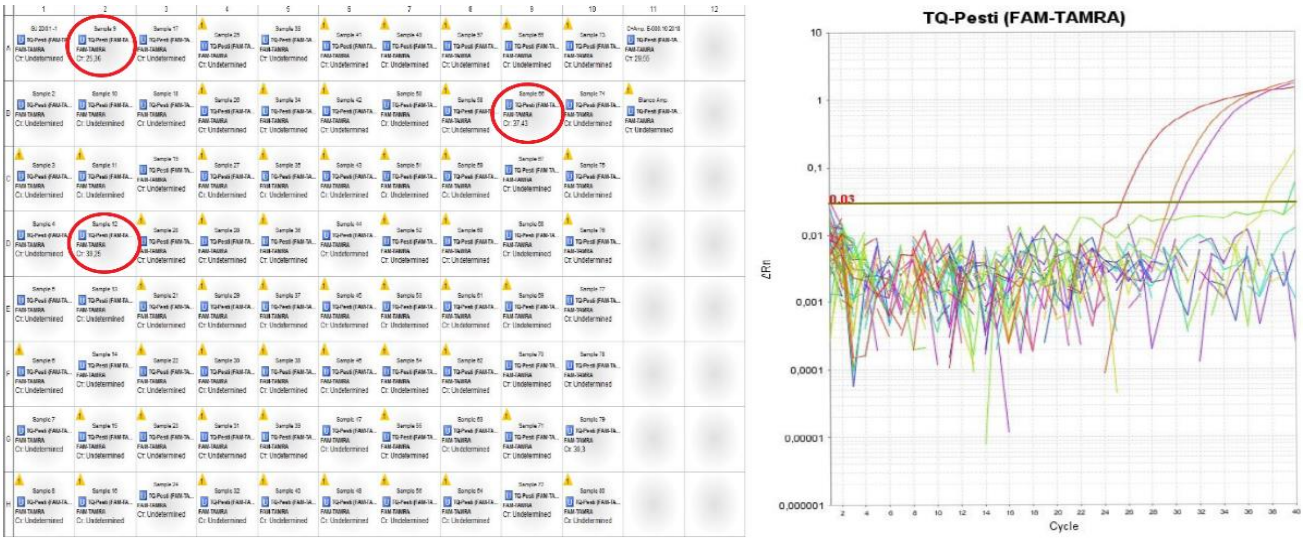


Figure 35. Reverse Transcription (RT) PCR on the 60 pools (234 serum)

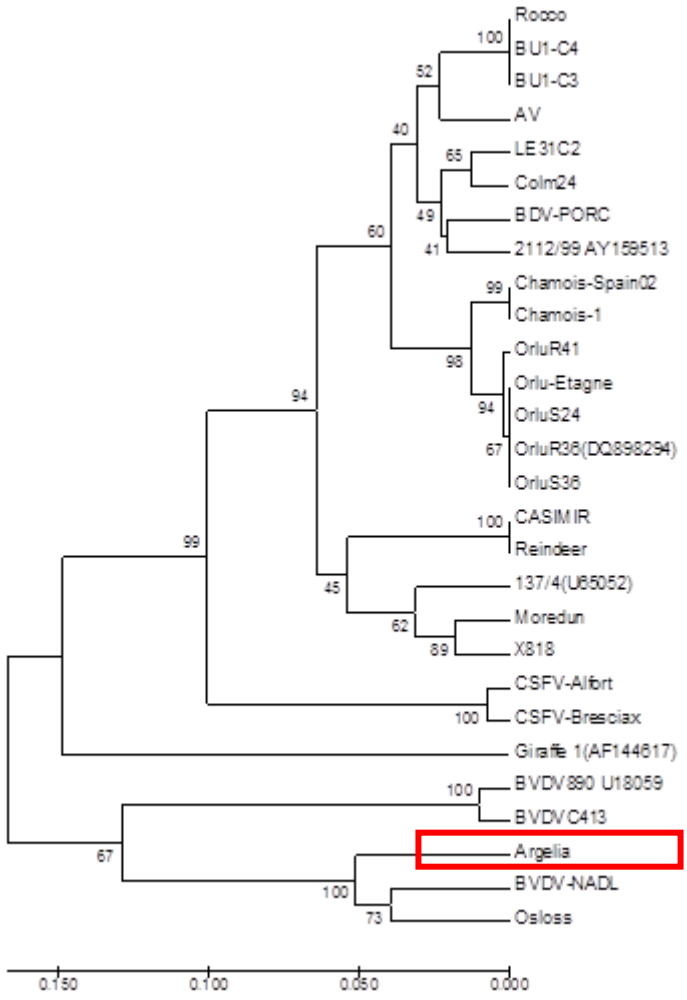


Figure 36. Phylogenetic analysis based on the nucleotide sequences of the 5'-UTR.

III. 3. 4. Discussion

The result of this study revealed that BVDV-1a as the major pathogen of cattle are prevalent in Tiaret region of Algeria. This study shows for the first time the spectrum of BVDV subtype diversity in non-vaccinated cattle within the study population. The results show high prevalence of antibodies against *Pestivirus* in cattle analysed in the present study, animal level was 58.9% (138 out of 234) with a 95% CI [52.4%, 65.3%]. The overall seroprevalence of infected herds was 93.5% (29 out of 31) with a 95% CI [78.6%, 99.2%] and the within-herd seroprevalence ranged from 0.00% to 100.00%.

The presence of BVDV-specific antibodies shows and indicates a natural exposure of cattle to wild BVDV because of the non-use of vaccination globally in Algeria. Consequently, the seropositive status in cattle reveals infection by BVDV once, or multiple infections. Our analysis also indicates a tendency to rise of BVDV seroprevalence and, also, possibly, increasing the importance of the virus throughout the study region in Algeria. The best-fitting model included the following factors as significantly associated ($p<0.05$) to the risk of *Pestivirus* infection: presence of sheep (OR=10.67; 95% CI [4.2, 27.4]), herd size ($n<20$) (OR=0.91; 95% CI [0.8, 1.0]), and age (cows vs heifer) (OR=2.53; 95% CI [1.0, 6.4]).

Cross-sectional study design associated with serological investigations, as used in the study, is widely used in veterinary epidemiology and used to assess the burden of a particular disease in a defined population (Dohoo et al., 2009). The advantage of cross-sectional design is that it is unambiguous, straightforward, inexpensive, and needs only one sampling occasion. It provides descriptive characteristics of a population at a particular point in time and includes both old and new cases (Dohoo et al., 2009). Nevertheless, it is less suitable for determining when the disease occurred or for how long it has lasted. The finding of antibodies in a single serum sample only indicates that infection has occurred sometime in the past which make its diagnostic value as indicator of present active infection limited (Levin, 2006). It is impossible to determine the sequence of events, namely whether exposure occurred before, during, or after the onset of disease outcome (Levin, 2006). In some type of ELISA's against some diseases, a high level of antibodies could indicate a possible acute phase of the diseases. One disadvantage of cross-sectional design compared to, for example, longitudinal study design is consequently the weakness in determining cause-effect relationships(Dohoo, 2009). The association between seropositivity and reproductive disorders found in the present study is therefore not necessarily

causal even though it is statistically significant. Other study designs, such as longitudinal study or case-control study, would have been stronger field visits.

Sample-size determination is often an important step in planning an epidemiological study. There are several approaches to determining sample size. It depends on the type of the study. Descriptive, observational and randomized controlled studies have different formulas to calculate sample size. An adequate sample size helps guarantee that the study will yield reliable information, regardless of whether the ultimate data suggest a clinically important difference between the treatment being studied, or the study is intended to measure the accuracy of a diagnostic test or the incidence of a disease. Ideally, to get a true prevalence estimate of a given infection in a population with good precision, all animals should be included (census). Due to the insufficiency of resources, only a fraction (sample) of the population is used to represent the whole population. This fraction needs to be optimal and representative to allow inferences to be made about the target population (Dohoo, 2009), which necessitates a random sampling strategy.

The calculation of sample size needed was complicated by several factors: unknown prevalence and heterogeneity in management systems. Pertinent literature on seroprevalence in Algeria is scarce and concerns studies conducted in other parts of the country with different management systems, study design, and laboratory techniques, limiting its relevance. The minimum sample size was increased to take into account the mentioned challenges. It is therefore likely that the sample size in the present study allows implications to be made about the target population; furthermore, the herds included were generally typical and are very likely representative of other herds in the study areas.

There are no pathognomonic clinical signs of infection with Bovine Viral Diarrhoea Virus in cattle. A wide range of test methods is available, but only a few tests are usually used in BVDV control at the herd, regional, or national level: antibody-Enzyme Linked ImmunoSorbent Assays (ELISA), antigen-ELISA, and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) tests (Jozef Laureyns, 2014). ELISA is now established as a sensitive, rapid, reliable and economical test for the study of the circulation of BVDV in cattle (Edwards, 1990). In unvaccinated dairy herds (like in study area), serological testing of bulk milk or blood is a convenient method for BVDV prevalence screening. Alternatively, serological testing of young

stock may indicate if BVDV is present in a herd. In BVDV positive herds, animals PI with BVDV can be identified by combined use of serological and virological tests for examination of blood samples (Sandvik, 1999). ELISAs have been used for rapid detection of both BVDV antibodies and antigens in blood, but should preferably be backed up by other methods such as virus neutralization, virus isolation in cell cultures or amplification of viral nucleic acid. Due to serological cross-reactivity of *Pestivirus*, serological surveillance of BVD by ELISA does not distinguish between BVD and BD virus as source of infection (Kaiser et al., 2017).

Despite the present study was performed in a different region (north-western) than the other studies (*Neospora caninum* and *Toxoplamsa gonndii, Coxiella burnetii, Chlamydia abortus* and *Brucella spp* seroprevalence and risk factors) and different breeders were interviewed, the same questionnaire was used with a similar level of underestimation risk in some cases. Additionally, BVDV mainly results in early embryonic losses, which are usually not observed by farmers and therefore this study might have underestimated the impact of BVDV on reproductive performance/disorders.

Ruminant *Pestivirus* are neglected pathogens in North Africa. However, the scarce data available suggest an endemic epidemiological scenario of *Pestivirus* in livestock. The antibody seroprevalence at population and farm levels obtained in the present study concur with the majority of the epidemiological data reported in cattle and dromedary camels in neighbouring North-African countries (Table 48). Surprisingly, the present study is in contrast to Derdour et al., (2017) that reported a very low prevalence of antibodies (1.4%) in cattle in Algeria, probably due to a sampling performed exclusively in intensive production systems. The hypothesis of an endemic and heterogenous *Pestivirus* scenario in North-Africa is reinforced by the studies performed in small ruminants, that show the presence of a third *Pestivirus*, *Pestivirus* D (Border Disease Virus – BDV) in these species, with similar high antibody prevalence (17.7% to 68.2%) (Table 48). Additionally, the present study reported the first description of a BVDV-1a in North Africa, whereas BVDV-2a and BVDV-1b had been isolated from cattle in Tunisia (Thabti et al., 2005b). Although the three PCR-positive animals could not be confirmed as PI individuals (i.e. two PCR-positive samples separated between 15 days), their presence together with the reported seroprevalences of antibodies in some farms, is highly suggestive of the presence of PI cattle in Algeria. Detection and elimination of PI individuals, and characterization of

circulating viruses are cornerstones for eradication programs. On the other side, the current Algerian seroprevalence is lower than that recorded in Kars district in Turkey by (Yilmaz, 2016), the seroprevalence were 89.58% and 83.85% in blood serum and milk respectively and also in Ankara 70.89% (Aslan Azkur and Gazyagci, 2015).

The risk factors detected in the present study (large herd size [n>20], mixed herd [presence of sheep], parity [cow]) have been previously related to *Pestivirus* infections' worldwide (Schweizer and Peterhans, 2014). However, the specific risk factors for *Pestivirus* infection in ruminants in North Africa have not been analysed in depth, and the few studies show a high heterogeneity of risk factors (Table 48), hindering the possibility of improving livestock production. In our study, the presence of sheep in the herd increased significantly the risk of *Pestivirus* infection (OR=10.67), which may be explained by the inter-specific infectious ability of *Pestivirus*, that facilitate their geographic dispersion and persistence in ruminant populations (Schweizer and Peterhans, 2014). Seroconversion in cattle after exposure to infected sheep has been the subject of several reports (Braun et al., 2019). Cohabitation between species may thus lead to bovine virus transmission to sheep and vice-versa. Furthermore, several studies reported that the disease may be caused in sheep by bovine viral diarrhoea virus (BVDV), transmitted from PI cattle to sheep which will present similar symptoms of BD (Paton et al., 1998; Braun et al., 2013). Other investigations have indicated that BDV can be transmitted to cattle on farms where the two species are kept together (Ueli Braun et al. 2013; 2014; Ueli Braun et al. 2019). These factors may explain the relatively high BVDV/BDV cattle seroprevalence levels observed in this study.

Many studies reported the fact that large herd size was a risk factors than small herd as previously reported (Viltro et al., 2002; Williams and Winden, 2014; Sun et al., 2015; Graham et al., 2016; Byrne et al., 2017; Amelung et al., 2018; Olmo et al., 2019; Akagami et al., 2020) while no such association was found for BVDV in northern Ireland (Cowley et al., 2014). Larger size herd had higher seropositivity than smaller herds and statistically significant. Self-clearance mechanism performs major role in smaller herd and automatically BVDV will be cleared by itself and chances of introduction of PI animals in early pregnancy stage and this phenomenon will be lower in smaller herds. The process of self-clearance is mainly altered by herd size, contact period and movement of animals within the farm(Kampa et al. 2009; Akagami

et al. 2020; Kumar et al. 2018). Smaller herds will allow the self-clearance mechanism than larger herds. Higher seroprevalence in larger farms is due to probable transmission of BVDV infection between animals by nose to nose contact and it depends on dose of virus, means of contact, also by airborne transmission of BVD from PI animals and self-clearance mechanism(Kampa et al., 2009). Kaiser et al. (2017) reported that the Swiss eradication program encompasses only bovines, but not sheep and goats. Thus, the mean BDV seroprevalence in *Pestivirus*-antibody positive cattle of at least 6.7% with an increasing trend between 2012 and 2014 indicates that the serological surveillance by ELISA, which does not differentiate BVDV from BDV infections, might be critical. Even though discrimination by cross-SNT (cross-serum neutralisation tests) as described in his study is laborious, it adds to classical epidemiological investigations and allows the identification of possible sources of infection, which is of particular importance in the late phase of an eradication program. In summary, they determined for the first time the prevalence of BDV in *Pestivirus*-positive cattle in Switzerland, and they provide strong evidence that common housing of cattle and sheep is the most significant risk factor for the interspecies transmission of BD virus from small ruminants to cattle (Kaiser et al., 2017). It was demonstrated by Danuser et al. (2009) by means of cross-serum neutralisation tests (cross-SNT) that 9% of the sheep and 6% of the goats were infected with BDV. However, 31% and 66% of the seropositive sheep and goats, respectively, could not be assigned to BVDV or BDV leaving the source of infection unidentified (Danuser et al., 2009).

Our results show that cows have a higher risk of *Pestivirus* infection as compared to heifers (OR=2.53), which coincides with previous studies (Schweizer and Peterhans, 2014; A. M. Selim et al., 2018), and explained because of the higher the age of cows versus heifers, that increases the probability to be exposed *Pestivirus* (cumulative infection with age). Following transient infection, specific anti-BVDV antibodies can be detected within 3 weeks of infection (Vilcek et al., 1994) and animals will remain antibody positive for life, so antibody prevalence reflects the proportion of animals previously exposed to BVDV at any point in life (Hans Houe, 1995). The increase in antibody prevalence by increasing age and consequently by the number of births is probably due to the fact that BVDV antibodies in most cases are long-term. So the older the animal, the higher is the probability that it has been infected during its life (Mockeliūniene et al., 2004; Selim et al., 2018; Erfani et al., 2019).

It has not been demonstrated an association between BVDV seropositive and reproductive disorders in the studied herds while repeat breeding was common (p=0.034) among BVDV exposed cows in Ethiopia (Kassahun Asmare et al., 2018). Infection with BVDV has been associated with a decline in the fertility of affected cattle (Robert et al., 2004; Burgstaller et al., 2016). Muñoz-Zanzi Thurmond and Hietala, (2004) considered the overall impact of endemic BVDV infection on fertility of dairy heifers to depend on the type and timing of infection relative to reproductive development. These reproductive losses vary from insidious reduction in reproductive performance at the herd level to devastating abortion storms (Grooms Ward and Brock, 1996). Occurrence of infection before the third trimester of pregnancy may do not causing reproductive disorders such as abortion (Anderson, 2007). Most BVDV abortions occur when previously unexposed dams are infected during gestation and probably this may be explained by the epidemiology of BVDV in that the infection is highly contagious and the within herd prevalence could rise to over 60% in a short time and most animals in transient infection clear the virus and remain with solid immunity for an extended period (Hans Houe, 2003; Talafha et al., 2009).

The role of wildlife as reservoir of *Pestivirus* has been proved in several wild species worldwide, being a risk factor for livestock. *Pesitivirus* A and D were reported to have a sylvatic cycle in white-tailed deer (*Odocoileus virginianus*; USA) and Pyrenean chamois (*Rupicapra pyrenaica*; Spain) respectively (Fernández-Sirera et al., 2012; Passler Ditchkoff and Walz, 2016). Also, *Pestivirus* D was reported to produce high mortality rates in chamois, entailing a threat for its conservation (Fernández-Sirera et al., 2012). There is not any available research on the presence of *Pestivirus* in North-African wildlife. Seven free-ranging wild ruminant species inhabit North-African countries, sharing territories with livestock like camels, dromedary and goat (IUCN, 2020). However, the conservation status is of all North-African wild ruminants is of concern, and two species are considered as critically endangered (Addax [*Addax nasomaculatus*], Dama Gazelle [*Nanger dama*]), one as endangered (Slender-horned Gazelle [*Gazella leptoceros*]), and four as vulnerable (Cuvier's Gazelle [*Gazella cuvieri*], Nubian Ibex [*Capra nubiana*]; Aoudad [*Ammotragus lervia*]; Dorcas Gazelle [*Gazella dorcas*]). In this sense, *Pestivirus* circulation in domestic ruminants should be of concern both for its economic impact but also from a wildlife conservation perspective.

In the present study, we have reported the circulation of BVDV-1a in north-western Algeria. Studies exploring the molecular epidemiology of BVDV can offer precious information about the diversity of viral strains present in a population and, in turn, inform control programs, drive vaccine development and determine likely infection sources(Richard Booth et al., 2013). Although the real circulation could have been underestimated due to the fluctuation of viremia and PCR-negative results in some PI animals (Bauermann et al., 2014). The experience with eradication program against the disease in a number of countries has shown that the RT-PCR method is a useful tool to reveal PI animals in cattle herds (Falcone et al., 2003; Hurtado et al., 2003; Moennig Houe and Lindberg, 2005; Wernike et al., 2017). Nevertheless, confirmation of PI status of a ruminant requires identification of virus in two separate samples with minimum of 15-21 days (Nettleton et al., 1998).

In the current study, RT-PCR resulted positive in 3 out of the 234 analysed animals. Only one of these three positive samples (a seronegative heifer of 20 months) could be sequenced targeting the *Pestivirus* 5'UTR region, confirming the presence of BVDV-1a in cattle from Algeria (Tiaret_2019; GenBank Acc. No. MT157227; Figure 36). Previously reported phylogenetic analyses of BVDV revealed the existence of at least 21 subtypes within BVDV-1 (1a-1u) (M. Deng et al., 2015). BVDV 1a has been reported in several countries, including Canada, France, Germany, New Zealand, Mozambique, Spain, Sweden, the UK, and the USA (Walz et al., 2010). As stated by Luzzago et al., (2014) the most prevalent subtypes are BVDV-1e in France, BVDV-1e and 1h in Switzerland, BVDV-1h in Austria, and BVDV-1d and 1f in Slovenia (Jackova et al., 2008; Bachofen et al., 2008; Toplak et al., 2004; Hornberg et al., 2009). Four frequency and distribution patterns of BVDV-1 subtypes were identified in Italy: high prevalent subtypes with a wide temporal-spatial distribution (BVDV-1b and 1e), low prevalent subtypes with a widespread geographic distribution (BVDV-1a, 1d, 1g, 1h, and 1k), low prevalent subtypes in restricted geographic areas (BVDV-1f in the North), and sporadic subtypes detected only in single herds (BVDV-1c, 1j, and 1l).

To our knowledge, this is the first phylogenic study of *Pestivirus* using 5'UTR region in cattle from Algeria. It suggests that an immediate survey regarding the genetic diversity of BVDV in cow flocks, including in PI cattle, is highly necessary for reducing the prevalence of BVDV base on administration of intervention measures including culling strategy and animal

vaccination, if necessary (Newcomer Chamorro and Walz, 2017). The current data on BVDV prevalence in these regions suggests that continuous epidemiological monitoring including small ruminants and comprehensive intervention strategy against BVDV infection in dairy cattle should be conducted.

III. 3. 5. Conclusion

This present study, in comparison with other regional studies, emphasis that any control programme needs to be initially assessed with the rate of infection in a specific region. Knowledge of herd management and environmental factors which enhance the risk of BVDV infection would improve the ability to control and prevent the transmission, reducing the detrimental impacts of BVDV infection on herd health and productivity. Furthermore, this study underlines that, BVDV-1a are among the important possible causes of abortion and infertility in dairy cattle in North-Western Algeria. Thus, authors would like to recommend for in-depth studies on *Pestiviruses* to launch strategic intervention considering both the economic and public health importance of diseases affecting the dairy sector. In summary, *Pestivirus* are widespread in livestock in North Africa. However, there is a significant lack of both cross-sectional and long-term transboundary studies about the epidemiology and molecular variability of ruminant *Pestivirus* in livestock and wildlife in North Africa. This is of concern for livestock health and wildlife conservation, and needs to be addressed.

IV. Conclusions and recommendations

The current study allows us to conclude that many efforts have to be accomplish in order to protect and reach the national needs concerning of livestock welfare. The present investigation underlines that the six studied diseases are among the important causes of abortion and infertility in dairy cattle in all Algeria. A global vision of the structure management of farms is necessary including the control and eradication programs of infectious diseases. To prevent the presence and spread of reproductive pathogens within cattle population, the knowledge of epidemiology of these infectious diseases must be improved in Algeria. Particularly we highlight the need for long term

The present studies emphasize that any control programme needs to be initially assessed with the rate of infection in a specific region. In addition, the knowledge of herd management and environmental factors which enhance the risk of abortive diseases would improve the ability to control and prevent the transmission, reducing the detrimental impacts of diseases on herd health and productivity. This experimental study made it possible to draw up an initial assessment of the seroprevalence of six abortive diseases, *Neospora Caninum, Toxoplasma Gondii, Chlamydia abortus, Coxiella burnetiid, Brucella abortus and Bovine Viral Diarrhea Virus* (BVDV) in unvaccinated cattle. Infections by BVDV, *Brucella* spp. *N. caninum* and *Coxiella burnetii* were found to be prevalent in the study area.

Direct and indirect factors were found as effectiveness tools for the spread and propagation of pathogens through dairy herds in the study localisation. The seroconversion risk increases with time or calving number, indicating a greater possibility of horizontal transmission of the disease possibly owing to the increased risk of *Brucella spp, Chlamydia abortus* and BVDV infection respectively by horizontal transmission.

The findings of the current study showed that the use of artificial insemination increases considerably the *N. caninum* and *Brucella spp* seropositive infection cattle in herds using this mode of reproduction. Consequently, AI represent a potential source of direct (semen) or indirect (Artificial insemination gun) infection.

The mixed herd or the visit of foreign farmers was one of the most important risk factors in which the presence of sheep or horse or even wild animals are a risk for Neospora caninum, BVDV, Chlamydia abortus, and Coxiella burnetii infection respectively. Larger size herd had

higher seropositivity than smaller herds and statistically significant regarding BVDV infection. While large size herd was recognized as a protective factor.

Brucela spp and *Toxoplasma gondi* were found to cause reproductive disorders in the study area. However, the impact of the remain pathogens appeared to be low in the study area, with no association with reproductive disorders detected.

Thus, the PhD candidate would like to recommend for in-depth study on these and other infectious causes of reproductive disorders to launch strategic intervention considering both the economic and public health importance of diseases affecting the dairy sector.
In addition, surveillance and complementary scientific research should be done in several regions (provinces) aimed to identify the possible presence of pathogenic agents in the sperm used for veterinary inseminators. This research, together with the control of artificial insemination practiced by veterinarians, will act in the right direction and effectively prevent the spread of infections.

Additionally, *Pestivirus* positive amplicons were sequenced and the 5' untranslated region (5'-UTR) was characterized. Studies exploring the molecular epidemiology of BVDV can offer precious information about the diversity of viral strains present in a population and, in turn, inform control programs, drive vaccine development and determine likely infection sources

Once controlled, all risk factors for the entrance of the studied abortive pathogens, it would be necessary to reduce/eradicate the pathogens through vaccination programs at national level (when the vaccine is available) and eliminating from flocks and herds the positive individuals.

Finally, there is a significant lack of both cross-sectional and long-term transboundary studies about the epidemiology and molecular variability of ruminant diseases in livestock and wildlife in North Africa. This is of concern for livestock health and wildlife conservation and needs to be addressed. Improve epidemiological data would be mandatory.

V. References

Abbasi-Doulatshahi, Ehsan, Hassan Hajian-Bidar, Jamal Gharekhani, and A. Rezaei. 2015. 'Q Fever in Domestic Ruminants: A Seroepidemiological Survey'. In .

Abdelbaset, Abdelbaset E., Mostafa F. N. Abushahba, Maha I. Hamed, and Mohamed S. Rawy. 2018. 'Sero-Diagnosis of Brucellosis in Sheep and Humans in Assiut and El-Minya Governorates, Egypt'. *International Journal of Veterinary Science and Medicine* 6 (January): S63–67. https://doi.org/10.1016/j.ijvsm.2018.01.007.

Abdel-Latif, Ahmed, Sagar Goyal, Yogesh Chander, Ahmed Abdel-Moneim, Sabry Tamam, and Hanafy Madbouly. 2013. 'Isolation and Molecular Characterisation of a Pestivirus from Goats in Egypt'. *Acta Veterinaria Hungarica* 61 (2): 270–80. https://doi.org/10.1556/AVet.2013.007.

Abdel-Moein, Khaled A., and Dalia A. Hamza. 2017. 'The Burden of *Coxiella Burnetii* among Aborted Dairy Animals in Egypt and Its Public Health Implications'. *Acta Tropica* 166 (February): 92–95. https://doi.org/10.1016/j.actatropica.2016.11.011.

Abdessalem Rekiki, F. Thabti, I. Dlissi, P. Russo, A. Rodolakis, Salah Hammami, R. Sanchis, and Michel PEPIN. 2005. 'Seroprevalence Survey of Major Infectious Abortive Diseases in Small Ruminants in Tunisia'. *Médecine Vétérinaire* 7 (156): 395–401.

Abernethy, D. A., F. D. Menzies, S. J. McCullough, S. W. J. McDowell, K. E. Burns, R. Watt, A. W. Gordon, M. Greiner, and D. U. Pfeiffer. 2012. 'Field Trial of Six Serological Tests for Bovine Brucellosis'. *The Veterinary Journal* 191 (3): 364–70. https://doi.org/10.1016/j.tvjl.2011.03.008.

Abutarbush, Sameeh M. 2010. 'Veterinary Medicine — A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats, 10th Edition'. *The Canadian Veterinary Journal* 51 (5): 541.

Achour, K., M.-H. Ben-Mahdi, M. Akkou, and R. Teniou. 2012. 'Séroprévalence de *Neospora caninum* dans les élevages bovins laitiers de la région centre nord de l'Algérie: -EN- Seroprevalence of *Neospora caninum* in dairy cattle farms in Algeria's central northern region -FR- -ES- Seroprevalencia de *Neospora caninum* en las granjas de bovinos lecheros de la región centro norte de Argelia'. *Revue Scientifique et Technique de l'OIE* 31 (3): 953–58. https://doi.org/10.20506/rst.31.3.2178.

Adams, Heather A., Tad S. Sonstegard, Paul M. VanRaden, Daniel J. Null, Curt P. Van Tassell, Denis M. Larkin, and Harris A. Lewin. 2016. 'Identification of a Nonsense Mutation in APAF1 That Is Likely Causal for a Decrease in Reproductive Efficiency in Holstein Dairy Cattle'. *Journal of Dairy Science* 99 (8): 6693–6701. https://doi.org/10.3168/jds.2015-10517.

Ae, Marsh, Barr Bc, Madigan J, Lakritz J, Nordhausen R, and Conrad Pa. 1996. 'Neosporosis as a Cause of Equine Protozoal Myeloencephalitis.' *Journal of the American Veterinary Medical Association* 209 (11): 1907–13.

Agerholm, Jørgen S. 2013. '*Coxiella Burnetii* Associated Reproductive Disorders in Domestic Animals--a Critical Review'. *Acta Veterinaria Scandinavica* 55 (February): 13. https://doi.org/10.1186/1751-0147-55-13.

Agerholm, Jørgen S., Tim K. Jensen, Jens F. Agger, Marc Y. Engelsma, and Hendrik I. J. Roest. 2017. 'Presence of *Coxiella Burnetii* DNA in Inflamed Bovine Cardiac Valves'. *BMC Veterinary Research* 13 (1): 69. https://doi.org/10.1186/s12917-017-0988-5.

Aggad, H., and L. Boukraa. 2006. 'Prevalence of Bovine and Human Brucellosis in Western Algeria: Comparison of Screening Tests'. *Eastern Mediterranean Health Journal = La Revue De Sante De La Mediterranee Orientale = Al-Majallah Al-Sihhiyah Li-Sharq Al-Mutawassit* 12 (1–2): 119–28.

Agger, Jens, Suman Paul, Anna-Bodil Christoffersen, and Jørgen Agerholm. 2013. 'Risk Factors for *Coxiella Burnetii* Antibodies in Bulk Tank Milk from Danish Dairy Herds'. *Acta Veterinaria Scandinavica* 55 (1): 80.

Ahmadian, Afshin, Maria Ehn, and Sophia Hober. 2006. 'Pyrosequencing: History, Biochemistry and Future'. *Clinica Chimica Acta*, From Real-Time PCR to Nanotechnology: Rapid and/or High-Throughput Diagnostic Methods for Nucleic Acids, 363 (1): 83–94. https://doi.org/10.1016/j.cccn.2005.04.038.

Ahmed, Sahar, Mohamed Abd El-Fatah Mahmoud, Cyril Viarouge, Corinne Sailleau, Stephan Zientara, and Emmanuel Breard. 2019. 'Presence of Bluetongue and Epizootic Hemorrhagic Disease Viruses in Egypt in 2016 and 2017'. *Infection, Genetics and Evolution* 73 (September): 221–26. https://doi.org/10.1016/j.meegid.2019.04.033.

Aitken, I. D., and D. Longbottom. 2008. 'Chlamydial Abortion'. In *Diseases of Sheep*, 105–12. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470753316.ch16.

Akagami, Masataka, Satoko Seki, Yuki Kashima, Kaoru Yamashita, Shoko Oya, Yuki Fujii, Mariko Takayasu, et al. 2020. 'Risk Factors Associated with the Within-Farm Transmission of Bovine Viral Diarrhea Virus and the Incidence of Persistently Infected Cattle on Dairy Farms from Ibaraki Prefecture of Japan'. *Research in Veterinary Science*, February. https://doi.org/10.1016/j.rvsc.2020.02.001.

Aldomy, F. M. M. (Ministry of Agriculture, A. J. Wilsmore, and S. H. Safi. 1998. 'Q Fever and Abortion in Sheep and Goats in Jordan'. *Pakistan Veterinary Journal (Pakistan)*. http://agris.fao.org/agris-search/search.do?recordID=PK2001000531.

Alencar Mota, Ana Lourdes Arrais de, Fernando Ferreira, José Soares Ferreira Neto, Ricardo Augusto Dias, Marcos Amaku, José Henrique Hildebrand Grisi-Filho, Evelise Oliveira Telles, and Vítor Salvador Picão Gonçalves. 2016. 'Large-Scale Study of Herd-Level Risk Factors for Bovine Brucellosis in Brazil'. *Acta Tropica* 164 (December): 226–32. https://doi.org/10.1016/j.actatropica.2016.09.016.

Allen, Adrian, Eleanor Breadon, Andrew Byrne, Thomas Mallon, Robin Skuce, Pauline Groussaud, Amanda Dainty, et al. 2015. 'Molecular Epidemiology of *Brucella Abortus* in Northern Ireland—1991 to 2012'. *PLOS ONE* 10 (9): e0136721. https://doi.org/10.1371/journal.pone.0136721.

Almendra, Cláudia, Teresa Luísa Silva, Albano Beja-Pereira, Ana Cristina Ferreira, Lénia Ferrão-Beck, Maria Inácia Corrêa de Sá, Betsy J. Bricker, and Gordon Luikart. 2009. '"HOOF-Print" Genotyping and Haplotype Inference Discriminates among *Brucella Spp*. Isolates from a Small Spatial Scale'. *Infection, Genetics and Evolution* 9 (1): 104–7. https://doi.org/10.1016/j.meegid.2008.10.007.

Almería, S., R. Araujo, W. Tuo, F. López-Gatius, J. P. Dubey, and L. C. Gasbarre. 2010. 'Fetal Death in Cows Experimentally Infected with *Neospora Caninum* at 110 Days of Gestation'. *Veterinary Parasitology* 169 (3–4): 304–11. https://doi.org/10.1016/j.vetpar.2009.12.044.

Almería, S., and F. López-Gatius. 2013. 'Bovine Neosporosis: Clinical and Practical Aspects'. *Research in Veterinary Science* 95 (2): 303–9. https://doi.org/10.1016/j.rvsc.2013.04.008.

Almeria, S., T. De Marez, H. Dawson, R. Araujo, J. P. Dubey, and L. C. Gasbarre. 2003. 'Cytokine Gene Expression in Dams and Foetuses after Experimental *Neospora Caninum* Infection of Heifers at 110 Days of Gestation'. *Parasite Immunology* 25 (7): 383–92. https://doi.org/10.1046/j.1365-3024.2003.00645.x.

Almería, Sonia, and Fernando López-Gatius. 2015. 'Markers Related to the Diagnosis and to the Risk of Abortion in Bovine Neosporosis'. *Research in Veterinary Science* 100 (June): 169–75. https://doi.org/10.1016/j.rvsc.2015.03.022.

Almería, Sonia, Beatriz Serrano-Pérez, and Fernando López-Gatius. 2017. 'Immune Response in Bovine Neosporosis: Protection or Contribution to the Pathogenesis of Abortion'. *Microbial Pathogenesis* 109 (August): 177–82. https://doi.org/10.1016/j.micpath.2017.05.042.

Alton, G. G. 1990. '*Brucella Melitensis*'. *Animal Brucellosis*, 384–404.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. 'Basic Local Alignment Search Tool'. *Journal of Molecular Biology* 215 (3): 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Alvarez, J., A. Perez, F. O. Mardones, M. Pérez-Sancho, T. García-Seco, E. Pagés, F. Mirat, R. Díaz, J. Carpintero, and L. Domínguez. 2012. 'Epidemiological Factors Associated with the Exposure of Cattle to *Coxiella Burnetii* in the Madrid Region of Spain'. *Veterinary Journal (London, England: 1997)* 194 (1): 102–7. https://doi.org/10.1016/j.tvjl.2012.02.022.

Alvarez-García, Gema, Alicia García-Culebras, Daniel Gutiérrez-Expósito, Vanesa Navarro-Lozano, Iván Pastor-Fernández, and Luis Miguel Ortega-Mora. 2013. 'Serological Diagnosis of Bovine Neosporosis: A Comparative Study of Commercially Available ELISA Tests'. *Veterinary Parasitology* 198 (1): 85–95. https://doi.org/10.1016/j.vetpar.2013.07.033.

Álvarez-García, G., J. Pereira-Bueno, M. Gómez-Bautista, and L. M. Ortega-Mora. 2002. 'Pattern of Recognition of *Neospora Caninum* Tachyzoite Antigens by Naturally Infected Pregnant Cattle and Aborted Foetuses'. *Veterinary Parasitology* 107 (1): 15–27. https://doi.org/10.1016/S0304-4017(02)00091-2.

Amdouni, Yosra, Mariem Rouatbi, Narjess Lassoued, Mourad Rekik, and Mohamed Gharbi. 2019. '*Neospora Caninum* Natural Infection in Tunisian Rams: Serological Study and Molecular Identification of Infection in Semen'. *Acta Parasitologica*, August. https://doi.org/10.2478/s11686-019-00105-0.

Amelung, Silke, Maria Hartmann, Ludwig Haas, and Lothar Kreienbrock. 2018. 'Factors Associated with the Bovine Viral Diarrhoea (BVD) Status in Cattle Herds in Northwest Germany'. *Veterinary Microbiology* 216 (March): 212–17. https://doi.org/10.1016/j.vetmic.2018.01.018.

Ammam, Abdelkader, Karima Grele, and Ahmed Reda Belmamoun. 2018. 'Prevalence of Human Brucellosis in the Southern Zone of Sidi-Bel-Abbes, Algeria'. *Archives of Clinical and Medical Case Reports 2* 2: 56–64.

Anderson, Alicia D., Deanna Kruszon-Moran, Amanda D. Loftis, Geraldine McQuillan, William L. Nicholson, Rachel A. Priestley, Amanda J. Candee, Nicole E. Patterson, and Robert F. Massung. 2009. 'Seroprevalence of Q Fever in the United States, 2003-2004'. *The American Journal of Tropical Medicine and Hygiene* 81 (4): 691–94. https://doi.org/10.4269/ajtmh.2009.09-0168.

Anderson, Mark L. 2007. 'Infectious Causes of Bovine Abortion during Mid- to Late-Gestation'. *Theriogenology*, Proceedings of the Annual Conference of the Society for Theriogenology, 68 (3): 474–86. https://doi.org/10.1016/j.theriogenology.2007.04.001.

Andrade, L. H., F. D. S. Mendes, J. C. Espindola, and M. C. S. Amaral. 2014. 'Nanofiltration as Tertiary Treatment for the Reuse of Dairy Wastewater Treated by Membrane Bioreactor'. *Separation and Purification Technology* 126 (April): 21–29. https://doi.org/10.1016/j.seppur.2014.01.056.

Angelakis, Emmanouil, and Didier Raoult. 2010. 'Q Fever'. *Veterinary Microbiology* 140 (3–4): 297–309. https://doi.org/10.1016/j.vetmic.2009.07.016.

Animal and Plant Health Agency (APHA). 2013. 'Veterinary Investigation Diagnosis Analysis (VIDA) Report'.

Aprizal Panus, Monayae Eakkatat, Peter Black, Liliek Indrayani, and Safrison Idris. 2018. 'Seroprevalence and Risk Factors for Bovine Brucellosis in Daerah Khusus Ibukota Jakarta Province, Indonesia'. In , 1–3.

Arias, P., M. Orlich, M. Prieto, S. Cedillo Rosales, H. -J. Thiel, M. Álvarez, and P. Becher. 2003. 'Genetic Heterogeneity of Bovine Viral Diarrhoea Viruses from Spain'. *Veterinary*

Microbiology, Pestiviruses ii: Contributions from the 5th Pestivirus Symposium of the European Society for Veterinary Virology, 96 (4): 327–36. https://doi.org/10.1016/j.vetmic.2003.09.009.

Aslan, Muhammet Eren, Ahmet Kursat Azkur, and Serkal Gazyagci. 2015. 'Epidemiology and Genetic Characterization of BVDV, BHV-1, BHV-4, BHV-5 and *Brucella Spp*. Infections in Cattle in Turkey'. *The Journal of Veterinary Medical Science* 77 (11): 1371–77. https://doi.org/10.1292/jvms.14-0657.

Asmare, K., Asfaw, Y., Gelaye, E., and Ayelet, G. 2010. 'Brucellosis in Extensive Management System of Zebu Cattle in Sidama Zone, Southern Ethiopia.' *African Journal of Agricultural Research* 5 (3): 257–63.

Asmare, K., F. Regassa, L. J. Robertson, and E. Skjerve. 2013. 'Seroprevalence of *Neospora Caninum* and Associated Risk Factors in Intensive or Semi-Intensively Managed Dairy and Breeding Cattle of Ethiopia'. *Veterinary Parasitology* 193 (1): 85–94. https://doi.org/10.1016/j.vetpar.2012.11.025.

Asmare, Kassahun, Berhanu Sibhat, Gelagay Ayelet, Endrias Zewdu Gebremedhin, Kassaye Aragaw Lidete, and Eystien Skjerve. 2018. 'Serological Evidence of Bovine Herpesvirus-1, Bovine Viral Diarrhea Virus and Schmallenberg Virus Infections in Relation to Reproductive Disorders in Dairy Cattle in Ethiopia'. *Acta Tropica* 178 (February): 236–41. https://doi.org/10.1016/j.actatropica.2017.12.005.

Astobiza, I., F. Ruiz-Fons, A. Piñero, J. F. Barandika, A. Hurtado, and A. L. García-Pérez. 2012. 'Estimation of *Coxiella Burnetii* Prevalence in Dairy Cattle in Intensive Systems by Serological and Molecular Analyses of Bulk-Tank Milk Samples'. *Journal of Dairy Science* 95 (4): 1632–38.

Ayayi Justin Akakpo, Assiongbon Têko-Agbo, and Philippe Koné. 2009. 'L'impact de La Brucellose Sur l'economie et La Santé Publique En Afrique'. *Conf. OIE*, 71–84.

B, Bonfoh, Kasymbekov J, Dürr S, Toktobaev N, Doherr Mg, Schueth T, Zinsstag J, and Schelling E. 2011. 'Representative Seroprevalences of Brucellosis in Humans and Livestock in Kyrgyzstan.' *Ecohealth* 9 (2): 132–38. https://doi.org/10.1007/s10393-011-0722-x.

Bachofen, Claudia, Hanspeter Stalder, Ueli Braun, Monika Hilbe, Felix Ehrensperger, and Ernst Peterhans. 2008. 'Co-Existence of Genetically and Antigenically Diverse Bovine Viral Diarrhoea Viruses in an Endemic Situation'. *Veterinary Microbiology* 131 (1–2): 93–102. https://doi.org/10.1016/j.vetmic.2008.02.023.

Barati, Sara, Naghmeh Moori-Bakhtiari, Masoud Ghorbanpoor Najafabadi, Hassan Momtaz, and Leili Shokuhizadeh. 2017. 'The Role of Zoonotic Chlamydial Agents in Ruminants Abortion'. *Iranian Journal of Microbiology* 9 (5): 288–94.

Bari, A. S. M., F. Yeasmin, and M. G. S. Alam. 1993. 'Pathology of Naturally Occurring Toxoplasma Abortion and Neonatal Mortality in Black Bengal Goat'. *Small Ruminant Research* 10 (4): 349–55. https://doi.org/10.1016/0921-4488(93)90139-9.

Barkallah, Mohamed, Yaakoub Gharbi, Mariam Hmani, Zouhir Mallek, Michel Gautier, Radhouane Gdoura, and Imen Fendri. 2016. 'Locked Nucleic Acid Probe-Based Real-Time PCR for the Diagnosis of Listeria Monocytogenes in Ruminants'. *Molecular and Cellular Probes* 30 (3): 138–45. https://doi.org/10.1016/j.mcp.2016.02.010.

Barkallah, Mohamed, Yaakoub Gharbi, Sonia Zormati, Nesrine Karkouch, Zouhir Mallek, Michel Gautier, Radhouane Gdoura, and Imen Fendri. 2017. 'A Mixed Methods Study of Ruminant Brucellosis in Central-Eastern Tunisia'. *Tropical Animal Health and Production* 49 (1): 39–45. https://doi.org/10.1007/s11250-016-1155-x.

Bartley, Paul M., Frank Katzer, Mara S. Rocchi, Stephen W. Maley, Julio Benavides, Mintu Nath, Yvonne Pang, et al. 2013. 'Development of Maternal and Foetal Immune Responses in Cattle Following Experimental Challenge with *Neospora Caninum* at Day 210 of Gestation'. *Veterinary Research* 44 (October): 91. https://doi.org/10.1186/1297-9716-44-91.

Bauermann, F. V., S. M. Falkenberg, B. Vander Ley, N. Decaro, B. W. Brodersen, A. Harmon, B. Hessman, E. F. Flores, and J. F. Ridpath. 2014. 'Generation of Calves Persistently Infected with HoBi-like Pestivirus and Comparison of Methods for Detection of These Persistent Infections'. *Journal of Clinical Microbiology* 52 (11): 3845–52. https://doi.org/10.1128/JCM.01563-14.

Beaudeau, F., C. Fourichon, A. Robert, A. Joly, and H. Seegers. 2005. 'Bulk Milk Somatic Cell Counts and Bovine Viral Diarrhoea Virus (BVDV) Infection in 7252 Dairy Herds in Brittany (Western France)'. *Preventive Veterinary Medicine*, Bovine virus diarrhoea virus (BVDV)control, 72 (1): 163–67. https://doi.org/10.1016/j.prevetmed.2005.08.017.

Belák, S., and A. Ballagi-Pordány. 1993. 'Experiences on the Application of the Polymerase Chain Reaction in a Diagnostic Laboratory'. *Molecular and Cellular Probes* 7 (3): 241–48. https://doi.org/10.1006/mcpr.1993.1035.

Bellil, Kousseila, and Moussa Boukrif. 2015. 'Analyse Comparative de La Rentabilité Économique Des Différents Systèmes d'élevages Laitiers à Bejaia'. *Revue Agriculture*. 10: 59–66. https://www.researchgate.net/publication/332343700_Analyse_comparative_de_la_rentabilite_economique_des_differents_systemes_d'elevages_laitiers_a_Bejaia.

Bello, N. M., J. S. Stevenson, and R. J. Tempelman. 2012. 'Invited Review: Milk Production and Reproductive Performance: Modern Interdisciplinary Insights into an Enduring Axiom'. *Journal of Dairy Science* 95 (10): 5461–75. https://doi.org/10.3168/jds.2012-5564.

Belluco, Simone, Ilaria Patuzzi, and Antonia Ricci. 2018. 'Bovine Meat versus Pork in *Toxoplasma Gondii* Transmission in Italy: A Quantitative Risk Assessment Model'. *International Journal of Food Microbiology* 269 (March): 1–11. https://doi.org/10.1016/j.ijfoodmicro.2017.12.026.

Benaissa, Mohammed H., Samir Ansel, Abdallah Mohamed-Cherif, Karima Benfodil, Djamel Khelef, Curtis R. Youngs, Rachid Kaidi, and Khatima Ait-Oudhia. 2017. 'Seroprevalence and Risk Factors for *Coxiella Burnetii*, the Causative Agent of Q Fever in the Dromedary Camel (*Camelus Dromedarius*) Population in Algeria'. *The Onderstepoort Journal of Veterinary Research* 84 (1): e1–7. https://doi.org/10.4102/ojvr.v84i1.1461.

Benkirane, Abdelali, Soukaina Essamkaoui, Ahmed El Idrissi, Laura Lucchese, and Alda Natale. 2015. 'A Sero-Survey of Major Infectious Causes of Abortion in Small Ruminants in Morocco'. *Veterinaria Italiana* 51 (1): 25–30. https://doi.org/10.12834/VetIt.389.1814.1.

Benyettou Mohamed Abdelkader, and Bouklikha Abdellah. 2017. 'Variations et tendances des températures et des précipitations journalières en Algérie.' MASTER ACADIMIQUE, Telemcen: Université ABOU BEKR BELKAID. http://dspace.univ-tlemcen.dz/bitstream/112/11879/1/Ms.HydBenyettou%2BBouklikha.pdf.

Berger, D. S. 2016. 'Brucellosis: Global Status, GIDEON Informatics Inc'. GIDEON - Global Infectious Diseases and Epidemiology Online Network. 2016. https://www.gideononline.com/ebooks/disease/brucellosis-global-status/.

Berri, M., F. Bernard, A. Lecu, F. Ollivet-Courtois, and A. Rodolakis. 2004. 'Molecular Characterisation and Ovine Live Vaccine 1B Evaluation toward a Chlamydophila Abortus Strain Isolated from Springbok Antelope Abortion'. *Veterinary Microbiology* 103 (3): 231–40. https://doi.org/10.1016/j.vetmic.2004.08.009.

Berry, D. P., R. D. Evans, and S. Mc Parland. 2011. 'Evaluation of Bull Fertility in Dairy and Beef Cattle Using Cow Field Data'. *Theriogenology* 75 (1): 172–81. https://doi.org/10.1016/j.theriogenology.2010.08.002.

Biesenkamp-Uhe, Carolin, Yihang Li, Hans-Robert Hehnen, Konrad Sachse, and Bernhard Kaltenboeck. 2007. 'Therapeutic Chlamydophila Abortus and C. Pecorum Vaccination Transiently Reduces Bovine Mastitis Associated with Chlamydophila Infection'. *Infection and Immunity* 75 (2): 870–77. https://doi.org/10.1128/IAI.00691-06.

Bildfell, R. J., G. W. Thomson, D. M. Haines, B. J. McEwen, and N. Smart. 2000. '*Coxiella Burnetii* Infection Is Associated with Placentitis in Cases of Bovine Abortion'. *Journal of Veterinary*

Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 12 (5): 419–25. https://doi.org/10.1177/104063870001200505.

Billinis, C., L. Leontides, G. S. Amiridis, V. Spyrou, P. Kostoulas, and M. Sofia. 2005. 'Prevalence of BVDV Infection in Greek Dairy Herds'. *Preventive Veterinary Medicine*, Bovine virus diarrhoea virus (BVDV)control, 72 (1): 75–79. https://doi.org/10.1016/j.prevetmed.2005.05.011.

Blumer, S., G. Greub, A. Waldvogel, M. Hässig, R. Thoma, A. Tschuor, A. Pospischil, and N. Borel. 2011. 'Waddlia, Parachlamydia and Chlamydiaceae in Bovine Abortion'. *Veterinary Microbiology* 152 (3–4): 385–93. https://doi.org/10.1016/j.vetmic.2011.05.024.

BonDurant, Robert H. 2005. 'Venereal Diseases of Cattle: Natural History, Diagnosis, and the Role of Vaccines in Their Control'. *Veterinary Clinics of North America: Food Animal Practice*, Bovine Theriogenology, 21 (2): 383–408. https://doi.org/10.1016/j.cvfa.2005.03.002.

Boni, Raffaele. 2019. 'Heat Stress, a Serious Threat to Reproductive Function in Animals and Humans'. *Molecular Reproduction and Development* 86 (10): 1307–23. https://doi.org/10.1002/mrd.23123.

Booth, R. E., M. P. Cranwell, and J. Brownlie. 2013. 'Monitoring the Bulk Milk Antibody Response to BVDV: The Effects of Vaccination and Herd Infection Status'. *Veterinary Record* 172 (17): 449–449. https://doi.org/10.1136/vr.101195.

Booth, Richard E., Carole J. Thomas, Laila M. R. El-Attar, George Gunn, and Joe Brownlie. 2013. 'A Phylogenetic Analysis of Bovine Viral Diarrhoea Virus (BVDV) Isolates from Six Different Regions of the UK and Links to Animal Movement Data'. *Veterinary Research* 44 (June): 43. https://doi.org/10.1186/1297-9716-44-43.

Borel, N., R. Thoma, P. Spaeni, R. Weilenmann, K. Teankum, E. Brugnera, D. R. Zimmermann, L. Vaughan, and A. Pospischil. 2006. '*Chlamydia*-Related Abortions in Cattle from Graubunden, Switzerland'. *Veterinary Pathology* 43 (5): 702–8. https://doi.org/10.1354/vp.43-5-702.

Borel, Nicole, Caroline F. Frey, Bruno Gottstein, Monika Hilbe, Andreas Pospischil, Francesca D. Franzoso, and Andreas Waldvogel. 2014. 'Laboratory Diagnosis of Ruminant Abortion in Europe'. *Veterinary Journal (London, England: 1997)* 200 (2): 218–29. https://doi.org/10.1016/j.tvjl.2014.03.015.

Borel, Nicole, Adam Polkinghorne, and Andreas Pospischil. 2018. 'A Review on Chlamydial Diseases in Animals: Still a Challenge for Pathologists?' *Veterinary Pathology* 55 (3): 374–90. https://doi.org/10.1177/0300985817751218.

Böttcher, Jens, Annette Vossen, Britta Janowetz, Michaela Alex, Armin Gangl, Andreas Randt, and Norbert Meier. 2011. 'Insights into the Dynamics of Endemic *Coxiella Burnetii* Infection in Cattle by Application of Phase-Specific ELISAs in an Infected Dairy Herd'. *Veterinary Microbiology* 151 (3–4): 291–300. https://doi.org/10.1016/j.vetmic.2011.03.007.

Bottieau, E., H. De Raeve, R. Colebunders, J. Van den Ende, T. Vervoort, and E. Van Marck. 2000. 'Q Fever after a Journey in Syria: A Diagnosis Suggested by Bone Marrow Biopsy'. *Acta Clinica Belgica* 55 (1): 30–33. https://doi.org/10.1080/17843286.2000.11754269.

Bouamra, M, F Ghozlane, and M K Ghozlane. 2016. 'Facteurs Influençant Les Performances de Reproduction de Vaches Laitières En Algérie'. *Livestock Research for Rural Development* 28 (4).

Boukary, Abdou Razac, Claude Saegerman, Emmanuel Abatih, David Fretin, Rianatou Alambédji Bada, Reginald De Deken, Halimatou Adamou Harouna, Alhassane Yenikoye, and Eric Thys. 2013. 'Seroprevalence and Potential Risk Factors for *Brucella Spp*. Infection in Traditional Cattle, Sheep and Goats Reared in Urban, Periurban and Rural Areas of Niger'. *PLoS ONE* 8 (12). https://doi.org/10.1371/journal.pone.0083175.

Boukhechem, Said, Nassim Moula, Nedjoua Lakhdara, and Rachid Kaidi. 2019. 'Feeding Practices of Dairy Cows in Algeria: Characterization, Typology, and Impact on Milk Production and

Fertility'. *JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH* 6 (4): 567–74.

Bouras, Ahlam. 2015. 'CONTRIBUTION A LA CONNAISSANCE DES SYSTEMES D'ELEVAGE BOVIN DANS LA REGION DE OUARGLA'. MASTER ACADIMIQUE, ouaregla: KASDI MERBAH, OUARGLA.

Bouzebda, Z. 2007. 'Gestion zootechnique de la reproduction dans des élevages bovins laitiers dans l'Est algérien.' PhD, DEPARTEMENT DES SCIENCES VETERINAIRES constantine: UNIVERSITE MENTOURI. CONSTANTINE.

Braun, U., C. Bachofen, B. Schenk, M. Hässig, and E. Peterhans. 2013. 'Investigation of Border Disease and Bovine Virus Diarrhoea in Sheep from 76 Mixed Cattle and Sheep Farms in Eastern Switzerland'. *Schweizer Archiv Fur Tierheilkunde* 155 (5): 293–98. https://doi.org/10.1024/0036-7281/a000460.

Braun, U., S. F. Reichle, C. Reichert, M. Hässig, H. P. Stalder, C. Bachofen, and E. Peterhans. 2014. 'Sheep Persistently Infected with Border Disease Readily Transmit Virus to Calves Seronegative to BVD Virus'. *Veterinary Microbiology* 168 (1): 98–104. https://doi.org/10.1016/j.vetmic.2013.11.004.

Braun, Ueli, Monika Hilbe, Ernst Peterhans, and Matthias Schweizer. 2019. 'Border Disease in Cattle'. *The Veterinary Journal* 246 (April): 12–20. https://doi.org/10.1016/j.tvjl.2019.01.006.

Brownlie, J., M. C. Clarke, C. J. Howard, and D. H. Pocock. 1987. 'Pathogenesis and Epidemiology of Bovine Virus Diarrhoea Virus Infection of Cattle'. *Annales De Recherches Veterinaires. Annals of Veterinary Research* 18 (2): 157–66.

Brownlie J, Clarke Mc, Howard Cj, and Pocock Dh. 1987. 'PATHOGENESIS AND EPIDEMIOLOGY OF BOVINE VIRUS DIARRHOEA VIRUS INFECTION OF CATTLE.' *Annales de Recherches Vétérinaires, INRA Editions*, 2, (18): pp.157-166.

Bruin, A. de, I. Janse, M. Koning, L. de Heer, R. Q. J. van der Plaats, J. P. G. van Leuken, and B. J. van Rotterdam. 2013. 'Detection of *Coxiella Burnetii* DNA in the Environment during and after a Large Q Fever Epidemic in the Netherlands'. *Journal of Applied Microbiology* 114 (5): 1395–1404. https://doi.org/10.1111/jam.12163.

Bugeza, James, Adrian Muwonge, Musso Munyeme, Phillip Lasuba, Jacques Godfroid, and Clovice Kankya. 2019. 'Seroprevalence of Bovine Brucellosis and Associated Risk Factors in Nakasongola District, Uganda'. *Tropical Animal Health and Production* 51 (7): 2073–76. https://doi.org/10.1007/s11250-018-1631-6.

Burgstaller, Johann, Walter Obritzhauser, Sabrina Kuchling, Ian Kopacka, Beate Pinior, and Josef Köfer. 2016. 'The Effect of Bovine Viral Diarrhoea Virus on Fertility in Dairy Cows: Two Case-Control Studies in the Province of Styria, Austria'. *Berliner Und Munchener Tierarztliche Wochenschrift* 129 (3–4): 103–10.

Burnard, Delaney, and Adam Polkinghorne. 2016. 'Chlamydial Infections in Wildlife–Conservation Threats and/or Reservoirs of "Spill-over" Infections?' *Veterinary Microbiology* 196 (November): 78–84. https://doi.org/10.1016/j.vetmic.2016.10.018.

Burrells, Alison, Alessandra Taroda, Marieke Opsteegh, Gereon Schares, Julio Benavides, Cecile Dam-Deisz, Paul M. Bartley, et al. 2018. 'Detection and Dissemination of *Toxoplasma Gondii* in Experimentally Infected Calves, a Single Test Does Not Tell the Whole Story'. *Parasites & Vectors* 11 (January). https://doi.org/10.1186/s13071-018-2632-z.

Butler, W. R. 2000. 'Nutritional Interactions with Reproductive Performance in Dairy Cattle'. *Animal Reproduction Science* 60–61 (July): 449–57. https://doi.org/10.1016/s0378-4320(00)00076-2.

Butler, W. R. 2003. 'Energy Balance Relationships with Follicular Development, Ovulation and Fertility in Postpartum Dairy Cows'. *Livestock Production Science* 83 (2): 211–18. https://doi.org/10.1016/S0301-6226(03)00112-X.

Butler, W. R., R. W. Everett, and C. E. Coppock. 1981. 'The Relationships between Energy Balance, Milk Production and Ovulation in Postpartum Holstein Cows'. *Journal of Animal Science* 53 (3): 742–48. https://doi.org/10.2527/jas1981.533742x.

Buxton, D., and J. Finlayson. 1986. 'Experimental Infection of Pregnant Sheep with *Toxoplasma Gondii*: Pathological and Immunological Observations on the Placenta and Foetus'. *Journal of Comparative Pathology* 96 (3): 319–33. https://doi.org/10.1016/0021-9975(86)90052-6.

Buxton, D., J. S. Gilmour, K. W. Angus, D. A. Blewett, and J. K. Miller. 1982. 'Perinatal Changes in Lambs Infected with *Toxoplasma Gondii*'. *Research in Veterinary Science* 32 (2): 170–76.

Buxton, D., and E. A. Innes. 1995. 'A Commercial Vaccine for Ovine Toxoplasmosis'. *Parasitology* 110 Suppl: S11-16. https://doi.org/10.1017/s003118200000144x.

Byrne, A. W., M. Guelbenzu-Gonzalo, S. A. J. Strain, S. McBride, J. Graham, A. Lahuerta-Marin, R. Harwood, D. A. Graham, and S. McDowell. 2017. 'Assessment of Concurrent Infection with Bovine Viral Diarrhoea Virus (BVDV) and Mycobacterium Bovis: A Herd-Level Risk Factor Analysis from Northern Ireland'. *Preventive Veterinary Medicine* 141 (June): 38–47. https://doi.org/10.1016/j.prevetmed.2017.04.007.

Cabell, Emma. 2007. 'Bovine Abortion: Aetiology and Investigations'. *In Practice* 29 (8): 455–63. https://doi.org/10.1136/inpract.29.8.455.

Cabrera, V. E., D. Solís, and J. del Corral. 2010. 'Determinants of Technical Efficiency among Dairy Farms in Wisconsin'. *Journal of Dairy Science* 93 (1): 387–93. https://doi.org/10.3168/jds.2009-2307.

Calistri, Paolo, Simona Iannetti, Marcello Atzeni, Calogero Di Bella, Pietro Schembri, and Armando Giovannini. 2013. 'Risk Factors for the Persistence of Bovine Brucellosis in Sicily from 2008 to 2010'. *Preventive Veterinary Medicine* 110 (3): 329–34. https://doi.org/10.1016/j.prevetmed.2012.12.008.

Canada, Nuno, Carla S. Meireles, A. Rocha, J. M. Correia da Costa, M. W. Erickson, and J. P. Dubey. 2002. 'Isolation of Viable *Toxoplasma Gondii* from Naturally Infected Aborted Bovine Fetuses'. *The Journal of Parasitology* 88 (6): 1247–48. https://doi.org/10.1645/0022-3395(2002)088[1247:IOVTGF]2.0.CO;2.

Cantas, Hasan, Adrian Muwonge, Baris Sareyyupoglu, Hakan Yardimci, and Eystein Skjerve. 2011. 'Q Fever Abortions in Ruminants and Associated On-Farm Risk Factors in Northern Cyprus'. *BMC Veterinary Research* 7 (1): 13. https://doi.org/10.1186/1746-6148-7-13.

Cárdenas, Liliana, Mario Peña, Oscar Melo, and Jordi Casal. 2019. 'Risk Factors for New Bovine Brucellosis Infections in Colombian Herds'. *BMC Veterinary Research* 15 (March). https://doi.org/10.1186/s12917-019-1825-9.

Cardinale, Eric, Olivier Esnault, Marina Beral, Florence Naze, and Alain Michault. 2014. 'Emergence of *Coxiella Burnetii* in Ruminants on Reunion Island? Prevalence and Risk Factors'. *PLoS Neglected Tropical Diseases* 8 (8): e3055.

Cardoso, José Márcio Sbruzzi, Marcos Amaku, Ana Julia Urias dos Santos Araújo, and Solange Maria Gennari. 2012. 'A Longitudinal Study of *Neospora Caninum* Infection on Three Dairy Farms in Brazil'. *Veterinary Parasitology* 187 (3–4): 553–57. https://doi.org/10.1016/j.vetpar.2012.01.019.

Carman, S., T. van Dreumel, J. Ridpath, M. Hazlett, D. Alves, E. Dubovi, R. Tremblay, S. Bolin, A. Godkin, and N. Anderson. 1998. 'Severe Acute Bovine Viral Diarrhea in Ontario, 1993-1995'. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 10 (1): 27–35. https://doi.org/10.1177/104063879801000106.

Carvalho, P. D., V. G. Santos, J. O. Giordano, M. C. Wiltbank, and P. M. Fricke. 2018. 'Development of Fertility Programs to Achieve High 21-Day Pregnancy Rates in High-Producing Dairy

Cows'. *Theriogenology* 114 (July): 165–72. https://doi.org/10.1016/j.theriogenology.2018.03.037.

Cavirani, S., C. S. Cabassi, G. Donofrio, B. De Iaco, S. Taddei, and C. F. Flammini. 2001. 'Association between *Chlamydia Psittaci* Seropositivity and Abortion in Italian Dairy Cows'. *Preventive Veterinary Medicine* 50 (1): 145–51. https://doi.org/10.1016/S0167-5877(01)00197-0.

Cetinkaya, B., H. Kalender, H. B. Ertas, A. Muz, N. Arslan, H. Ongor, and M. Gurçay. 2000. 'Seroprevalence of Coxiellosis in Cattle, Sheep and People in the East of Turkey'. *The Veterinary Record* 146 (5): 131–36. https://doi.org/10.1136/vr.146.5.131.

Çetinkaya, B., H. Kalender, H. B. Ertas, A. Muz, N. Arslan, H. Ongor, and M. Gurçay. 2000. 'Seroprevalence of Coxiellosis in Cattle, Sheep and People in the East of Turkey'. *Veterinary Record* 146 (5): 131–36. https://doi.org/10.1136/vr.146.5.131.

Chand, Puran, and Rajesh Chhabra. 2013. 'Herd and Individual Animal Prevalence of Bovine Brucellosis with Associated Risk Factors on Dairy Farms in Haryana and Punjab in India'. *Tropical Animal Health and Production* 45 (6): 1313–19. https://doi.org/10.1007/s11250-013-0362-y.

Chandrawathani, P., R. Nurulaini, C. M. Zanin, B. Premaalatha, M. Adnan, O. Jamnah, S. K. Khor, et al. 2008. 'Seroprevalence of *Toxoplasma Gondii* Antibodies in Pigs, Goats, Cattle, Dogs and Cats in Peninsular Malaysia'. *Tropical Biomedicine* 25 (3): 257–58.

Charlier, Carole, Wanbo Li, Chad Harland, Mathew Littlejohn, Wouter Coppieters, Frances Creagh, Steve Davis, et al. 2016. 'NGS-Based Reverse Genetic Screen for Common Embryonic Lethal Mutations Compromising Fertility in Livestock'. *Genome Research* 26 (10): 1333–41. https://doi.org/10.1101/gr.207076.116.

Chase, Christopher C. L., David J. Hurley, and Adrian J. Reber. 2008. 'Neonatal Immune Development in the Calf and Its Impact on Vaccine Response'. *The Veterinary Clinics of North America. Food Animal Practice* 24 (1): 87–104. https://doi.org/10.1016/j.cvfa.2007.11.001.

Chemma, Nawal. 2017. 'LA DEPENDANCE LAITIERE : OÙ EN EST L'ALGERIE?' *Revue d'Etudes en Management et Finance d'Organisation* 0 (5). https://revues.imist.ma/index.php?journal=REMFO&page=article&op=view&path%5B%5D=8426.

Chon, S. K., D. R. Perez, and R. O. Donis. 1998. 'Genetic Analysis of the Internal Ribosome Entry Segment of Bovine Viral Diarrhea Virus'. *Virology* 251 (2): 370–82. https://doi.org/10.1006/viro.1998.9425.

Climat Batna. 2020. 'Climat Batna: Pluviométrie et Température Moyenne Batna, Diagramme Ombrothermique Pour Batna'. Climate-Data.Org. 13 April 2020. https://fr.climate-data.org/afrique/algerie/batna/batna-3686/.

Climat Sétif. 2020. 'Climat Sétif: Pluviométrie et Température Moyenne Sétif, Diagramme Ombrothermique Pour Sétif'. 2020. https://fr.climate-data.org/afrique/algerie/setif/setif-3595/.

Clothier, K., and M. Anderson. 2016. 'Evaluation of Bovine Abortion Cases and Tissue Suitability for Identification of Infectious Agents in California Diagnostic Laboratory Cases from 2007 to 2012'. *Theriogenology* 85 (5): 933–38. https://doi.org/10.1016/j.theriogenology.2015.11.001.

'CNIAAG'. 2020. 2020. http://www.cniaag.dz/.

Coelho, Beatriz, Bruno Veigas, Elvira Fortunato, Rodrigo Martins, Hugo Águas, Rui Igreja, and Pedro V. Baptista. 2017. 'Digital Microfluidics for Nucleic Acid Amplification'. *Sensors (Basel, Switzerland)* 17 (7). https://doi.org/10.3390/s17071495.

Colett, M. S., R. Larson, C. Gold, D. Strick, D. K. Anderson, and A. F. Purchio. 1988. 'Molecular Cloning and Nucleotide Sequence of the Pestivirus Bovine Viral Diarrhea Virus'. *Virology* 165 (1): 191–99. https://doi.org/10.1016/0042-6822(88)90672-1.

Corbel, Michael J., and World Health Organization. 2006. *Brucellosis in Humans and Animals*. World Health Organization.

Costa, Gustavo Henrique Nogueira, Alvimar José da Costa, Welber Daniel Zanetti Lopes, Katia Denise Saraiva Bresciani, Thais Rabelo dos Santos, César Roberto Esper, and Áureo Evangelista Santana. 2011. '*Toxoplasma Gondii*: Infection Natural Congenital in Cattle and an Experimental Inoculation of Gestating Cows with Oocysts'. *Experimental Parasitology* 127 (1): 277–81. https://doi.org/10.1016/j.exppara.2010.08.005.

Costa, Paulo Sérgio Gonçalves da, Marcos Emilio Brigatte, and Dirceu Bartolomeu Greco. 2005. 'Antibodies to Rickettsia Rickettsii, Rickettsia Typhi, *Coxiella Burnetii*, Bartonella Henselae, Bartonella Quintana, and Ehrlichia Chaffeensis among Healthy Population in Minas Gerais, Brazil'. *Memorias Do Instituto Oswaldo Cruz* 100 (8): 853–59. https://doi.org/10.1590/s0074-02762005000800006.

Courcoul, Aurélie, Hervé Monod, Mirjam Nielen, Don Klinkenberg, Lenny Hogerwerf, François Beaudeau, and Elisabeta Vergu. 2011. 'Modelling the Effect of Heterogeneity of Shedding on the within Herd *Coxiella Burnetii* Spread and Identification of Key Parameters by Sensitivity Analysis'. *Journal of Theoretical Biology* 284 (1): 130–41. https://doi.org/10.1016/j.jtbi.2011.06.017.

Cowley, D. J. Bosco, David A. Graham, Maria Guelbenzu, Michael L. Doherty, and Simon J. More. 2014. 'Aspects of Bovine Herpesvirus 1 and Bovine Viral Diarrhoea Virus Herd-Level Seroprevalence and Vaccination in Dairy and Beef Herds in Northern Ireland'. *Irish Veterinary Journal* 67 (1): 18. https://doi.org/10.1186/2046-0481-67-18.

Cremoux, Renée de, Elodie Rousset, Anne Touratier, Ghislain Audusseau, Philippe Nicollet, Danièle Ribaud, Valérie David, and Marilyne Le Pape. 2012. 'Assessment of Vaccination by a Phase I *Coxiella Burnetii*-Inactivated Vaccine in Goat Herds in Clinical Q Fever Situation'. *FEMS Immunology and Medical Microbiology* 64 (1): 104–6. https://doi.org/10.1111/j.1574-695X.2011.00892.x.

Crowe, M. A., M. G. Diskin, and E. J. Williams. 2014. 'Parturition to Resumption of Ovarian Cyclicity: Comparative Aspects of Beef and Dairy Cows'. *Animal: An International Journal of Animal Bioscience* 8 Suppl 1 (May): 40–53. https://doi.org/10.1017/S1751731114000251.

Crowe, M. A., and E. J. Williams. 2012. 'TRIENNIAL LACTATION SYMPOSIUM: Effects of Stress on Postpartum Reproduction in Dairy Cows',. *Journal of Animal Science* 90 (5): 1722–27. https://doi.org/10.2527/jas.2011-4674.

Croxatto, Antony, Nadia Rieille, Tahar Kernif, Idir Bitam, Sébastien Aeby, Olivier Péter, and Gilbert Greub. 2014. 'Presence of Chlamydiales DNA in Ticks and Fleas Suggests That Ticks Are Carriers of Chlamydiae'. *Ticks and Tick-Borne Diseases* 5 (4): 359–65. https://doi.org/10.1016/j.ttbdis.2013.11.009.

Cummins, S. B., P. Lonergan, A. C. O. Evans, D. P. Berry, R. D. Evans, and S. T. Butler. 2012. 'Genetic Merit for Fertility Traits in Holstein Cows: I. Production Characteristics and Reproductive Efficiency in a Pasture-Based System'. *Journal of Dairy Science* 95 (3): 1310–22. https://doi.org/10.3168/jds.2011-4742.

Cummins, S. B., P. Lonergan, A. C. O. Evans, and S. T. Butler. 2012. 'Genetic Merit for Fertility Traits in Holstein Cows: II. Ovarian Follicular and Corpus Luteum Dynamics, Reproductive Hormones, and Estrus Behavior'. *Journal of Dairy Science* 95 (7): 3698–3710. https://doi.org/10.3168/jds.2011-4976.

Cummins, S. B., S. M. Waters, A. C. O. Evans, P. Lonergan, and S. T. Butler. 2012. 'Genetic Merit for Fertility Traits in Holstein Cows: III. Hepatic Expression of Somatotropic Axis Genes during Pregnancy and Lactation'. *Journal of Dairy Science* 95 (7): 3711–21. https://doi.org/10.3168/jds.2011-4977.

Cutler, Sally J., Maha Bouzid, and Ronald R. Cutler. 2007. 'Q Fever'. *The Journal of Infection* 54 (4): 313–18. https://doi.org/10.1016/j.jinf.2006.10.048.

Dabaja, M. F., G. Greco, S. Villari, G. Vesco, A. Bayan, B. El Bazzal, E. Ibrahim, et al. 2019. 'Occurrence and Risk Factors of *Coxiella Burnetii* in Domestic Ruminants in Lebanon'. *Comparative Immunology, Microbiology and Infectious Diseases* 64 (June): 109–16. https://doi.org/10.1016/j.cimid.2019.03.003.

Danuser, R., H.-R. Vogt, Th Kaufmann, E. Peterhans, and R. Zanoni. 2009. 'Seroprevalence and Characterization of Pestivirus Infections in Small Ruminants and New World Camelids in Switzerland'. *Schweizer Archiv Fur Tierheilkunde* 151 (3): 109–17. https://doi.org/10.1024/0036-7281.151.3.109.

De Biase, Davide, Alessandro Costagliola, Fabio Del Piero, Rossella Di Palo, Domenico Coronati, Giorgio Galiero, Barbara Degli Uberti, et al. 2018. '*Coxiella Burnetii* in Infertile Dairy Cattle With Chronic Endometritis'. *Veterinary Pathology* 55 (4): 539–42. https://doi.org/10.1177/0300985818760376.

De Marez, T, S Liddell, J.P Dubey, M.C Jenkins, and L Gasbarre. 1999. 'Oral Infection of Calves with *Neospora Caninum* Oocysts from Dogs: Humoral and Cellular Immune Responses'. *International Journal for Parasitology* 29 (10): 1647–57. https://doi.org/10.1016/S0020-7519(99)00154-X.

De Vries. 2006. 'Economic Value of Pregnancy in Dairy Cattle.' *Journal of Dairy Science* 10 (89): 3876–85.

Dean, Anna S., Bassirou Bonfoh, Abalo E. Kulo, G. Aboudou Boukaya, Moussa Amidou, Jan Hattendorf, Paola Pilo, and Esther Schelling. 2013. 'Epidemiology of Brucellosis and Q Fever in Linked Human and Animal Populations in Northern Togo'. *PLOS ONE* 8 (8): e71501. https://doi.org/10.1371/journal.pone.0071501.

Decaro, Nicola, Maria Stella Lucente, Viviana Mari, Rossana Sciarretta, Pierfrancesco Pinto, Domenico Buonavoglia, Vito Martella, and Canio Buonavoglia. 2012. 'Hobi-Like Pestivirus in Aborted Bovine Fetuses'. *Journal of Clinical Microbiology* 50 (2): 509–12. https://doi.org/10.1128/JCM.05887-11.

Deng, Mingliang, Sukun Ji, Wentao Fei, Sohail Raza, Chenfei He, Yingyu Chen, Huanchun Chen, and Aizhen Guo. 2015. 'Prevalence Study and Genetic Typing of Bovine Viral Diarrhea Virus (BVDV) in Four Bovine Species in China'. *PLOS ONE* 10 (4): e0121718. https://doi.org/10.1371/journal.pone.0121718.

Deng, R, and K V Brock. 1993. '5' and 3' Untranslated Regions of Pestivirus Genome: Primary and Secondary Structure Analyses.' *Nucleic Acids Research* 21 (8): 1949–57.

Derdour, Salima-Yamina, Fella Hafsi, Naouelle Azzag, Safia Tennah, Abdelouahab Laamari, Bernard China, and Farida Ghalmi. 2017. 'Prevalence of The Main Infectious Causes of Abortion in Dairy Cattle in Algeria'. *Journal of Veterinary Research* 61 (3): 337–43. https://doi.org/10.1515/jvetres-2017-0044.

Dijkstra, T., H. W. Barkema, M. Eysker, and W. Wouda. 2001. 'Evidence of Post-Natal Transmission of *Neospora Caninum* in Dutch Dairy Herds'. *International Journal for Parasitology* 31 (2): 209–15. https://doi.org/10.1016/s0020-7519(00)00160-0.

Dirar, Bashahun Gebremichael, George William Nasinyama, and Benti Deresa Gelalcha. 2015. 'Seroprevalence and Risk Factors for Brucellosis in Cattle in Selected Districts of Jimma Zone, Ethiopia'. *Tropical Animal Health and Production* 47 (8): 1615–19. https://doi.org/10.1007/s11250-015-0910-8.

Diskin, M. G., S. M. Waters, M. H. Parr, and D. A. Kenny. 2016. 'Pregnancy Losses in Cattle: Potential for Improvement'. *Reproduction, Fertility, and Development* 28 (1–2): 83–93. https://doi.org/10.1071/RD15366.

Djebbara. 2008. 'Durabilité et Politique de l'élevage En Algérie. Le Cas Du Bovin Laitier.' In *Développement Durable Des Productions Animales : Enjeux, Évaluations et Perspective,*. Alger,.

Djellata, N, A Yahimi, C Hanzen, C. Saegerman, and R Kaidi. 2019. 'Prevalence and Factors Associated with a Highter or Lower Risk of Exposure to *Coxiella Burnetii*, *Chlamydia Abortus* and *Toxoplasma Gondii* in Dairy Cowsthat Have Aborted in Algeria'. *Scientic and Technical* 3 (38).

Djermoun, Abdelkader, Foued Chehat, and Abdelhamid Bencharif. 2017. 'Stratégies Des Éleveurs Laitiers Du Cheliff (Algérie).' *Food Agricultural Organisation*. http://www.fao.org/family-farming/detail/fr/c/880772/.

Djønne, Berit. 2007. 'Infections and Perinatal Diseases – a Comparative Overview'. *Acta Veterinaria Scandinavica* 49 (1): 1–6. https://doi.org/10.1186/1751-0147-49-S1-S10.

Dohoo, Ian, Wayne Martin, and Henrik Stryhn. 2009. *Veterinary Epidemiologic Research*. 2nd edition. Charlottetown, CA: VER Inc.

Dohoo Ian, Stryhn HE, and Martin W,. 2003. 'Veterinary Epidemiologic Research'. *Third Printing of the Second Edition* (blog). 2003. http://projects.upei.ca/ver/.

Doosti, Abbas, Faham Khamesipour, Shahin Nekoei, and Ismar Lutvikadic. 2015. 'Survey for the Presence of *Neospora Caninum* in Frozen Bull's Semen Samples by PCR Assay'. *Asian Pacific Journal of Tropical Disease* 5 (1): 7–12. https://doi.org/10.1016/S2222-1808(14)60618-6.

Dorneles, Elaine Maria Seles, Jordana Almeida Santana, Telma Maria Alves, Rebeca Barbosa Pauletti, Juliana Pinto da Silva Mol, Marcos Bryan Heinemann, and Andrey Pereira Lage. 2014. 'Genetic Stability of *Brucella Abortus* Isolates from an Outbreak by Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA16)'. *BMC Microbiology* 14 (1): 186. https://doi.org/10.1186/1471-2180-14-186.

Dubey, J. P. 1986. 'A Review of Toxoplasmosis in Cattle'. *Veterinary Parasitology* 22 (3–4): 177–202. https://doi.org/10.1016/0304-4017(86)90106-8.

Dubey, J. P. 2009. 'Toxoplasmosis in Pigs—The Last 20 Years'. *Veterinary Parasitology* 164 (2): 89–103. https://doi.org/10.1016/j.vetpar.2009.05.018.

Dubey, J. P., M. R. Lappin, and P. Thulliez. 1995. 'Long-Term Antibody Responses of Cats Fed *Toxoplasma Gondii* Tissue Cysts'. *The Journal of Parasitology* 81 (6): 887–93.

Dubey, J. P., and D. S. Lindsay. 1996a. 'A Review of *Neospora Caninum* and Neosporosis'. *Veterinary Parasitology* 67 (1–2): 1–59.

Dubey, J. P., D. S. Lindsay, W. J. Saville, S. M. Reed, D. E. Granstrom, and C. A. Speer. 2001. 'A Review of Sarcocystis Neurona and Equine Protozoal Myeloencephalitis (EPM)'. *Veterinary Parasitology* 95 (2–4): 89–131. https://doi.org/10.1016/s0304-4017(00)00384-8.

Dubey, J. P., M. D. Ruff, M. E. Camargo, S. K. Shen, G. L. Wilkins, O. C. Kwok, and P. Thulliez. 1993. 'Serologic and Parasitologic Responses of Domestic Chickens after Oral Inoculation with *Toxoplasma Gondii* Oocysts'. *American Journal of Veterinary Research* 54 (10): 1668–72.

Dubey, J. P., and G. Schares. 2006a. 'Diagnosis of Bovine Neosporosis'. *Veterinary Parasitology* 140 (1–2): 1–34. https://doi.org/10.1016/j.vetpar.2006.03.035.

Dubey, J. P. 2006b. 'Diagnosis of Bovine Neosporosis'. *Veterinary Parasitology* 140 (1–2): 1–34. https://doi.org/10.1016/j.vetpar.2006.03.035.

Dubey, J. P. 2011. 'Neosporosis in Animals--the Last Five Years'. *Veterinary Parasitology* 180 (1–2): 90–108. https://doi.org/10.1016/j.vetpar.2011.05.031.

Dubey, J. P., G. Schares, and L. M. Ortega-Mora. 2007a. 'Epidemiology and Control of Neosporosis and *Neospora Caninum*'. *Clinical Microbiology Reviews* 20 (2): 323–67. https://doi.org/10.1128/CMR.00031-06.

Dubey, J. P. 2007b. 'Epidemiology and Control of Neosporosis and *Neospora Caninum*'. *Clinical Microbiology Reviews* 20 (2): 323–67. https://doi.org/10.1128/CMR.00031-06.

Dubey, J. P., C. A. Speer, and R. Fayer. 1989. 'Sarcocystosis of Animals and Man.' *Sarcocystosis of Animals and Man*. https://www.cabdirect.org/cabdirect/abstract/19910869228.

Dubey, J. P., and Joanne P Webster. 2010. 'Toxoplasmosis of Animals and Humans'. *Parasites & Vectors* 3 (November): 112. https://doi.org/10.1186/1756-3305-3-112.

Dubey, J.P., M.C. Jenkins, C. Rajendran, K. Miska, L.R. Ferreira, J. Martins, O.C.H. Kwok, and S. Choudhary. 2011. 'Gray Wolf (Canis Lupus) Is a Natural Definitive Host for *Neospora Caninum'*. *Veterinary Parasitology* 181 (2–4): 382–87. https://doi.org/10.1016/j.vetpar.2011.05.018.

Dubey, J.P., and D.S. Lindsay. 1996b. 'A Review of *Neospora Caninum* and Neosporosis'. *Veterinary Parasitology* 67 (1–2): 1–59. https://doi.org/10.1016/S0304-4017(96)01035-7.

Ducrotoy, Marie J., Wilson J. Bertu, Reuben A. Ocholi, Amahyel M. Gusi, Ward Bryssinckx, Sue Welburn, and Ignacio Moriyón. 2014. 'Brucellosis as an Emerging Threat in Developing Economies: Lessons from Nigeria'. *PLOS Neglected Tropical Diseases* 8 (7): e3008. https://doi.org/10.1371/journal.pntd.0003008.

Dumetre, A, D Ajzenberg, L Rozette, A Mercier, and M Darde. 2006. '*Toxoplasma Gondii* Infection in Sheep from Haute-Vienne, France: Seroprevalence and Isolate Genotyping by Microsatellite Analysis'. *Veterinary Parasitology* 142 (3–4): 376–79. https://doi.org/10.1016/j.vetpar.2006.07.005.

E. Rousset, V. Duquesne, P. Russo, and M. F. Aubert,. 2010. 'Accès en ligne: OIE - World Organisation for Animal Health'. 2010. https://www.oie.int/fr/normes/manuel-terrestre/acces-en-ligne/.

Eaglesome M.D., and Garcia M. M. 1993. 'Disease Risks to Animal Health from Artificial Insemination with Bovine Semen'. *Rev. Sci. Tech. Off. Int. Epiz* 16 (1): 215–25.

Edwards, S. 1990. 'The Diagnosis of Bovine Virus Diarrhoea-Mucosal Disease in Cattle'. *Rey. Xi. Tech. Off. Int. Epiz.*, 9 (1): 115–30. https://www.oie.int/doc/ged/d8247.pdf.

El Bahgy, Halla E. K., Hala K. Abdelmegeed, and Marawan A. Marawan. 2018. 'Epidemiological Surveillance of Bovine Viral Diarrhea and Rift Valley Fever Infections in Camel'. *Veterinary World* 11 (9): 1331–37. https://doi.org/10.14202/vetworld.2018.1331-1337.

El-Bagoury G.F, El-Habbaa A.S, Nawal M.A, and Khadr K.A. 2012. 'Prevalence of Bovine Viral Diarrhea Virus (BVDV) in Cattle from Some Governorates in Egypt'. *Benha Veterinary Medical Journal* 23 (1): 123–30.

Eldin, Carole, Emmanouil Angelakis, Aurélie Renvoisé, and Didier Raoult. 2013. '*Coxiella Burnetii* DNA, but Not Viable Bacteria, in Dairy Products in France'. *The American Journal of Tropical Medicine and Hygiene* 88 (4): 765–69. https://doi.org/10.4269/ajtmh.12-0212.

Elfahal, Abdelghafar M., Amira M. Elhassan, Mohammed O. Hussien, Khalid A. Enan, Azza B. Musa, and Abdelrahim M. El Hussein. 2013a. 'Seroprevalence of *Toxoplasma Gondii* in Dairy Cattle with Reproductive Problems in Sudan'. *ISRN Veterinary Science* 2013: 895165. https://doi.org/10.1155/2013/895165.

Elfahal, Abdelghafar M., Amira M. Elhassan, Mohammed O. Hussien, Khalid A. Enan, Azza B. Musa, and Abdelrahim M. El Hussein. 2013b. 'Seroprevalence of *Toxoplasma Gondii* in Dairy Cattle with Reproductive Problems in Sudan'. *ISRN Veterinary Science* 2013 (September). https://doi.org/10.1155/2013/895165.

Elhassan, Amira M., M. A. Fadol El-Hussein, and A.M. El-Hussein. 2011. 'Seroprevalence of Bovine Herpes Virus-1, Bovine Herpes Virus-4 and Bovine Viral Diarrhea Virus in Dairy Cattle in Sudan'. *Pakistan Veterinary Journal* 31 (4): 317–20.

El-Tarabany, Mahmoud Salah. 2015. 'Impact of Stillbirth and Abortion on the Subsequent Fertility and Productivity of Holstein, Brown Swiss and Their Crosses in Subtropics'. *Tropical Animal Health and Production* 47 (7): 1351–56. https://doi.org/10.1007/s11250-015-0870-z.

Emery, Michelle P., Eileen N. Ostlund, Mohamed Ait Ichou, Jeff D. Ballin, David McFarling, and Luanne McGonigle. 2014. '*Coxiella Burnetii* Serology Assays in Goat Abortion Storm'. *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 26 (1): 141–45. https://doi.org/10.1177/1040638713517233.

Entrican, G. 2002. 'Immune Regulation during Pregnancy and Host-Pathogen Interactions in Infectious Abortion'. *Journal of Comparative Pathology* 126 (2–3): 79–94. https://doi.org/10.1053/jcpa.2001.0539.

Erfani, Amir Mehdi, Mehran Bakhshesh, Mohammad Hosein Fallah, and Majid Hashemi. 2019. 'Seroprevalence and Risk Factors Associated with Bovine Viral Diarrhea Virus and Bovine Herpes Virus-1 in Zanjan Province, Iran'. *Tropical Animal Health and Production* 51 (2): 313–19. https://doi.org/10.1007/s11250-018-1687-3.

Esteban-Redondo, I., and E. A. Innes. 1997. '*Toxoplasma Gondii* Infection in Sheep and Cattle'. *Comparative Immunology, Microbiology and Infectious Diseases* 20 (2): 191–96.

Esteban-Redondo, Irma, and Elisabeth A. Innes. 1997. '*Toxoplasma Gondii* Infection in Sheep and Cattle'. *Comparative Immunology, Microbiology and Infectious Diseases* 20 (2): 191–96. https://doi.org/10.1016/S0147-9571(96)00039-2.

Evans, R. D., M. Wallace, L. Shalloo, D. J. Garrick, and P. Dillon. 2006. 'Financial Implications of Recent Declines in Reproduction and Survival of Holstein-Friesian Cows in Spring-Calving Irish Dairy Herds'. *Agricultural Systems* 89 (1): 165–83. https://doi.org/10.1016/j.agsy.2005.08.008.

Evans, Tim J. 2011. 'Diminished Reproductive Performance and Selected Toxicants in Forages and Grains'. *The Veterinary Clinics of North America. Food Animal Practice* 27 (2): 345–71, viii. https://doi.org/10.1016/j.cvfa.2011.03.001.

Evans, Tim J.. 2012. 'Chapter 19 - Reproductive Toxicity and Endocrine Disruption'. In *Veterinary Toxicology (Second Edition)*, edited by Ramesh C. Gupta, 278–318. Boston: Academic Press. https://doi.org/10.1016/B978-0-12-385926-6.00019-3.

Faix, Dennis J., Dustin J. Harrison, Mark S. Riddle, Andrew F. Vaughn, Samuel L. Yingst, Kenneth Earhart, and Glenn Thibault. 2008. 'Outbreak of Q Fever among US Military in Western Iraq, June-July 2005'. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 46 (7): e65-68. https://doi.org/10.1086/528866.

Falcone, E., P. Cordioli, M. Tarantino, M. Muscillo, G. La Rosa, and M. Tollis. 2003. 'Genetic Heterogeneity of Bovine Viral Diarrhoea Virus in Italy'. *Veterinary Research Communications* 27 (6): 485–94. https://doi.org/10.1023/a:1025793708771.

Fassi Fihri, Ouafaa, Noâma Jammar, Nadia Amrani, Ikhlass El Berbri, and Said Alali. 2019. 'Sheep Pestivirus in Morocco: Sero-Epidemiological and Molecular Study'. *Veterinary Record Open* 6 (1): e000324. https://doi.org/10.1136/vetreco-2018-000324.

Fediaevsky, A., B. Dufour, and B. Garin-bastuji. 2011. 'Maintaining Vigilance against Bovine Brucellosis in France in 2010'. *Bull. Épidémiol. Santé Anim. ? Aliment,* vol.46, pp.10–14, 2011.: 10–14.

Feknous, Naouel, Jean-Baptiste Hanon, Marylène Tignon, Hamza Khaled, Abdallah Bouyoucef, and Brigitte Cay. 2018. 'Seroprevalence of Border Disease Virus and Other Pestiviruses in Sheep in Algeria and Associated Risk Factors'. *BMC Veterinary Research* 14 (1): 339. https://doi.org/10.1186/s12917-018-1666-y.

Fereig, Ragab M., Hassan Y. A. H. Mahmoud, Samy G. A. Mohamed, Mahmoud Rezk AbouLaila, Azza Abdel-Wahab, Salama Ahmed Osman, Sherif Abdallah Zidan, Sabry A. El-Khodary, Adel Elsayed Ahmed Mohamed, and Yoshifumi Nishikawa. 2016. 'Seroprevalence and Epidemiology of *Toxoplasma Gondii* in Farm Animals in Different Regions of Egypt'. *Veterinary Parasitology: Regional Studies and Reports* 3–4 (June): 1–6. https://doi.org/10.1016/j.vprsr.2016.05.002.

Ferguson, James D. 2003. 'Imapct Economic de La Performance de Reproduction Du Troupeau'. In . hotel des seigneurs saint hyacinthe.: CRAAQ centre de reference en agriculture et egroalimentaire en quebec.

Ferguson, JD, and DT Galligan. 1999. 'Veterinary Reproductive Programs. In: .: American Association of Bovine Practitioners'; In , 133-7. Nashville (TN).

Fernández-Sirera, Laura, Oscar Cabezón, Alberto Allepuz, Rosa Rosell, Cristina Riquelme, Emmanuel Serrano, Santiago Lavín, and Ignasi Marco. 2012. 'Two Different Epidemiological Scenarios of Border Disease in the Populations of Pyrenean Chamois (Rupicapra p. Pyrenaica) after the First Disease Outbreaks'. *PloS One* 7 (12): e51031. https://doi.org/10.1371/journal.pone.0051031.

Ferrah. A. 2000. 'L'élevage Bovin Laitier En Algerie, Problèmatique, Questions et Hypothèses de Recherche.' In *Séminaire-Atelier Sur Restructurations et Stratégies Des Acteurs de La Filière Lait En Algérie*. Université Mouloud Mammeri (UMMTO): Institut technique des élevages, département systèmes et filières d'élevage.

Ferre, Ignacio, Gorka Aduriz, Itziar Del-Pozo, Javier Regidor-Cerrillo, Raquel Atxaerandio, Esther Collantes-Fernández, Ana Hurtado, Carlos Ugarte-Garagalza, and Luis Miguel Ortega-Mora. 2005. 'Detection of *Neospora Caninum* in the Semen and Blood of Naturally Infected Bulls'. *Theriogenology* 63 (5): 1504–18. https://doi.org/10.1016/j.theriogenology.2004.07.002.

F.G. Da Silva, J.C. De Freitas, and E.E. Müller. 2006. 'Chlamydophila Abortus in Production Animals'. *January Ciência Rural 36(1):342-348* 36 (1): 342–48.

Flores, E. F., L. H. Gil, S. A. Botton, R. Weiblen, J. F. Ridpath, L. C. Kreutz, C. Pilati, D. Driemeyer, V. Moojen, and A. C. Wendelstein. 2000. 'Clinical, Pathological and Antigenic Aspects of Bovine Viral Diarrhea Virus (BVDV) Type 2 Isolates Identified in Brazil'. *Veterinary Microbiology* 77 (1–2): 175–83. https://doi.org/10.1016/s0378-1135(00)00274-1.

Franco C. Mufinda, Fernando S. Boinas, and , Carla S. Nunes. 2015. 'Prevalence and Factors Associated with Cattle Brucellosis in Animal Herds of the Namibe Province in Angola'. *Alexandria Journal of Veterinary Sciences* 47: 7–17. https://doi.org/10.5455/ajvs.188809.

Fred Unger, Susanne Münstermann, Alphonse Goumou, Correia Nunes Apia, Mamady Konte, and Michaela Hempen. 2003. 'Risk Associated with Bovine Brucellosis in Selected Study Herds and Market Places in Four Countries of West Africa'. *Animal HealthWorking* 5 (8).

F.Z. Abdelhadi, D. Mahmoud, N.L. Smail, S. Meliani, B. Benallou, A. Niar, and S.A. Abdelhadi. 2015. 'Abortions in Cattle on the Level of Tiaret Area (Algeria)'. *Global Veterinaria* 14 (5): 638–45.

Gädicke, P., R. Vidal, and G. Monti. 2010. 'Economic Effect of Bovine Abortion Syndrome in Commercial Dairy Herds in Southern Chile'. *Preventive Veterinary Medicine* 97 (1): 9–19. https://doi.org/10.1016/j.prevetmed.2010.07.008.

Galvão, K. N., P. Federico, A. De Vries, and G. M. Schuenemann. 2013. 'Economic Comparison of Reproductive Programs for Dairy Herds Using Estrus Detection, Timed Artificial Insemination, or a Combination'. *Journal of Dairy Science* 96 (4): 2681–93. https://doi.org/10.3168/jds.2012-5982.

Garcell, Humberto G., Elias G. Garcia, Pedro V. Pueyo, Isis R. Martín, Ariadna V. Arias, and Ramon N. Alfonso Serrano. 2016. 'Outbreaks of Brucellosis Related to the Consumption of Unpasteurized Camel Milk'. *Journal of Infection and Public Health* 9 (4): 523–27. https://doi.org/10.1016/j.jiph.2015.12.006.

Garnsworthy, P. C., K. D. Sinclair, and R. Webb. 2008. 'Integration of Physiological Mechanisms That Influence Fertility in Dairy Cows*'. *Animal* 2 (8): 1144–52. https://doi.org/10.1017/S1751731108002358.

Ghalmi, Farida, Bernard China, Asma Ghalmi, Darifa Hammitouche, and Bertrand Losson. 2012a. 'Study of the Risk Factors Associated with *Neospora Caninum* Seroprevalence in Algerian Cattle Populations'. *Research in Veterinary Science* 93 (2): 655–61. https://doi.org/10.1016/j.rvsc.2011.12.015.

Ghalmi, Farida, Bernard China, Asma Ghalmi, Darifa Hammitouche, and Bertrand Losson. 2012b. 'Study of the Risk Factors Associated with *Neospora Caninum* Seroprevalence in Algerian

Cattle Populations'. *Research in Veterinary Science* 93 (2): 655–61. https://doi.org/10.1016/j.rvsc.2011.12.015.

Ghasemi, Ahmad, Mohammad Reza Hajinezhad, Saber Esmaeili, Ehsan Mostafavi, and Veterinary Medicine Faculty Department of Basic Science. 2018. 'Seroprevalence of Q Fever and Brucellosis in Domestic and Imported Cattle of Southeastern Iran'. *Journal of Medical Microbiology and Infectious Diseases* 6 (2): 48–52.

Ghozlane F, Bousbia A, Benyoucef MT, and Yakhlef H. 2009. 'Technicaleconomic Impact of the Concentrate/Fodder Ratio on the Bovine Dairy Production: Case of the Farming of Constantine.' *Livest Res Rural Dev* 21 (6). https://doi.org/; http://www.lrrd.org/lrrd21/6/ghoz21094.htm.

Ghozlane, M K, A Atia, D Miles, and D Khellef. 2010. 'Insémination Artificielle En Algérie: Etude de Quelques Facteurs d'influence Chez La Vache Laitière'. *Livestock Research for Rural Development 22 (2) 2010* 22 (2).

Gilbert, P., F. Holst, J. Rossbach, and H. K. Pauli. 1991. '[Brucellosis-induced granulomatous non-puerperal mastitis--a case report]'. *Geburtshilfe Und Frauenheilkunde* 51 (9): 747–49. https://doi.org/10.1055/s-2007-1023826.

Gindri, Patrícia Carvalho, Bruna Mion, Jorgea Pradieé, Tatiane Senna Bialves, Guilherme Nunes de Souza, Odir Antônio Dellagostin, Augusto Schneider, et al. 2018. 'Seroprevalence Estimate and Associated Risk Factors for Neosporosis in Dairy Cattle in the Northwest Region of Rio Grande Do Sul State, Brazil'. *Ciência Rural* 48 (7). https://doi.org/10.1590/0103-8478cr20170921.

Giordano, J. O., A. S. Kalantari, P. M. Fricke, M. C. Wiltbank, and V. E. Cabrera. 2012. 'A Daily Herd Markov-Chain Model to Study the Reproductive and Economic Impact of Reproductive Programs Combining Timed Artificial Insemination and Estrus Detection'. *Journal of Dairy Science* 95 (9): 5442–60. https://doi.org/10.3168/jds.2011-4972.

Givens, M. Daniel. 2006. 'A Clinical, Evidence-Based Approach to Infectious Causes of Infertility in Beef Cattle'. *Theriogenology* 66 (3): 648–54. https://doi.org/10.1016/j.theriogenology.2006.04.021.

Givens, M. Daniel, and M. S. D. Marley. 2008. 'Pathogens That Cause Infertility of Bulls or Transmission via Semen'. *Theriogenology* 70 (3): 504–7. https://doi.org/10.1016/j.theriogenology.2008.05.033.

Godfroid, Jacques, Sascha Al Dahouk, Georgios Pappas, Felix Roth, Gift Matope, John Muma, Tanguy Marcotty, Dirk Pfeiffer, and Eystein Skjerve. 2013. 'A "One Health" Surveillance and Control of Brucellosis in Developing Countries: Moving Away from Improvisation'. *Comparative Immunology, Microbiology and Infectious Diseases*, Special issue: One Health, 36 (3): 241–48. https://doi.org/10.1016/j.cimid.2012.09.001.

Godin, Ann-Charlotte, Camilla Björkman, Stina Englund, Karl-Erik Johansson, Rauni Niskanen, and Stefan Alenius. 2008. 'Investigation of Chlamydophila Spp. in Dairy Cows with Reproductive Disorders'. *Acta Veterinaria Scandinavica* 50 (September): 39. https://doi.org/10.1186/1751-0147-50-39.

Gondim, L. F P., M. M. McAllister, R. C. Anderson-Sprecher, C. Björkman, T. F. Lock, L. D. Firkins, L. Gao, and W. R. Fischer. 2004. 'Transplacental transmission and abortion in cows administered *Neospora Caninum* oocysts'. *Journal of Parasitology* 90 (6): 1394–1400. https://doi.org/10.1645/GE-359R.

Goodswen, Stephen J., Paul J. Kennedy, and John T. Ellis. 2013. 'A Review of the Infection, Genetics, and Evolution of *Neospora Caninum*: From the Past to the Present'. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 13 (January): 133–50. https://doi.org/10.1016/j.meegid.2012.08.012.

Grace, Delia, F. Mutua, P. Ochungo, R. L. Kruska, K. Jones, L. Brierley, Ma Lucila Lapar, et al. 2012. 'Mapping of Poverty and Likely Zoonoses Hotspots'. Report. ILRI. https://cgspace.cgiar.org/handle/10568/21161.

Graham, D. A., T. A. Clegg, H. -H. Thulke, P. O'Sullivan, G. McGrath, and S. J. More. 2016. 'Quantifying the Risk of Spread of Bovine Viral Diarrhoea Virus (BVDV) between Contiguous Herds in Ireland'. *Preventive Veterinary Medicine* 126 (April): 30–38. https://doi.org/10.1016/j.prevetmed.2016.01.017.

Gröhn, Y. T., and P. J. Rajala-Schultz. 2000. 'Epidemiology of Reproductive Performance in Dairy Cows'. *Animal Reproduction Science* 60–61 (July): 605–14. https://doi.org/10.1016/s0378-4320(00)00085-3.

Grooms, D. L., L. A. Ward, and K. V. Brock. 1996. 'Morphologic Changes and Immunohistochemical Detection of Viral Antigen in Ovaries from Cattle Persistently Infected with Bovine Viral Diarrhea Virus'. *American Journal of Veterinary Research* 57 (6): 830–33.

Guatteo, Raphaël. 2011. 'Prevalence of *Coxiella Burnetii* Infection in Domestic Ruminants: A CriticalReview'.*Veterinary*.https://www.academia.edu/11708774/Prevalence_of_*Coxiella_burnetii*_infection_in_domestic_ruminants_A_critical_review.

Guatteo, Raphaël, François Beaudeau, Mustapha Berri, Annie Rodolakis, Alain Joly, and Henri Seegers. 2006. 'Shedding Routes of *Coxiella Burnetii* in Dairy Cows: Implications for Detection and Control'. *Veterinary Research* 37 (6): 827–33. https://doi.org/10.1051/vetres:2006038.

Guatteo, Raphaël, François Beaudeau, Alain Joly, and Henri Seegers. 2007. '*Coxiella Burnetii* Shedding by Dairy Cows'. *Veterinary Research* 38 (6): 849–60. https://doi.org/10.1051/vetres:2007038.

Guest editorial. 2006. 'What Is the Prevalence and Economic Impact of Chlamydial Infections in Cattle? The Need to Validate and Harmonise Existing Methods of Detection'. *The Veterinary Journal* 172 (1): 3–5. https://doi.org/10.1016/j.tvjl.2005.05.001.

Guido, Stefano, Frank Katzer, Ian Nanjiani, Elspeth Milne, and Elisabeth A. Innes. 2016. 'Serology-Based Diagnostics for the Control of Bovine Neosporosis'. *Trends in Parasitology* 32 (2): 131–43. https://doi.org/10.1016/j.pt.2015.11.014.

Guimarães, J.S., S.L.P. Souza, D.P. Bergamaschi, and S.M. Gennari. 2004. 'Prevalence of *Neospora Caninum* Antibodies and Factors Associated with Their Presence in Dairy Cattle of the North of Paraná State, Brazil'. *Veterinary Parasitology* 124 (1–2): 1–8. https://doi.org/10.1016/j.vetpar.2004.07.002.

Gyuranecz, Miklós, Béla Dénes, Sándor Hornok, Péter Kovács, Gábor Horváth, Viktor Jurkovich, Tamás Varga, et al. 2012. 'Prevalence of *Coxiella Burnetii* in Hungary: Screening of Dairy Cows, Sheep, Commercial Milk Samples, and Ticks'. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)* 12 (8): 650–53. https://doi.org/10.1089/vbz.2011.0953.

Hailu, Degefu, Mohamud, M., Hailemelekot, M., and Yohannes, M.,. 2011. 'Seroprevalence of Bovine Brucellosis in Agro Pastoral Areas of Jijjiga Zone of Somali National Regional State, Eastern Ethiopia.' *Ethiopian Veterinary Journal* 15 (1).

Halil Ibrahim Gokce, Oktay Genç, Mahmut Sozmen, and Cihan Kaçar. 2007. 'Seroprevalance of Chlamydophila Abortus in Aborting Ewes and Dairy Cattle in the North-East Part of Turkey'. *Veterinary Institute in Pulawy* 1 (51): 9–13.

Hamidinejat, Hossein, Masoud Ghorbanpour, Leily Nabavi, Mohammad Rahim Haji Hajikolaie, and Mohammad Hossein Razi Jalali. 2010. 'Occurrence of Anti-*Toxoplasma Gondii* Antibodies in Female Cattle in South-West of Iran'. *Tropical Animal Health and Production* 42 (5): 899–903. https://doi.org/10.1007/s11250-009-9505-6.

Hanon, Jean-Baptiste, Miet De Baere, Camille De la Ferté, Sophie Roelandt, Yves Van der Stede, and Brigitte Cay. 2017. 'Evaluation of 16 Commercial Antibody ELISAs for the Detection of Bovine Viral Diarrhea Virus–Specific Antibodies in Serum and Milk Using Well-Characterized

Sample Panels'. *Journal of Veterinary Diagnostic Investigation* 29 (6): 833–43. https://doi.org/10.1177/1040638717724839.

Hansen, Mette S., Annie Rodolakis, Denis Cochonneau, Jens F. Agger, Anna-Bodil Christoffersen, Tim K. Jensen, and Jørgen S. Agerholm. 2011. '*Coxiella Burnetii* Associated Placental Lesions and Infection Level in Parturient Cows'. *Veterinary Journal (London, England: 1997)* 190 (2): e135-139. https://doi.org/10.1016/j.tvjl.2010.12.021.

Hassani, Sawsan Kacimi El. 2013. 'La Dépendance Alimentaire En Algérie: Importation de Lait En Poudre versus Production Locale, Quelle Evolution?' *Mediterranean Journal of Social Sciences* 4 (11): 152.

Hellmann, E., C. Staak, and M. Baumann. 1984. 'Bovine Brucellosis among Two Different Cattle Populations in Bahr El Ghazal Province of Southern Sudan'. *Tropenmedizin Und Parasitologie* 35 (2): 123–26.

Hemphill, A., and B. Gottstein. 2000. 'A European Perspective on *Neospora Caninum*'. *International Journal for Parasitology* 30 (8): 877–924. https://doi.org/10.1016/S0020-7519(00)00072-2.

Herbut, Piotr, Sabina Angrecka, and Jacek Walczak. 2018. 'Environmental Parameters to Assessing of Heat Stress in Dairy Cattle—a Review'. *International Journal of Biometeorology* 62 (12): 2089–97. https://doi.org/10.1007/s00484-018-1629-9.

Hermanowicz, Slawomir W. 2008. 'Sustainability in Water Resources Management: Changes in Meaning and Perception'. *Sustainability Science* 3 (2): 181–88. https://doi.org/10.1007/s11625-008-0055-z.

Hide, Geoff. 2016. 'Role of Vertical Transmission of *Toxoplasma Gondii* in Prevalence of Infection'. *Expert Review of Anti-Infective Therapy* 14 (3): 335–44. https://doi.org/10.1586/14787210.2016.1146131.

Hill, D., and J.P. Dubey. 2002. '*Toxoplasma Gondii*: Transmission, Diagnosis and Prevention'. *Clinical Microbiology and Infection* 8 (10): 634–40. https://doi.org/10.1046/j.1469-0691.2002.00485.x.

Hireche, Sana, Mustafa Mohammed Kheir Ababneh, Omar Bouaziz, and Sabrina Boussena. 2016. 'Seroprevalence and Molecular Characterization of *Chlamydia Abortus* in Frozen Fetal and Placental Tissues of Aborting Ewes in Northeastern Algeria'. *Tropical Animal Health and Production* 48 (2): 255–62. https://doi.org/10.1007/s11250-015-0944-y.

Hobson, J. C., T. F. Duffield, D. Kelton, K. Lissemore, S. K. Hietala, K. E. Leslie, B. McEwen, and A. S. Peregrine. 2005. 'Risk Factors Associated with *Neospora Caninum* Abortion in Ontario Holstein Dairy Herds'. *Veterinary Parasitology* 127 (3): 177–88. https://doi.org/10.1016/j.vetpar.2004.09.025.

Hoffmann, Karolin, Franziska Schott, Manuela Donati, Antonietta Di Francesco, Michael Hässig, Sabrina Wanninger, Xaver Sidler, and Nicole Borel. 2015. 'Prevalence of Chlamydial Infections in Fattening Pigs and Their Influencing Factors'. *PLOS ONE* 10 (11): e0143576. https://doi.org/10.1371/journal.pone.0143576.

Holliman, A., R. G. Daniel, J. G. Parr, P. C. Griffiths, B. J. Bevan, T. C. Martin, R. G. Hewinson, M. Dawson, and R. Munro. 1994. 'Chlamydiosis and Abortion in a Dairy Herd'. *Veterinary Record* 134 (19): 500–502. https://doi.org/10.1136/vr.134.19.500.

Homan, W. L, M Vercammen, J De Braekeleer, and H Verschueren. 2000. 'Identification of a 200- to 300-Fold Repetitive 529 Bp DNA Fragment in *Toxoplasma Gondii*, and Its Use for Diagnostic and Quantitative PCR1Note: Nucleotide Sequence Data Reported in This Paper Have Been Submitted to GenBankTM Database with the Accession Number AF146527 (*Toxoplasma Gondii* Genomic Repetitive 529 Bp Fragment).1'. *International Journal for Parasitology* 30 (1): 69–75. https://doi.org/10.1016/S0020-7519(99)00170-8.

Hornberg, Andrea, Sandra Revilla Fernández, Claus Vogl, Stefan Vilcek, Monika Matt, Maria Fink, Josef Köfer, and Karl Schöpf. 2009. 'Genetic Diversity of Pestivirus Isolates in Cattle from

Western Austria'. *Veterinary Microbiology* 135 (3–4): 205–13. https://doi.org/10.1016/j.vetmic.2008.09.068.

Hotzel, Helmut, Angela Berndt, Falk Melzer, and Konrad Sachse. 2004. 'Occurrence of Chlamydiaceae Spp. in a Wild Boar (Sus Scrofa L.) Population in Thuringia (Germany)'. *Veterinary Microbiology* 103 (1–2): 121–26. https://doi.org/10.1016/j.vetmic.2004.06.009.

Houe, H., A. Lindberg, and V. Moennig. 2006. 'Test Strategies in Bovine Viral Diarrhea Virus Control and Eradication Campaigns in Europe'. *Journal of Veterinary Diagnostic Investigation* 18 (5): 427–36. https://doi.org/10.1177/104063870601800501.

Houe, Hans. 1995. 'Epidemiology of Bovine Viral Diarrhea Virus'. *Veterinary Clinics of North America: Food Animal Practice* 11 (3): 521–47. https://doi.org/10.1016/S0749-0720(15)30465-5.

Houe, Hans. 2003. 'Economic Impact of BVDV Infection in Dairies'. *Biologicals*, Detecting and Controlling BVDV Infections, 31 (2): 137–43. https://doi.org/10.1016/S1045-1056(03)00030-7.

Hovingh E. 2002. 'Abortions in Dairy Cattle. Common Causes of Abortions.' *Virginia Polytechnic Institute and State University, Blacksburg* 404-288.

Hurtado, A., A. L. García-Pérez, G. Aduriz, and R. A. Juste. 2003. 'Genetic Diversity of Ruminant Pestiviruses from Spain'. *Virus Research* 92 (1): 67–73. https://doi.org/10.1016/s0168-1702(02)00315-5.

Ibrahim, Nuraddis, Kelay Belihu, Fikre Lobago, and Merga Bekana. 2010. 'Sero-Prevalence of Bovine Brucellosis and Its Risk Factors in Jimma Zone of Oromia Region, South-Western Ethiopia'. *Tropical Animal Health and Production* 42 (1): 35–40. https://doi.org/10.1007/s11250-009-9382-z.

'IDvet | Innovative Diagnostic Kits'. n.d. IDVet. Accessed 7 July 2020. https://www.id-vet.com/.

IDvet innovative diagnostic. 2018. 'ID Screen® Brucellosis Serum Indirect Multi-Species'. Internal validation report 613. Grabels: ID.VET.

Intisar, Kamil Saeed, Yahia H. Ali, Abdelmelik I. Khalafalla, E. A. Rahman Mahasin, Adil S. Amin, and Khalid M. Taha. 2010. 'The First Report on the Prevalence of Pestivirus Infection in Camels in Sudan'. *Tropical Animal Health and Production* 42 (6): 1203–7. https://doi.org/10.1007/s11250-010-9549-7.

Isken, Leslie D., Marleen Kraaij-Dirkzwager, Patricia E. Vermeer-de Bondt, Hans C. Rümke, Clementine Wijkmans, Wim Opstelten, and Aura Timen. 2013. 'Implementation of a Q Fever Vaccination Program for High-Risk Patients in the Netherlands'. *Vaccine* 31 (23): 2617–22. https://doi.org/10.1016/j.vaccine.2013.03.062.

IUCN. 2020. 'The IUCN Red List of Threatened Species'. IUCN Red List of Threatened Species. 2020. https://www.iucnredlist.org/en.

J, He P, Yu Y, Du L, Gong P, Zhang G, and Zhang X. 2014. 'Detection of *Neospora Caninum*-DNA in Feces Collected from Dogs in Shenyang (China) and ITS1 Phylogenetic Analysis.' *Veterinary Parasitology* 205 (1–2): 361–64. https://doi.org/10.1016/j.vetpar.2014.06.036.

Jackova, A., M. Novackova, C. Pelletier, C. Audeval, E. Gueneau, A. Haffar, E. Petit, L. Rehby, and S. Vilcek. 2008. 'The Extended Genetic Diversity of BVDV-1: Typing of BVDV Isolates from France'. *Veterinary Research Communications* 32 (1): 7–11. https://doi.org/10.1007/s11259-007-9012-z.

Jamaluddin, Aziz A., James T. Case, David W. Hird, Patricia C. Blanchard, John R. Peauroi, and Mark L. Anderson. 2016. 'Dairy Cattle Abortion in California: Evaluation of Diagnostic Laboratory Data': *Journal of Veterinary Diagnostic Investigation*, June. https://doi.org/10.1177/104063879600800211.

Jennings, Gregory J., Rana A. Hajjeh, Fouad Y. Girgis, Moustafa A. Fadeel, Mohamed A. Maksoud, Momtaz O. Wasfy, Nasr El Sayed, et al. 2007. 'Brucellosis as a Cause of Acute Febrile Illness

in Egypt'. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101 (7): 707–13. https://doi.org/10.1016/j.trstmh.2007.02.027.

Jensen, Tim K., Donald L. Montgomery, Paula T. Jaeger, Tina Lindhardt, Jørgen S. Agerholm, Vivi Bille-Hansen, and Mette Boye. 2007. 'Application of Fluorescent in Situ Hybridisation for Demonstration of *Coxiella Burnetii* in Placentas from Ruminant Abortions'. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 115 (4): 347–53. https://doi.org/10.1111/j.1600-0463.2007.apm_591.x.

Jiménez-Ruiz, Elena, Gema Alvarez-García, Adriana Aguado-Martínez, Hesham Salman, Juan M. Irache, Virginia Marugán-Hernández, and Luis M. Ortega-Mora. 2012. 'Low Efficacy of NcGRA7, NcSAG4, NcBSR4 and NcSRS9 Formulated in Poly-ε-Caprolactone against *Neospora Caninum* Infection in Mice'. *Vaccine* 30 (33): 4983–92. https://doi.org/10.1016/j.vaccine.2012.05.033.

Jittapalapong, S, A Sangwaranond, T Inpankaew, C Phasuk, N Pinyopanuwat, W Chimnoi, C Kengradomkij, P Arunwipat, and S Maruyama. 2008. 'Seroprevalence of *Toxoplasma Gondii* Infection in dairy cows in Northeastern Thailand'. *southeast asian j trop med public health* 39: 5.

Johanna F. Lindahl, Jatinder Paul Singh Gill, , Razibuddin Ahmed Hazarika, Nadeem Mohamed Fairoze, , Jasbir S. Bedi, , Ian Dohoo, , Abhimanyu Singh Chauhan, Delia Grace, and Manish Kakkar. 2019. 'Risk Factors for *Brucella* Seroprevalence in Peri-Urban Dairy Farms in Five Indian Cities'. *Trop. Med. Infect. Dis.* 4 (70). https://doi.org/10.3390/tropicalmed4020070.

Johansen, Maria Vang, Susan Christina Welburn, Pierre Dorny, and Norbert W. Brattig. 2017. 'Control of Neglected Zoonotic Diseases'. *Acta Tropica* 165 (January): 1–2. https://doi.org/10.1016/j.actatropica.2016.11.036.

John Matthews. 2016. 'Diseases of the Goat'. In *Diseases of the Goat*, 18–34. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119073543.ch2.

Jones, Bryony A., Delia Grace, Richard Kock, Silvia Alonso, Jonathan Rushton, Mohammed Y. Said, Declan McKeever, et al. 2013. 'Zoonosis Emergence Linked to Agricultural Intensification and Environmental Change'. *Proceedings of the National Academy of Sciences of the United States of America* 110 (21): 8399–8404. https://doi.org/10.1073/pnas.1208059110.

Jones, Rachael M., Mark Nicas, Alan E. Hubbard, and Arthur L. Reingold. 2016. 'The Infectious Dose of *Coxiella Burnetii* (Q Fever)': *Applied Biosafety*, June. https://doi.org/10.1177/153567600601100106.

Jorritsma, Ruurd, Theo Wensing, Theo A. M. Kruip, Peter L. A. M. Vos, and Jos P. T. M. Noordhuizen. 2003. 'Metabolic Changes in Early Lactation and Impaired Reproductive Performance in Dairy Cows'. *Veterinary Research* 34 (1): 11–26. https://doi.org/10.1051/vetres:2002054.

Jose M. Díaz-Cao, Alberto Prieto, Gonzalo López-Lorenzo, Pablo Díaz-Fernández, Ceferino López-Sández, Patrocinio Morrondo, and Gonzalo Fernández-Rodríguez. 2018. 'Short Communication: Broadening the Diagnosis Panel of Reproductive Pathogens Associated with Abortion in Ruminants'. *Spanish Journal of Agricultural Research* 16 (2): 05–01. https://doi.org/10.5424/sjar/2018162-12180.

Jozef Laureyns. 2014. *Challenges in the Control of Bovine Viral Diarrhoea Virus – Implications for a Belgian Eradication Programme*. PhD Thesis. Ghent University.

J.P. Dubey, A. Hemphill, R. Calero-Bernal, and G. Schares. 2017. 'Neosporosis in Animals, (2017)',. *CRC Press, NY* 1st Edition: 530.

Justo, Riciely Vanessa, Janaina Bacon Manfio, Juliana Arena Galhardo, João Luis Garcia, and Artur Kanadani Campos. 2013. 'Seroepidemiological Inquiry on Bovine Neosporosis in Northern Mato Grosso State, Brazil'. *Semina: Ciências Agrárias* 34 (6Supl2): 3897–3902. https://doi.org/10.5433/1679-0359.2013v34n6Supl2p3897.

Kaaboub, El Aid, Nassim Ouchene, Nadjet Amina Ouchene-Khelifi, and Djamel Khelef. 2019. 'Serological and Histopathological Investigation of Brucellosis in Cattle in Medea Region, Northern Algeria'. *Veterinary World* 12 (5): 713–18. https://doi.org/10.14202/vetworld.2019.713-718.

Kadi SA, Djellal F, and Berchiche M. 2007. 'Characterization of Feeding Practices of the Dairy Cows in Tizi-Ouzou Area, Algeria. 2007; 19(4)'; *Livest Res Rural Dev* 19 (4). https://www.researchgate.net/publication/259008731_Characterization_of_feeding_practices_of_the_dairy_cows_in_Tizi-Ouzou_area_Algeria.

Kadir, Yeşilbağ, Förster Christine, Bank-Wolf Barbara, Yılmaz Zeki, Alkan Feray, Ozkul Aykut, Burgu Ibrahim, Rosales Sibilina Cedillo, Thiel Heinz-Jürgen, and König Matthias. 2008. 'Genetic Heterogeneity of Bovine Viral Diarrhoea Virus (BVDV) Isolates from Turkey: Identification of a New Subgroup in BVDV-1'. *Veterinary Microbiology* 130 (3): 258–67. https://doi.org/10.1016/j.vetmic.2008.01.016.

Kadzere, C. T, M. R Murphy, N Silanikove, and E Maltz. 2002. 'Heat Stress in Lactating Dairy Cows: A Review'. *Livestock Production Science* 77 (1): 59–91. https://doi.org/10.1016/S0301-6226(01)00330-X.

Kaiser, V., L. Nebel, G. Schüpbach-Regula, R. G. Zanoni, and M. Schweizer. 2017. 'Influence of Border Disease Virus (BDV) on Serological Surveillance within the Bovine Virus Diarrhea (BVD) Eradication Program in Switzerland'. *BMC Veterinary Research* 13 (January). https://doi.org/10.1186/s12917-016-0932-0.

Kalantari, A.S., Mehrabani-Yeganeh, and H., Moradi M. 2008. 'Evaluation of Economic Losses Due to Abortion and Decision Making Process for Culling Aborted Cow.' Master thesis in Animal Science, Iran: University of Tehran,.

Kali S, Benidir M, Ait Kaci K, Belkheir B, and Benyoucef MT. 2011. 'Situation de La Filière Lait En Algérie : Approche Analytique d'amont En Aval. 23 (08).' *Livestock Research for Rural Development* 22 (2).

Kaltenboeck, B., H. -R. Hehnen, and A. Vaglenov. 2005. 'Bovine Chlamydophila Spp. Infection: Do We Underestimate the Impact on Fertility?' *Veterinary Research Communications* 29 (1): 1–15. https://doi.org/10.1007/s11259-005-0832-4.

Kampa, Jaruwan, Stefan Alenius, Ulf Emanuelson, Aran Chanlun, and Suneerat Aiumlamai. 2009. 'Bovine Herpesvirus Type 1 (BHV-1) and Bovine Viral Diarrhoea Virus (BVDV) Infections in Dairy Herds: Self Clearance and the Detection of Seroconversions against a New Atypical Pestivirus'. *Veterinary Journal (London, England: 1997)* 182 (2): 223–30. https://doi.org/10.1016/j.tvjl.2008.07.006.

Kardjadj, M. 2016. 'The Epidemiology of Human and Animal Brucellosis in Algeria'. *Journal of Bacteriology and Mycology* 3 (2): 1025.

Kardjadj, Moustafa. 2017. 'Did Rev-1 Small Ruminants Vaccination Helped Improve Cattle Brucellosis Prevalence Status in Algeria?' *Tropical Animal Health and Production* 49 (8): 1783–85. https://doi.org/10.1007/s11250-017-1370-0.

Kardjadj, Moustafa. 2018. 'The Epidemiology of Cattle Abortion in Algeria'. *Tropical Animal Health and Production* 50 (2): 445–48. https://doi.org/10.1007/s11250-017-1430-5.

Karlsen, F., H. B. Steen, and J. M. Nesland. 1995. 'SYBR Green I DNA Staining Increases the Detection Sensitivity of Viruses by Polymerase Chain Reaction'. *Journal of Virological Methods* 55 (1): 153–56. https://doi.org/10.1016/0166-0934(95)00053-w.

Kato, Kentaro. 2018. 'How Does *Toxoplama Gondii* Invade Host Cells?' *Journal of Veterinary Medical Science* advpub. https://doi.org/10.1292/jvms.18-0344.

Kent A. Weigel, Kent A. 2006. 'Prospects for Improving Reproductive Performance through Genetic Selection'. *Animal Reproduction Science*, Special Issue: Nutrition and Fertility in Dairy Cattle, 96 (3): 323–30. https://doi.org/10.1016/j.anireprosci.2006.08.010.

Keyserlingk, M. A. G von, and M. J. Hötzel. 2015. 'The Ticking Clock: Addressing Farm Animal Welfare in Emerging Countries.' *J. Agric. Environ. Ethics* 28: 179–95.

Khaled, H., K. Sidi-Boumedine, S. Merdja, P. Dufour, A. Dahmani, R. Thiéry, E. Rousset, and A. Bouyoucef. 2016. 'Serological and Molecular Evidence of Q Fever among Small Ruminant Flocks in Algeria'. *Comparative Immunology, Microbiology and Infectious Diseases* 47 (August): 19–25. https://doi.org/10.1016/j.cimid.2016.05.002.

Khalili, Mohammad, Ehsanollah Sakhaee, and Homayoon Babaei. 2012. 'Frequency of Anti-*Coxiella Burnetii* Antibodies in Cattle with Reproductive Disorders'. *Comparative Clinical Pathology* 21 (5): 917–19.

Khamassi Khbou, Médiha, Samaher Htira, Kaouther Harabech, and M'hammed Benzarti. 2017. 'First Case-Control Study of Zoonotic Brucellosis in Gafsa District, Southwest Tunisia'. *One Health* 5 (December): 21–26. https://doi.org/10.1016/j.onehlt.2017.12.001.

Khames, Mammar, Virginie Mick, M. Jesús de Miguel, Guillaume Girault, Raquel Conde-Álvarez, Djamel Khelef, Mustapha Oumouna, Ignacio Moriyón, Pilar M. Muñoz, and Amaia Zúñiga-Ripa. 2017. 'The Characterization of *Brucella* Strains Isolated from Cattle in Algeria Reveals the Existence of a *B. Abortus* Lineage Distinct from European and Sub-Saharan Africa Strains'. *Veterinary Microbiology* 211 (November): 124–28. https://doi.org/10.1016/j.vetmic.2017.10.008.

Khames, Mammar, Feriel Yekkour, Celia Fernández-Rubio, Dominique Aubert, Paul Nguewa, and Isabelle Villena. 2018. 'Serological Survey of Cattle Toxoplasmosis in Medea, Algeria'. *Veterinary Parasitology: Regional Studies and Reports* 12 (May): 89–90. https://doi.org/10.1016/j.vprsr.2018.02.009.

Khames, Mammar, Feriel Yekkour, CeliaFernández Rubio, Dominique Aubert, Paul Nguewa, and Isabelledubey Villena. 2018. 'Serological Survey of Cattle Toxoplasmosis in Medea, Algeria'. *Veterinary Parasitology: Regional Studies and Reports* 12 (May): 89–90. https://doi.org/10.1016/j.vprsr.2018.02.009.

Khan, Muhammad Zahoor, and Muhammad Zahoor. 2018. 'An Overview of Brucellosis in Cattle and Humans, and Its Serological and Molecular Diagnosis in Control Strategies'. *Tropical Medicine and Infectious Disease* 3 (2). https://doi.org/10.3390/tropicalmed3020065.

Khatima, hocine, and Mohamed-Cherif Abdellah. 2015. 'Sero-Epidemiological Survey of Toxoplasmosis in Cattle, Sheep and Goats in Algeria'. *Journal of Bacteriology & Parasitology*, August. https://doi.org/10.4172/2155-9597.S1.014.

Kherzat Bahidja. 2006. 'Essai d'évaluation de La Politique Laitière En Perspective de l'adhésion de l'Algérie à l'Organisation Mondiale Du Commerce et à La Zone de Libre Echange Avec l'Union Européenne.' Magister en sciences agronomiques, Institut National Agronomique - ELHARRACH: Institut National Agronomique -ELHARRACH.

Kim, Sung G., Renee R. Anderson, Jin Z. Yu, Nancy C. Zylich, Hailu Kinde, Suzanne Carman, Daniela Bedenice, and Edward J. Dubovi. 2009. 'Genotyping and Phylogenetic Analysis of Bovine Viral Diarrhea Virus Isolates from BVDV Infected Alpacas in North America'. *Veterinary Microbiology* 136 (3–4): 209–16. https://doi.org/10.1016/j.vetmic.2008.10.029.

Kirat, S. 2007. *Les Conditions d'émergence d'un Système d'élevage Spécialisé En Engraissement et Ses Conséquences Sur La Redynamisation de l'exploitation Agricole et La Filière Des Viandes Rouges Bovines : Cas de La Wilaya de Jijel En Algérie*. Master of Science. Montpellier: CIHEAM-IAMM. http://www.iamm.fr/ressources/opac_css/doc_num.php?explnum_id=4370.

Kirk, J. H. 2003. 'Infectious Abortions in Dairy Cows.' *Vet. Med. Ext. Fact Sheet, Univ. of California, Davis.* http://www.vetmed.ucdavis.edu/vetext/INF-DA/Abortion.pdf.

Kirk, Martyn D., Sara M. Pires, Robert E. Black, Marisa Caipo, John A. Crump, Brecht Devleesschauwer, Dörte Döpfer, et al. 2015. 'World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases,

2010: A Data Synthesis'. *PLOS Medicine* 12 (12): e1001921. https://doi.org/10.1371/journal.pmed.1001921.

Klemmer, Jessica, John Njeru, Aya Emam, Ahmed El-Sayed, Amira A. Moawad, Klaus Henning, Mohamed A. Elbeskawy, et al. 2018. 'Q Fever in Egypt: Epidemiological Survey of *Coxiella Burnetii* Specific Antibodies in Cattle, Buffaloes, Sheep, Goats and Camels'. *PLOS ONE* 13 (2): e0192188. https://doi.org/10.1371/journal.pone.0192188.

Knobel, Darryn L., Alice N. Maina, Sally J. Cutler, Eric Ogola, Daniel R. Feikin, Muthoni Junghae, Jo E. B. Halliday, et al. 2013. '*Coxiella Burnetii* in Humans, Domestic Ruminants, and Ticks in Rural Western Kenya'. *The American Journal of Tropical Medicine and Hygiene* 88 (3): 513–18. https://doi.org/10.4269/ajtmh.12-0169.

Koestner, A., and C. R. Cole. 1961. 'Neuropathology of Ovine and Bovine Toxoplasmosis'. *American Journal of Veterinary Research* 22 (January): 53–66.

Koutinhouin. B, Youssao. A.K.I., Houehou. A.E., and Agbadje. P.M. 2003. 'Prévalence de la brucellose bovine dans les élevages traditionnels encadrés par le Projet pour le Développement de l'Elevage (PDE) au Bénin'. *Revue Méd. Vét.* 154 (4): 271–76.

Kovácová, E., J. Kazár, and A. Simková. 1998. 'Clinical and Serological Analysis of a Q Fever Outbreak in Western Slovakia with Four-Year Follow-Up'. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 17 (12): 867–69. https://doi.org/10.1007/s100960050209.

Kraus, Thomas A., Stephanie M. Engel, Rhoda S. Sperling, Lisa Kellerman, Yungtai Lo, Sylvan Wallenstein, Maria M. Escribese, et al. 2012. 'Characterizing the Pregnancy Immune Phenotype: Results of the Viral Immunity and Pregnancy (VIP) Study'. *Journal of Clinical Immunology* 32 (2): 300–311. https://doi.org/10.1007/s10875-011-9627-2.

Kul, Oğuz, Nalan Kabakci, Kader Yildiz, Naci Öcal, Hakan Kalender, and N. Aycan İlkme. 2009. '*Neospora Caninum* Associated with Epidemic Abortions in Dairy Cattle: The First Clinical Neosporosis Report in Turkey'. *Veterinary Parasitology* 159 (1): 69–72. https://doi.org/10.1016/j.vetpar.2008.10.019.

Kumar, Subbiah Krishna, K. M. Palanivel, K. Sukumar, B. Samuel Masilamoni Ronald, G. Selvaraju, and G. Ponnudurai. 2018. 'Herd-Level Risk Factors for Bovine Viral Diarrhea Infection in Cattle of Tamil Nadu'. *Tropical Animal Health and Production* 50 (4): 793–99. https://doi.org/10.1007/s11250-017-1497-z.

Lacheheb, A., and D. Raoult. 2009. 'Seroprevalence of Q-Fever in Algeria'. *Clinical Microbiology and Infection* 15 (December): 167–68. https://doi.org/10.1111/j.1469-0691.2008.02211.x.

Lachkhem, Arwa, Darine Slama, Wahiba Sakly, Najoua Haouas, Mohamed Gorcii, Alex, er W. Pfaff, et al. 2015. 'Prevalence of Toxoplasmosis in Sheep, Goats and Cattle in Southern Tunisia'. *Journal of Bacteriology & Parasitology* 6 (5): 1–4. https://doi.org/10.4172/2155-9597.1000245.

Lamming, G. E., D. C. Wathes, and A. R. Peters. 1981. 'Endocrine Patterns of the Post-Partum Cow'. *Journal of Reproduction and Fertility. Supplement* 30: 155–70.

Lanave, G., N. Decaro, M. S. Lucente, A. Guercio, N. Cavaliere, G. Purpari, I. Padalino, et al. 2017. 'Circulation of Multiple Subtypes of Bovine Viral Diarrhoea Virus Type 1 with No Evidence for HoBi-like Pestivirus in Cattle Herds of Southern Italy'. *Infection, Genetics and Evolution* 50 (June): 1–6. https://doi.org/10.1016/j.meegid.2017.02.009.

Lee, K. M., and J. H. Gillespie. 1957. 'Propagation of Virus Diarrhea Virus of Cattle in Tissue Culture'. *American Journal of Veterinary Research* 18 (69): 952–53.

Lefkaditis, M., R. Mpairamoglou, A. Sossidou, K. Spanoudis, and M. Tsakiroglou. 2020. '*Neospora Caninum*, A Potential Cause of Reproductive Failure in Dairy Cows from Northern Greece'. *Veterinary Parasitology: Regional Studies and Reports* 19 (January): 100365. https://doi.org/10.1016/j.vprsr.2019.100365.

Lefkowitz, Elliot J, Donald M Dempsey, Robert Curtis Hendrickson, Richard J Orton, Stuart G Siddell, and Donald B Smith. 2018. 'Virus Taxonomy: The Database of the International Committee on Taxonomy of Viruses (ICTV)'. *Nucleic Acids Research* 46 (D1): D708–17. https://doi.org/10.1093/nar/gkx932.

Levin, Kate Ann. 2006. 'Study Design III: Cross-Sectional Studies'. *Evidence-Based Dentistry* 7 (1): 24–25. https://doi.org/10.1038/sj.ebd.6400375.

Li, Zhaocai, Ping Liu, Xiaoan Cao, Zhongzi Lou, Kinga Zaręba-Marchewka, Monika Szymańska-Czerwińska, Krzysztof Niemczuk, Bo Hu, Xue Bai, and Jizhang Zhou. 2018. 'First Report of *Chlamydia Abortus* in Farmed Fur Animals'. Research Article. BioMed Research International. Hindawi. 26 November 2018. https://doi.org/10.1155/2018/4289648.

Løken, T., J. Krogsrud, and I. Bjerkås. 1991. 'Outbreaks of Border Disease in Goats Induced by a Pestivirus-Contaminated Orf Vaccine, with Virus Transmission to Sheep and Cattle'. *Journal of Comparative Pathology* 104 (2): 195–209. https://doi.org/10.1016/S0021-9975(08)80103-X.

Lopez-Gatius, F., P. Santolaria, J. L. Yaniz, J. M. Garbayo, and S. Almeria. 2005. 'The Use of Beef Bull Semen Reduced the Risk of Abortion in Neospora-Seropositive Dairy Cows'. *Journal of Veterinary Medicine Series B* 52 (2): 88–92. https://doi.org/10.1111/j.1439-0450.2004.00818.x.

Lounes, Nedjma, Moulay-Ali Cherfa, Gilles Le Carrou, Abdellah Bouyoucef, Maryne Jay, Bruno Garin-Bastuji, and Virginie Mick. 2014. 'Human Brucellosis in Maghreb: Existence of a Lineage Related to Socio-Historical Connections with Europe'. *PloS One* 9 (12): e115319. https://doi.org/10.1371/journal.pone.0115319.

Lucchese, Laura, Abdelali Benkirane, Imane Hakimi, Ahmed El Idrissi, and Alda Natale. 2016. 'Seroprevalence Study of the Main Causes of Abortion in Dairy Cattle in Morocco'. *Veterinaria Italiana* 52 (1): 13–19. https://doi.org/10.12834/VetIt.388.1813.1.

Luzzago, Camilla, Stefania Lauzi, Erika Ebranati, Monica Giammarioli, Ana Moreno, Vincenza Cannella, Loretta Masoero, et al. 2014. 'Extended Genetic Diversity of Bovine Viral Diarrhea Virus and Frequency of Genotypes and Subtypes in Cattle in Italy between 1995 and 2013'. *BioMed Research International* 2014. https://doi.org/10.1155/2014/147145.

Maass, M., J. Gieffers, E. Krause, P. M. Engel, C. Bartels, and W. Solbach. 1998. 'Poor Correlation between Microimmunofluorescence Serology and Polymerase Chain Reaction for Detection of Vascular *Chlamydia Pneumoniae* Infection in Coronary Artery Disease Patients'. *Medical Microbiology and Immunology* 187 (2): 103–6. https://doi.org/10.1007/s004300050080.

Madani T, Yakhlef H, and Marie M. 2008. 'Effect of Age at First Calving on Lactation and Reproduction of Dairy Cows Reared in Semi Arid Region of Algeria.' *Retrieved January 10, 2020* 20 (6): 92.

MADR. 2013. 'Statistiques Agricoles, Superficies et Production, Séries A et B 2013; 2014:2015.' 2014:2015 2013.

MADR. 2017a. 'Lancement de la campagne de vaccination contre la Fièvre aphteuse et la Rage –'. Ministery of Agriculture and Rural Develpment of Algeria. 2017a. http://madrp.gov.dz/lancement-de-la-campagne-de-vaccination-contre-la-fievre-aphteuse-et-la-rage/.

MADR. 2017b. 'Statistiques Agricoles, ministere algerien de l'agriculture et du developpement rural'. Ministery of Agriculture and Rural Develpment of Algeria. 2017b. http://madrp.gov.dz/agriculture/statistiques-agricoles/.

Mahin, L., M. Chadli, J. Briouga, M. Hamidi, and G. Wellemans. 1982. 'Clinical Bovine Virus Diarrhoea in Morocco. First Report'. *Zentralblatt Fur Veterinarmedizin. Reihe B. Journal of Veterinary Medicine. Series B* 29 (10): 789–93. https://doi.org/10.1111/j.1439-0450.1982.tb01199.x.

Mahin, L., G. Wellemans, and A. Shimi. 1985. 'Prevalence of Antibodies to Bovid Herpesvirus 1 (IBR-IPV), Bovine Virus Diarrhoea, Bovine Respiratory Syncytial Parainfluenza 3, Adeno A and

Adeno B Viruses in Indigenous and Imported Moroccan Cattle'. *Annales De Recherches Veterinaires. Annals of Veterinary Research* 16 (3): 279–83.

Mainar-Jaime, R. C, B Berzal-Herranz, P Arias, and F. A Rojo-Vázquez. 2001. 'Epidemiological Pattern and Risk Factors Associated with Bovine Viral-Diarrhoea Virus (BVDV) Infection in a Non-Vaccinated Dairy-Cattle Population from the Asturias Region of Spain'. *Preventive Veterinary Medicine* 52 (1): 63–73. https://doi.org/10.1016/S0167-5877(01)00239-2.

Maley, S. W., D. Buxton, C. N. Macaldowie, I. E. Anderson, S. E. Wright, P. M. Bartley, I. Esteban-Redondo, C. M. Hamilton, A. K. Storset, and E. A. Innes. 2006. 'Characterization of the Immune Response in the Placenta of Cattle Experimentally Infected with *Neospora Caninum* in Early Gestation'. *Journal of Comparative Pathology* 135 (2–3): 130–41. https://doi.org/10.1016/j.jcpa.2006.07.001.

Markusfeld-Nir, O. 1997. 'Epidemiology of Bovine Abortions in Israeli Dairy Herds'. *Preventive Veterinary Medicine* 31 (3–4): 245–55. https://doi.org/10.1016/s0167-5877(96)01142-7.

Martins, Adriano Augusto, Thiago de Oliveira Zamprogna, Thays Mizuki Lucas, Ivo Alexandre Leme da Cunha, João Luis Garcia, and Aristeu Vieira da Silva. 2013. 'FREQUENCY AND RISK FACTORS FOR INFECTION BY *Neospora Caninum* IN DAIRY FARMS OF UMUARAMA, PR, BRAZIL'. *Arquivos de Ciências Veterinárias e Zoologia Da UNIPAR* 15 (2). http://revistas.unipar.br/index.php/veterinaria/article/view/4217.

Masala, Giovanna, Rosaura Porcu, Giovanna Sanna, Giovanna Chessa, Grazia Cillara, Valentina Chisu, and Sebastiana Tola. 2004. 'Occurrence, Distribution, and Role in Abortion of *Coxiella Burnetii* in Sheep and Goats in Sardinia, Italy'. *Veterinary Microbiology* 99 (3–4): 301–5. https://doi.org/10.1016/j.vetmic.2004.01.006.

Mauger, Guillaume, Yoram Bauman, Tamilee Nennich, and Eric Salathé. 2015. 'Impacts of Climate Change on Milk Production in the United States'. *The Professional Geographer* 67 (1): 121–31. https://doi.org/10.1080/00330124.2014.921017.

Maurin, M., and D. Raoult. 1999. 'Q Fever'. *Clinical Microbiology Reviews* 12 (4): 518–53. https://doi.org/10.1128/CMR.12.4.518.

Maxam, A. M., and W. Gilbert. 1977. 'A New Method for Sequencing DNA'. *Proceedings of the National Academy of Sciences of the United States of America* 74 (2): 560–64. https://doi.org/10.1073/pnas.74.2.560.

Mayer, Nathalie. 2011. 'Effet Peltier'. Futura. 2011. https://www.futura-sciences.com/sciences/definitions/physique-effet-peltier-16151/.

Mazeri, Stella, Francesca Scolamacchia, Ian G. Handel, Kenton L. Morgan, Vincent N. Tanya, and Barend M. deC. Bronsvoort. 2013. 'Risk Factor Analysis for Antibodies to *Brucella, Leptospira and C. Burnetii* among Cattle in the Adamawa Region of Cameroon: A Cross-Sectional Study'. *Tropical Animal Health and Production* 45 (2): 617–23. https://doi.org/10.1007/s11250-012-0268-0.

Mazuz, Monica L., Leah Fish, Dror Reznikov, Ricardo Wolkomirsky, Benjamin Leibovitz, Igor Savitzky, Jacob Golenser, and Varda Shkap. 2014. 'Neosporosis in Naturally Infected Pregnant Dairy Cattle'. *Veterinary Parasitology* 205 (1–2): 85–91. https://doi.org/10.1016/j.vetpar.2014.06.009.

McAllister, Milton M, J.P Dubey, David S Lindsay, William R Jolley, Rebecca A Wills, and Angela M McGuire. 1998. 'Dogs Are Definitive Hosts of *Neospora Caninum*'. *International Journal for Parasitology* 28 (September): 1473–79. https://doi.org/10.1016/s0020-7519(98)00138-6.

McAloon, Conor G., Paul Whyte, Simon J. More, Martin J. Green, Luke O'Grady, AnaBelen Garcia, and Michael L. Doherty. 2016. 'The Effect of Paratuberculosis on Milk Yield—A Systematic Review and Meta-Analysis'. *Journal of Dairy Science* 99 (2): 1449–60. https://doi.org/10.3168/jds.2015-10156.

McArt, J. a. A., D. V. Nydam, and G. R. Oetzel. 2012. 'Epidemiology of Subclinical Ketosis in Early Lactation Dairy Cattle'. *Journal of Dairy Science* 95 (9): 5056–66. https://doi.org/10.3168/jds.2012-5443.

McCaughey, C., L. J. Murray, J. P. McKenna, F. D. Menzies, S. J. McCullough, H. J. O'Neill, D. E. Wyatt, C. R. Cardwell, and P. V. Coyle. 2010. '*Coxiella Burnetii* (Q Fever) Seroprevalence in Cattle'. *Epidemiology and Infection* 138 (1): 21–27. https://doi.org/10.1017/S0950268809002854.

McCauley, L. M. E., M. J. Lancaster, K. L. Butler, and C. G. V. Ainsworth. 2010. 'Serological Analysis of Chlamydophila Abortus in Australian Sheep and Implications for the Rejection of Breeder Sheep for Export'. *Australian Veterinary Journal* 88 (1–2): 32–38. https://doi.org/10.1111/j.1751-0813.2009.00536.x.

McDermott, J., D. Grace, and J. Zinsstag. 2013. 'Economics of Brucellosis Impact and Control in Low-Income Countries'. *Revue Scientifique Et Technique (International Office of Epizootics)* 32 (1): 249–61. https://doi.org/10.20506/rst.32.1.2197.

McDermott, John J, and S. M Arimi. 2002. 'Brucellosis in Sub-Saharan Africa: Epidemiology, Control and Impact'. *Veterinary Microbiology*, Brucellosis S.I., 90 (1): 111–34. https://doi.org/10.1016/S0378-1135(02)00249-3.

McDonald, W. L., R. Jamaludin, G. Mackereth, M. Hansen, S. Humphrey, P. Short, T. Taylor, et al. 2006. 'Characterization of a *Brucella Sp*. Strain as a Marine-Mammal Type despite Isolation from a Patient with Spinal Osteomyelitis in New Zealand'. *Journal of Clinical Microbiology* 44 (12): 4363–70. https://doi.org/10.1128/JCM.00680-06.

McGiven, J. A., J. D. Tucker, L. L. Perrett, J. A. Stack, S. D. Brew, and A. P. MacMillan. 2003. 'Validation of FPA and CELISA for the Detection of Antibodies to *Brucella Abortus* in Cattle Sera and Comparison to SAT, CFT, and IELISA'. *Journal of Immunological Methods* 278 (1): 171–78. https://doi.org/10.1016/S0022-1759(03)00201-1.

Megersa, Bekele, Demelash Biffa, Fekadu Niguse, Tesfaye Rufael, Kassahun Asmare, and Eystein Skjerve. 2011. 'Cattle Brucellosis in Traditional Livestock Husbandry Practice in Southern and Eastern Ethiopia, and Its Zoonotic Implication'. *Acta Veterinaria Scandinavica* 53 (1): 24. https://doi.org/10.1186/1751-0147-53-24.

Merdja, Salah Eddine, Hamza Khaled, Ali Dahmani, and Abdallah Bouyoucef. 2015. 'Chlamydial Abortion in Algerian Small Ruminants'. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine* 72 (1): 23–26. https://doi.org/10.15835/buasvmcn-vm:10283.

Meriem Essayagh, Touria Essayagh, Khalid Khallayoune, Sanah Essayagh, and Badreeddine Lmimouni. 2017. 'Seroprevalence of *Toxoplasma Gondii* in Ruminants in Morocco'. *Veterinary Research* 10: 1–5. https://doi.org/10.36478/vr.2017.1.5.

Messier, V., B. Lévesque, J.-F. Proulx, L. Rochette, B. Serhir, M. Couillard, B. J. Ward, M. D. Libman, E. Dewailly, and S. Déry. 2012. 'Seroprevalence of Seven Zoonotic Infections in Nunavik, Quebec (Canada)'. *Zoonoses and Public Health* 59 (2): 107–17. https://doi.org/10.1111/j.1863-2378.2011.01424.x.

Mick, Virginie, Gilles Le Carrou, Yannick Corde, Yvette Game, Maryne Jay, and Bruno Garin-Bastuji. 2014. '*Brucella Melitensis* in France: Persistence in Wildlife and Probable Spillover from Alpine Ibex to Domestic Animals'. *PloS One* 9 (4): e94168. https://doi.org/10.1371/journal.pone.0094168.

Ministère du Commerce International. 2019. 'Les Echanges Extérieurs de l'Algérie Au Cours de l'année 2018'. *Ministere Du Commerce Internationnal* (blog). 2019. http://www.mae.gov.dz/LesEchangesextrieursdelAlgrie-au-cours-du-1er-semestre--2013.aspx.

Mockeliūniene, Violeta, Algirdas Salomskas, Raimundas Mockeliūnas, and Saulius Petkevicius. 2004. 'Prevalence and Epidemiological Features of Bovine Viral Diarrhoea Virus Infection in

Lithuania'. *Veterinary Microbiology* 99 (1): 51–57. https://doi.org/10.1016/j.vetmic.2003.11.008.

Moeller, Robert B. 2011. 'Disorders of Sheep and Goats'. In *Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals*, 49–87. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119949053.ch3.

Moennig, Volker, Hans Houe, and Ann Lindberg. 2005. 'BVD Control in Europe: Current Status and Perspectives'. *Animal Health Research Reviews* 6 (1): 63–74. https://doi.org/10.1079/ahr2005102.

Mohammad, Alavi Seyed, and Motlagh Mohammad Esmaeil. 2012. 'A REVIEW OF EPIDEMIOLOGY, DIAGNOSIS AND MANAGEMENT OF BRUCELLOSIS FOR GENERAL PHYSICIANS WORKING IN THE IRANIAN HEALTH NETWORK' 5 (216): 384–87.

Mohammed, Farouk U., Salisu Ibrahim, Ikwe Ajogi, and Bale J. O. Olaniyi. 2011. 'Prevalence of Bovine Brucellosis and Risk Factors Assessment in Cattle Herds in Jigawa State'. Research Article. ISRN Veterinary Science. Hindawi. 27 December 2011. https://doi.org/10.5402/2011/132897.

Moncada, Pablo A., and Jose G. Montoya. 2012. 'Toxoplasmosis in the Fetus and Newborn: An Update on Prevalence, Diagnosis and Treatment'. *Expert Review of Anti-Infective Therapy* 10 (7): 815–28. https://doi.org/10.1586/eri.12.58.

Monteiro, Francielle Liz, Bruno Martins, Juliana F. Cargnelutti, Jessica G. Noll, Rudi Weiblen, and Eduardo F. Flores. 2019. 'Genetic Identification of Pestiviruses from Beef Cattle in Southern Brazil'. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology]* 50 (2): 557–63. https://doi.org/10.1007/s42770-019-00058-6.

Mostert, P. F., E. a. M. Bokkers, C. E. van Middelaar, H. Hogeveen, and I. J. M. de Boer. 2018. 'Estimating the Economic Impact of Subclinical Ketosis in Dairy Cattle Using a Dynamic Stochastic Simulation Model'. *Animal: An International Journal of Animal Bioscience* 12 (1): 145–54. https://doi.org/10.1017/S1751731117001306.

Mouffok Charef-Eddine. 2014. *Performance de l'élevage bovin dans la région semi aride. Approche globale : Systémique, zootechnique et métabolique. PhD Thesis*. PhD Thesis. Université Ferhat Abbas Sétif 1 Faculté des Sciences de la Nature et de la Vie. http://dspace.univ-setif.dz:8888/jspui/handle/123456789/1082.

Mouffok, Charef-Eddine. 2018. 'Performance de l'élevage Bovin Dans La Région Semi Aride : Approche Globale : Systémique Zootechnique et Métabolique'. Thesis. http://dspace.univ-setif.dz:8888/jspui/handle/123456789/1082.

Muflihanah, Hanah, Mochammad Hatta, Ente Rood, Pauline Scheelbeek, Theresia H. Abdoel, and Henk L. Smits. 2013. 'Brucellosis Seroprevalence in Bali Cattle with Reproductive Failure in South Sulawesi and *Brucella Abortus* Biovar 1 Genotypes in the Eastern Indonesian Archipelago'. *BMC Veterinary Research* 9 (1): 233. https://doi.org/10.1186/1746-6148-9-233.

Mukasa-Mugerwa, E., and International Livestock Centre for Africa. 1989. *A Review of a Reproductive Performance of Female Bos Indicus (Zebu) Cattle*. ILRI (aka ILCA and ILRAD).

Müller, N, V Zimmermann, B Hentrich, and B Gottstein. 1996. 'Diagnosis of *Neospora Caninum* and *Toxoplasma Gondii* Infection by PCR and DNA Hybridization Immunoassay.' *Journal of Clinical Microbiology* 34 (11): 2850–52.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. 'Specific Enzymatic Amplification of DNA in Vitro: The Polymerase Chain Reaction'. *Cold Spring Harbor Symposia on Quantitative Biology* 51 Pt 1: 263–73. https://doi.org/10.1101/sqb.1986.051.01.032.

Muma, J. B., K. L. Samui, J. Oloya, M. Munyeme, and E. Skjerve. 2007. 'Risk Factors for Brucellosis in Indigenous Cattle Reared in Livestock-Wildlife Interface Areas of Zambia'. *Preventive Veterinary Medicine* 80 (4): 306–17. https://doi.org/10.1016/j.prevetmed.2007.03.003.

Muma, J. B., K. L. Samui, V. M. Siamudaala, J. Oloya, G. Matope, M. K. Omer, M. Munyeme, C. Mubita, and E. Skjerve. 2006. 'Prevalence of Antibodies to *Brucella Spp*. and Individual Risk Factors of Infection in Traditional Cattle, Goats and Sheep Reared in Livestock–Wildlife Interface Areas of Zambia'. *Tropical Animal Health and Production* 38 (3): 195–206. https://doi.org/10.1007/s11250-006-4320-9.

Munday, B. L. 1978. 'Bovine Toxoplasmosis: Experimental Infections'. *International Journal for Parasitology* 8 (4): 285–88. https://doi.org/10.1016/0020-7519(78)90092-9.

Muñoz-Zanzi, Claudia A., Mark C. Thurmond, and Sharon K. Hietala. 2004. 'Effect of Bovine Viral Diarrhea Virus Infection on Fertility of Dairy Heifers'. *Theriogenology* 61 (6): 1085–99. https://doi.org/10.1016/j.theriogenology.2003.06.003.

Musallam, I. I., M. Abo-Shehada, M. Omar, and J. Guitian. 2015. 'Cross-Sectional Study of Brucellosis in Jordan: Prevalence, Risk Factors and Spatial Distribution in Small Ruminants and Cattle'. *Preventive Veterinary Medicine* 118 (4): 387–96. https://doi.org/10.1016/j.prevetmed.2014.12.020.

Muskens, J., E. van Engelen, C. van Maanen, C. Bartels, and T. J. G. M. Lam. 2011. 'Prevalence of *Coxiella Burnetii* Infection in Dutch Dairy Herds Based on Testing Bulk Tank Milk and Individual Samples by PCR and ELISA'. *The Veterinary Record* 168 (3): 79. https://doi.org/10.1136/vr.c6106.

Muskens, J., W. Wouda, T. von Bannisseht-Wijsmuller, and C. van Maanen. 2012. 'Prevalence of *Coxiella Burnetii* Infections in Aborted Fetuses and Stillborn Calves'. *The Veterinary Record* 170 (10): 260. https://doi.org/10.1136/vr.100378.

Neta, Alcina V. Carvalho, Juliana P. S. Mol, Mariana N. Xavier, Tatiane A. Paixão, Andrey P. Lage, and Renato L. Santos. 2010. 'Pathogenesis of Bovine Brucellosis'. *The Veterinary Journal* 184 (2): 146–55. https://doi.org/10.1016/j.tvjl.2009.04.010.

Nettleton, P. F., J. A. Gilray, P. Russo, and E. Dlissi. 1998. 'Border Disease of Sheep and Goats'. *Veterinary Research* 29 (3–4): 327–40.

Neuvians, T. P., D. Schams, B. Berisha, and M. W. Pfaffl. 2004. 'Involvement of Pro-Inflammatory Cytokines, Mediators of Inflammation, and Basic Fibroblast Growth Factor in Prostaglandin F2alpha-Induced Luteolysis in Bovine Corpus Luteum'. *Biology of Reproduction* 70 (2): 473–80. https://doi.org/10.1095/biolreprod.103.016154.

Newcomer, Benjamin W., Manuel F. Chamorro, and Paul H. Walz. 2017. 'Vaccination of Cattle against Bovine Viral Diarrhea Virus'. *Veterinary Microbiology*, Recent Advances in Vaccine Research Against Economically Important Viral Diseases of Food Animals, 206 (July): 78–83. https://doi.org/10.1016/j.vetmic.2017.04.003.

Nicoletti, P. 1980. 'The Epidemiology of Bovine Brucellosis'. *Advances in Veterinary Science and Comparative Medicine* 24: 69–98.

Nicoletti, Paul. 2002. 'A Short History of Brucellosis'. *Veterinary Microbiology*, Brucellosis S.I., 90 (1): 5–9. https://doi.org/10.1016/S0378-1135(02)00209-2.

Nielsen, K. H., L. Kelly, D. Gall, P. Nicoletti, and W. Kelly. 1995. 'Improved Competitive Enzyme Immunoassay for the Diagnosis of Bovine Brucellosis'. *Veterinary Immunology and Immunopathology* 46 (3–4): 285–91. https://doi.org/10.1016/0165-2427(94)05361-u.

Nielsen, Klaus. 2002. 'Diagnosis of Brucellosis by Serology'. *Veterinary Microbiology*, Brucellosis S.I., 90 (1): 447–59. https://doi.org/10.1016/S0378-1135(02)00229-8.

Niemczuk, Krzysztof, Monika Szymańska-Czerwińska, Krzysztof Śmietanka, and Łukasz Bocian. 2014. 'Comparison of Diagnostic Potential of Serological, Molecular and Cell Culture Methods for Detection of Q Fever in Ruminants'. *Veterinary Microbiology* 171 (1–2): 147–52. https://doi.org/10.1016/j.vetmic.2014.03.015.

Niskanen, R., A. Lindberg, and M. Tråvén. 2002. 'Failure to Spread Bovine Virus Diarrhoea Virus Infection from Primarily Infected Calves Despite Concurrent Infection with Bovine Coronavirus'. *The Veterinary Journal* 163 (3): 251–59. https://doi.org/10.1053/tvjl.2001.0657.

Nokhodian, Zary, Awat Feizi, Abdolreza Moradi, Majid Yaran, Shervin Ghaffari Hoseini, Behrooz Ataei, and Mohammad Hosseini. 2016. 'Detection and Risk Factors of *Coxiella Burnetii* Infection in Dairy Cattle Based on Bulk Tank Milk Samples in Center of Iran'. *Preventive Veterinary Medicine* 134 (November): 139–44. https://doi.org/10.1016/j.prevetmed.2016.10.003.

Norman, H. D., R. H. Miller, J. R. Wright, J. L. Hutchison, and K. M. Olson. 2012. 'Factors Associated with Frequency of Abortions Recorded through Dairy Herd Improvement Test Plans'. *Journal of Dairy Science* 95 (7): 4074–84. https://doi.org/10.3168/jds.2011-4998.

Norman, H. D., J. R. Wright, S. M. Hubbard, R. H. Miller, and J. L. Hutchison. 2009. 'Reproductive Status of Holstein and Jersey Cows in the United States'. *Journal of Dairy Science* 92 (7): 3517–28. https://doi.org/10.3168/jds.2008-1768.

Nusinovici, S., J. Frossling, S. Widgren, F. Beaudeau, and A. Lindberg. 2015. 'Q Fever Infection in Dairy Cattle Herds: Increased Risk with High Wind Speed and Low Precipitation'. *Epidemiology and Infection* 143 (15): 3316–26. https://doi.org/10.1017/S0950268814003926.

Obaidat, Mohammad, and Gilbert J. Kersh. 2017. 'Prevalence and Risk Factors of *Coxiella Burnetii* Antibodies in Bulk Milk from Cattle, Sheep, and Goats in Jordan'. *Journal of Food Protection* 80 (4): 561–66. https://doi.org/10.4315/0362-028X.JFP-16-377.

O'Connell, Catherine M., and Morgan E. Ferone. 2016. '*Chlamydia Trachomatis* Genital Infections'. *Microbial Cell (Graz, Austria)* 3 (9): 390–403. https://doi.org/10.15698/mic2016.09.525.

Ogugua, Akwoba Joseph, Victor Oluwatoyin Akinseye, Eniola O. Cadmus, Emmanuel A. Jolaoluwa Awosanya, Peter Ibukun Alabi, Olugbenga S. Idowu, Samson A. Akinade, et al. 2018. 'Prevalence and Risk Factors Associated with Bovine Brucellosis in Herds under Extensive Production System in Southwestern Nigeria'. *Tropical Animal Health and Production* 50 (7): 1573–82. https://doi.org/10.1007/s11250-018-1597-4.

OIE. 2008. *MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS (Mammals, Birds and Bees)*. Sixth Edition. Vol. 2. https://www.oie.int/doc/ged/D7709.PDF.

OIE. 2009. 'Bovine Brucellosis, in MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS'. *Office International Des Epizooties, Paris*, 2009, fiveth Edition edition.

OIE. 2019. 'OIE - World Organisation for Animal Health. Manual of Diagnostic Tests and Vaccinesfor Terrestrial Animals 2019.' 2019. https://www.oie.int/en/standard-setting/terrestrial-manual/access-online/.

OIE. 2020. 'World Organisation for Animal Health'. 2020. https://www.oie.int/fr/.

Olmo, Luisa, Michael P. Reichel, Sonevilay Nampanya, Syseng Khounsy, Lloyd C. Wahl, Bethanie A. Clark, Peter C. Thomson, Peter A. Windsor, and Russell D. Bush. 2019. 'Risk Factors for *Neospora Caninum*, Bovine Viral Diarrhoea Virus, and Leptospira Interrogans Serovar Hardjo Infection in Smallholder Cattle and Buffalo in Lao PDR'. *PLOS ONE* 14 (8): e0220335. https://doi.org/10.1371/journal.pone.0220335.

Oloffs, A. 1996. 'Bovine Brucellosis Investigations on Selected Dairy Farms in Rukungiri District and Mukono County, Uganda.' Diploma Thesis, Freie Universität, Fachbereich Veterinärmedizin, Berlin, Germany.

O'Neill, L. M., Á. O'Driscoll, and B. Markey. 2018. 'Comparison of Three Commercial Serological Tests for the Detection of *Chlamydia Abortus* Infection in Ewes'. *Irish Veterinary Journal* 71 (May). https://doi.org/10.1186/s13620-018-0124-2.

Opsteegh, Marieke, Peter Teunis, Lothar Züchner, Ad Koets, Merel Langelaar, and Joke van der Giessen. 2011. 'Low Predictive Value of Seroprevalence of *Toxoplasma Gondii* in Cattle for

Detection of Parasite DNA'. *International Journal for Parasitology* 41 (3–4): 343–54. https://doi.org/10.1016/j.ijpara.2010.10.006.

Ornoy, A., K. Crone, and G. Altshuler. 1976. 'Pathological Features of the Placenta in Fetal Death'. *Archives of Pathology & Laboratory Medicine* 100 (7): 367–71.

Ortega-Mora, Luis. 2007. *Protozoal Abortion in Farm Ruminants: Guidelines for Diagnosis and Control*. CABI.

Ortega-Mora, Luis Miguel, Ignacio Ferre, Itziar del-Pozo, Andrea Caetano-da-Silva, Esther Collantes-Fernández, Javier Regidor-Cerrillo, Carlos Ugarte-Garagalza, and Gorka Aduriz. 2003. 'Detection of *Neospora Caninum* in Semen of Bulls'. *Veterinary Parasitology* 117 (4): 301–8. https://doi.org/10.1016/j.vetpar.2003.09.015.

Oshiro, Leandra Marla, Ana Rita Coimbra Motta-Castro, Solange Zacalusni Freitas, Rodrigo Casquero Cunha, Rosangela Locatelli Dittrich, Andréa Christine Ferreira Meirelles, Renato Andreotti, et al. 2015. '*Neospora Caninum* and *Toxoplasma Gondii* Serodiagnosis in Human Immunodeficiency Virus Carriers'. *Revista Da Sociedade Brasileira de Medicina Tropical* 48 (5): 568–72. https://doi.org/10.1590/0037-8682-0151-2015.

Osman, K. M., H. A. Ali, J. A. ElJakee, and H. M. Galal. 2011. 'Chlamydophila Psittaci and Chlamydophila Pecorum Infections in Goats and Sheep in Egypt'. *Revue Scientifique Et Technique (International Office of Epizootics)* 30 (3): 939–48. https://doi.org/10.20506/rst.30.3.2088.

Ozbek, Ahmet, Elvan Ozbek, Yildiray Kalkan, Ahmet Temur, and Omer Faruk Küçükkalem. 2008. 'Can *Chlamydia trachomatis* human biovars cause abortion in cattle? An immunohistochemical study on a new host-pathogen relationship'. *Mikrobiyoloji Bulteni* 42 (4): 599–605.

Paiba, G. A., L. E. Green, G. Lloyd, D. Patel, and K. L. Morgan. 1999. 'Prevalence of Antibodies to *Coxiella Burnetii* (Q Fever) in Bulk Tank Milk in England and Wales'. *The Veterinary Record* 144 (19): 519–22. https://doi.org/10.1136/vr.144.19.519.

Pandey, G.S., Kobayashi, K., Nomura, Y., Nambota, A., Mwima, H.K., and Suzuki, A.K.,. 1999. 'Studies on Sero-Prevalence of Brucellosis in Kafue Lechwe (Kobus Lechwe Kafuensis) in Zambia.' *Ind. Vet. J.* 76: 275–78.

Pandit, Pranav, Thierry Hoch, Pauline Ezanno, François Beaudeau, and Elisabeta Vergu. 2016. 'Spread of *Coxiella Burnetii* between Dairy Cattle Herds in an Enzootic Region: Modelling Contributions of Airborne Transmission and Trade'. *Veterinary Research* 47 (1): 48. https://doi.org/10.1186/s13567-016-0330-4.

Pappas, Georgios, Photini Papadimitriou, Nikolaos Akritidis, Leonidas Christou, and Epameinondas V. Tsianos. 2006. 'The New Global Map of Human Brucellosis'. *The Lancet. Infectious Diseases* 6 (2): 91–99. https://doi.org/10.1016/S1473-3099(06)70382-6.

Parisi, Antonio, Rosa Fraccalvieri, Mariassunta Cafiero, Angela Miccolupo, Iolanda Padalino, Cosimo Montagna, Federico Capuano, and Roldano Sottili. 2006. 'Diagnosis of *Coxiella Burnetii*-Related Abortion in Italian Domestic Ruminants Using Single-Tube Nested PCR'. *Veterinary Microbiology* 118 (1–2): 101–6. https://doi.org/10.1016/j.vetmic.2006.06.023.

Parkinson, Timothy J. 2019. '24 - Specific Infectious Diseases Causing Infertility and Subfertility in Cattle'. In *Veterinary Reproduction and Obstetrics (Tenth Edition)*, edited by David E. Noakes, Timothy J. Parkinson, and Gary C. W. England, 434–66. St. Louis (MO): W.B. Saunders. https://doi.org/10.1016/B978-0-7020-7233-8.00024-0.

Passler, Thomas, Stephen S. Ditchkoff, and Paul H. Walz. 2016. 'Bovine Viral Diarrhea Virus (BVDV) in White-Tailed Deer (Odocoileus Virginianus)'. *Frontiers in Microbiology* 7: 945. https://doi.org/10.3389/fmicb.2016.00945.

Paton, D. J., K. Christiansen, S. Alenius, M. P. Cranwell, G. C. Pritchard, and T. W. Drew. 1998. 'Prevalence of Antibodies to Bovine Virus Diarrhoea Virus and Other Viruses in Bulk Tank

Milk in England and Wales'. *Veterinary Record* 142 (15): 385–91. https://doi.org/10.1136/vr.142.15.385.

Paul, Suman, Jens F. Agger, Bo Markussen, Anna-Bodil Christoffersen, and Jørgen S. Agerholm. 2012. 'Factors Associated with *Coxiella Burnetii* Antibody Positivity in Danish Dairy Cows'. *Preventive Veterinary Medicine* 107 (1–2): 57–64. https://doi.org/10.1016/j.prevetmed.2012.05.015.

Pereira, Gabriel Ribas, Fernanda Silveira Flores Vogel, Rodrigo Camponogara Bohrer, Janduí Escarião da Nóbrega, Gustavo Freitas Ilha, Paulo Roberto Antunes da Rosa, Werner Giehl Glanzner, et al. 2014. '*Neospora Caninum* DNA Detection by TaqMan Real-Time PCR Assay in Experimentally Infected Pregnant Heifers'. *Veterinary Parasitology* 199 (3–4): 129–35. https://doi.org/10.1016/j.vetpar.2013.10.018.

Perin, Géssica, Juscivete F. Fávero, Diego R. T. Severo, Anielen D. Silva, Gustavo Machado, Hugo L. Araújo, Walter Lilenbaum, et al. 2017. 'Occurrence of Oxidative Stress in Dairy Cows Seropositives for *Brucella Abortus*'. *Microbial Pathogenesis* 110 (September): 196–201. https://doi.org/10.1016/j.micpath.2017.06.043.

Peter, Allison. 2000. 'Abortions in Dairy Cows: New Insights and Economic Impact'. In .

Peter, A.T. 2000. 'Abortions in Dairy Cows: New Insights and Economic Impact.' *Adv. Dairy Technol.* 12: 233–44.

Petersson, K.-J., B. Berglund, E. Strandberg, H. Gustafsson, A. P. F. Flint, J. A. Woolliams, and M. D. Royal. 2007. 'Genetic Analysis of Postpartum Measures of Luteal Activity in Dairy Cows'. *Journal of Dairy Science* 90 (1): 427–34. https://doi.org/10.3168/jds.S0022-0302(07)72644-9.

Petit, Tom, Joachim Spergser, Jörg Aurich, and Renate Rosengarten. 2008. 'Prevalence of Chlamydiaceae and Mollicutes on the Genital Mucosa and Serological Findings in Dairy Cattle'. *Veterinary Microbiology* 127 (3–4): 325–33. https://doi.org/10.1016/j.vetmic.2007.08.022.

Petty, Lindsay A., Helen S. Te, and Kenneth Pursell. 2017. 'A Case of Q Fever after Liver Transplantation'. *Transplant Infectious Disease: An Official Journal of the Transplantation Society* 19 (5). https://doi.org/10.1111/tid.12737.

Pinedo, P. J., A. De Vries, and D. W. Webb. 2010. 'Dynamics of Culling Risk with Disposal Codes Reported by Dairy Herd Improvement Dairy Herds'. *Journal of Dairy Science* 93 (5): 2250–61. https://doi.org/10.3168/jds.2009-2572.

Pittman, Kelly J., and Laura J. Knoll. 2015. 'Long-Term Relationships: The Complicated Interplay between the Host and the Developmental Stages of *Toxoplasma Gondii* during Acute and Chronic Infections'. *Microbiology and Molecular Biology Reviews* 79 (4): 387–401. https://doi.org/10.1128/MMBR.00027-15.

Plummer, Paul J., J. Trenton McClure, Paula Menzies, Paul S. Morley, René Van den Brom, and David C. Van Metre. 2018. 'Management of *Coxiella Burnetii* Infection in Livestock Populations and the Associated Zoonotic Risk: A Consensus Statement'. *Journal of Veterinary Internal Medicine* 32 (5): 1481–94. https://doi.org/10.1111/jvim.15229.

Pluta, Silvia, Kathrin Hartelt, Rainer Oehme, Ute Mackenstedt, and Peter Kimmig. 2010. 'Prevalence of *Coxiella Burnetii* and Rickettsia Spp. in Ticks and Rodents in Southern Germany'. *Ticks and Tick-Borne Diseases* 1 (3): 145–47. https://doi.org/10.1016/j.ttbdis.2010.04.001.

Poester, F.P., K. Nielsen, L.E. Samartino, and W.L. Yu. 2010. 'Diagnosis of Brucellosis'. *Open Vet Sci J* 4 (4): 46–60.

Polsky, Liam, and Marina A. G. von Keyserlingk. 2017. 'Invited Review: Effects of Heat Stress on Dairy Cattle Welfare'. *Journal of Dairy Science* 100 (11): 8645–57. https://doi.org/10.3168/jds.2017-12651.

Powers, Ann M., and Stephen H. Waterman. 2017. 'A Decade of Arboviral Activity—Lessons Learned from the Trenches'. *PLOS Neglected Tropical Diseases* 11 (4): e0005421. https://doi.org/10.1371/journal.pntd.0005421.

Psaroulaki, A., C. Hadjichristodoulou, F. Loukaides, E. Soteriades, A. Konstantinidis, P. Papastergiou, M. C. Ioannidou, and Y. Tselentis. 2006. 'Epidemiological Study of Q Fever in Humans, Ruminant Animals, and Ticks in Cyprus Using a Geographical Information System'. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 25 (9): 576–86. https://doi.org/10.1007/s10096-006-0170-7.

Qin, Si-Yuan, Si-Yang Huang, Ming-Yang Yin, Qi-Dong Tan, Guang-Xue Liu, Dong-Hui Zhou, Xing-Quan Zhu, Ji-Zhang Zhou, and Ai-Dong Qian. 2015. 'Seroprevalence and Risk Factors of *Chlamydia Abortus* Infection in Free-Ranging White Yaks in China'. *BMC Veterinary Research* 11 (January). https://doi.org/10.1186/s12917-015-0323-y.

Quintanilla-Gozalo, A., J. Pereira-Bueno, E. Tabarés, E. A. Innes, R. González-Paniello, and L. M. Ortega-Mora. 1999. 'Seroprevalence of *Neospora Caninum* Infection in Dairy and Beef Cattle in Spain'. *International Journal for Parasitology* 29 (8): 1201–8. https://doi.org/10.1016/s0020-7519(99)00084-3.

Racloz, V., E. Schelling, N. Chitnis, F. Roth, and J. Zinsstag. 2013. 'Persistence of Brucellosis in Pastoral Systems'. *Revue Scientifique Et Technique (International Office of Epizootics)* 32 (1): 61–70. https://doi.org/10.20506/rst.32.1.2186.

Rády, M., R. Glávits, and G. Nagy. 1985. 'Demonstration in Hungary of Q Fever Associated with Abortions in Cattle and Sheep'. *Acta Veterinaria Hungarica* 33 (3–4): 169–76.

Rahal K, Bennadji A, A. Dahmani, Dechicha A, Khaled H, and S. Merdja. 2011. 'Séroprévalence Apparente de La Brucellose, Chlamydiose et Fièvre Q Chez Les Ovins de La Région de Ksar Boukhari','. *Recueil 4èmes Journées Vétérinaires de Blida* 4: 1–16.

Rahal, M., D. Tahir, C. Eldin, I. Bitam, D. Raoult, and P. Parola. 2018. 'Genotyping of *Coxiella Burnetii* Detected in Placental Tissues from Aborted Dairy Cattle in the North of Algeria'. *Comparative Immunology, Microbiology and Infectious Diseases* 57 (April): 50–54. https://doi.org/10.1016/j.cimid.2018.06.001.

Raoult, D., Tj Marrie, and Jl Mege. 2005. 'Natural History and Pathophysiology of Q Fever'. *The Lancet. Infectious Diseases* 5 (4): 219–26. https://doi.org/10.1016/S1473-3099(05)70052-9.

Ray, Katrina, Benoit Marteyn, Philippe J. Sansonetti, and Christoph M. Tang. 2009. 'Life on the inside: The Intracellular Lifestyle of Cytosolic Bacteria'. *Nature Reviews. Microbiology* 7 (5): 333–40. https://doi.org/10.1038/nrmicro2112.

Refai, Mohamed. 2002. 'Incidence and Control of Brucellosis in the Near East Region'. *Veterinary Microbiology*, Brucellosis S.I., 90 (1): 81–110. https://doi.org/10.1016/S0378-1135(02)00248-1.

Reichel, Michael P., Lloyd C. Wahl, and Fraser I. Hill. 2018. 'Review of Diagnostic Procedures and Approaches to Infectious Causes of Reproductive Failures of Cattle in Australia and New Zealand'. *Frontiers in Veterinary Science* 5. https://doi.org/10.3389/fvets.2018.00222.

Reinhold, Petra, Julia Jaeger, Elisabeth Liebler-Tenorio, Angela Berndt, Ruediger Bachmann, Evelyn Schubert, Falk Melzer, Mandy Elschner, and Konrad Sachse. 2008. 'Impact of Latent Infections with Chlamydophila Species in Young Cattle'. *The Veterinary Journal* 175 (2): 202–11. https://doi.org/10.1016/j.tvjl.2007.01.004.

Reinhold, Petra, Konrad Sachse, and Bernhard Kaltenboeck. 2011. 'Chlamydiaceae in Cattle: Commensals, Trigger Organisms, or Pathogens?' *The Veterinary Journal* 189 (3): 257–67. https://doi.org/10.1016/j.tvjl.2010.09.003.

Reisberg, Kerstin, Abdelfattah M. Selim, and Wolfgang Gaede. 2013. 'Simultaneous Detection of *Chlamydia Spp.*, *Coxiella Burnetii*, and *Neospora Caninum* in Abortion Material of Ruminants

by Multiplex Real-Time Polymerase Chain Reaction': *Journal of Veterinary Diagnostic Investigation*, August. https://doi.org/10.1177/1040638713497483.

Reiter-Owona I, Petersen E, Joynson D, Aspöck H, Dardé ML, Disko R, Dreazen O, Dumon H, Grillo R, and Gross U, Hayde M, Holliman R, Ho-Yen DO, Janitschke K, Jenum PA, Naser K, Olszewski M, Thulliez P, Seitz HM. 1999. 'The Past and Present Role of the Sabin-Feldman Dye Test in the Serodiagnosis of Toxoplasmosis.' *World Health Organ* 77 (11): 929–35.

Renaudeau, D., A. Collin, S. Yahav, V. de Basilio, J. L. Gourdine, and R. J. Collier. 2012. 'Adaptation to Hot Climate and Strategies to Alleviate Heat Stress in Livestock Production'. *Animal* 6 (5): 707–28. https://doi.org/10.1017/S1751731111002448.

Reviriego, F. J, M. A Moreno, and L Domínguez. 2000. 'Risk Factors for Brucellosis Seroprevalence of Sheep and Goat Flocks in Spain'. *Preventive Veterinary Medicine* 44 (3): 167–73. https://doi.org/10.1016/S0167-5877(00)00108-2.

Ribeiro, E. S, K. N Galvão, W. W Thatcher, and J.E.P. Santos. 2012. 'Economic Aspects of Applying Reproductive Technologies to Dairy Herds.' *Anim Reprod,* 9 (3): 370–87.

Richter, Veronika, Karin Lebl, Walter Baumgartner, Walter Obritzhauser, Annemarie Käsbohrer, and Beate Pinior. 2017. 'A Systematic Worldwide Review of the Direct Monetary Losses in Cattle Due to Bovine Viral Diarrhoea Virus Infection'. *The Veterinary Journal* 220 (February): 80–87. https://doi.org/10.1016/j.tvjl.2017.01.005.

Ridpath, Julia F. 2005. 'Practical Significance of Heterogeneity among BVDV Strains: Impact of Biotype and Genotype on U.S. Control Programs'. *Preventive Veterinary Medicine* 72 (1–2): 17–30; discussion 215-219. https://doi.org/10.1016/j.prevetmed.2005.08.003.

Rinaldi, L., G. Fusco, V. Musella, V. Veneziano, A. Guarino, R. Taddei, and G. Cringoli. 2005. '*Neospora Caninum* in Pastured Cattle: Determination of Climatic, Environmental, Farm Management and Individual Animal Risk Factors Using Remote Sensing and Geographical Information Systems'. *Veterinary Parasitology* 128 (3–4): 219–30. https://doi.org/10.1016/j.vetpar.2004.12.011.

Rizzo, Francesca, Nicoletta Vitale, Marco Ballardini, Vitaliano Borromeo, Camilla Luzzago, Laura Chiavacci, and Maria Lucia Mandola. 2016. 'Q Fever Seroprevalence and Risk Factors in Sheep and Goats in Northwest Italy'. *Preventive Veterinary Medicine* 130 (August): 10–17. https://doi.org/10.1016/j.prevetmed.2016.05.014.

Robert, A, F Beaudeau, H Seegers, A Joly, and J. M Philipot. 2004. 'Large Scale Assessment of the Effect Associated with Bovine Viral Diarrhoea Virus Infection on Fertility of Dairy Cows in 6149 Dairy Herds in Brittany (Western France)'. *Theriogenology* 61 (1): 117–27. https://doi.org/10.1016/S0093-691X(03)00182-1.

Robinson, A. 2003. 'Guidelines for Coordinated Human and Animal Brucellosis Surveillance for the Animal Production and Health Division FAO Agriculture Department. EMERGENCY PREVENTION SYSTEM FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS Rome'. 2003. http://www.fao.org/3/y4723e/y4723e00.htm.

Robinson, J. J., C. J. Ashworth, J. A. Rooke, L. M. Mitchell, and T. G. McEvoy. 2006. 'Nutrition and Fertility in Ruminant Livestock'. *Animal Feed Science and Technology,* Feed and Animal Health, 126 (3): 259–76. https://doi.org/10.1016/j.anifeedsci.2005.08.006.

Roche, J. F., and M. G. Diskin. 2001. 'Resumption of Reproductive Activity in the Early Postpartum Period of Cows'. *BSAP Occasional Publication* 26 (1): 31–42. https://doi.org/10.1017/S0263967X00033577.

Roche, J. R., A. W. Bell, T. R. Overton, and J. J. Loor. 2013. 'Nutritional Management of the Transition Cow in the 21st Century-a Paradigm Shift in Thinking'. *Animal Production Science* 53 (9): 1000–1023. https://doi.org/10.1071/AN12293.

Roche, James F. 2006. 'The Effect of Nutritional Management of the Dairy Cow on Reproductive Efficiency'. *Animal Reproduction Science* 96 (3–4): 282–96. https://doi.org/10.1016/j.anireprosci.2006.08.007.

Rodning, S. P., M. D. Givens, M. S. D. Marley, Y. Zhang, K. P. Riddell, P. K. Galik, T. L. Hathcock, J. A. Gard, J. W. Prevatt, and W. F. Owsley. 2012. 'Reproductive and Economic Impact Following Controlled Introduction of Cattle Persistently Infected with Bovine Viral Diarrhea Virus into a Naive Group of Heifers'. *Theriogenology* 78 (7): 1508–16. https://doi.org/10.1016/j.theriogenology.2012.05.031.

Rodolakis, Annie. 2009. 'Q Fever in Dairy Animals'. *Annals of the New York Academy of Sciences* 1166 (May): 90–93. https://doi.org/10.1111/j.1749-6632.2009.04511.x.

Rodolakis, Annie, and Khalil Yousef Mohamad. 2010. 'Zoonotic Potential of Chlamydophila'. *Veterinary Microbiology* 140 (3–4): 382–91. https://doi.org/10.1016/j.vetmic.2009.03.014.

Rodríguez, María Cruz, Cristina Viadas, Asunción Seoane, Félix Javier Sangari, Ignacio López-Goñi, and Juan María García-Lobo. 2012. 'Evaluation of the Effects of Erythritol on Gene Expression in *Brucella Abortus*'. *PloS One* 7 (12): e50876. https://doi.org/10.1371/journal.pone.0050876.

Roelofs, J., F. López-Gatius, R. H. F. Hunter, F. J. C. M. van Eerdenburg, and Ch Hanzen. 2010. 'When Is a Cow in Estrus? Clinical and Practical Aspects'. *Theriogenology* 74 (3): 327–44. https://doi.org/10.1016/j.theriogenology.2010.02.016.

Roescher, Annemiek M., Albert Timmer, Jan Jaap H. M. Erwich, and Arend F. Bos. 2014. 'Placental Pathology, Perinatal Death, Neonatal Outcome, and Neurological Development: A Systematic Review'. *PloS One* 9 (2): e89419. https://doi.org/10.1371/journal.pone.0089419.

Roth, Zvi. 2017. 'Effect of Heat Stress on Reproduction in Dairy Cows: Insights into the Cellular and Molecular Responses of the Oocyte'. *Annual Review of Animal Biosciences* 5 (1): 151–70. https://doi.org/10.1146/annurev-animal-022516-022849.

Rotz, C. A., D. L. Zartman, and K. L. Crandall. 2005. 'Economic and Environmental Feasibility of a Perennial Cow Dairy Farm'. *Journal of Dairy Science* 88 (8): 3009–19. https://doi.org/10.3168/jds.S0022-0302(05)72981-7.

Rouatbi, Mariem, Safa Amairia, Yosra Amdouni, Mohamed Anis Boussaadoun, Ouarda Ayadi, Amira Adel Taha Al-Hosary, Mourad Rekik, et al. 2019. '*Toxoplasma Gondii* Infection and Toxoplasmosis in North Africa: A Review'. *Parasite* 26. https://doi.org/10.1051/parasite/2019006.

Roulis, Eileen, Adam Polkinghorne, and Peter Timms. 2013. '*Chlamydia Pneumoniae*: Modern Insights into an Ancient Pathogen'. *Trends in Microbiology* 21 (3): 120–28. https://doi.org/10.1016/j.tim.2012.10.009.

Ruiz-Fons, Francisco, Ianire Astobiza, Jesús F. Barandika, Ana Hurtado, Raquel Atxaerandio, Ramón A. Juste, and Ana L. García-Pérez. 2010. 'Seroepidemiological Study of Q Fever in Domestic Ruminants in Semi-Extensive Grazing Systems'. *BMC Veterinary Research* 6 (January): 3. https://doi.org/10.1186/1746-6148-6-3.

Ryan, E. D., M. Kirby, D. M. Collins, R. Sayers, J. F. Mee, and T. Clegg. 2011. 'Prevalence of *Coxiella Burnetii* (Q Fever) Antibodies in Bovine Serum and Bulk-Milk Samples'. *Epidemiology and Infection* 139 (9): 1413–17. https://doi.org/10.1017/S0950268810002530.

S. Eicker, and J. Fetrow. 2003. 'New Tools for Deciding When to Replace Used Dairy Cows Proc.' In *Kentucky Dairy Conference*, 33–46. Lexington, KY, University of Kentucky,.

Sachse, Konrad, Patrik M. Bavoil, Bernhard Kaltenboeck, Richard S. Stephens, Cho-Chou Kuo, Ramon Rosselló-Móra, and Matthias Horn. 2015. 'Emendation of the Family Chlamydiaceae: Proposal of a Single Genus, *Chlamydia*, to Include All Currently Recognized Species'. *Systematic and Applied Microbiology* 38 (2): 99–103. https://doi.org/10.1016/j.syapm.2014.12.004.

Saeed, Intisar Kamil, Yahia Hassan Ali, Magdi Badawi AbdulRahman, Zakia Abas Mohammed, Halima Mohammed Osman, Khalid Mohammed Taha, Mohammed Zain Musa, and AbdelMelik

Ibrahim Khalafalla. 2015. 'Mixed Infection of Peste Des Petits Ruminants Virus (PPRV) and Other Respiratory Viruses in Dromedary Camels in Sudan, an Abattoir Study'. *Tropical Animal Health and Production* 47 (5): 995–98. https://doi.org/10.1007/s11250-015-0798-3.

Saegerman, C., L. De Waele, D. Gilson, J. Godfroid, P. Thiange, P. Michel, B. Limbourg, et al. 2004. 'Evaluation of Three Serum I-ELISAs Using Monoclonal Antibodies and Protein G as Peroxidase Conjugate for the Diagnosis of Bovine Brucellosis'. *Veterinary Microbiology* 100 (1–2): 91–105. https://doi.org/10.1016/j.vetmic.2004.02.003.

Sagamiko, F. D., J. B. Muma, E. D. Karimuribo, A. M. Mwanza, C. Sindato, and B. M. Hang'ombe. 2018. 'Sero-Prevalence of Bovine Brucellosis and Associated Risk Factors in Mbeya Region, Southern Highlands of Tanzania'. *Acta Tropica* 178 (February): 169–75. https://doi.org/10.1016/j.actatropica.2017.11.022.

Şahin, Mitat, Oktay Genç, Ahmet Ünver, and Salih Otlu. 2008. 'Investigation of Bovine Brucellosis in the Northeastern Turkey'. *Tropical Animal Health and Production* 40 (4): 281–86. https://doi.org/10.1007/s11250-007-9092-3.

Said, B., K. D. Halsby, C. M. O'Connor, J. Francis, K. Hewitt, N. Q. Verlander, E. Guy, and D. Morgan. 2017. 'Risk Factors for Acute Toxoplasmosis in England and Wales'. *Epidemiology and Infection* 145 (1): 23–29. https://doi.org/10.1017/S0950268816002235.

Saidi, Radhwane, Amina Bessas, Idir Bitam, Yaşar Ergün, and Veysel Soydal Ataseven. 2018. 'Bovine Herpesvirus-1 (BHV-1), Bovine Leukemia Virus (BLV) and Bovine Viral Diarrhea Virus (BVDV) Infections in Algerian Dromedary Camels (Camelus Dromaderius)'. *Tropical Animal Health and Production* 50 (3): 561–64. https://doi.org/10.1007/s11250-017-1469-3.

Sakaguchi, Minoru. 2011. 'Practical Aspects of the Fertility of Dairy Cattle'. *The Journal of Reproduction and Development* 57 (1): 17–33. https://doi.org/10.1262/jrd.10-197e.

Sala, Giulia, Alessia Gazzonis, Antonio Boccardo, Eleonora Coppoletta, Chiara Galasso, Maria Teresa Manfredi, and Davide Pravettoni. 2018. 'Using Beef-Breed Semen in Seropositive Dams for the Control of Bovine Neosporosis'. *Preventive Veterinary Medicine* 161 (December): 127–33. https://doi.org/10.1016/j.prevetmed.2018.10.024.

Salinas, J., M. R. Caro, J. Vicente, F. Cuello, A. R. Reyes-Garcia, A. J. Buendía, A. Rodolakis, and C. Gortázar. 2009. 'High Prevalence of Antibodies against Chlamydiaceae and Chlamydophila Abortus in Wild Ungulates Using Two "in House" Blocking-ELISA Tests'. *Veterinary Microbiology* 135 (1–2): 46–53. https://doi.org/10.1016/j.vetmic.2008.10.001.

Sanderson, M. W., J. M. Gay, and T. V. Baszler. 2000. *'Neospora Caninum* Seroprevalence and Associated Risk Factors in Beef Cattle in the Northwestern United States'. *Veterinary Parasitology* 90 (1–2): 15–24. https://doi.org/10.1016/s0304-4017(00)00234-x.

Sandvik, Torstein. 1999. 'Laboratory Diagnostic Investigations for Bovine Viral Diarrhoea Virus Infections in Cattle'. *Veterinary Microbiology* 64 (2): 123–34. https://doi.org/10.1016/S0378-1135(98)00264-8.

Sanger, F., S. Nicklen, and A. R. Coulson. 1992. 'DNA Sequencing with Chain-Terminating Inhibitors. 1977'. *Biotechnology (Reading, Mass.)* 24: 104–8.

Santolaria, P., S. Almería, D. Martínez-Bello, C. Nogareda, M. Mezo, M. Gonzalez-Warleta, J. A. Castro-Hermida, M. Pabón, J. L. Yániz, and F. López-Gatius. 2011. 'Different Humoral Mechanisms against *Neospora Caninum* Infection in Purebreed and Crossbreed Beef/Dairy Cattle Pregnancies'. *Veterinary Parasitology* 178 (1–2): 70–76. https://doi.org/10.1016/j.vetpar.2010.12.018.

Santos, J. E. P., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. 'Applying Nutrition and Physiology to Improve Reproduction in Dairy Cattle'. *Society of Reproduction and Fertility Supplement* 67: 387–403. https://doi.org/10.7313/upo9781907284991.030.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sá Filho. 2009. 'Risk Factors for Resumption of Postpartum Estrous Cycles and Embryonic Survival in Lactating Dairy Cows'. *Animal Reproduction Science* 110 (3–4): 207–21. https://doi.org/10.1016/j.anireprosci.2008.01.014.

Santos JEP. 2008. 'Implementation of Reproductive Programs in Dairy Herds, 16:5-14.' *Cattle Pract* 16: 6–14.

Sarrazin, S., J. Dewulf, E. Mathijs, J. Laureyns, L. Mostin, and A. B. Cay. 2014. 'Virulence Comparison and Quantification of Horizontal Bovine Viral Diarrhoea Virus Transmission Following Experimental Infection in Calves'. *The Veterinary Journal* 202 (2): 244–49. https://doi.org/10.1016/j.tvjl.2014.07.010.

Sayers, R. G., N. Byrne, E. O'Doherty, and S. Arkins. 2015. 'Prevalence of Exposure to Bovine Viral Diarrhoea Virus (BVDV) and Bovine Herpesvirus-1 (BoHV-1) in Irish Dairy Herds'. *Research in Veterinary Science* 100 (June): 21–30. https://doi.org/10.1016/j.rvsc.2015.02.011.

Schaik, Erin J. van, Chen Chen, Katja Mertens, Mary M. Weber, and James E. Samuel. 2013. 'Molecular Pathogenesis of the Obligate Intracellular Bacterium *Coxiella Burnetii*'. *Nature Reviews Microbiology* 11 (8): 561–73. https://doi.org/10.1038/nrmicro3049.

Schär, Christoph, Pier Luigi Vidale, Daniel Lüthi, Christoph Frei, Christian Häberli, Mark A. Liniger, and Christof Appenzeller. 2004. 'The Role of Increasing Temperature Variability in European Summer Heatwaves'. *Nature* 427 (6972): 332–36. https://doi.org/10.1038/nature02300.

Schimmer, B., N. Schotten, E. van Engelen, J.L.A. Hautvast, P.M. Schneeberger, and Y.T.H.P. van Duijnhoven. 2014. '*Coxiella Burnetii* Seroprevalence and Risk for Humans on Dairy Cattle Farms, the Netherlands, 2010–2011'. *Emerging Infectious Diseases* 20 (3): 417–25. https://doi.org/10.3201/eid2003.131111.

Schlafer, D. H., P. J. Fisher, and C. J. Davies. 2000. 'The Bovine Placenta before and after Birth: Placental Development and Function in Health and Disease'. *Animal Reproduction Science* 60–61 (July): 145–60. https://doi.org/10.1016/s0378-4320(00)00132-9.

Scholz, H. C., and G. Vergnaud. 2013. 'Molecular Characterisation of *Brucella* Species'. *Revue Scientifique Et Technique (International Office of Epizootics)* 32 (1): 149–62. https://doi.org/10.20506/rst.32.1.2189.

Schoonman, Luuk B., T. Wilsmore, and Emmanuel S. Swai. 2010. 'Sero-Epidemiological Investigation of Bovine Toxoplasmosis in Traditional and Smallholder Cattle Production Systems of Tanga Region, Tanzania'. *Tropical Animal Health and Production* 42 (4): 579–87. https://doi.org/10.1007/s11250-009-9460-2.

Schweizer, Matthias, and Ernst Peterhans. 2014. 'Pestiviruses'. *Annual Review of Animal Biosciences* 2 (1): 141–63. https://doi.org/10.1146/annurev-animal-022513-114209.

Scientific report of EFSA and ECDC. 2012. 'The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Borne Outbreaks in 2010'. *EFSA Journal* 10 (3): 2597. https://doi.org/10.2903/j.efsa.2012.2597.

See, Wade, William H. Edwards, Stacey Dauwalter, Claudia Almendra, Martin D. Kardos, Jennifer L. Lowell, Rick Wallen, Steven L. Cain, William E. Holben, and Gordon Luikart. 2012. 'Yersinia Enterocolitica: An Unlikely Cause of Positive Brucellosis Tests in Greater Yellowstone Ecosystem Bison (Bison Bison)'. *Journal of Wildlife Diseases* 48 (3): 537–41. https://doi.org/10.7589/0090-3558-48.3.537.

Selim, Abdelfattah, Kotb Attia, Eman Ramadan, Yaser M. Hafez, and Alamery Salman. 2019. 'Seroprevalence and Molecular Characterization of *Brucella* Species in Naturally Infected Cattle and Sheep'. *Preventive Veterinary Medicine* 171 (November): 104756. https://doi.org/10.1016/j.prevetmed.2019.104756.

Selim, Abdelfattah M., Mahmoud M. Elhaig, Sherif A. Moawed, and Ehab El-Nahas. 2018. 'Modeling the Potential Risk Factors of Bovine Viral Diarrhea Prevalence in Egypt Using Univariable and

Multivariable Logistic Regression Analyses'. *Veterinary World* 11 (3): 259–67. https://doi.org/10.14202/vetworld.2018.259-267.

Selmi, Rachid, Aymen Mamlouk, Houcine Ben Yahia, Hedi Abdelaali, Mourad Ben Said, Khalil Sellami, Monia Daaloul-Jedidi, Mohamed Habib Jemli, and Lilia Messadi. 2018. '*Coxiella Burnetii* in Tunisian Dromedary Camels (Camelus Dromedarius): Seroprevalence, Associated Risk Factors and Seasonal Dynamics'. *Acta Tropica* 188 (December): 234–39. https://doi.org/10.1016/j.actatropica.2018.09.008.

Senoussi A. 2008. 'Caractérisation de l'élevage Bovin Laitier Dans Le Sahra : Situation et Perspectives de Développement. Cas de Région de Guerra-'. In *Colloque International « Développement Durable Des Productions Animales : Enjeux, Évaluation et Perspectives », Alger*.

Sergeant, ESG. 2018. 'Epitools Epidemiological Calculators. Ausvet.' 2018. https://epitools.ausvet.com.au/.

Serrano, E., I. Ferre, K. Osoro, G. Aduriz, A. Mateos-Sanz, A. Martínez, R. Atxaerandio, C.O. Hidalgo, and L.M. Ortega-Mora. 2006. 'Intrauterine *Neospora Caninum* Inoculation of Heifers'. *Veterinary Parasitology* 135 (3–4): 197–203. https://doi.org/10.1016/j.vetpar.2005.10.003.

Sharifzadeh, Ali, Abbas Doosti, and Payam Ghasemi Dehkordi. 2012. 'PCR Assay for Detection of *Neospora Caninum* in Fresh and Frozen Semen Specimens of Iranian Bulls'. *Wallingford, Oxfordshire, UK : CABI, 2007* 309 pages: 8.

Shome, Rajeswari. 2014. 'Bovine Brucellosis in Organized Farms of India - An Assessment of Diagnostic Assays and Risk Factors'. *Advances in Animal and Veterinary Sciences* 2 (10): 557–64. https://doi.org/10.14737/journal.aavs/2014/2.10.557.564.

Sidi-Boumedine, K., E. Rousset, K. Henning, M. Ziller, K. Niemczuck, H. I. J. Roest, and R. Thiéry. 2010. 'Development of Harmonised Schemes for the Monitoring and Reporting of Q-Fever in Animals in the European Union'. *EFSA Supporting Publications* 7 (5): 48E. https://doi.org/10.2903/sp.efsa.2010.EN-48.

Skarzynski, Dariusz J., Jerzy J. Jaroszewski, and Kiyoshi Okuda. 2005. 'Role of Tumor Necrosis Factor-Alpha and Nitric Oxide in Luteolysis in Cattle'. *Domestic Animal Endocrinology* 29 (2): 340–46. https://doi.org/10.1016/j.domaniend.2005.02.005.

Smirnova, Ekaterina A., Andrey V. Vasin, Nurlan T. Sandybaev, Sergey A. Klotchenko, Marina A. Plotnikova, Olga V. Chervyakova, Abylai R. Sansyzbay, and Oleg I. Kiselev. 2013. 'Current Methods of Human and Animal Brucellosis Diagnostics'. *Advances in Infectious Diseases* 3 (3): 177–84. https://doi.org/10.4236/aid.2013.33026.

Smith H, Williams AE, Pearce JH, Keppie J, and Harris-Smith PW. 1962. 'Foetal Erythritol: A Cause of the Localization of *Brucella Abortus* in Bovine Contagious Abortion.' *Nature* 193: 47–49.

Smith, R. L., R. L. Strawderman, Y. H. Schukken, S. J. Wells, A. K. Pradhan, L. A. Espejo, R. H. Whitlock, et al. 2010. 'Effect of Johne's Disease Status on Reproduction and Culling in Dairy Cattle'. *Journal of Dairy Science* 93 (8): 3513–24. https://doi.org/10.3168/jds.2009-2742.

Softic, Adis, Kassahun Asmare, Erik Georg Granquist, Jacques Godfroid, Nihad Fejzic, and Eystein Skjerve. 2018. 'The Serostatus of *Brucella Spp.*, *Chlamydia Abortus*, *Coxiella Burnetii* and *Neospora Caninum* in Cattle in Three Cantons in Bosnia and Herzegovina'. *BMC Veterinary Research* 14 (1): 40. https://doi.org/10.1186/s12917-018-1361-z.

Sperry, J. F., and D. C. Robertson. 1975. 'Erythritol Catabolism by *Brucella Abortus.*' *Journal of Bacteriology* 121 (2): 619–30.

Sraïri, M. T., R. Benjelloun, M. Karrou, S. Ates, and M. Kuper. 2016. 'Biophysical and Economic Water Productivity of Dual-Purpose Cattle Farming'. *Animal* 10 (2): 283–91. https://doi.org/10.1017/S1751731115002360.

Sraïri, Mohamed Taher, Mohammed Tahar Benyoucef, and Khemais Kraiem. 2013. 'The Dairy Chains in North Africa (Algeria, Morocco and Tunisia): From Self Sufficiency Options to Food Dependency?' *SpringerPlus* 2 (1): 162. https://doi.org/10.1186/2193-1801-2-162.

Sraïri MT, and Farit Y. 2001. 'Studies on Dairy Cattle Reproduction Performances in Morocco Based on Analysis of Artificial Insemination Data.' *Tropicultura*, no. 19: 151-155.

Sraïri MT, Rjafallah H, Kuper M, and Le Gal P-Y. 2009. 'Water Productivity through Dual Purpose (Milk and Meat) Herds in the Tadla Irrigation Scheme. Morocco',. *Irrig Drainage* 58: S334–45.

Srinivas, Sindhu K., Yujie Ma, Mary D. Sammel, Doris Chou, Cindy McGrath, Samuel Parry, and Michal A. Elovitz. 2006. 'Placental Inflammation and Viral Infection Are Implicated in Second Trimester Pregnancy Loss'. *American Journal of Obstetrics and Gynecology* 195 (3): 797–802. https://doi.org/10.1016/j.ajog.2006.05.049.

Sting R, Kopp J, Mandl J, Seeh C, and Seemann G. 2003. 'Comparative Serological Studies in Dairy Herds of *Chlamydia* and *Coxiella Burnetti* Infections Using CFT and ELISA.' *Tierarzt Umschau* 58: 218–28.

Stott, A. W., R. W. Humphry, and G. J. Gunn. 2010. 'Modelling the Effects of Previous Infection and Re-Infection on the Costs of Bovine Viral Diarrhoea Outbreaks in Beef Herds'. *The Veterinary Journal* 185 (2): 138–43. https://doi.org/10.1016/j.tvjl.2009.05.020.

Sun, Wu-Wen, Qing-Feng Meng, Wei Cong, Xiao-Feng Shan, Chun-Feng Wang, and Ai-Dong Qian. 2015. 'Herd-Level Prevalence and Associated Risk Factors for *Toxoplasma Gondii*, *Neospora Caninum*, *Chlamydia Abortus* and Bovine Viral Diarrhoea Virus in Commercial Dairy and Beef Cattle in Eastern, Northern and Northeastern China'. *Parasitology Research* 114 (11): 4211–18. https://doi.org/10.1007/s00436-015-4655-0.

Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. 'Prevalence of Subclinical Ketosis and Relationships with Postpartum Diseases in European Dairy Cows'. *Journal of Dairy Science* 96 (5): 2925–38. https://doi.org/10.3168/jds.2012-6035.

Szymańska-Czerwińska, Monika, Krzysztof Niemczuk, and Elżbieta Monika Galińska. 2013. 'Serological and Nested PCR Survey to Determine the Occurrence of *Chlamydia* Infections in the Polish Cattle Population'. *Annals of Agricultural and Environmental Medicine: AAEM* 20 (4): 682–86.

Tajima, M., H. R. Frey, O. Yamato, Y. Maede, V. Moennig, H. Scholz, and I. Greiser-Wilke. 2001. 'Prevalence of Genotypes 1 and 2 of Bovine Viral Diarrhea Virus in Lower Saxony, Germany'. *Virus Research* 76 (1): 31–42. https://doi.org/10.1016/s0168-1702(01)00244-1.

Talafha, A. Q., S. M. Hirche, M. M. Ababneh, A. M. Al-Majali, and M. M. Ababneh. 2009. 'Prevalence and Risk Factors Associated with Bovine Viral Diarrhea Virus Infection in Dairy Herds in Jordan'. *Tropical Animal Health and Production* 41 (4): 499–506. https://doi.org/10.1007/s11250-008-9214-6.

Talafha, Abdelsalam Q., Mohammed M. Ababneh, Mustafa M. Ababneh, and Ahmad M. Al-Majali. 2012. 'Prevalence and Risk Factors Associated with Chlamydophila Abortus Infection in Dairy Herds in Jordan'. *Tropical Animal Health and Production* 44 (8): 1841–46. https://doi.org/10.1007/s11250-012-0146-9.

Tao, S., and G. E. Dahl. 2013. 'Invited Review: Heat Stress Effects during Late Gestation on Dry Cows and Their Calves'. *Journal of Dairy Science* 96 (7): 4079–93. https://doi.org/10.3168/jds.2012-6278.

Tasiame, W., B. O. Emikpe, R. D. Folitse, C. O. Fofie, V. Burimuah, S. Johnson, J. A. Awuni, E. Afari, N. Yebuah, and F. Wurapa. 2016. 'The Prevalence of Brucellosis in Cattle and Their Handlers in North Tongu District of Volta Region, Ghana'. *African Journal of Infectious Diseases* 10 (2): 111. https://doi.org/10.21010/ajid.v10i2.6.

Taurel, Anne-Frieda, Raphaël Guatteo, Alain Joly, Henri Seegers, and François Beaudeau. 2011. 'Seroprevalence of Q Fever in Naturally Infected Dairy Cattle Herds'. *Preventive Veterinary Medicine* 101 (1–2): 51–57. https://doi.org/10.1016/j.prevetmed.2011.05.005.

Taylor, M. A, R. L Coop, and R. L Wall. 2013. *Veterinary Parasitology*. Chicester: Wiley. http://public.eblib.com/choice/publicfullrecord.aspx?p=1158636.

Tenter, Astrid M, Anja R Heckeroth, and Louis M Weiss. 2000. '*Toxoplasma Gondii*: From Animals to Humans'. *International Journal for Parasitology*, Thematic Issue: Emerging Parasite Zoonoses, 30 (12): 1217–58. https://doi.org/10.1016/S0020-7519(00)00124-7.

Terefe, Yitagele, Sisay Girma, Negesse Mekonnen, and Biruhtesfa Asrade. 2017. 'Brucellosis and Associated Risk Factors in Dairy Cattle of Eastern Ethiopia'. *Tropical Animal Health and Production* 49 (3): 599–606. https://doi.org/10.1007/s11250-017-1242-7.

Thabti F, Hammami M. PEPIN, L. Bakkali Kassimi, A. M'zah, P. Russo, M.S. Be, Said, S.Hammami, and M. Pepin,. 2005b. 'First Detection and Genetic Characterization of Bovine Viral Diarrhoea Viruses (BVDV) Types 1 and 2 in Tunisia'. *Revue de Médecine Vétérinaire* 156 (8–9): 419–22.

Thabti F, C. Letellier, S. Hammami, M. Pépin, M. Ribière, A. Mesplède, P. Kerkhofs, and P. Russo. 2005a. 'Detection of a Novel Border Disease Virus Subgroup in Tunisian Sheep'. *Archives of Virology* 150 (2): 215–29. https://doi.org/10.1007/s00705-004-0427-3.

Thabti, Fatma, Laure Fronzaroli, Elyess Dlissi, Jean-Michel Guibert, Salah Hammami, Michel Pepin, and Pierre Russo. 2002. 'Experimental Model of Border Disease Virus Infection in Lambs: Comparative Pathogenicity of Pestiviruses Isolated in France and Tunisia'. *Veterinary Research* 33 (1): 35–45. https://doi.org/10.1051/vetres:2001004.

Thatcher, W. W., and C. J. Wilcox. 1973. 'Postpartum Estrus as an Indicator of Reproductive Status in the Dairy Cow'. *Journal of Dairy Science* 56 (5): 608–10. https://doi.org/10.3168/jds.S0022-0302(73)85227-0.

Thilsted, John P., and J. P. Dubey. 1989. 'Neosporosis-Like Abortions in a Herd of Dairy Cattle'. *Journal of Veterinary Diagnostic Investigation* 1 (3): 205–9. https://doi.org/10.1177/104063878900100301.

Thomann, B., A. Tschopp, I. Magouras, M. Meylan, G. Schüpbach-Regula, and B. Häsler. 2017. 'Economic Evaluation of the Eradication Program for Bovine Viral Diarrhea in the Swiss Dairy Sector'. *Preventive Veterinary Medicine* 145 (September): 1–6. https://doi.org/10.1016/j.prevetmed.2017.05.020.

Thurmond, M. C., A. J. Branscum, W. O. Johnson, E. J. Bedrick, and T. E. Hanson. 2005. 'Predicting the Probability of Abortion in Dairy Cows: A Hierarchical Bayesian Logistic-Survival Model Using Sequential Pregnancy Data'. *Preventive Veterinary Medicine* 68 (2): 223–39. https://doi.org/10.1016/j.prevetmed.2005.01.008.

Thurmond, Mark C., and John P. Picanso. 1990. 'A Surveillance System for Bovine Abortion'. *Preventive Veterinary Medicine* 8 (1): 41–53. https://doi.org/10.1016/0167-5877(90)90021-9.

Tialla, D., P. Koné, M. C. Kadja, A. Kamga-Waladjo, C. B. Dieng, N. Ndoye, K. G. G. Kouame, S. Bakou, and A. J. Akakpo. 2014. 'Prévalence de la brucellose bovine et comportements à risque associés à cette zoonose dans la zone périurbaine de Dakar au Sénégal'. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 67 (2): 67–72. https://doi.org/10.19182/remvt.10186.

Tilahun, Berhanu, Yacob Hailu Tolossa, Getachew Tilahun, Hagos Ashenafi, and Shihun Shimelis. 2018. 'Seroprevalence and Risk Factors of *Toxoplasma Gondii* Infection among Domestic Ruminants in East Hararghe Zone of Oromia Region, Ethiopia'. *Veterinary Medicine International* 2018 (May). https://doi.org/10.1155/2018/4263470.

To, H., K. K. Htwe, N. Kako, H. J. Kim, T. Yamaguchi, H. Fukushi, and K. Hirai. 1998. 'Prevalence of *Coxiella Burnetii* Infection in Dairy Cattle with Reproductive Disorders'. *The Journal of Veterinary Medical Science* 60 (7): 859–61. https://doi.org/10.1292/jvms.60.859.

Tonin, Alexandre A., Aleksandro S. da Silva, Gustavo R. Thomé, Guilherme V. Bochi, Maria R. C. Schetinger, Rafael N. Moresco, Giovana Camillo, Gustavo Toscan, Fernanda F. Vogel, and Sonia T. A. Lopes. 2014. 'Oxidative Stress in Brain Tissue of Gerbils Experimentally Infected

with *Neospora Caninum*'. *Journal of Parasitology* 100 (1): 154–56. https://doi.org/10.1645/13-310.1.

Toplak, Ivan, Torstein Sandvik, Darja Barlic-Maganja, Joze Grom, and David J. Paton. 2004. 'Genetic Typing of Bovine Viral Diarrhoea Virus: Most Slovenian Isolates Are of Genotypes 1d and 1f'. *Veterinary Microbiology* 99 (3–4): 175–85. https://doi.org/10.1016/j.vetmic.2003.12.004.

Tozer, S. J., S. B. Lambert, T. P. Sloots, and M. D. Nissen. 2011. 'Q Fever Seroprevalence in Metropolitan Samples Is Similar to Rural/Remote Samples in Queensland, Australia'. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 30 (10): 1287–93. https://doi.org/10.1007/s10096-011-1225-y.

Tramuta, Clara, Daniela Lacerenza, Simona Zoppi, Mariella Goria, Alessandro Dondo, Ezio Ferroglio, Patrizia Nebbia, and Sergio Rosati. 2011. 'Development of a Set of Multiplex Standard Polymerase Chain Reaction Assays for the Identification of Infectious Agents from Aborted Bovine Clinical Samples'. *Journal of Veterinary Diagnostic Investigation* 23 (4): 657–64. https://doi.org/10.1177/1040638711407880.

Van den Brom, René, Barbara Schimmer, Peter M. Schneeberger, Wim A. Swart, Wim van der Hoek, and Piet Vellema. 2013. 'Seroepidemiological Survey for *Coxiella Burnetii* Antibodies and Associated Risk Factors in Dutch Livestock Veterinarians'. *PLoS ONE* 8 (1). https://doi.org/10.1371/journal.pone.0054021.

VanRaden, P. M., A. H. Sanders, M. E. Tooker, R. H. Miller, H. D. Norman, M. T. Kuhn, and G. R. Wiggans. 2004. 'Development of a National Genetic Evaluation for Cow Fertility'. *Journal of Dairy Science* 87 (7): 2285–92. https://doi.org/10.3168/jds.S0022-0302(04)70049-1.

Vicari, N., S. Faccini, M. Ricchi, C. Garbarino, L. Decastelli, M. Boldini, C. Rosignoli, A. Dalmasso, V. Bronzo, and M. Fabbi. 2013. 'Occurrence of *Coxiella Burnetii* in Bulk Tank Milk from Northwestern Italy'. *Veterinary Record* 172 (26): 687–687. https://doi.org/10.1136/vr.101423.

Vilcek, S., A. J. Herring, J. A. Herring, P. F. Nettleton, J. P. Lowings, and D. J. Paton. 1994. 'Pestiviruses Isolated from Pigs, Cattle and Sheep Can Be Allocated into at Least Three Genogroups Using Polymerase Chain Reaction and Restriction Endonuclease Analysis'. *Archives of Virology* 136 (3–4): 309–23. https://doi.org/10.1007/bf01321060.

Vilček, Š., A. J. Herring, J. A. Herring, P. F. Nettleton, J. P. Lowings, and D. J. Paton. 1994. 'Pestiviruses Isolated from Pigs, Cattle and Sheep Can Be Allocated into at Least Three Genogroups Using Polymerase Chain Reaction and Restriction Endonuclease Analysis'. *Archives of Virology* 136 (3): 309–23. https://doi.org/10.1007/BF01321060.

Vilcek, S., D. J. Paton, B. Durkovic, L. Strojny, G. Ibata, A. Moussa, A. Loitsch, et al. 2001. 'Bovine Viral Diarrhoea Virus Genotype 1 Can Be Separated into at Least Eleven Genetic Groups'. *Archives of Virology* 146 (1): 99–115. https://doi.org/10.1007/s007050170194.

Viltro, A., J. Alaots, M. Pärn, and K. Must. 2002. 'Natural Changes in the Spread of Bovine Viral Diarrhoea Virus (BVDV) among Estonian Cattle'. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health* 49 (6): 263–69. https://doi.org/10.1046/j.1439-0450.2002.00560.x.

Wada, Yoshihide, L. P. H. van Beek, and Marc F. P. Bierkens. 2012. 'Nonsustainable Groundwater Sustaining Irrigation: A Global Assessment'. *Water Resources Research* 48 (6). https://doi.org/10.1029/2011WR010562.

Walsh, S. W., E. J. Williams, and A. C. O. Evans. 2011. 'A Review of the Causes of Poor Fertility in High Milk Producing Dairy Cows'. *Animal Reproduction Science* 123 (3): 127–38. https://doi.org/10.1016/j.anireprosci.2010.12.001.

Walz, P. H., D. L. Grooms, T. Passler, J. F. Ridpath, R. Tremblay, D. L. Step, R. J. Callan, M. D. Givens, and American College of Veterinary Internal Medicine. 2010. 'Control of Bovine Viral Diarrhea

Virus in Ruminants'. *Journal of Veterinary Internal Medicine* 24 (3): 476–86. https://doi.org/10.1111/j.1939-1676.2010.0502.x.

Walz, P. H., T. Montgomery, T. Passler, K. P. Riddell, T. D. Braden, Y. Zhang, P. K. Galik, and S. Zuidhof. 2015. 'Comparison of Reproductive Performance of Primiparous Dairy Cattle Following Revaccination with Either Modified-Live or Killed Multivalent Viral Vaccines in Early Lactation'. *Journal of Dairy Science* 98 (12): 8753–63. https://doi.org/10.3168/jds.2015-9760.

Wang, Fun-In, Helen Shieh, and Yung-Kung Liao. 2001. 'Prevalence of *Chlamydophila Abortus* Infection in Domesticated Ruminants in Taiwan'. *Journal of Veterinary Medical Science* 63 (11): 1215–20. https://doi.org/10.1292/jvms.63.1215.

Wareth, Gamal, Falk Melzer, Mandy C. Elschner, Heinrich Neubauer, and Uwe Roesler. 2014. 'Detection of *Brucella Melitensis* in Bovine Milk and Milk Products from Apparently Healthy Animals in Egypt by Real-Time PCR'. *The Journal of Infection in Developing Countries* 8 (10): 1339–43. https://doi.org/10.3855/jidc.4847.

Wasmuth, James D., Viviana Pszenny, Simon Haile, Emily M. Jansen, Alexandra T. Gast, Alan Sher, Jon P. Boyle, Martin J. Boulanger, John Parkinson, and Michael E. Grigg. 2012. 'Integrated Bioinformatic and Targeted Deletion Analyses of the SRS Gene Superfamily Identify SRS29C as a Negative Regulator of Toxoplasma Virulence'. *MBio* 3 (6). https://doi.org/10.1128/mBio.00321-12.

Webster, Joanne P. 2010. 'Review of "Toxoplasmosis of Animals and Humans (Second Edition)" by J.P. Dubey'. *Parasites & Vectors* 3: 112. https://doi.org/10.1186/1756-3305-3-112.

Wehrend, Axel, Klaus Failing, Bernhard Hauser, Cornelie Jäger, and H. Bostedt. 2005. 'Production, Reproductive, and Metabolic Factors Associated with Chlamydial Seropositivity and Reproductive Tract Antigens in Dairy Herds with Fertility Disorders'. *Theriogenology* 63 (3): 923–30. https://doi.org/10.1016/j.theriogenology.2004.05.009.

Weigel, K. A., R. W. Palmer, and D. Z. Caraviello. 2003. 'Investigation of Factors Affecting Voluntary and Involuntary Culling in Expanding Dairy Herds in Wisconsin Using Survival Analysis'. *Journal of Dairy Science* 86 (4): 1482–86. https://doi.org/10.3168/jds.S0022-0302(03)73733-3.

Wernike, Kerstin, Jörn Gethmann, Horst Schirrmeier, Ronald Schröder, Franz J. Conraths, and Martin Beer. 2017. 'Six Years (2011-2016) of Mandatory Nationwide Bovine Viral Diarrhea Control in Germany-A Success Story'. *Pathogens (Basel, Switzerland)* 6 (4). https://doi.org/10.3390/pathogens6040050.

Whatmore, Adrian M., Nicholas Davison, Axel Cloeckaert, Sascha Al Dahouk, Michel S. Zygmunt, Simon D. Brew, Lorraine L. Perrett, et al. 2014. '*Brucella Papionis Sp.* Nov., Isolated from Baboons (Papio Spp.)'. *International Journal of Systematic and Evolutionary Microbiology*, 64 (12): 4120–28. https://doi.org/10.1099/ijs.0.065482-0.

Wheelhouse, Nicholas, Frank Katzer, Frank Wright, and David Longbottom. 2010. 'Novel *Chlamydia*-like Organisms as Cause of Bovine Abortions, UK'. *Emerging Infectious Diseases* 16 (8): 1323–24. https://doi.org/10.3201/eid1608.091878.

White, Michael W., and Elena S. Suvorova. 2018. 'Apicomplexa Cell Cycles: Something Old, Borrowed, Lost, and New'. *Trends in Parasitology* 34 (9): 759–71. https://doi.org/10.1016/j.pt.2018.07.006.

Whitney, Ellen A. S., Robert F. Massung, Amanda J. Candee, Elizabeth C. Ailes, Lee M. Myers, Nicole E. Patterson, and Ruth L. Berkelman. 2009. 'Seroepidemiologic and Occupational Risk Survey for *Coxiella Burnetii* Antibodies among US Veterinarians'. *Clinical Infectious Diseases* 48 (5): 550–57.

WHO. 2015. ‘Estimates of the Global Burden of Foodborne Diseases. Foodborne Diseases Burden Epidemiology Reference Group 2007-2015’. *255 Publication*, 3 December 2015, World Health Organization edition.

Wilkowsky, Silvina Elizabeth, Guillermo Gimenez Bareiro, María Laura Mon, Dadin Prando Moore, Gastón Caspe, Carlos Campero, Marcelo Fort, and María Isabel Romano. 2011. ‘An Applied Printing Immunoassay with Recombinant Nc-SAG1 for Detection of Antibodies to *Neospora Caninum* in Cattle’. *Journal of Veterinary Diagnostic Investigation* 23 (5): 971–76. https://doi.org/10.1177/1040638711416845.

Williams, D., and S. V. Winden. 2014. ‘Risk Factors Associated with High Bulk Milk Antibody Levels to Common Pathogens in UK Dairies’. *The Veterinary Record* 174 (23): 580. https://doi.org/10.1136/vr.102049.

Williams, R. H., E. K. Morley, J. M. Hughes, P. Duncanson, R. S. Terry, J. E. Smith, and G. Hide. 2005. ‘High Levels of Congenital Transmission of *Toxoplasma Gondii* in Longitudinal and Cross-Sectional Studies on Sheep Farms Provides Evidence of Vertical Transmission in Ovine Hosts’. *Parasitology* 130 (3): 301–7. https://doi.org/10.1017/S0031182004006614.

Wiltbank, M. C., A. Gümen, and R. Sartori. 2002. ‘Physiological Classification of Anovulatory Conditions in Cattle’. *Theriogenology* 57 (1): 21–52. https://doi.org/10.1016/s0093-691x(01)00656-2.

Wiltbank, Milo C., Giovanni M. Baez, Alvaro Garcia-Guerra, Mateus Z. Toledo, Pedro L. J. Monteiro, Leonardo F. Melo, Julian C. Ochoa, José E. P. Santos, and Roberto Sartori. 2016. ‘Pivotal Periods for Pregnancy Loss during the First Trimester of Gestation in Lactating Dairy Cows’. *Theriogenology* 86 (1): 239–53. https://doi.org/10.1016/j.theriogenology.2016.04.037.

Wittenbrink, M. M., H. A. Schoon, W. Bisping, and A. Binder. 1993. ‘Infection of the Bovine Female Genital Tract with *Chlamydia Psittaci* as a Possible Cause of Infertility’. *Reproduction in Domestic Animals* 28 (2): 129–36. https://doi.org/10.1111/j.1439-0531.1993.tb00734.x.

Woldehiwet, Zerai. 2004. ‘Q Fever (Coxiellosis): Epidemiology and Pathogenesis’. *Research in Veterinary Science* 77 (2): 93–100. https://doi.org/10.1016/j.rvsc.2003.09.001.

Wolf-Jäckel, Godelind Alma, Mette Sif Hansen, Gitte Larsen, Elisabeth Holm, Jørgen Steen Agerholm, and Tim Kåre Jensen. 2020. ‘Diagnostic Studies of Abortion in Danish Cattle 2015–2017’. *Acta Veterinaria Scandinavica* 62 (1): 1. https://doi.org/10.1186/s13028-019-0499-4.

Wright, I. A., S. M. Rhind, T. K. Whyte, A. J. Smith, S. R. McMillen, and R. Prado. 1990. ‘Circulating Concentrations of LH and FSH and Pituitary Responsiveness to GnRH in Intact and Ovariectomized Suckled Beef Cows in Two Levels of Body Condition’. *Animal Science* 51 (1): 93–101. https://doi.org/10.1017/S0003356100005183.

www.aps.dz. 2015. ‘Tiaret : collecte de lait insuffisante malgré une abondance de la production - Algerie360’. 9 May 2015. https://www.algerie360.com/tiaret-collecte-de-lait-insuffisante-malgre-une-abondance-de-la-production/.

Xiao, Pei, Hongxia Yang, Dongdong Di, Dongri Piao, Qiuxiang Zhang, Ruie Hao, Suxia Yao, et al. 2015. ‘Genotyping of Human *Brucella Melitensis* Biovar 3 Isolated from Shanxi Province in China by MLVA16 and HOOF’. *PLOS ONE* 10 (1): e0115932. https://doi.org/10.1371/journal.pone.0115932.

Yağcı Yücel, Sükran, Mehmet Yaman, Cemal Kurt, Cahit Babür, Bekir Celebi, Selçuk Kılıç, and Doğukan Ozen. 2014. ‘Seroprevalance of brucellosis, listeriosis and toxoplasmosis in cattle in Adana province of Turkey’. *Turkiye Parazitolojii Dergisi* 38 (2): 91–96. https://doi.org/10.5152/tpd.2014.3454.

Yahia, A., K. Hamrat, K. Saidani, and R. Kaidi. 2018. ‘Seroprevalence and Risk Factors of Bovine Brucellosis in the Province of Djelfa (ALGERIA)’. *Indian Journal Of Animal Research*, June. https://doi.org/10.18805/ijar.B-893.

Yahiaoui, W.I, Dahmani, A., Bouzebda, Z., and Afri-Bouzebda, F., ,. 2013. 'Sondage Sérologique de La Fièvre Q Chez Les Ovins Par La Méthode ELISA et Prévalence Des Avortements Dans La Région de Ksar El Boukhari (Algérie)','. *TROPICULTURA 32(1), 22–27*. 32 (1): 22–27.

Yahyaoui Azami, Hind, Marie J. Ducrotoy, Mohammed Bouslikhane, Jan Hattendorf, Mike Thrusfield, Raquel Conde- Álvarez, Ignacio Moriyón, et al. 2018. 'The Prevalence of Brucellosis and Bovine Tuberculosis in Ruminants in Sidi Kacem Province, Morocco'. *PLoS ONE* 13 (9). https://doi.org/10.1371/journal.pone.0203360.

Yekhlef, H. 1989. 'La Production Extensive de Lait En Algérie.' Options Méditerranéennes, Série Séminaires, (6) pp 135-139.

Yilmaz, Huseyin, Eda Altan, Julia Ridpath, and Nuri Turan. 2012. 'Genetic Diversity and Frequency of Bovine Viral Diarrhea Virus (BVDV) Detected in Cattle in Turkey'. *Comparative Immunology, Microbiology and Infectious Diseases* 35 (5): 411–16. https://doi.org/10.1016/j.cimid.2012.03.006.

Yilmaz, Volkan. 2016. 'Prevalence of Antibodies to Bovine Viral Diarrhea Virus (BVDV) in Blood and Milk Serum in Dairy Cattle in Kars District of Turkey'. *Indian Journal Of Animal Research* 50 (5): 811–15. https://doi.org/10.18805/ijar.9497.

Yoo, Han Sang. 2010. 'Infectious Causes of Reproductive Disorders in Cattle'. *The Journal of Reproduction and Development* 56 Suppl (January): S53-60. https://doi.org/10.1262/jrd.1056s53.

Yu, Jinhai, Zhaofei Xia, Qun Liu, Jing Liu, Jun Ding, and Wei Zhang. 2007. 'Seroepidemiology of *Neospora Caninum* and *Toxoplasma Gondii* in Cattle and Water Buffaloes (Bubalus Bubalis) in the People's Republic of China'. *Veterinary Parasitology* 143 (1): 79–85. https://doi.org/10.1016/j.vetpar.2006.07.031.

Yves Leforban, Karim Ben Jebara, Youssef Lhor, John Ryan, and Ali Benkirane. 1999. 'On the 1999 Epidemic, History of Recent Epidemics and Suggestions for Control.' History of recent epidemics and suggestions for control. on the 1999 epidemic,. FOOD AND AGRIGULTURE ORGANISATION FAO.

Zaeim, I, D Tainturier, A Amara, M Zmerli, J Chemli, and V Moquay. 1993. 'A Propos d'un Cas de Border Disease Dans Un Troupeau Ovin En Tunisie'. *Revue de Medecine Vétérinaire* 12 (144): 975–79.

Zaghawa, A. 1998. 'Prevalence of Antibodies to Bovine Viral Diarrhoea Virus and/or Border Disease Virus in Domestic Ruminants'. *Journal of Veterinary Medicine, Series B* 45 (1–10): 345–51. https://doi.org/10.1111/j.1439-0450.1998.tb00803.x.

Zhou, Mo, Shinuo Cao, Ferda Sevinc, Mutlu Sevinc, Onur Ceylan, Mingming Liu, Guanbo Wang, et al. 2017. 'Enzyme-Linked Immunosorbent Assays Using Recombinant TgSAG2 and NcSAG1 to Detect *Toxoplasma Gondii* and *Neospora Caninum*-Specific Antibodies in Domestic Animals in Turkey'. *The Journal of Veterinary Medical Science* 78 (12): 1877–81. https://doi.org/10.1292/jvms.16-0234.

Zubairu, A., M. B. Ardo, and H. M. Mai. 2014. 'Seroprevalence of Ruminant Brucellosis in Three Selected Local Government Areas of Taraba State'. *Sokoto Journal of Veterinary Sciences* 12 (1): 51–56. https://doi.org/10.4314/sokjvs.v12i1.8.

VI. Appendix

Appendix 1 Questionnaire used in the study

→ ***Section identification herd and farmer***

Date of interview----------
Season----------
Farm/Herd Number----------
Farmer Name ----------
Localisation ----------
Zones agro –écologiques ----------
Propriétaire----------
Phone Number----------

→ ***Section livestock***

What is the total number of cattle in your farm?

Category	Bull (>1 year)	Young Bull	Cow		Heifer> 1 year		calves		Total
			Pregnant	empty	Pregnant	empty	Male	female	
effective									

What type of production system do you practice?

Kind of production system	Intensive	Extensive	Semi-intensive
Yes / No			

If Cats are present **1. Occasional presence of neighborhood cats •**
2. Regular presence (cats feeding on the spot) •
Do you see kittens **1. Yes** **2. No**
Presence of stray dogs in pastures or stables **1. Yes** **2. No**
Use of disinfectant **1. Yes** **2. No**
Frequency of disinfection **1. Once a week**
2. Once a month
3. Once a quarter
4. Once a semester
5. Once a year
6. Other
Cleaning method **1. Scanning**
2. Piping
3. Both
4. Not practical
Vaccination **1. Yes** **2. No**
Against which diseases? **1. Rabies**
2. Foot-and-mouth disease

Regular presence of a veterinarian **1. Yes** **2. No**
Mixture between cattle other species **1. Yes** **2. No**
If yes which species **1. Sheep**
2. Goats
3. Monkey
4. Horses
5. Kitchen

Animal	Total	The same building	The same pasture
Sheep			
Goat			
Horse			
Monkey			
cats			
Dogs			
Chicken			
Rabbit			

Visit of the farm by foreign breeders **1. Yes** **2. No**
Food sources **1. mixed on the farm**
2. From grazing land
3. Bought
4. Both
Drinking Water sources **1. Tap**
2.Well or drilling
4. River water
Do animals have access to running water (river, stream) to drink **1. Yes** **2. No**
Do animals have access to a standing water point (pond) to drink **1. Yes** **2. No**
Quarantine practice **1. Yes** **2. No**
Are there special calving box **1. Yes** **2. No**
Brucellosis screening **1. Yes** **2. No** date of last screening----------
tuberculosis screening **1. Yes** **2. No** date of last screening----------

Wildlife animals	To the building Yes/No	In the pasture Yes/No
Boar		
Fox		
wolf		
Other		

Rate the production efficiency/performance of your animals **1. Very good**
2. Good
3. Poor

Presence of other livestock buildings around the farm **1. 0**
2. 1 or 2
3. More than 2

Do workers use special clothing for the farm **1. Yes** **2. No**

Presence of rodents **1. Yes** **2. No**

Presence of ticks and flies **1. Yes** **2. No**

Do you sell milk **1.Yes** **2. No**

To whom do you sell your milk

1. Neighbors

2. Milk vendor

3. Primary co-operative milk collection center

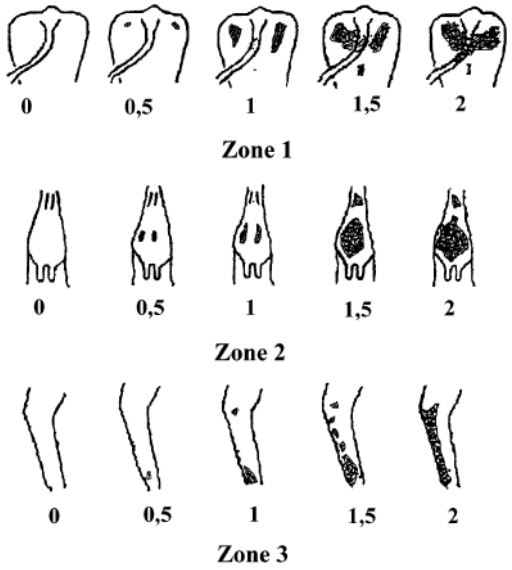
4. Private milk collection center

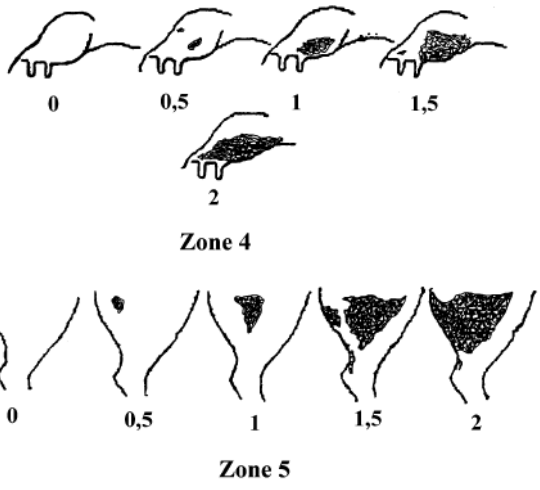
5. Restaurant/hotel

6. Processing factory

7. Milk kiosk

8. Others (state)





Zone 1 : / 02

Zone 2 : / 02

Zone 3 : /02

Zone 4 : / 02

Zone 5 : / 02

Note : / 10

0-<2 _ stabulation très propre

2-<4 _ stabulation propre

4-<6 _ stabulation un peu sale

6-<8 _ stabulation sale

8-10 _ stabulation très sale

→ *Reproduction performance section*

Mode of reproduction **1. natural**
2. artificial

If you are using natural breeding what is the source of the bull **1. From own farm**
2. Neighbourhoods
3. Other farms

Abortion rate/year----------

How do you dispose the after-birth **1. Buried**
2. In the pit
3. Burn
4. Others (Please specify)

Are there cats at home or from the neighbourhoods **1. Yes 2. No**

Are there dogs at home or from the neighbourhoods **1. Yes 2. No**

Have you seen any rodents around your farm/ home **1. Yes 2. No**

Cattle identification	Age (months)	Breed	Type (Cow/Heifer)	Stage of gestation (months)	Number of pregnancies	Number of calving	Inter calving period (Expected" or "Prolonged) *	Number of abortions in the last 5 years **	Stillbirth (Yes/No)	Retained foetal membrane (Yes/No)	Endometritis (Yes/No)	Weak calf (Yes/No)	Anoestrus (Yes/No)	Repeat breeding (Yes/No) ***

* Expected refers to calving every 12–18 months while prolonged refers to over 18 months.

** abortion was defined as loss of the foetus between 42 and 260 days of gestation, and stillbirth was defined as a calf that was born dead between 260 days and full-term or died within 24 h following birth.

*** Cows reported requiring 3 or more services per pregnancy were categorized as repeat breeders.

Appendix 2. RNA extraction from serum using IndiMag® Pathogen Kit with BioSprint 96.

Equipment and reagents to be supplied by user

- If applicable: Magnetic head for use with Large 96-Rod Covers
- Pipettors and disposable pipette tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipette tips with aerosol barriers
- Multidispenser
- Ethanol (96-100%)*
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Vortexer
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the used worktable

Important points before starting

- Ensure that you are familiar with the correct operation of the workstation. Refer to the respective user manual for operating instructions.
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and Carrier RNA have been prepared according to the instructions.
- Check that Buffer VXL or Buffer ACB does not contain a white precipitate. If necessary, incubate Buffer VXL or Buffer ACB for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- If using IndiMag Pathogen Kit (SP947457): The 96-rod covers are supplied as packets of 2. When using a new packet of 2, store the second 96-rod cover on another S-block or plate. Care should be taken to not bend the 96-rod covers.

Things to do before starting.

- Thaw and equilibrate samples at room temperature (15-25°C).
- If the volume of the sample is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- Prepare the Buffer VXL mixture according to Table 3 on page 26, for use in step 3 of the procedure.

Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time or 1 minute before subsequent uses.

Important: Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates. Follow the procedure as described below (pipetting Proteinase K into the wells, followed by sample and then Buffer VXL mixture).

Buffer VXL mixture preparation.

	Number of samples		
Reagent	1	48	96
Buffer VXL	100 µl	4.8 ml	9.6 ml
Buffer ACB	400 µl	19.2 ml	38.4 ml
MagAttract Suspension G	25 µl	1.2 ml	2.4 ml
Carrier RNA (1 µg/µl)	1 µl	48 µl	96 µl

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

Procedure for use with magnetic particle processors (e.g., KingFisher Flex, BioSprint 96 or equivalent)

1. Label and prepare 4 x 96-well deep well plates (S-Block) and 1 x 96-well microplate (slots 2-6) according to Table 2.

Instrument setup and reagent volumes.

Slot	**Loading message**	**Format**	**Item to add**	**Volume per well (µl)**
6	Load rod cover	S-Block	Large 96-rod cover	-
5	Load elution	96-well microplate MP	Buffer AVE	100
4	Load wash 3	S-Block	Buffer (96-100%)	750
3	Load wash 2	S-Block	Buffer AW2	700
2	Load wash 1	S-Block	Buffer AW1	700
1	Load lysate	S-Block	Lysate*	720

* Includes 20 µl Proteinase K, 200 µl sample and 500 µl Buffer VXL mixture.

2. Ensure to have prepared enough Buffer VXL mixture.
3. Pipet 20 µl Proteinase K into the bottom of a new well of the 96-well deep plate or S-Block and add 200 µl sample. Note: If your sample volume is less than 200 µl, bring it to 200 µl by adding PBS.
4. Mix Buffer VXL mixture thoroughly for 30 s and add 500 µl Buffer VXL mixture to each sample in the 96-well deep well plate.

Immediately load the prepared plates onto the processor and start the respective protocol as demonstrated in the following link on how using of the DNA extraction on Biosprint 96 instrument at CAGE.

https://www.youtube.com/watch?v=g-ko9SqDSN4

Appendix 3: ERRATA.

ERRATA for the defense of the doctoral thesis

Date: 14/06/2021.

Doctorate candidate: GUIDOUM Khaled Azzeddine

Veterinary Institute of Ibn Khaldoun-Tiaret university-Algeria

Title of thesis: Seroprevalence and risk factors of the main abortive infectious agents of cattle in Batna.

Despite the vigilance given when writing the final thesis, a mistake has, escaped my notice. The only and main correction to be made are presented below:

Instead of the title of the thesis "**Seroprevalence and risk factors of the main abortive infectious agents of cattle in Batna**" read "**Seroprevalence and risk factors of the main abortive infectious agents of cattle in Algeria**"

Initially the experimental study was programmed to cover only the region of Batna (northeast Algeria).

Following the support provided by veterinary practitioners working in other regions of eastern Algeria, in particular Setif and Khanchela, sampling could be carried out effectively in these latter localities.

In the same way, the sampling could also cover the province of Tiaret.

Appendix 4: Published paper

Ruminant *Pestiviruses* in North Africa

Guidoum, K. A., B. Benallou, L. Pailler, J. Espunyes, S. Napp, and O. Cabezón. 2020. 'Ruminant *Pestiviruses* in North Africa'. *Preventive Veterinary Medicine* 184 (November): 105156. https://doi.org/10.1016/j.prevetmed.2020.105156.

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Ruminant pestiviruses in North Africa

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ABSTRACT

Ruminant pestiviruses are widely distributed worldwide, causing congenital disease and massive economic losses. Although ruminant production is an important economic sector in North Africa, the knowledge about pestiviruses is scarce. The present study aimed at assessing the presence of *Pestivirus* in cattle in Algeria, and to review the data available on ruminant pestiviruses in North Africa. A cross-sectional study was conducted on dairy farms from North-Western Algeria. Blood samples from 234 dairy cattle from 21 herds were collected. All sera were analysed for the presence of antibodies using a commercial iELISA. The presence of *Pestivirus* RNA was also assessed by using a Reverse Transcription-PCR, and PCR-positive samples were sequenced. Risk factors related to *Pestivirus* infection were also analysed. The review of the presence of ruminant pestiviruses in North Africa was performed using a systematic search and compilation methodology of the peer-reviewed literature available in order to identify gaps of knowledge for future research. The seroprevalence at population and farm levels obtained in the present study (59.9% and 90.5%, respectively) concur with data reported in neighbouring countries. Risk factors associated with *Pestivirus* infection in cattle were the presence of sheep in the herd and the animal category (cow vs heifer). Furthermore, we confirmed the presence of BVDV-1a in Algeria. The scarce data suggest an endemic epidemiological scenario of *pestivirus* in livestock. The lack of studies about the epidemiology and molecular variability of ruminant pestiviruses in livestock and wildlife in North Africa is of concern for animal health and wildlife conservation, and needs to be addressed.

1. Introduction

The genus *Pestivirus* comprises four traditionally recognized species of enveloped, positive-sense single-stranded RNA (12.3 kb) viruses: Bovine Viral Diarrhoea Virus (BVDV) -1, BVDV-2, Border Disease Virus (BDV) and Classical Swine Fever Virus (CSFV) (Schweizer and Peterhans, 2014). Recently, the International Committee on Taxonomy of Viruses (ICTV) has recognized eleven species within this Genus (*Pestivirus* A to K) (ICTV - International Committee on Taxonomy of Viruses (ICTV, 2018). In adult ruminants, horizontal *Pestivirus* infection usually causes a mild disease and short viremia (7–15 days) that ends with the outcome of humoral response. Vertical transmission of the virus will cause fetal resorption, abortion, stillbirths or malformations of the fetus. However, if the infection by *Pestivirus* A (BVDV-1) or *Pestivirus* B (BVDV-2) occurs between the second and fourth month of gestation in cattle, or before the second month in sheep in the case of *Pestivirus* D (BDV), the infection may lead to the birth of Persistently Infected (PI) animals that are immunotolerant to the virus (Schweizer and Peterhans, 2014). These PI individuals are the main source of spread and persistence of ruminant *Pestivirus* in livestock. Ruminant pestiviruses are distributed worldwide causing congenital disease and entailing massive economic losses (OIE, 2019).

Algeria faces a huge deficit in dairy and meat production, triggering a significant annual import expenditure, which amounted to US$ 2.045 billion for milk and US$ 0.307 billion for meat in 2014 (Kardjadj and Luka, 2016). A massive surge in food demand has occurred in Maghreb countries, particularly in animal products (meat and milk), implying the need for a "Livestock Revolution" (Srairi et al., 2013). Red meat marketed and consumed in Algeria consists essentially of mutton and beef. According to the official cattle census of Algeria (MADR, 2017a) there

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was a total of 1.89 million bovine heads in 2017. The bovine production is one of the main sources of both meat and milk in Algeria, playing a vital role in food security. The 33% of the national consumption is provided by national production. Cattle farming is generally structured in small to medium-sized workshops dedicated mainly to milk production (Mouffok, 2018). The cattle population is composed of imported cattle (mainly Holstein and Montbéliarde from Europe), local cattle (the local breed "Brune de l'Atlas") and crossbreed cattle (crosses between the local and the introduced breeds). The population of small ruminants in Algeria comprises more than 31.2 million heads (26.4 million sheep and 4.8 million goats) (MADR, 2017b). Sheep meat production amounts to 325,000 tons (Business France, 2018). Animal health improvement is necessary in Algeria, especially regarding reproductive disorders (abortive pathogens). *Pestivirus* seroprevalence in ruminants has been rarely assessed in the country and to our knowledge there is only one study in cattle, one in sheep and one in dromedary camels (Derdour et al., 2017; Felcoun et al., 2018; Saidi et al., 2018). The only study in cattle reported a seroprevalence of 1.39% in the North-centre of the country (Derdour et al., 2018). In fact, cattle are not routinely vaccinated against BVDV in Algeria and the knowledge about epidemiology and molecular epidemiology of *Pestivirus*, especially *Pestivirus* A–B, in the whole North-African region is scarce. The present study aimed to study the genetic diversity of *Pestivirus* circulating in cattle herds in Algeria, to determine the risk factors for *Pestivirus* A–B infection in Algeria, and to review the available data of ruminant pestiviruses in North Africa.

2. Materials and methods

2.1. Animals and samples

A two-stage sampling survey was carried out in North-Western Algeria (Tiaret province; 35°22'10.1"N 1°19'47.7"E) between June 2018 and August 2019. The Tiaret province is located approximately 160 Km from the Mediterranean coasts and covers an area of 20,399 km2 including part of the Tell Atlas in the North and the highlands in the centre and South. Temperature ranges from 6 to 25 °C and precipitations from 4 to 69 mm through the year. As in the whole country, smallholder dairy systems are the dominant organization in the region. The Tiaret Veterinary Office provided a list of all cattle herds registered in the province, which included information of the herd owner, the address or number of animals. The sampling frame included 209 dairy cattle herds.

For the first stage of sampling (sampling of herds), the sample size for disease detection was calculated based on the following formula (Dohoo et al., 2003):

$$n_1 = \left(1 - (1-\alpha_1)^{\frac{1}{D_1}}\right) \times \left(N_1 - \frac{D_1 - 1}{2}\right)$$

where α_1 was the confidence level (set at 95%), D_1 was the minimum number of infected herds (estimated as $D_1 = Prev_1 \times N_1$), where $Prev_1$ was the minimum herd prevalence to be detected (set at 10%), and N_1 was the population of herds (which in our case were 209 dairy herds). The estimate of n_1 was 29 herds. No formal random process was used for the selection of herds. Instead, from the list, a herd was randomly selected, and the herd owner was contacted, and asked, first a) whether they complied with the inclusion criteria, and then b) whether they were willing to participate. The process was repeated until the number of herds needed for the first stage was completed. The inclusion criteria comprised that the herd had at least one female animal above six months, and that the milk was not only for own consumption (i.e. some of the milk was sold). This age category was selected to avoid interference as much as possible the detection of maternal antibodies in the seroprevalence studies (Chase et al., 2008).

For the second stage (sampling of animals within herds), the sample sizes for disease detection were also calculated based on the formula by Dohoo et al. (2003):

$$n_2 = \left(1 - (1-\alpha_2)^{\frac{1}{D_2}}\right) \times \left(N_2 - \frac{D_2 - 1}{2}\right)$$

where α_2 was the confidence level (set at 95%), D_2 was the minimum number of infected animals in herd i (estimated as $D_2 = Prev_2 \times N_i$), where $Prev_2$ was the minimum within-herd prevalence to be detected (set at 30%), and N_2 was the population size of herd i (size of herds selected in stage 1 varied between 7 and 62). The estimate of n_2 varied between 4 and 8. The sampling of animals within herds (second stage) was also random, although because of the lack of proper sampling frames, no formal random process was used either. Random animals in the herd were selected until the number of animals needed for the second stage was completed. However, because of logistics problems, the number of samples per herd could not always be completed, and therefore some extra convenience samples were collected in some of the remaining herds, and also a few extra herds were sampled. Sample sizes for the two stages were calculated using Epitools (Sergeant, 2018).

The sampled population was composed of 224 dairy cattle aged between 9 and 180 months, in semi-intensive husbandry system. Holstein, Montbéliard and crossbreed were the most common breed and the size of the sampled herds varied from ten to a hundred individuals. Blood samples (5 mL) were collected by qualified private and state veterinarians, from the coccygian vein on sterile dry vacutainer tubes, using disposable needles, and was immediately sent on ice to the local laboratory. The sera were extracted by centrifuging the samples at 1200 g for 10 min and were then stored at −20 °C, until tested. All samples were then sent on dry ice to the Animal Health Research Centre (CReSA, IRTA-UAB, Bellaterra, Spain) where the serological and molecular analyses were performed. The study was approved by the ethical review board of the Veterinary Institute of the University Ibn Khaldoun (Tiaret, Algeria).

2.2. Serological and virological analyses

The presence of antibodies against *Pestivirus* p80 protein was determined using a commercial ELISA (IDEXX, Montpellier, France) in accordance with manufacturer's instructions. All sera were also analysed for the presence of *Pestivirus* RNA using a Reverse transcription-PCR (RT-PCR). Total viral RNA was extracted directly from 200 µL of sera using the commercial kit IndiMag® Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany) according to the manufacturer's instructions. The RT-PCR was performed using primers 324 and 326 (Vilček et al., 1994) and a commercial kit (One-Step PCR kit, Qiagen Inc., Hilden Germany). *Pestivirus* positive amplicons were sequenced, and the 5' untranslated region (5'-UTR) was characterized.

2.3. Prevalence and risk factors

In order to account for effect of the two-stage sampling design in the calculation of prevalence and confidence intervals, the R package survey (Lumley, 2020) was used.

Also, to evaluate the effects of the sensitivity and specificity of the commercial iELISA on the prevalence estimation, the true prevalence (TP) of disease (and corresponding 95% CI) were calculated according to the following formula (Dohoo et al., 2003):

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

where AP was the apparent prevalence, Sp was the specificity of the test (0.97; Hanon et al., 2017), and Se was the sensitivity of the test (0.60).

Risk factors related to *Pestivirus* infection were evaluated by studying

several individual and herd traits, such as breed category (pure for Montbéliarde, Holstein, Brown of the Alps and Fleckvieh; and crossbreed for crosses between local breed and imported dairy cow, commonly, Holstein and Montbéliarde), animal category (heifers and cows), herd size, mixed herd (cattle mixed with sheep, poultry or horses), food source (purchased or mixed on the farm), water source (network, well or surface water), presence of standing water and reproductive disorders (repeat breeding, anoestrus, still birth, birth of weak calves, calving interval, abortion, number of calving and pregnancy, endometritis and retained fetal membrane). A bivariate analysis was performed to study the crude association between *Pestivirus* infection and the variables of interest. For numeric variables, we used the Student's *t*-test. For categorical variables, we used the Chi-squared test, except when the sample size for any of the categories was small (i.e. lower than 5), in which case we used the Fisher's exact test. The association between *Pestivirus* infection (binary response) and the variables was further evaluated using a mixed-effects logistic regression model. To account for the two-stage sampling design, and therefore the lack of independence of samples, due to the fact that animals were grouped in herds, the herd was included in the model as a random effect.

Model building strategy: we started by including all the variables that complied with the inclusion criteria ($p < 0.25$). To avoid the multicollinearity the Variance Inflation Factor (VIF) was evaluated. The fixed effect with high VIF were removed and the regression model was re-run. When two or more covariates had similar high VIFs, those with lowest significance in the univariate analysis were removed. The process was repeated until all variables had VIFs lower than or equal five (Dohoo et al., 2003). Then, one by one, variables were removed starting with the less significant, and the Aikake information criteria (AIC) was checked. If the AIC of the reduced model was lower, then the variable was permanently excluded, and we proceeded to eliminate the next less significant variable. Once all the remaining variables were significant, we computed the correlation matrix and excluded the variables that were strongly correlated (according to the criteria rho>0.5). Removal of the later variables caused some changes in the significance of the remaining ones, and further selection was needed, pursuing to obtain the lowest AIC. Finally, all the possible two-way interactions were evaluated, but none of them was significant. There is no satisfactory test to evaluate the goodness of fit in the case categorical multi-level/hierarchical data (Perera et al., 2016). Therefore, we used the Hosmer-Lemeshow method combined with the estimation of the model accuracy. To obtain the model accuracy, the data was splitted into training and testing datasets. Then, a logistic regression model was built with the training data. The model was used to predict the response of the testing data based on the values of independent variables, and those predictions were compared with the observed values. The process was repeated 10 times and the mean was the final model accuracy. As the response is binary, the coefficients obtained are interpreted in terms of odds ratio (OR). All statistical analyses were carried out using R statistical software (R core team, 2020).

2.4. *Review of the presence of ruminant pestiviruses in North Africa*

The review of the presence of ruminant pestiviruses in North Africa was performed using a systematic search and compilation methodology of peer-reviewed literature available in order to identify gaps of knowledge for future research. North Africa is the UN subregion comprised by Algeria, Egypt, Libya, Morocco, Sudan, Tunisia and Western Sahara. We searched Web of Science: All Databases (WoS; Thomson Reuters) literature database using "topic" searcher. We used the words "(Pestivirus AND Algeria OR Egypt OR Libya OR Morocco OR Sudan OR Tunisia OR Western Sahara)" (44 articles) and then we discarded research papers on Classical Swine Fever Virus (44 *Pestivirus* articles - 21 CSFV articles = 23 Ruminant *Pestivirus* articles from North-African countries). Finally, we added any relevant literature that was not originally included in WoS (2 articles).

3. Results

3.1. *Serology, molecular characterization, and risk factors*

The prevalence of antibodies against *Pestivirus* in cattle found in the present study, adjusted for the two-stage design, was 59.9% (138 out of 234) with a 95% CI [49.0–70.7%]. The overall seroprevalence of infected herds was 93.5% (29 out of 31) with a 95% CI [78.6%–99.2%] and the within-herd seroprevalence ranged from 0.0%–100.0%. Considering the sensitivity and specificity of the IDEXX p80 test, 60% and 97%, respectively (Hanon et al., 2017), and an estimated apparent prevalence of 59.9%, the true prevalence of disease would be 99.8%.

The model with all the factors that were significantly associated ($p < 0.05$) with the presence of *Pestivirus* infection included: presence of sheep, size of herd, animal category, breed, presence of standing water and number of calving. After studying the correlation, the best-fitting model included: presence of sheep (OR = 5.64; 95% CI [2.0, 15.9]; $p = 0.001$) and animal category (cow, OR = 3.80; 95% CI [1.6, 8.9]; $p = 0.002$). The variance of the random effect was 0.48, therefore evidencing the heterogeneity among herds. Model validation suggests that the model did not provide a good fit to the data ($p < 0.001$), while the estimated model accuracy was only 60%. RT-PCR resulted positive in 3 out of the 234 analysed animals. Only one of these three positive samples (a heifer of 20 months) could be sequenced targeting the *Pestivirus* 5'UTR region, confirming the presence of BVDV-1a in cattle from Algeria (Tiaret_2019; GenBank Acc. No. MT157227).

3.2. *Review of ruminant pestiviruses in North Africa*

Our literature review (25 research articles) confirmed the presence of ruminant pestiviruses in all North African countries, except in Libya and Western Sahara, where no data was available. A summary of the studies on *Pestivirus* in livestock in North Africa is presented in Table 1. The most studied and reported pestiviruses were *Pestivirus* A and B (BVDV-1, -2) in cattle and dromedary camels. However, the few studies on *Pestivirus* D (BDV) reported high seroprevalences in Algeria, Morocco and Tunisia, and an outbreak of severe clinical Border Disease in Tunisia in small ruminants. The review of the main risk factors for the presence of pestiviruses in livestock in North Africa found them to be heterogeneous (Table 1). On the other hand, no information about *Pestivirus* in wild ruminants in North African countries was recorded.

4. Discussion

Ruminant pestiviruses are neglected pathogens in North Africa. However, the scarce data available suggest an endemic epidemiological scenario of *Pestivirus* in livestock. The antibody seroprevalence at population and farm levels obtained in the present study concur with the majority of the epidemiological data reported in cattle and dromedary camels in neighbouring North-African countries (Table 1). Surprisingly, the present study is in contrast to Derdour et al. (2017) that reported a very low prevalence of antibodies (1.4%) in cattle in Algeria, probably due to a sampling performed exclusively in intensive production systems, where the "hit and run" transmission strategy of *Pestivirus* (horizontal transmission between individuals) can be favored (Peterhans and Schweizer, 2010). The hypothesis of an endemic and heterogeneous *Pestivirus* scenario in North-Africa is reinforced by the studies performed in small ruminants that showed the presence of a third *Pestivirus*, *Pestivirus* D (Border Disease Virus – BDV) in these species, with similar high antibody prevalences (17.7%–68.2%) (Table 1). Additionally, the present study reported the first description of a BVDV-1a in North Africa, whereas BVDV-2a and BVDV-1b had been isolated from cattle in Tunisia (Thabti F et al., 2005b). Although the three PCR-positive animals could not be confirmed as persistently infected (i.e. two PCR-positive samples separated between 15 days), their presence together with the reported seroprevalence of antibodies in some farms, is highly suggestive of the

Table 1
Results of studies investigating the seroprevalence of Pestiviruses in ruminants in North African countries.

Country	Year of sampling	Pestivirus	Species	Diagnostic method	N	Prevalence	Risk factors	Ref.
Algeria	2011– 2013	BVDV	Cattle	Ab-ELISA	360	1.4%	Abortions	(Zenbour et al., 2017)
	2019	BVDV-1a	Cattle	Ab-ELISA RT-qPCR	204	59.9% 1.2%	Presence of sheep Increase of age	Present Study
	2015– 2016	BDV/ BVDV	Sheep	Ab-ELISA	576	68.2%	Climate: arid vs Mediterranean	(Yakhlef et al., 2018)
				VNT	197	68.2%	Landscape: mountain vs plateau	
				Ag-ELISA	669	0%	Flock management: sedentary vs transhumant	
				RT-PCR	669	0%	Presence of goats	
	2016– 2017	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA	113	9.0% 41.4%	Sheep, goat, cattle in mixed herd	(Saidi et al., 2018)
Morocco	1984	BVDV	Cattle	IPP	324	48.5%	Extensive management system Local ruminants	(Mahin et al., 1985)
	NA	BDV	Sheep	Ab-ELISA qPCR Ag-ELISA	760 543 150	28.9% 0% 0%	Ruminants without apparent respiratory symptoms Extensive farming Presence of cattle	(Pearl Pilot et al., 2019)
	NA (1980?)	BVDV	Cattle	Disease	1			(Mahin et al., 1982)
	NA	BVDV	Cattle	Ab-ELISA	42	37.7%		(Lecollinet et al., 2016)
Tunisia	1995	BDV	Sheep	Disease Sequencing	NA 9	NA	Vaccine contamination	(Thabti et al., 2003a)
	1995	BDV	Sheep from 1 flock with BD clinical history	Abortion VNT	2826 50 aborted sheep	17.7% 100%		(Darkin et al., 1995)
		BVDV2a	Cattle from 2	Ab-ELISA	P1– 169	92%		
	2001– 2002	BVDV1b	farms (P1, P2) with BVD clinical history	PCR Sequencing	P2– 920 P1 P2	92% 2.6% 0.2% BVDV2a BVDV1b	Importation of infected cattle/ semen	(Thabti et al., 2003b)
Egypt	NA	BVDV	Cattle Buffalo Sheep Goats Dromedary camel	VNT (BVDV strains)	128 150 178 120 50	49.2% 52.0% 27.5% 21.4% 52.5%	Species: Cattle/buffalo vs sheep/goat/dromedary	(Zaghawa, 1998)
			Sheep	IHC	5 4	0%		
	2011	BDV/ BVDV	Goat	MAbs RT-PCR MDBK Sequencing		50% 0% 25% BVDV1b BVDV1a	Neurological signs	(Abdel-Latif et al., 2012)
	2012– 2013	BVDV	Cattle Buffalo	Ab-ELISA	480 200	40% 22%	Species: Cattle vs Buffalo	(Selim et al., 2018)
	2011	BVDV	Cattle Buffalo	Ab-ELISA Ag-ELISA MDBK IPAT IPMA	151 97 22 21 19 3	100% 62.2% 14.5% 13.9% 12.5% 1.9%	- NA	(El-Bagoury et al., 2012)
	2017	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA RT-PCR	80 80 10	11.2% 7.0% 0%	Camels from Sudan	(El Balagy et al., 2018)
Libya Western Sahara		No Data No Data						
Sudan	2017	BVDV	Dromedary camel-smuggler in to Egypt	Ab-ELISA Ag-ELISA RT-PCR	120 120 7	47.5% 31.6% 42.8%		(El Balagy et al., 2018)
	2000– 2006	BVDV	Dromedary camel	Ab-ELISA Ag-ELISA RT-PCR	260 186 10	84.6% 7% 100%		(Ezzeldin et al., 2010)
	2000– 2012	BVDV	Dromedary camel	i-ELISA	474	9.0%	• Mixed virus infection • Pneumonia • Lactination	(Saeed et al., 2015)
	2005– 2008	BVDV	Cattle	Ab-ELISA	668	25.7%	• Khartoum state	(Elhassan et al., 2011)

(continued on next page)

Table 1 (continued)

Country	Year of sampling	Pestivirus	Species	Diagnostic method	N	Prevalence	Risk factors	Ref.
							• Rainy season (July to October) • Females • Old cattle • Abortions • Neonatal deaths	

presence of PI cattle in Algeria. Detection and elimination of PI individuals, and characterization of circulating viruses are cornerstones for eradication programs.

The risk factors detected in the present study (mixed herd [presence of sheep], and animal category [cow]) have been previously associated with *Pestivirus* infections worldwide (Schweizer and Peterhans, 2014). However, the specific risk factors for *Pestivirus* infection in ruminants in North Africa have not been analysed in depth, and the few studies show a high heterogeneity of risk factors (Table 1), hindering the possibility of improving livestock production. In our study, the presence of sheep in the herd increased significantly the risk of *Pestivirus* infection (OR = 5.65), which may be explained by the inter-specific infectious ability of pestiviruses, that facilitate their geographic dispersion and persistence in ruminant populations (Schweizer and Peterhans, 2014). Multi-species herds are a common practice in Algeria and even if there is a lack of information around their proportion in our study area, Mouffok (2018) reported 42% of mixed herds in the Northeastern part of the country. In our study, the proportion of herds of cattle mixed with sheep reached 40%. This practice allows a diversification of revenues for the farmer but should be avoided or at least cattle should be kept separated from other ruminants, in order to limit interspecific *Pestivirus* infection. Our results show that cows have a higher risk of *Pestivirus* infection as compared to heifers (OR = 3.80), which coincides with previous studies (Schweizer and Peterhans, 2014; Selim et al., 2018), and is explained by the higher the age of cows that increases the probability of having been exposed to pestiviruses. We tried to minimize any selection bias in the herds and animals chosen, and therefore we consider the herds and animals selected were representative of the population. However, some sources of bias cannot be ruled out (e.g. if herds not officially registered are different from those registered, or if herds in which herd owners willing to participate are different from those which are not).

The role of wildlife as reservoir of pestiviruses has been proved in several wild species worldwide, being a risk factor for livestock. Pestiviruses A and D were reported to have a sylvatic cycle in white-tailed deer (*Odocoileus virginianus*; USA) and Pyrenean chamois (*Rupicapra pyrenaica*; Spain) respectively (Fernández-Sirera et al., 2012; Passler et al., 2016). However, there is no research available on the presence of pestiviruses in North-African wildlife even though seven free-ranging wild ruminant species share territories with livestock like camels, dromedary and goat in North-African countries (IUCN, 2020). Additionally, *Pestivirus* D has produced high mortality rates in chamois, entailing a threat for its conservation (Fernández-Sirera et al., 2012). The conservation status of all North-African wild ruminants is of concern; two species being considered as critically endangered (Addax [*Addax nasomaculatus*], Dama Gazelle [*Nanger dama*]), one as endangered (Slender-horned Gazelle [*Gazella leptoceros*]), and four as vulnerable (Cuvier's Gazelle [*Gazella cuvieri*]; Nubian Ibex [*Capra nubiana*]; Aoudad [*Ammotragus lervia*]; Dorcas Gazelle [*Gazella dorcas*]). In that sense, *Pestivirus* circulation in domestic and wild ruminants should be of concern both for its economic impact but also from a wildlife conservation perspective.

5. Conclusions

In summary, pestiviruses are widespread in livestock in North Africa. However, there is a significant lack of both cross-sectional and longitudinal transboundary studies about the epidemiology and molecular variability of ruminant pestiviruses in livestock and wildlife in North Africa. This is of concern for livestock health and wildlife conservation, and needs to be addressed.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.105156.

References

Abdel-Latif, A.O., Goyal, S.M., Chander, Y., Abdel-Moneim, A.S., Tamam, S.M., Madbouly, H.M., 2013. Isolation and molecular characterisation of a pestivirus from goats in Egypt. Acta Vet. Hung. 61, 270–280.
Business France, 2018. Algérie - La Production Algérienne De Viande Rouge a Dépassé Les 500000 Tonnes En 2017. https://www.businessfrance.fr/algerie-la-production-algerienne-d-e-viande-rouge-a-depasse-les-500-000-tonnes-en-2017.
Chase, C., Hurley, D., Reber, A., 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. North Am. Food Anim. Pract. 24 (1), 87–104.
Derdour, S.Y., Hafsi, F., Azzag, N., Tennah, S., Laamari, A., China, B., Ghalmi, F., 2017. Prevalence of the main infectious causes of abortion in dairy cattle in Algeria. J. Vet. Res. 61 (3), 337–343.
Dohoo, I.R., Martin, W., Stryhn, H.E., 2003. Veterinary Epidemiologic Research. Charlottetown, P.E.I.
El Bahgy, H.E.K., Abdelmegeed, H.K., Marawan, M.A., 2018. Epidemiological surveillance of bovine viral diarrhea and rift valley fever infections in camel. Vet. World 11, 1331–1337.
El-Bagoury, G.F., El-Habbaa, A.S., Nawal, M.A., Khadr, K.A., 2013. Prevalence of Bovine Viral Diarrhea Virus (BVDV) in cattle from some governorates in Egypt. Benha Vet. Med. J. 25, 125–133.
Elhassan, A.M., El-Hussein, M.A.F., El-Hussein, A.M., 2011. Seroprevalence of Bovine Herpes Virus-1, Bovine Herpes Virus-4 and Bovine Viral Diarrhea Virus in Dairy Cattle in Sudan. Pak. Vet. J. 31, 317–320.
Fassi Fihri, O., Jazouar, N., Azouzi, N., El Berbri, I., Alali, S., 2019. Sheep pestivirus in Morocco: sero-epidemiological and molecular study. Vet. Rec. Open 6, e000324.
Feknous, N., Hanon, J.B., Tignon, M., Khaled, H., Bouyoucef, A., Cay, B., 2018. Seroprevalence of border disease virus and other pestiviruses in sheep in Algeria and associated risk factors. BMC Vet. Res. 14, 339.
Fernández-Sirera, L., Cabezón, O., Allepuz, A., Rosell, R., Riquelme, C., Serrano, E., Lavín, S., Marco, I., 2012. Two different epidemiological scenarios of border disease in the populations of Pyrenean Chamois (Rupicapra p. pyrenaica) after the first disease outbreaks. PLoS One 7, e51031.
Hanon, J.B., De Baere, M., De la Ferté, C., Roelandt, S., Van der Stede, Y., Cay, B., 2017. Evaluation of 16 commercial antibody ELISAs for the detection of bovine viral diarrhea virus-specific antibodies in serum and milk using well-characterized sample panels. J. Vet. Diagn. Invest. 29 (6), 833–843.
ICTV - International Committee on Taxonomy of Viruses (ICTV), 2018. ICTV - International Committee on Taxonomy of Viruses (ICTV) (accessed 5th January 2020). https://talk.ictvonline.org/taxonomy/.
Intisar, K.S., Ali, Y.H., Khalafalla, A.I., Rahman Mahasin, E.A., Amin, A.S., Taha, K.M., 2010. The first report on the prevalence of pestivirus infection in camels in Sudan. Trop. Anim. Health Prod. 42, 1203–1207.
IUCN - The International Union for Conservation of Nature, 2020. The IUCN Red List of Threatened Species (accessed 10th March 2020). https://www.iucnredlist.org/en.

Kardjadj, M., Luka, P.D., 2015. Current situation of milk and red meat industry in Algeria. J. Nutr. Food Sci. 6, 1–2.

Lucchese, L., Benkirane, A., Hakimi, I., El Idrissi, A., Natale, A., 2016. Seroprevalence study of the main causes of abortion in dairy cattle in Morocco. Vet. Ital. 52, 13–19.

Lumley, T., 2020. "Survey: Analysis of Complex Survey Samples" R Package Version 4.0..

MADR, 2017a. Lancement De La Campagne De Vaccination Contre La Fièvre Aphteuse Et La Rage. Ministery of Agriculture and Rural Development of Algeria. http://madrp.gov.dz/lancement-de-la-campagne-de-vaccination-contre-la-fievre-aphteuse-et-la-rage/.

MADR, 2017b. Statistiques Agricoles, Ministère Algérien De l'agriculture Et Du Développement Rural. Ministery of Agriculture and Rural Development of Algeria. http://madrp.gov.dz/agriculture/statistiques-agricoles/.

Mahin, L., Chadli, M., Bréhaga, J., Hamidi, M., Wellemans, G., 1982. Clinical bovine virus diarrhoea in Morocco. First Report. J. Vet. Med. Series B. 29, 789–792.

Mahin, L., Wellemans, G., Shimi, A., 1985. Prevalence of antibodies to bovid herpesvirus 1 (IBR-IPV), bovine virus diarrhoea, bovine respiratory syncytial parainfluenza 3, Adeno A and Adeno B viruses in indigenous and imported Moroccan cattle. Ann. Vet. Res. 16, 279–283.

Mouffok, C.E., 2018. Performance De l'Élevage Bovin Dans La Région Semi Aride: Approche Globale: Systémique Zootechnique Et Métabolique (Doctoral Dissertation, Université Ferhat Abbas Sétif 1).

OIE, 2019. Access Online: OIE - World Organisation for Animal Health. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019 (accessed 5th January 2020). https://www.oie.int/en/standard-setting/terrestrial-manual/access-online/.

Passler, T., Ditchkoff, S.S., Walz, P.H., 2016. Bovine viral diarrhea virus (BVDV) in white-tailed deer (Odocoileus Virginianus). Front. Microbiol. 7, 945.

Perera, A., Sooriyarachchi, M.R., Wickramasuriya, S.L., 2016. A goodness of fit test for the multilevel logistic model. Commun. Stat.-Simul. Comput. 45 (2), 643–659.

Peterhans, E., Schweizer, M., 2010. Pestiviruses: how to outmaneuver your hosts. Vet. Microbiol. 142 (1–2), 18–25.

R Core Team, 2020. R: a Language and Environment for Statistical Computing. URL. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Saeed, I.K., Ali, Y.H., AbdulRahman, M.B., Mohammed, Z.A., Osman, H.M., Taha, K.M., Musa, M.Z., Khalafalla, A.I., 2015. Mixed infection of peste des petits ruminants virus (PPRV) and other respiratory viruses in dromedary camels in Sudan, an Abattoir study. Trop. Anim. Health Prod. 47, 995–998.

Saidi, R., Bessas, A., Bitam, I., Ergün, Y., Ataseven, V.S., 2018. Bovine herpesvirus-1 (BHV-1), bovine leukemia virus (BLV) and bovine viral diarrhea virus (BVDV) infections in Algerian Dromedary camels (Camelus Dromaderius). Trop. Anim. Health Prod. 50, 551–564.

Schweizer, M., Peterhans, E., 2014. Pestiviruses. Annu. Rev. Anim. Biosci. 2, 141–163.

Selim, A.M., Elhaig, M.M., Moawed, S.A., El-Nahas, E., 2018. Modeling the potential risk factors of bovine viral diarrhea prevalence in Egypt using univariable and multivariable logistic regression analyses. Vet. World 11, 259–267.

Sergeant, E.S.G., 2018. Epitools epidemiological calculators. Ausvet.

Sraïri, M.T., Benyoucef, M.T., Kraiem, K., 2013. The dairy chains in North Africa (Algeria, Morocco and Tunisia): from self sufficiency options to food dependency? SpringerPlus 2, 162.

Thabti, F., Letellier, C., Hammami, S., Pépin, M., Ribière, M., Mesplède, A., Kerkhofs, P., Russo, P., 2005a. Detection of a novel border disease virus subgroup in Tunisian sheep. Arch. Virol. 150, 215–229.

Thabti, F., Pépin, S.M., Kassimi, L.B., M'zah, A., Russo, P., Ben Said, M.S., Hammami, S., Pépin, M., 2005b. First detection and genetic characterization of bovine viral diarrhoea viruses (BVDV) types 1 and 2 in Tunisia. Rev. Med. Vet. 156, 419–422.

Vilcek, S., Herring, A.J., Herring, J.A., Nettleton, P.F., Lowings, J.P., Paton, D.J., 1994. Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch. Virol. 136, 309–323.

Zaehm, I., Tabtouker, D., Amara, A., Zouari, M., Chenaf, J., Moquay, V., 1993. A propos d'un cas de Border Disease dans un troupeau ovin en Tunisie. Rev. Med. Vet. 12, 975–979.

Zaghawa, A., 1998. Prevalence of antibodies to bovine viral diarrhoea virus and/or border disease virus in domestic ruminants. J. Vet. Med. B Infect. Dis. Vet. Public Health 45, 345–351.