ORIGINAL ARTICLE

Ovine abortion and stillbirth investigations in Australia

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PRODUCTION ANIMALS

Fetal loss and lamb mortality between mid-pregnancy and weaning are important economic and welfare issues for the Australian sheep industry. The aim of this study was to determine common causes of ovine abortion and stillbirths based on submissions to veterinary laboratories and identify factors that impact the determination of an aetiological diagnosis. Data for 529 investigations on abortion or stillbirth between 2000 and 2018 were retrieved from four state veterinary laboratories in Western Australia, South Australia, Victoria and Tasmania. An aetiological diagnosis was made for 57% of investigations. Investigations that included placental tissue samples were more than twice as likely to have an aetiological diagnosis compared to investigations without placenta (P = 0.017, 95% confidence interval 1.1, 4.5). Of the investigations where an aetiological diagnosis was made, 81% involved infectious abortion, with Campylobacter spp. (32%), Listeria spp. (25%) and Toxoplasma gondii (9%) being the three most common abortigenic pathogens implicated. The remaining 19% of investigations with an aetiological diagnosis included a wide range of infectious and non-infectious diseases. Diagnoses made varied year to year and between states. No evidence of exotic abortigenic pathogens were reported. Veterinary practitioners can improve the probability of an aetiological diagnosis by emphasising to farmers the importance of collecting any aborted material, especially placenta, and appropriate storage of the tissues until they can be submitted to the laboratory. Some diseases that cause abortion in Australian sheep have zoonotic potential, and veterinary practitioners play an important role in educating clients about appropriate hygiene when handling pregnant and lambing ewes or any aborted material.

Keywords *Campylobacter*; lamb survival; *Listeria*; mortality; sheep; *Toxoplasma gondii*

Abbreviations CI, confidence interval; PCR, polymerase chain reaction; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia

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F etal loss and lamb mortality between mid-pregnancy and weaning is an important economic and welfare problem for the Australian sheep industry.^{1,2} A number of diseases endemic in Australia can cause abortion and the birth of lambs with poor viability. *Campylobacter* spp., *Listeria* spp. and *Toxoplasma gondii* are reported as the leading causes of ovine abortion.³⁻¹⁰ However, most historical studies only report on localised geographical regions or specific putative causes and are considerably dated. To our knowledge, no systematic comprehensive surveys of the causes of ovine abortion across major Australian sheep production areas have been reported.

Identifying the aetiological agent involved in an abortion outbreak has important implications for disease management and control, managing public health risks with zoonotic infections and preventing further outbreaks. Abortion investigations also play an important role in the surveillance of exotic diseases, including *Chlamydia abortus*, *Brucella melitensis* and *Salmonella enterica* serotype Abortusovis, which are associated with abortion in other countries.^{11–13} Exclusion of these exotic diseases in abortion investigations has implications for trade by demonstrating freedom from these diseases.

Reaching an aetiological diagnosis for ovine abortion investigations can be challenging. This relies heavily on laboratory methods for the demonstration of the presence or absence of infectious agents, which can be hindered by the type and quality of samples submitted, the availability of diagnostic tests and difficulty in identifying non-infectious causes of lamb mortality. Most Australian studies reporting causes of ovine abortion predate the widespread availability of molecular diagnostic tests.^{3–9} It is not clear if the introduction of more sensitive tests such as PCR and quantitative PCR has changed the range of diseases diagnosed in abortion or neonatal mortality investigations or the proportion of investigations where an aetiological diagnosis is made.

A sound understanding of the most common causes of abortion and neonatal mortality would allow veterinary practitioners to develop appropriate protocols for field investigations that ensure appropriate samples are collected. Understanding factors that impact the ability of pathologists to make an aetiological diagnosis in these investigations will inform these protocols and allow practitioners to counsel clients on the likelihood of the investigation yielding an aetiological diagnosis.

The aims of this study were to identify the most common causes of ovine abortion and stillbirths reported in Australia based on submissions to state veterinary laboratories and to determine factors that influenced whether aetiological diagnoses could be made for investigations.

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State		Timeframe					
	Investigations (n)	Years	Duration (years)	Investigations per year	Farms (n) ^a	Mean farms with investigation per year (% farms)	
WA	65	2008–2018 ^b	11	5.9	5596	0.11 ^a	
SA	72	2006-2017	12	6	6414	0.09 ^a	
VIC	248	2010-2018	9	27.6	9855	0.28 ^b	
TAS	144	2000-2018	19	7.6	1503	0.50 ^b	
Total	529	-	_	-	-	-	

 Table 1. Abortion and stillbirth investigations conducted by state government laboratories in Western Australia, South Australia, Victoria and Tas

 mania between 2000 and 2018

^a Mean number of sheep farms per state over specified timeframe derived from Australian Bureau of Statistics.

^b 2018 data for WA incudes January–June 2018 only.

Values (proportion) in column with different letters are significantly different (P < 0.05).

SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

Materials and methods

Data were collated for ovine abortion and stillbirth investigations conducted by the state government or associated veterinary diagnostic laboratories in Western Australia (Department of Primary Industry and Regional Development Diagnostic Laboratory Services, formerly Department of Agriculture and Food Western Australia Animal Health Laboratory), South Australia (VETLAB, with testing performed by Gribbles Veterinary Pathology), Victoria (Agriculture Victoria, with testing performed by AgriBio Laboratories or Gribbles Veterinary Pathology) and Tasmania (Animal Health Laboratory). These laboratories are National Association of Testing Authorities (NATA) accredited under ISO 17025 for veterinary testing, although it was not determined if all the specific tests performed were covered by NATA accreditation in each laboratory. Submissions to veterinary laboratories were made by government and private veterinarians. Diagnostic tests performed by each laboratory included gross pathology, histopathology, microbial culture, molecular diagnostic tests and serology. Specific testing procedures varied between submissions, and different database software was used by each laboratory. Retrieval of data for investigations was conducted by staff from each laboratory using the following search terms: 'abortion', 'stillbirth/ born' or 'infertility'. 'Infertility' cases were predominately associated with ram infertility investigations and subsequently omitted from analyses. The time period included for datasets varied between laboratories due to constraints in the ability to retrieve results from the databases (Table 1).

Datasets were provided as spreadsheets with summary information for each investigation, including submission identification code, date of submission, farm location (postcode or geographical coordinates), summary of case history, sample (specimen) type, diagnostic tests performed and diagnosis reported for each investigation. The level of detail available for samples varied between laboratories. In some cases, the number of fetuses or stillborn lambs submitted for each investigation was not clear, and in some cases, it was not possible to determine if more than one submission was made for the same farm within the same year. Detailed pathology reports for each investigation were not available. The approximate number of ovine abortion investigations as a proportion of all ovine disease investigations (not including faecal egg counts) was provided for laboratories in Victoria and Tasmania.

Statistical analyses

Datasets were consolidated, and cases that included the submission of specimens of more than one fetus/lamb from the same farm in the same lambing period were considered a single investigation. Each investigation was evaluated to determine if a diagnosis was made, if infectious agents were identified by diagnostic testing and – where possible – submission characteristics such as type and quality of tissue submitted and outcomes for specific tests. The mean annual number of investigations was compared to the number of farms in each state based on the average of the annual number of sheep businesses reported by the Australian Bureau of Statistics during the time period from which the datasets were derived.¹⁴

Statistical analyses were performed using IBM SPSS Statistics (version 24). Proportions (e.g. investigations performed, diagnoses made or specific diagnoses) were compared using Chi-square analyses, with a two-tailed Pearson test for significance. Associations between submission of placenta (yes/no) and aetiological diagnosis (yes/no) were compared using Chi-square analyses with a two-tailed Pearson test for significance, plus odds ratio and relative risk with a 95% confidence interval.

Results

Abortion and stillbirth investigations

A total of 529 investigations were analysed for the period 2000–2018, although the period of reporting by laboratories varied over this time (Table 1). Most reports were received from Victoria (n = 248, years 2010–2018) and Tasmania (n = 144, 2000–2018), and abortion was investigated more often from sheep flocks in these two states. Abortion investigations represented approximately 5%–10% of total annual sheep disease investigations (excluding worm

 Table 2. Abortion and stillbirth investigations with aetiological diagnosis between 2000 and 2018

		Investigations with diagnosis		
State	Total investigations (n)	(n)	(%)	
WA	65	32	49.2 ^a	
SA	72	36	50.0 ^a	
VIC	248	156	62.9 ^b	
TAS	144	76	52.8 ^a	
Total	529	300	56.7	

Values (proportion) in column with different letters are different (P < 0.05).

SA South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

egg counts) performed at state government veterinary laboratories for Victoria and Tasmania.

A diagnosis was made for 57% investigations (n = 300), in a range of 49%–63% across the four states (Table 2). The proportion of investigations with a diagnosis was higher in Victoria compared to the three other states (Table 2).

Datasets from Tasmania and Victoria included details of diagnostic tests used for each investigation. Histopathology results were available for 139 investigations from Tasmania and 133 investigations from Victoria. Six investigations included only ewe serology or biochemistry, with 1–16 ewes sampled per investigation. A diagnosis was made for two of the investigations that used only dam serology or biochemistry, specifically a diagnosis of leptospirosis and copper deficiency. Seroconversion for *T. gondii* was demonstrated in 75%– 100% serology samples submitted per investigation; however, an aetiological diagnosis of toxoplasmosis was not made without supportive histopathological evidence, paired serology or isolation of organism in fetal tissues. Two further investigations included only vaginal swabs without serology or fetal tissue, and no diagnosis was made for these investigations.

Aetiological diagnoses made for abortion and stillbirth investigations

An infectious aetiology was determined in 81.3% of cases where an aetiological diagnosis was determined (Table 3). *Campylobacter* spp. (32.3%), *Listeria* spp. (25.7%) and *T. gondii* (9.3%) were the most commonly diagnosed infectious agents, with other infectious agents making up 14% of cases with aetiological diagnosis (Table 4). Most investigations had a single diagnosis, except for one farm in Tasmania with *Campylobacter coli* isolated concurrently with *Campylobacter jejuni* from one aborted fetus and one farm in Victoria with *Campylobacter fetus* and *Listeria ivanovii* isolated from two separate aborted fetuses during the same lambing period.

Leptospirosis was diagnosed in 4% of investigations with a diagnosis, with 83% of these cases diagnosed in Victorian flocks in 2018.

Table 3. Diagnoses made for abortion and stillborn lamb disease investigations in WA (2008–2018), SA (2006–2017), VIC (2010–2018) and TAS (2000–2018)

		Diagnoses	Overall			
	WA	SA	VIC	TAS	n	% investigations with diagnosis
Campylobacter (not speciated)	1	0	22	5	32	10.7
C. fetus	4	5	21 ^a	16	42	14.0
C. jejuni	0	1	4	18	23	7.7
C. coli	0	0	0	1 ^b	1	0.3
TOTAL Campylobacter spp.	5	6	47	39	97	32.3 ^a
Listeria (not speciated)	0	11	26	0	42	14.0
L. ivanovii	2	0	25 ^a	7	32	10.7
L. monocytogenes	3	0	2	1	3	1.0
L. innocula	0	0	1	0	1	0.3
TOTAL Listeria spp.	5	11	54	8	77	25.7 ^a
Toxoplasma gondii	1	4	8	16	28	9.3 ^b
Other infectious	6	7	23	6	42	14.0 ^b
TOTAL infectious diagnoses	17	28	131 ^c	69 ^c	244	81.3
TOTAL non-infectious diagnoses	15	8	26	7	56	18.7

^a *L. ivanovii* and *C. fetus* isolated from two separate fetuses from one farm in the same lambing period.

^b C. coli isolated concurrently with C. jejuni from an aborted fetus.

^c Total number diagnoses accounts for mixed infections (i.e. more than one infectious aetiology for investigation).

Values for diagnoses (*Campylobacter*, *Listeria*, *Toxoplasma*, other) with different letters are significantly different (P < 0.05).

SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

Non-infectious aetiology (n) ^b
Maternal illness (17)
Dystocia (13)
Congenital abnormality/anomaly (7)
Goitre (7)
Nutritional (7) ^c
Toxicity (4) ^d
Starvation-mismothering-exposure (1)

Table 4. Less frequently reported aetiological diagnoses made in ovineabortion investigations between 2000 and 2018

^a Uncommon infectious agents combined represent 14% investigations with diagnosis.

^b Non-infectious causes combined represent 19% investigations with diagnosis.

^c Includes cases of vitamin E, copper, selenium or cobalt deficiencies. ^d Toxicities included predominantly plant poisonings: phalaris, romulosis and toxic algae.

Leptospira interrogans serovar Hardjo was the only serovar reported (n = 2), although serovar was not reported for all cases. Leptospirosis was not diagnosed in Western Australia. An aetiological diagnosis of yersinosis was made for 3% of investigations with a diagnosis, with both *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* identified as aetiological agents either together or separately. Other infectious agents each represented less than 2% of diagnosed investigations. 'Maternal illness' and dystocia were the most common non-infectious cause identified. However, 'maternal illness' was a non-specific diagnosis and could have involved infectious diseases in some cases. Exotic disease agents were not identified in any of the investigations.

 Table 6. Association between submission of placenta samples and diagnostic success (% investigations with aetiological diagnosis) in ovine abortion investigations

	Submissions (n)	Aetiological diagnosis made	No aetiological diagnosis
Placenta available	77	48 (62%)	29 (38%)
Placenta not available	62	26 (42%)	36 (58%)

Data were available from all four states for the period 2010–2017 (Table 5), with some variation year on year in the frequency of diagnoses for the major infectious causes of abortion. The most frequently reported aetiological diagnoses over this period were listeriosis (30.6% investigations with aetiological diagnosis), campylobacteriosis (27.6% investigations with diagnosis) and toxoplasmosis (10.3% investigations with diagnosis).

Of the investigations where histopathological findings were provided (n = 272), 56% reported inflammatory lesions. Aetiological diagnoses were made in 76% of this sample, and 97% of these had an infectious cause. Of the investigations where no aetiological diagnosis was made, 30% also showed inflammatory changes. The most common lesions described, together or independently, were pneumonia (n = 42), placentitis (n = 38) and hepatitis (n = 27), although detailed information of inflammatory lesions was not available for all cases.

Factors associated with determination of aetiological diagnosis

Of 139 investigations where information on types of samples submitted was available, 55% included placenta. Investigations that included the submission of placenta were 2.3 (95% confidence interval [CI] 1.1 to 4.5) times more likely to have a diagnosis made compared to those investigations without placenta available (P = 0.017; Table 6).

Table 5. Number of diagnoses made for abortion and stillborn lamb disease investigations in Western Australia, South Australia, Victoria and Tas-
mania between 2010 and 2017

	Year of study							Overall		
	2010	2011	2012	2013	2014	2015	2016	2017	n	%
Investigations (n)	51	40	45	28	50	55	94	46	409	_
Diagnoses (n)										
Listeria	9	7	11	5	8	9	16	6	71	17.4ª
Campylobacter	10	0	6	7	14	12	10	5	64	15.6°
Toxoplasma	4	4	3	0	3	0	10	0	24	5.9 ^t
Other infectious causes	2	4	1	4	1	4	9	3	28	6.8 ^t
Non-infectious causes	5	5	9	5	3	4	8	6	45	11.0 ^a
No diagnosis made	21	20	15	7	21	26	41	26	177	43.3°

Values in column with different letters are significantly different (P < 0.05).

Autolytic changes were reported for 50% of investigations, yet an aetiological diagnosis was still reached in 57% of these, and autolytic changes was not associated with the likelihood of an aetiological diagnosis being made (P = 0.578).

Discussion

This study examined causes of abortion and stillbirths for 529 investigations submitted to state veterinary diagnostic laboratories between 2000 and 2018. Pathologists reported a diagnosis for 57% investigations, with a diagnosis more likely to be made for investigations that included the submission of placenta samples. Campylobacteriosis, listeriosis and toxoplasmosis were the most common diagnoses reported. There was no evidence of infection with *C. abortus*, *B. melitensis* or *S. enterica* serotype Abortusovis, which are considered exotic to Australia and are important causes of abortion in sheep and public health risks overseas.^{11–13}

An infectious aetiology was determined in 46% of investigations. In an additional 30% of cases without a diagnosis, morphological diagnoses suggestive of an infectious aetiology, including placentitis, fetal hepatitis and/or pneumonia, were reported, suggesting that infectious abortion could be underreported in this sample. This is consistent with previous studies where inflammatory lesions suggestive of infectious causes were found in 7%–15% of cases without isolation of an aetiological agent.^{15–19}

Abortion associated with *Listeria spp., Campylobacter spp.* and *T. gondii* accounted for more than two-thirds of investigations with a diagnosis reported. This is consistent with previous studies reporting causes of infectious abortion in Australian sheep.^{5,7,8,20,21} These pathogens are also associated with sheep reproductive losses in many other countries.^{22–25} Despite these diseases being endemic in Australia, the incidence of infectious abortion associated with these infections is poorly described.

Campylobacter fetus fetus has been recognised as a cause of ovine abortion in Australia,⁸ which is consistent with the observations of this study. However, in Tasmania, C. jejuni was reported more commonly in abortion investigations than C. fetus. Campylobacter jejuni is a commensal organism that is commonly shed in faeces by asymptomatic sheep.²⁶ Under some circumstances, C. jejuni can be associated with abortion in sheep and goats,²⁷⁻³⁰ but specific risk factors for C. jejuni-associated abortion are not well described. A clonal form of C. jejuni has become the predominant cause of ovine abortion in some parts of North America.³¹⁻³³ Abortigenic C. jejuni strains in Australia have not been studied with molecular characterisation, and it is unclear why C. jejuni-associated abortion was reported more frequently in Tasmania compared to other states given the widespread distribution of the organism in Western Australia, South Australia and Victoria.²⁶ A vaccine for Campylobacter has been commercially available in Australia since 2013. Given the limitations of the data available, it is not possible to determine if the availability of vaccination has impacted the incidence of campylobacter-associated abortion in Australia.

A retrospective evaluation of laboratory data is useful for determining the number of investigations being performed and what aetiological agents were diagnosed, which can subsequently identify changing disease trends and the introduction of novel pathogens.^{17,34,35} However, disease investigations reported in this study were derived from submissions by veterinarians to animal health laboratories, which rely on farmers identifying abortion in the flock, reporting this to veterinarians, suitable samples being available for submission to the laboratory and veterinarians making the decision to submit samples for testing. This is associated with the potential for bias and the risk of over- or underreporting specific diseases.³⁴ Therefore, the incidence of abortion in Australian sheep or the true incidence of specific diseases cannot be derived from data included in this study. Diseases associated with mid-pregnancy abortion are likely to be underrepresented as fetal tissue is less likely to be recovered.

In this study, an aetiological diagnosis was made in 57% of investigations. This was comparable with previous Australian studies conducted between 1966 and 1987, where an aetiological diagnosis was reported for 25%–76% cases.^{4–6,8,9} Similar findings have been reported in other countries.^{12,15,16,34} Differences in the number of submissions, predominant diseases, method of sample collection and diagnostic methodology, including the availability of molecular diagnostic tools, will contribute to variation in the proportion of investigations with aetiological diagnosis and the types of diagnoses made. Overall, determining an aetiological diagnosis in abortion investigations for ruminants remains a challenge in many countries despite the availability of molecular diagnostics.

The type and quality of aborted material submitted for laboratory analysis impacts the ability of pathologists to make an aetiological diagnosis for disease investigations. Inclusion of placental tissue in submissions increased the probability of making an aetiological diagnosis by 2.3 times, and this was consistent with previous studies.^{5,16,17,36} It was not possible to determine the impact of the number of animals included per submission, but it has been previously reported that the likelihood of detecting an infectious agent is improved where samples from more aborting ewes are available.^{8,37}

Autolysis of tissues was commonly reported for samples submitted for histopathology, and this was consistent with other abortion studies.^{16,17,38} Autolysis of specimens is often unavoidable and is a consequence of the disease process or maceration before expulsion from the uterus. Tissue autolysis may hinder diagnostic success by reducing the viability of causative agents for culture and affecting the interpretation of histopathology. Despite this, an aetiological diagnosis was achieved in over 57% of cases where autolysis was described, largely due to the use of molecular techniques and/or culture for the isolation of infectious agents. Submission of suitable tissue samples for histopathology is especially important for aetiological diagnosis, where it is suspected that pathogens could be involved which are both abortigenic and common gut inhabitants of asymptomatic sheep (e.g. C. jejuni, Listeria spp or Yersinia spp.) and where tissue contamination with soil or faeces is evident.³⁹ Veterinary practitioners can improve the likelihood of obtaining an aetiological diagnosis by encouraging farmers to collect any aborted or perinatal dead lambs and placenta (if available), even if there is evidence of predation, and to store these in temperatures of between 4 and 10°C before submission to the practitioner or veterinary diagnostic laboratory.

The inclusion of cultures and molecular diagnostic techniques is likely to improve the ability to make a diagnosis in cases with autolysis, but it is important to note that the detection of a pathogen may not be sufficient to determine causation for abortion, and in some cases, the detection of infectious agents may have been incidental findings. The detection of infectious agents in multiple cases from the same property and supportive histological findings increase confidence in the diagnosis. However, the recovery of appropriate samples is difficult on extensive livestock farms, and the autolysis of samples is common. Furthermore, the use of ewe serology for cases where fetal tissue is not available is unreliable unless paired samples collected close to the time of fetal loss are available to demonstrate active infection evidenced as rising titres. Consequently, determining an aetiological diagnosis can be challenging, particularly under conditions where abortion is not readily observed.

Abortion and stillbirth investigations were conducted for a low proportion of farms (up to 0.5% farms per year). It is not clear if low rates of submission are due to low levels of abortion being observed on Australian farms, low rates of voluntarily reporting of abortions to veterinarians by farmers or veterinarians choosing not to submit specimens for laboratory testing. A range of factors can influence the likelihood of disease investigation, including cost of investigation, access to veterinary services and willingness of farmers to seek veterinary services, veterinary investigative capacity and severity of disease outbreak.^{40–42} The ability of producers to identify an abortion problem in the first instance can be challenging on sheep farms with extensive management systems where the likelihood of observing abortion or locating aborted fetuses in the paddock is low. Furthermore, producers have variable sensitivity thresholds for livestock morbidity and mortality before seeking veterinary advice, and the sporadic nature of lowlevel abortion events and lack of obvious clinical signs of disease in ewes with many abortive disease syndromes in Australia likely contribute to under-reporting of abortions to veterinarians.^{43,44}

Infections with zoonotic potential were identified in this study, including *T. gondii*,⁴⁵ *Campylobacter* spp.,⁴⁶ *Leptospira* spp.⁴⁷ and *Salmonella* spp.⁴⁸ Human disease after exposure to ovine aborted material and lambing ewes has previously been reported.^{49–51} This reinforces the importance of appropriate personal protection and hygiene measures for farmers, veterinarians and laboratory workers when handling pregnant and lambing ewes and aborted material. Recommendations include limiting contact between pregnant or immunocompromised people and lambing ewes or aborted material. General hygiene precautions should be used to avoid ingesting infectious agents (e.g. bacteria, parasites), and if contact with lambing ewes and fetal tissues is unavoidable, open wounds should be covered with waterproof dressings, and the importance of effective hand washing after handling animals or tissues should be emphasised.⁵²

Conclusion

Infectious diseases are the most frequent cause of abortion in investigations submitted to state veterinary diagnostic laboratories in southern Australia. The most common diagnoses made are campylobacteriosis, listeriosis and toxoplasmosis, but a wide variety of other infectious and non-infectious causes of abortion is diagnosed in Australian sheep. Although an aetiological diagnosis in abortion and stillbirth investigations is sometimes not reached, veterinary practitioners can improve the probability of an aetiological diagnosis by emphasising to farmers the importance of collecting any aborted tissues, especially placenta; submitting multiple fetuses; and appropriate storage of the tissues until they can be submitted to the laboratory. Submission of appropriate tissue samples aids diagnosis, even when autolysis is evident. Many of the diseases that cause abortion and stillborn lambs in Australian sheep have zoonotic potential, and therefore, veterinary practitioners play an important role in educating clients about appropriate hygiene when handling pregnant and lambing ewes and any aborted material.

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Conflicts of interests

Sue Beetson is a current Editorial Board member for the Australian Veterinary Journal. The authors declare no other conflicts of interest for the work presented here.

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