RETROSPECTIVE STUDY OF BACTERIAL AND FUNGAL CAUSES OF ABORTION IN DOMESTIC RUMINANTS IN NORTHERN REGIONS OF SOUTH AFRICA (2006-2016)

Running title: ABORTION IN DOMESTIC RUMINANTS IN SOUTH AFRICA

Annelize Jonker*

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Old Soutpan Road, Onderstepoort, 0110, South Africa

Anita Luise Michel

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Old Soutpan Road, Onderstepoort, 0110, South Africa

***Corresponding author:** Annelize Jonker

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa,

Tel: 027 012 5298329, Fax: 012 5298312, E-mail: Annelize.Jonker@up.ac.za

ABSTRACT

This initial retrospective study presents information on bacterial and fungal causes of abortion in domestic ruminants in South Africa over 10 years (2006-2016). A complete set of data was collected at the Faculty of Veterinary Science by a keyword search of pathology and bacteriology laboratory registers. Additional electronic data was received from an external laboratory. A total of 288 cases were recorded from six provinces. Overall diagnostic rate was

35.1%. In 14.6% of cases histological evidence of an infectious process was found, but no aetiological agent was detected. Several cases did not include aetiological diagnoses because applicable diagnostic techniques were not available or not applied when necessary. Increased submission of placenta as well as additional conventional and molecular diagnostic methods can contribute to improved diagnostic rate. In addition, the study highlights the superior significance of *Brucella abortus* as a major bovine pathogen in South Africa.

KEYWORDS

Abortion, bacterial, fungal, ruminants

INTRODUCTION

A retrospective or historical study is defined as a study that collects and records information after the outcome of an exposure is known.[1] Retrospective studies of data on ruminant abortion over a period at selected veterinary laboratories covering all major farming areas can provide useful data for the development of herd health programmes and state veterinary disease control programmes to reduce future losses due to abortion.[2] Data on number of cases, diagnostic rate and regional incidence of causes of abortion and farming systems can be collected. In comparison with prospective field studies that are expensive, retrospective studies can produce valuable information at low cost.[3]

A significant number of retrospective studies covering causes of abortion have been published internationally.[3,4,5,6] Diagnostic rate[4] emerged as an important parameter for the evaluation of the efficiency of laboratory investigation. Several studies from different laboratories in the United States of America (USA) and Canada reported data on bovine, ovine or caprine abortion cases.[2,3,5,6,7,8] Diagnostic rate in bovine cases ranged from 29 to 56%.[2,3,5,6] Diagnostic rates of 44% and 47% were recorded for ovine and caprine abortions respectively.[7,8] In European studies, diagnostic rate was reported as 22 to 26% in bovine cases, 56 to 74% in ovine cases and 67% in caprine cases.[9,10,11]

Bacteria and fungi were often implicated as major causes of abortion. The detection of pathogens responsible for abortion will provide an indication of the mitigation or control method to address the problem such as test and slaughter, vaccination or improved feed management. In cases of bovine and ovine abortion, bacteria have been implicated in 14 to 67%; and fungi in 4 to 28% of all cases.[2,5-7,10] Studies of caprine abortion implicated bacteria in 30.5% and fungi in 0.5% of cases.[8]

Results of a retrospective study can indicate the most common infectious agents in a country as well as regional differences.[2] These differences occur for various reasons such as farming systems, climate and type of animals.[2] This information can prove useful in focussing control efforts. In selected developed countries in the northern hemisphere certain organisms have emerged as the most significant agents of abortion in cattle, sheep and goats including *Bacillus licheniformus* (Finland), *Campylobacter* species (Netherlands, United Kingdom and USA), *Chlamydia abortus* (UK, Netherlands, USA), *Coxiella burnetii* (USA)*, Listeria monocytogenes* (Canada, Netherlands), *Trueperella* (*Actinomyces*) *pyogenes* (Canada, Finland, USA) and *Ureaplasma diversum* (Finland).[2,3,9-12] In the southern hemisphere, the most common agents of abortion in sheep in New Zealand was reported to be *Campylobacter* and *Salmonella* species.[13] In addition, Reichel *et al*. (2018)[4] have reported that most mycotic abortions in cattle in these countries were due to *Aspergillus* species and *Mortierella wolfii*.

Placentitis and other pathological lesions without detection of a causative agent are commonly associated with cases of abortion. Such reports range from 4% to 40.6%.[2,3,5,9,11] These reports serve to highlight the fact that there are still cases where an aetiological diagnosis cannot be reached, hence the need for improvement in laboratory techniques.

Conventional bacterial and fungal culture methods are commonly employed to analyse products of abortion. Internationally, the composition of diagnostic abortion panels varies. Most studies reported at least aerobic culture in 4 to 7% CO_2 as well as microaerophilic culture on blood and/or MacConkey agar.[2,7-9] Some studies included chocolate agar.[2,8] Few studies included anaerobic culture.[9] Genus specific cultures such as *Brucella* [8,9] and *Mycoplasma* [9] were only

mentioned by a few researchers. Fungal culture was mostly done when indicated[2,3] except for Syrjäla *et al*. (2007)[9] who reported it as part of a routine conventional culture panel.

Additional history such as farming systems, abortion rate and feeding practices are not often mentioned although it can play an important part in reaching a diagnosis.[10] Anderson *et al*. (1990)[2] reported cases from intensive, zero-grazing dairy cattle farms. Another study by Clothier & Anderson (2016)[6] had information associating cases with dairy or beef cattle. Syrjälä *et al*. (2007)[9] received additional data in 242 (89.3%) cases indicating that 55% of submissions were from dairy and 45% of submissions from beef cattle.

The advantage of the retrospective studies mentioned above was that it gave animal health agencies, policy makers, researchers and diagnosticians an indication of the causative agents involved, relative prevalence and geographical diversities of these agents, as well as risk factors for abortion. Information generated is useful for planning purposes in several areas, for example at government level it can advise animal disease surveillance and control efforts. At farm level specific vaccines can be included in vaccine programmes to improve prophylaxis or treatment can be applied. At laboratory level the data can be used for financial planning since agents known to occur in the area can be targeted by inclusion of suitable assays in diagnostic panels.

The aims of the study were to exploit available retrospective laboratory data on cases of confirmed or suspected bacterial or fungal abortions in cattle, sheep and goats for the purpose of determining the diagnostic rate including factors that could influence it as well as to identify the most significant bacterial and fungal causative pathogens per animal species and province.

MATERIALS AND METHODS

The observational retrospective study attempted to use cases from multiple diagnostic laboratory systems. A case was defined as samples from one or more foetuses or products of abortion from a single herd submitted at the same time for bacterial and fungal culture. Case results were used in the count of infectious agents. If two foetuses registered as a single case

yielded different agents these were counted separately. Criteria for selection was foetus (not stillborn or neonate). Indications that the calf/ lamb/ kid was born alive such as aerated lungs, colostrum in the abomasum or worn golden slippers were criteria for exclusion. A full necropsy was recorded on all foetuses, including macroscopic and microscopic pathology. Samples were submitted for bacterial and fungal analysis at the discretion of the pathologist. A diagnosis was made on the combined findings of necropsy, histopathology, microbiology and other analyses such as immunohistochemical staining and polymerase chain reaction (PCR).

South African public and private veterinary diagnostic laboratories and their management (where appropriate) were approached for permission to collect data on cases received between 2006 and 2016. Data was recoded on a spreadsheet under the following headings: farming systems, feeding practices, geographical area of origin of foetuses/samples, condition of samples received, routine test methods used for foetuses/ abortion samples by the various laboratories, the number of abortion cases received (infectious and non-infectious), the number of confirmed diagnoses of infectious causes, the agents of abortion identified, the number of suspected diagnoses of infectious causes that could not be confirmed and the number of cases were there were macroscopic or microscopic indications of an infectious cause but no diagnosis.

At the Bacteriology laboratory, Department Veterinary Tropical Diseases and Histopathology laboratory, Section Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria a search was done manually and electronically. Registers were searched for the keywords: bovine, ovine, caprine, foetus, calf, lamb, kid, abomasal content and foetal fluid. Lists of possible abortion cases were compiled at the histopathology and bacteriology laboratories. These were used as reference to search files of pathology reports. Pathology reports were opened individually, and data recorded. Electronic pathology reports received from a private laboratory, Vetdiagnostix Pathology Laboratory Services, were also opened individually and data recorded.

The overall diagnostic rate (percentage) as well as the diagnostic rate per species was calculated by means of the following formula: (number of cases with confirmed diagnoses/

number of cases submitted) x 100. Diagnostic rate was also calculated for samples in good condition, autolytic samples, cases where a placenta was submitted, and the different panels of tests offered for conventional culture. The percentage of cases that recorded additional data was calculated as well as abortion rate. The formula for abortion rate was: number of abortions/ number of animals pregnant in the herd at the farm of origin of the samples.[14] Percentages with 95% confidence intervals (CI) were calculated and the association between diagnosis and completeness of submission was analysed by logistic regression using Epi info 7™ 7.2.3.1, (Centers for Disease Control and Prevention, Atlanta, Georgia).

RESULTS

The country-wide 10-year retrospective study from 2006 to 2016 that was initially planned was not achievable. In total, 288 cases were recorded from six provinces. Two hundred and thirty-five (235) cases were recorded over 10 years at the Pathology and Bacteriology laboratories of the Veterinary Faculty of University of Pretoria. In addition, data were obtained from a sponsored study on causes of abortion in sheep and goats (CEVA Animal Health) in 2013 which yielded 18 cases. For 2016 additional data on 35 cases were received from Vetdiagnostix Pathology Laboratory Services.

Cases consisted of 282 foetuses, of which 58 were complete sets consisting of foetuses with placenta and 224 comprised of foetuses only. Six cases were placenta only. One hundred and ninety-three (67%) were bovine, 57 (20%) ovine and 39 (14%) caprine cases. A diagnosis was made in 101 cases. The overall diagnostic rate was 35.1% (95% CI [85,118]); 35.2% (95% CI [55, 82]) in bovine, 33.3% (95% CI [12,27]) in ovine and 35.9% (95%CI [8,21]) in caprine cases.

Table 1 Microorganisms isolated arranged per animal species per province

Microorganisms	**Province**					
Bacteria	**FS**	**GT**	**KZN**	**LP**	**MP**	**NW**
Actinomyces israelii		B(1)				
Anaplasma marginale			B(2)			
Bacillus cereus					B(1)	
Bacillus licheniformus			O(1)			
Brucella abortus biovar 1	B(1)	B(3)			B(1)	B(1)
Brucella abortus biovar 2				B(1)		
Brucella species		B(5)	B(1)	B(1)	B(4)	B(3)
Burkholderia cepacia					B(1)	
Campylobacter species				O(1)		B(1)
Chlamydia abortus		C(1)				
Chlamydia pecorum		O(1)				
Chlamydia species		C(1)				
Corynebacterium species		B(1)				
Coxiella burnetii	O(1)					
Enterobacter cloacae		C(1)			B(1)	
Enterococcus faecalis				C(1)		
Escherichia coli		B(2);O(3);C(1)				B(1)
Fusobacterium species		B(1)				
Leptospira species		B(1)				
Listeria monocytogenes			O(1)	B(2)		
Nocardia asteroides		B(1)				
Salmonella species		B(1);C(1)				

Streptococcus canis				B(1)		
Trueperella pyogenes		B(1)	B(1)	B(3)	O(1)	
Fungi						
Aspergillus fumigatus		B(1)				
Penicillium species			O(1)			
Rhizopus species					C(1)	
Unidentified fungus		B(1)				

FS: Free State, GT: Gauteng, KZN: KwaZulu Natal, LP: Limpopo, MP: Mpumalanga, NW: North West, B: Bovine, O: Ovine, C: Caprine, (): Number of isolates

In 63 cases (21.9%, 95% CI [50,78]) a bacterial or fungal isolate was implicated taking into account presence of bacteria in the lesion as well as the type of lesion (Table 1). A bacterial aetiology was identified in 59 cases (20.5%, 95% CI [46,74]) and a fungal aetiology in four cases (1.38%, 95% CI [1,10]). *Brucella* species constituted the most common isolates in all provinces. It was detected in 21 cases (7.3%, 95% CI [13,31]) and only in bovine foetuses. *Trueperella pyogenes* was isolated from five bovine (1.73% 95% CI [2,12]) and one ovine foetus (0.34%, 95% CI [0,6]). Members of the Enterobacteriaceae were commonly isolated. Most common was *E. coli* (seven cases) (2.4%, 95% CI [3,14]) followed by *Salmonella* species (two cases) (0.69% 95% CI [0,7]).

Table 2 Number of case submissions with and without placenta.

	Number of cases	**Percentage (%) of cases**
Foetuses w/o placenta	224	77.8
Foetuses with placenta	58	20.1
Placenta only	6	2
Total placenta submitted	64	22.2

Only 58 (20.1%) cases were classified as complete (foetus with placenta) and six (2%) had only placenta (Table 2). In most cases (n=224) foetuses were submitted without placenta. Placenta submissions totalled 64 (22.2%). Diagnostic rate for cases where placenta was included was 40.6% (95% CI [18,34]). Thirty-two (50%) placentas had lesions indicative of an infectious

cause. Necrotic placentitis was recorded in 21 (32.8%) and necrotic placentitis with vasculitis in five (7.8%) cases. Samples in good condition returned 80 (79.2%, 95% CI [71,88]) diagnoses while autolytic samples returned 21 (20.8%, 95% CI [13,30]) diagnoses.

Table 3 Cases with pathological lesions indicative of infection, but no aetiological agent identified.

Macro- or microscopic lesion reported	Number of cases	Percentage of cases (%)
Abomasitis	1	2.4
Hepatitis/ Liver necrosis	5	11.9
Hepatosis	5	11.9
Kidney necrosis	3	7.1
Meningitis/ Meningoencephalitis	4	9.5
Nephritis	1	2.4
Placentitis	14	21.8
Pneumonia	10	23.8
Total	42	14.6%

Necropsy and/ or histopathological lesions indicative of an infectious process was recorded in 42 cases (14.6%) where no infectious agent was identified (Table 3). In these cases, the most common lesions recorded were placentitis 14 cases (21.8%) and pneumonia 10 cases (23.8%).

Abortion rate was available in 22.9% of case submissions. *Brucella* species, *T. pyogenes*, *L. monocytogenes*, *C. burnetii*, *B. licheniformus* and *Rhizopus* species were implicated in cases where abortion rates higher than 5 to 8% were reported. Six cases with abortion rates below 4% were associated with agents that become endemic in herds such as *B. abortus*, *Leptospira*, *Chlamydia pecorum* and *Campylobacter*. Farmers and veterinarians were more likely to report abortions over time than abortion rate. Four abortions in four months was associated with a *Brucella* species infection.

One hundred and twenty-nine cases (44.8%) in this study included information on production systems. Information received revealed that 17 cases were from beef production and 11 from dairy systems. Sheep and goat production systems were mostly extensive (11 cases) or semi-intensive (seven cases). Feeding practices were reported in 54 cases revealing that most herds were pasture fed with supplementary feeding such as lick (30 cases). Feed quality is often incriminated in cases of mycotic, *Listeria* and *Bacillus* species abortions. In this study, only one case involving these agents included a history of feeding practices.

DISCUSSION

The aim of the study was to exploit available retrospective laboratory data on cases of confirmed or suspected bacterial or fungal abortions in cattle, sheep and goats for the purpose of identifying the most significant bacterial and fungal causative pathogens per animal species and province, as well as the diagnostic rate and factors that could influence it.

The relatively small number of cases recorded (n=288) was expected as there were several limitations to this study. Submission of abortion cases is not compulsory in South Africa. Farmers and field diagnosticians will often only opt for an abortion investigation if the number of abortions in a herd is seen as a financial threat. Most cases originate from farms closer to the participating laboratories in the northern parts of South Africa, an indication that distance affects submission rate. In the few laboratories that offered to share their data, filing methods and man power proved to be a challenge. Electronic filing systems were created to search accounts not reports. Non-electronic filing systems had to be searched manually. Both required man power most laboratories could not assist with. The result was that data was collected from only two laboratories. In contrast to declining submission rates reported by van Engelen *et al.* (2014)[11], submission rates in this study appeared to increase from 2014 onward. Future investigation including data from veterinary laboratories in the southern parts of South Africa will be useful to identify similarities and differences between different parts of the country.

Common agents of bacterial or fungal abortion could be identified per species and per country, but not per province. This is most probably due to the low number of laboratories involved and

cases recorded. The most commonly detected bacterial species in all provinces was *Brucella* species in cattle. This was expected as South Africa has a high incidence of brucellosis.[17] What was surprising was the low rate of detection, only two to three positive cases per year. No *B. melitensis* isolates were recorded. However, isolation of *B. melitensis* from cattle samples collected at Gauteng abattoirs in 2016 and 2017 was reported by Kolo *et al*. (2019).[18] An increase in complete submissions, increased laboratory capacity, routine inclusion of *Brucella* isolation in conventional bacteriology panels as well as more sensitive methods of detection should lead to improved detection of these bacteria.

Abortions due to sporadic infections were common. *Trueperella pyogenes* was the second most common isolate at 10 (14.2%) bovine cases and one ovine case. It was implicated as the agent of abortion if histopathological findings indicated a suppurative placentitis and/ or if it was isolated in a pure or almost pure culture from the abomasal fluid and foetal tissues.[5] *T. pyogenes* is also a common isolate from bovine abortion cases in the USA and Canada.[2,3]

Salmonella was rarely detected. When detected, isolates were rarely identified to serovar level during the study period, most likely due to limited availability of serotyping and financial reasons. However, serotyping is important to determine whether vaccination is an option for control as vaccines are available in South Africa against the two most common *Salmonella* species found in cattle, e.g. *S*. Dublin and *S*. Typhimurium.

An important finding of this study was the bacteria that were not detected or detected in low numbers, such as the anaerobes, Chlamydiales, Campylobacteriales, *Leptospira*, *Listeria* species and Mycoplasmatales, as well as their identification to genus level only. Culture methods for fastidious bacteria are not routinely included in conventional abortion panels due to cost. Some of these bacteria fall in the difficult to culture category[19] due to an intracellular lifestyle, e.g. the Chlamydiales and *C. burnetii*; or due to their fastidious nature, e.g. Campylobacteriales, *Leptospira*, *Mycoplasma* and *Ureaplasma*. Others such as *Listeria* species are quickly overgrown by contaminating bacteria. However, inclusion of conventional and molecular detection methods is necessary to determine whether these bacteria are truly uncommon in South Africa. Identification to species level is preferable for bacteria such as

Campylobacter and *Chlamydia* as vaccines are only available for some species, e.g. *Campylobacter fetus* subspecies *venerealis* and *Chlamydia abortus*.

In this study only four mycotic abortions were recorded. Successful diagnosis of mycotic abortion is dependent on the availability of placenta as foetal tissues are often not involved.[5] Increased submission of placenta as well as inclusion of fungal isolation in conventional abortion panels should improve detection of fungi. Internationally, mycotic abortion appears to be rare in more recent years. In cases of bovine abortion, reports ranged from 0.5 to 6.8%.[2,3,9] The reason for this phenomenon is not clear, but improved feed management or drier climatic conditions due to climate change may play a role.

Placentas were received in only 22.2% of cases. Diagnostic rate for cases including placenta was 40.6%, but there was no statistically significant association between presence of placenta and diagnosis. Placenta associated bacteria and fungi detected were *B. licheniformis*, *Chlamydia*, *C. burnetii*, *Penicillium* and *Rhizopus*. These agents may have been missed if the placenta had not been included. Placenta was positively associated with diagnosis of agents of abortion in an American study by Moeller (2001)[8] and is considered the most useful sample for abortion investigation. However, the rate of submission is low worldwide at 12.5% to 26%, for various reasons.[6,9] In addition, placenta samples are often of poor quality due to contamination and autolysis reducing chances for isolation in the laboratory.[6,19] The importance of placenta samples has to be highlighted continually in laboratory reports to veterinarians and farmers and in presentations at educational events in an effort to increase submission rate.

Histological evidence of an infectious process was reported in 42 cases (14.6%) in which no cause could be identified. Reasons could be that the bacteria were not culturable by conventional methods or overgrown by fast growing non-pathogenic organisms.[11] This serves as an indication that improved diagnostic methods that are not reliant on viable bacteria are necessary to determine causes of abortion. Similar results were reported in studies in other countries where placentitis or other signs of inflammation and no aetiological agent was reported in 7.3% to 11.7% of bovine cases [3,6], 4 % of caprine cases and 11 to 15.4% of ovine cases.[5,11]

Additional clinical history could serve as an important component of a successful diagnosis. Half of the abortion rates in this study fell in the less than 5% category. Abortions in this category tend to be sporadic.[20] The reason for this may be that clients who can provide details on abortions in relation to herd size are also the ones who will submit abortions sooner. Abortion rates less than 2% is usually not seen as an indication for investigation.[14] However, an abortion rate of between 2% and 5% may be an indication of an endemic infectious agent.[14] The latter was evident in this study where such cases were associated with agents that become endemic in herds such as *B. abortus*, *Leptospira*, *Chlamydia pecorum* and *Campylobacter*. Abortion rates higher than 5 to 8% is usually seen as more than sporadic and an indication that investigation is necessary. In this study *Brucella* species, *T. pyogenes*, *L. monocytogenes*, *C. burnetii*, *B. licheniformus* and *Rhizopus* species infections were implicated in such cases. In this study a cluster of four abortions in four months was associated with a *Brucella* species infection indicating that reports of abortions over time can also be useful. A similar finding was reported by Menzies (2011).[14]

CONCLUSION

The results of this study emphasize the value of analysis of data collected over a long time. Increased submission of placenta and application of additional conventional and molecular diagnostic techniques may lead to increased successful diagnoses. *Brucella* species were the dominant isolates in all provinces included in this study. It would be prudent for all laboratories in these provinces to include *Brucella* detection in their routine abortion panels. The importance of submitting placenta, as well as recording abortion rate and other clinical information can be communicated to veterinarians and farmers at educational events.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of Dr RD Last, Dr L du Plessis, Ms N Timmerman, Ms T Twala and Ms S Malan during the data collection process. The project was funded by Red Meat Research and Development South Africa (Reference no 1395058846)

CONFLICT OF INTEREST STATEMENT

No conflict of interest is declared.

REFERENCES

1. Klebanoff MA, Snowden JM. Historical (retrospective) cohort studies and other epidemiologic study designs in perinatal research. *Am J Obstet Gynecol* 2018. https://doi.org/10.1016/j.ajog.2018.08.044

2. Anderson ML, Blanchard PC, Barr RL et al. A survey of causes of bovine abortion occurring in the San Joaquin Valley, California. *J Vet Diagn Invest* 1990;2:283-287.

3. Khodakaram-Tafti A, Ikede BO. A retrospective study of sporadic bovine abortions, stillbirths, and neonatal abnormalities in Atlantic Canada, from 1990 to 2001. *Canadian Vet J* 2005;46:635-637.

4. Reichel MP, Wahl LC & Hill FI. Review of diagnostic procedures and approaches to infectious causes of reproductive failures of cattle in Australia and New Zealand. *Front Vet Sci*, 2018;5:222. https://doi.org/10.3389/fvets.2018.00222

5. Kirkbride C. Etiologic agents detected in a 10-year study of bovine abortions and stillbirths. *J Vet Diagn Invest* 1992;4:175-180.

6. Clothier K, Anderson M. Evaluation of bovine abortion cases and tissue suitability for identification of infectious agents in California diagnostic laboratory cases from 2007 to 2012. *Theriogenology* 2016;85:5:933-938. http://dx.doi.org/10.1016/j.theriogenology.2015.11.001

7. Kirkbride C. Diagnoses in 1,784 ovine abortions and stillbirths. *J Vet Diagn Invest* 1993;5:398-402.

8. Moeller RB. Causes of caprine abortion: diagnostic assessment of 211 cases (1991-1998). *J of Vet Diagn Invest* 2001;13:265-270.

9. Syrjälä P, Anttila M, Dillard K et al. Causes of bovine abortion, stillbirth and neonatal death in Finland 1999-2006. *In* Perinatal Death in domestic animals. Proceedings of the 20th Symposium of the Nordic Committee for Veterinary Scientific Cooperation (NKVet); 2007 April

26-27; Reykjavik, Iceland: *Acta Vet Scand*: 2007;49:1:3. https://doi.org/10.1186/1751-0147-49-S1-S3.

10. Wheelhouse N & Dagleish M. Diagnosing the causes of ruminant abortion: Where are we now? *Vet J* 2014;201:3:243-244. http://dx.doi.org/10.1016/j.tvjl.2014.05.042

11. Van Engelen E, Luttikholt S, Peperkamp K, Vellema P & Van den Brom R. Small ruminant abortions in the Netherlands during lambing season 2012-2013. *Vet Rec* 2014. https://doi.org/10.1136/vr.102244

12. Pritchard GC, Smith RP, Errington J et al. Prevalence of *Coxiella burnetii* in livestock abortion material using PCR. *Vet Rec* 2011;169,391a. https://doi.org/10.1136/vr.d4693

13. West DM, Ovine abortion in New Zealand. *New Zeal Vet J* 2002;50:3:93-95. https://doi.org/10.1080/00480169.2002.36279

14. Menzies PI. Control of important causes of infectious abortion in sheep and goats. *Vet Clin Food Anim* 2011;27:81-93. https://doi.org/10.1016/j.cvfa.2010.10.011

15. Schnydrig P, Vidal S, Brodard I et al. Bacterial, fungal, parasitological and pathological analyses of abortions in small ruminants from 2012-2016. *Schweiz Arc Tierheilkd* 2017;12:647-656. https://doi.org/10.17236/sat00136

17. Mbizeni S. Brucellosis in South Africa: Progress and challenges. Presented at the Brucellosis research workshop, Faculty of Veterinary Science, University of Pretoria, 13-15 May 2015. http://repository.up.ac.za/dspace/handle/2263

18. Kolo FB, Adesiyun AA, Fasina FO, Katsande CT, Dogonyaro BB, Potts A, Matle I, Gelaw AK & van Heerden H. Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. Vet Med Sci 2019;5:545-555. http://dx.doi.org/10.1002/vms3.190.

19. Vidal S, Kegler K, Greub G, Aeby S, Borel N, Dagleish MP, Posthaus H, Perreten V & Rodriguez-Campos S. Neglected zoonotic agents in cattle abortion: tackling the difficult to grow bacteria. *BMC Vet Res* 2017;13:373. https://doi.org/10.1186/s12917-017-1294-y

20. Givens MD, Marley MSD. Infectious causes of embryonic and fetal mortality. *Theriogenology* 2008;70:3:270-285. http://dx.doi.org/10.1016/j.theriogenology.2008.04.018