Brucella ovis infection in deer

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Abstract

Brucella ovis was identified in the New Zealand farmed deer population in 1996. From 1998-2002, several experiments were undertaken to investigate this disease in deer. Studies demonstrated transmission from infected rams to stags, and between infected stags, when animals were grazing in the same paddock. Transmission did not occur between stags by indirect contact. Behavioural studies suggested possible routes of transmission between stags. Stags were initially very susceptible to B. ovis infection but the majority stopped shedding within 11 months of infection, suggesting resolution. During the shedding phase the majority produced semen that had poor sperm motility and morphology. However, following cessation of shedding semen characteristics were markedly improved. The sensitivity of the commercially available serological tests at detecting infection in deer decreased from about 60-100 days after infection. Detection of lesions of epididymitis by scrotal palpation of stags was an insensitive diagnostic method. Stags infected with B. ovis developed lesions in the epididymes, seminal vesicles and ampullae. Vaginal inoculation of hinds immediately prior to mating resulted in no measurable adverse effects on reproduction. Stags that mated vaginally-infected hinds became infected, demonstrating venereal transmission of the organism.

Introduction

Brucella ovis has been recognised causing epididymitis and reduced fertility of rams in New Zealand for almost 50 years (Buddle and Boyes, 1953), but it was not until 1996 that the organism was identified in New Zealand farmed deer (O'Neil, 1996). To date, infection has been identified in stags from 5 properties (Scott, 1998; 1999). Between 1998 and 2002 a number of experiments have been undertaken at Massey University to establish the transmission, effects and diagnosis of this disease in deer. This paper presents the key results of this research and discusses its relevance to *B. ovis* diagnosis, control and prevention for the New Zealand deer industry.

1. Transmission

1.1 Transmission between stags and from stags to rams (West et al., 1999)

Two stags artificially infected with *B. ovis* by intravenous inoculation and shedding *B. ovis* in semen were grazed with 8 non-infected stags and 11 non-infected rams for a 9-month period from October 1997-June 1998. At monthly intervals all animals were blood sampled for *B. ovis* serology (CFT). When infection was suspected, semen was collected for *B. ovis* culture. During April and May, 7 months after mixing, infection was identified in 4 of the 8 non-infected stags. The rams did not become infected. This experiment demonstrated transmission between stags when in direct contact with one another. Transmission appeared to occur during the rut period over a short time period. This suggests that all 4 stags became infected at a similar time, and that sexual activity is important in the transmission of this disease.

1.2 Transmission from rams to stags (Ridler et al., 2000a,c)

Five rams artificially infected with *B. ovis* by intravenous inoculation and shedding *B. ovis* in semen were grazed with 6 non-infected stags from 3 March-13 July 1999. Animals were blood sampled at 1-4 weekly intervals for *B. ovis* testing and when infection was suspected, semen was collected for *B. ovis* culture. On 4 June, 3 months after mixing, 5 of the 6 stags were infected. This confirmed that transmission occurs from infected rams to stags grazing in the same paddock. Again, transmission occurred over a short time period during the rut period, suggesting that all 5 stags became infected at about the same time and that sexual behaviour may be important for the transmission of this disease.

1.3 Transmission between stags by indirect contact (Ridler et al., 2000a;b).

Five stags were artificially infected with *B. ovis* by intravenous inoculation and shed *B. ovis* in semen. From 4 March-18 August 1999, these infected stags swapped paddocks 1-2 times per week with 6 non-infected stags. Thus, non-infected stags were shifted onto a paddock that had only just been vacated by infected stags for a total of 32 times. In addition, 6 non-infected stags were grazed in an adjacent paddock to the infected stags so that they had contact with them through the fence throughout the 5.5 month trial period. Animals were blood sampled at 1-4 weekly intervals for *B. ovis* testing (CFT). None of the non-infected stags became infected. Similar experiments with sheep have also failed to demonstrate infection by "indirect" contact with infected animals. This suggests that transmission of *B. ovis* requires animals to be in the same paddock or pen together.

1.4 Venereal transmission from hinds to stags (Ridler et al., in press)

In March 2000, 30 hinds were synchronised for oestrus, and on the day of expected oestrus each hind was inoculated with 1ml of *B. ovis* inoculum into the anterior vagina. Hinds were split into 4 groups of 5 and 1 group of 10, and each group was mated to a non-infected 16-month-old stag for a 55-day (~3 oestrous cycle) period. At the end of mating and again 3 weeks later each stag was blood sampled for *B. ovis* serology (CFT + ELISA) and semen sampled for *B. ovis* culture. Three of the 5 stags became infected with *B. ovis*, demonstrating venereal transmission from the vagina of the hind to the stag. This suggests that where more than 1 stag is mated to a group of hinds, transmission between stags could occur in this way if more than 1 stag mates the same hind. Similarly, even in a single-sire mating situation, if stags were swapped between hind groups during mating there is the potential for transmission to occur. *Brucella ovis* was isolated from the vagina of 1/17 hinds tested 338 days after inoculation, suggesting the organism could survive as a commensal in the vagina of some hinds. The impact of this on the epidemiology of the disease is unknown beyond these time intervals, but this observation suggests there may be a slight risk of transmission from season to season via the hind.

1.5 Transmission– general discussion (Ridler, 2002a)

These studies have demonstrated that transmission of *B. ovis* may occur:

- from stag to stag when in direct contact
- from ram to stag when in direct contact
- from stag to hind to stag by venereal transmission

It is probable that sexual behaviour is important in the transmission of the organism and that because of the defined breeding season of deer, the most transmission is likely to occur during the rut period. The mechanism of transmission is unknown but it has been observed that stags in all-male groups exhibit behaviours that are considered high-risk for the transmission of *B. ovis* including low mounting (non-ejculatory), preputial sniffing and licking, perineal sniffing and licking and spraying fluid from an extruded penis. High (ejaculatory) mounts have also been observed although these appear to be uncommon. Experimentally it has been demonstrated that stags may become infected by inoculation of the conjunctival, nasal and rectal mucous membranes (Ridler, 2002a). Thus it is probable that stags become infected by either sniffing or licking infected semen around the prepuce of another stag/ram, aerosol spray of infected urine/semen contacting the conjunctiva, sniffing or licking infected semen deposited on the perineal region of another stag/ram after it has been mounted by a stag/ram, or by rectal copulation. The latter route between rams and stags is improbable.

2. Persistence of infection in stags (Ridler, 2002a)

The persistence of *B. ovis* infection in rams has not been well defined but it has been reported as greater than 2 years (Hartley et al., 1955) or greater than 4 years (Buddle, 1956). To investigate this, 14 stags and 8 rams were artificially infected with *B. ovis* by intravenous inoculation on 23 December, 1999. A further 3 stags that became infected with *B. ovis* in a more "natural" way by mating vaginally-infected hinds (Section 1.4 above) were added to the stag group in March 2000. From the time of infection until October 2002, a period of almost 22 months, animals were blood sampled at approximately monthly intervals for *B. ovis* serology (CFT + ELISA, see later in this paper) and

semen was collected for *B. ovis* culture. At slaughter in October 2002, the reproductive organs were collected for *B. ovis* culture and histopathology (see later).

2.1 Stags intravenously infected

12 of the 14 stags initially shed *B. ovis* in semen but from 138 to 342 days after inoculation *B. ovis* was no longer isolated from the semen of 10 of these 12. The remaining 2 stags shed *B. ovis* until the trial ended on day 630 (Table 2.1).

Table 2.1: *B. ovis* culture from the semen of 14 stags artificially infected with *B. ovis* by intravenous inoculation on Day 0.

Stag	DAYS AFTER INOCULATION																	
ID	83	103	138	166	200	223	235	263	291	342	437	459	467	493	524	558	592	630
803																		
827				NS										NS				
842																		
852			NS												NS		NS	
854														NS				
855			NS										NS					
860													NS			NS		
864																		
866																		
869							NS			NS			NS					
865	NS			NS	NS		NS									NS		
867																NS		
826																NS		
861			NS															

B. ovis positive *B. ovis* negative NS no semen sample collected

2.2 Stags infected in a "natural" way by mating vaginally inoculated hinds

All 3 stags stopped shedding *B. ovis* in semen some time between days 134 and 253 after infection (Table 2.2).

Table 2.2: *B. ovis* culture from the semen of three stags infected with *B. ovis* by mating vaginally infected hinds on approximately Day 0.

Stag	DAYS AFTER EXPOSURE TO INFECTION														
ID	55	77	134	146	174	202	253	349	371	379	405	435	470	504	542
833														NS	
839															
850									NS			NS			

B. ovis positive *B. ovis* negative NS no semen sample collected

2.3 Rams intravenously infected

Of the 8 rams artificially infected, 6 shed *B. ovis* in semen throughout the trial period. *Brucella ovis* was not isolated from the semen of the remaining 2 (numbers 55, 56) at any time (Table 2.3).

Table 2.3: *B. ovis* culture from the semen of eight rams artificially infected with *B. ovis* by intravenous inoculation on Day 0.

Ram ID	DAYS AFTER INOCULATION																	
	83	103	138	166	200	223	235	263	291	342	437	459	467	493	524	558	592	630
55																		
56																		
57																		
58																		
59																		
60								NS										
61																		
69																		

B. ovis positive *B. ovis* negative NS no semen sample collected

2.4 Culture at slaughter

At slaughter, *B. ovis* was isolated from the reproductive tracts of the 2 stags and 6 rams that shed the organism in semen throughout the experiment, but not from the reproductive tracts of the remaining stags and rams.

Thus 13 of 15 stags stopped shedding *B. ovis* in semen by 342 days after inoculation. The organism was not isolated from the reproductive tract suggesting that these stags resolved the infection, or affected a "self-cure". In contrast, the rams kept shedding *B. ovis* in semen for at least 22 months.

3. Pathophysiological effects of infection in deer

3.1 Semen characteristics

Semen was collected for evaluation from 10 stags used in the above experiment. During the 2000 breeding season when all 10 stags were shedding *B. ovis*, 3 semen samples collected at monthly intervals were evaluated (Ridler and West, 2002). During the 2001 breeding season when all stags had stopped shedding *B. ovis*, 5 semen samples collected at 1-4 weekly intervals were evaluated. Semen was evaluated for gross appearance, percentage of sperm with forward motility, percentage of normal and abnormal sperm and relative number of leukocytes.

During the shedding phase, semen from 7 of the 10 stags had purulent material grossly visible and large numbers of leukocytes visible under microscopy. Semen from the remaining 3 stags did not contain purulent material. Semen from 2 had small numbers of leukocytes present while the other had none. Semen from 7 of the 10 stags (the same 7 that had purulent material visible) had poor motility and large numbers of abnormal sperm (Table 3.1).

In 2001, all of the 10 stags considered in this experiment had stopped shedding *B. ovis* in semen. There was no purulent material grossly visible in the semen. Semen from all 10 had good sperm motility and morphology (Table 3.1). However, leukocytes were still present in the semen of all stags (Table 3.1).

Thus, during the shedding phase the majority of stags had purulent material grossly visible in semen and low sperm motility with large numbers of abnormal sperm. It is probable that the fertility of these stags would have been reduced as a result of *B. ovis* infection. Following resolution of infection the semen of these stags improved to a level that would be considered acceptable for breeding.

Table 3.1 Semen characteristics of stags infected with *B. ovis* during the 2000 and 2001 breeding seasons. In 2000, all stags were shedding *B. ovis* in semen, in 2001 all stags had resolved the infection.

	% forward motility				% normal sperm				% major abnorm.				% minor abnorm.			
Stag ID	2000 (Shedding)		2001 (Resolved)		2000 (Shedding)		2001 (Resolved)		2000 (Shedding)		2001 (Resolved)		2000 (Shedding)		2001 (Resolved)	
	0	SD	0	SD	0	SD	0	SD	0	SD	0	SD	0	SD	0	SD
842	73	(5.8)	75	(12.2)	76	(13.1)	91	(2.8)	12	(2.6)	5[a]	(2.1)	12	(12.2)	2	(1.4)
854	53	(15.3)	77	(12.5)	71	(20.8)	91	(8.3)	10	(6.1)	1	(1.7)	19	(14.8)	5	(4.4)
867	67	(5.8)	81	(11.1)	86	(5.5)	87	(8.6)	18	(16.7)	5	(2.1)	4	(2.6)	3	(2.5)
852	3	(3.5)	82	(2.4)	42	(10.0)	92[a]	(2.4)	22	(1.5)	4[a]	(5.8)	32	(4.7)	8[a]	(2.9)
855	10	(14.1)	75	(7.1)	22	(9.9)	94[a]	(4.0)	54	(7.8)	5	(2.1)	25	(2.1)	1[a]	(0.6)
860	15	(8.7)	83	(8.5)	25	(11.5)	93[a]	(3.2)	58	(17.4)	5[a]	(2.7)	17	(9.0)	3	(3.0)
864	15	(5.0)	74	(13.6)	54	(13.6)	87[a]	(7.7)	17	(11.0)	13	(9.9)	29	(3.5)	6[a]	(3.8)
865	37	(12.6)	78	(8.3)	45	(13.6)	92[a]	(2.5)	41	(8.1)	11[a]	(4.9)	14	(11.3)	4	(5.1)
866	10	(14.1)	78	(13.1)	32	(9.1)	84[a]	(9.1)	30	(5.2)	4	(3.2)	35	(10.0)	10[a]	(6.7)
869	10	(10.0)	84	(4.1)	13	(12.1)	86[a]	(4.5)	15	(4.2)	6	(3.1)	71	(8.1)	4[a]	(1.0)

[a] significant difference, $p < 0.05$

3.2 Pathology in stags

During the course of the above experiments, 30 infected stags were examined. Three (10%) had lesions of epididymitis that could be detected by scrotal palpation. This contrasts with rams where 30-40% of infected animals have palpable lesions of epididymitis (Hughes and Claxton, 1968). Following slaughter, subtle enlargement of the epididymes and lesions within the epididymes were detected in a further 4 stags.

Histopathological examination of the epididymes, seminal vesicles and ampullae of 5 stags examined within a month of becoming infected showed severe lesions in all 3 organs. Lesions consisted of intraepithelial cysts and spermatic granulomas within the epididymes, and lymphocytic infiltration into the lamina propria in all 3 organs. These lesions are similar to those seen in infected rams (Kennedy et al., 1956; Biberstein et al., 1964; Foster et al., 1987). In contrast, the lesions in these organs from 13 stags that had resolved the infection were mild and consisted of occasional small foci of 5-10 lymphocytes.

3.3 Effects in hinds – Reproductive performance (Ridler et al., in press)

Thirty hinds were used in this experiment which started in March 2000. They were synchronised for oestrus and on the expected day of oestrus and 19 days later each hind received an intravaginal inoculation of *B. ovis*. Hinds were single sire mated in 6 groups of 5 hinds to non-infected stags and pregnancy scanned monthly. Twenty were retained until parturition and calves were identified and blood sampled at birth and again at weaning. A total of 13 hinds were slaughtered during this experiment for sample collection and analysis.

There were no detectable effects on hind reproductive performance that could be attributed to *B. ovis* infection. This result is similar to observations from ewes, where, following infection by a "natural" route, effects of infection are rare (Buddle, 1955; Hartley et al., 1955; Hughes, 1972).

4. Diagnosis of *B.ovis* in deer

4.1 Scrotal palpation

As stated in 3.2, only 3 out of 30 infected stags had lesions of epididymitis detectable by scrotal palpation, suggesting this is an insensitive method of diagnosis.

4.2 Serology

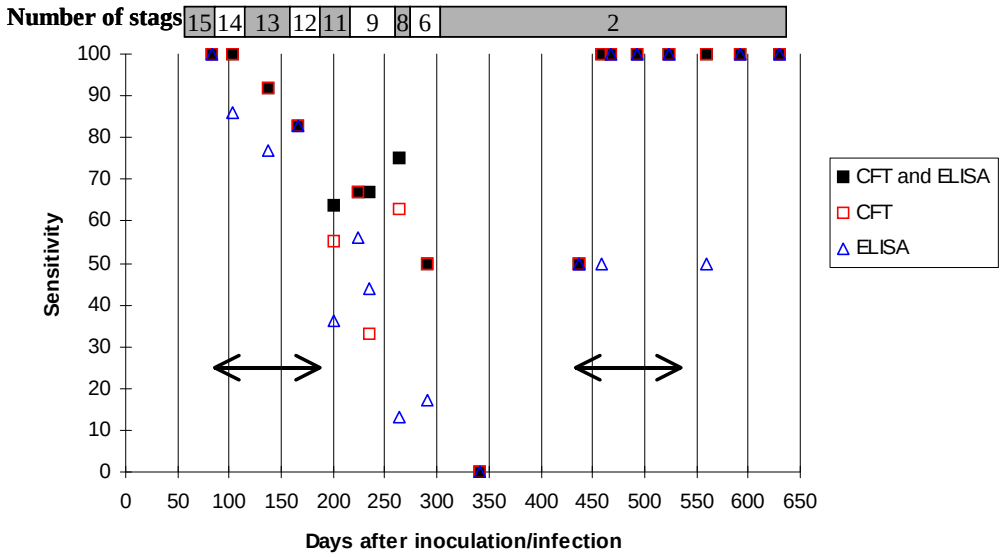
Sensitivity

Using the *B. ovis* CFT and ELISA tests commercially available in New Zealand for testing sheep sera and using the cut-off values described for sheep, within the first 20 to 60 days of infection the CFT and ELISA were 100% sensitive at detecting infection in 29 stags that became infected either by intravenous inoculation (17 stags) or by contact with infected animals (12 stags). It would therefore appear that during the early stages of infection both of these tests are highly sensitive at detecting infection.

However, over time the ability of both the CFT and ELISA to detect infection in stags that continued to be semen culture positive decreased. Up to 100 days after infection, the sensitivity of the CFT at detecting infection in 12 stags artificially infected by intravenous inoculation and 3 stags that became infected by mating vaginally-infected hinds was 100% and the sensitivity of the ELISA ranged from 85 to 100%. From 100 to 300 days after infection, the sensitivity of the CFT decreased to around 30 to 70% and the sensitivity of the ELISA decreased to 10 to 60%, despite those stags shedding *B.ovis* in semen. At the same time other stags were concurrently resolving the infection and were culture and serology negative (Figure 4.1).

In contrast, the sensitivity of these tests at detecting infection in rams remained at 100% throughout the 22-month trial period.

Figure 4.1: Sensitivity of the *B. ovis* CFT and ELISA at detecting infection in stags over time. Arrows indicate the breeding periods.



Specificity

During the course of these studies, 221 serum samples from 104 non-infected deer were tested in the CFT and 109 samples from 59 non-infected deer were tested in the ELISA. There has been one false-positive reaction in the CFT (Section 4.3) and none in the ELISA, suggesting a specificity of 99% and 100% respectively. Using the CFT, Kittelberger and Reichel (1998) tested serum from 1498 deer that were presumed to be non-infected, with a specificity of 99.6%. The specificity of the *B. ovis* CFT and ELISA in deer appears to be high.

4.3 Bacteriology

Isolation of *B. ovis* from the semen has proven an effective way of detecting infection in stags. Similarly, the organism can be isolated from the epididymes, seminal vesicles and ampullae but it is advisable to culture all 3 of these organs because the organism cannot be consistently isolated from any one of them.

5. Strain typing *B. ovis* isolates by pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis, a genetic technique used to generate a DNA profile specific to that strain-type, was performed on isolates of *B. ovis* from rams and stags in New Zealand. A total of 10 isolates from commercial flock rams from 10 separate properties, and the Type strain, were analysed. In addition, an isolate from the original stag infection identified in the South Island in 1996, and an isolate from a stag from a North Island property identified in 1997 were analysed. Two distinct strain types were identified. Each strain type was isolated from both rams and stags, demonstrating that the strain types are not specific to either animal species. The *B. ovis* isolates from the 2 deer farms were different strain types, demonstrating that the farm in the North Island had not become infected by movement of deer from the South Island. This suggests that both the infections on these deer properties originated from contact with infected rams, further emphasising the importance of rams in the epidemiology of this disease in deer.

6. Impact of *B. ovis* on the New Zealand farmed deer industry

6.1 Prevalence of disease in deer

Analysis of serology data from 1996-June 2000 showed that 1074 deer, representing less than 0.1% of the New Zealand farmed deer population, were tested serologically for *B. ovis* using the CFT (Ridler, 2001). Using the cut-off values described for sheep, 11% of these samples were positive and the positive samples came from deer from 10 different properties. On 5 of these properties infection has been confirmed by culture of *B. ovis* from semen or epididymes (Scott, 1999). The remaining 5 properties had only 1-3 positive deer from each, and it is unknown whether they are true or false-positive serological reactions. Since 1998, only 1 new case has been identified by serology and this was not confirmed by *B. ovis* culture. Scrotal palpation of stags to detect lesions of epididymitis, with investigation of any lesions identified, is a routine post-mortem inspection procedure at deer slaughter premises. While the exact prevalence of *B. ovis* in the New Zealand farmed deer population is not known it is likely to be low.

However, in spite of a voluntary accreditation scheme for *B. ovis* in sheep that has been in place since 1986 (Reichel and West, 1997), *B. ovis* is still a relatively common disease of sheep in some areas of New Zealand. It appears that stags can readily become infected from rams and it is probable that stag infection from contact with infected rams will continue to occur sporadically.

Of particular concern would be if infection occurred within a stud deer herd, and infected stags were sold to other properties to be used for breeding.

6.2 Impact of infection within deer herds

Infection of breeding stags is likely to result in reduced semen quality, and affect fertility. It is not uncommon for deer farmers to mate only 1 stag to a large group of hinds and if the stag were infected this could have serious consequences on the pregnancy rates of those hinds.

Infection of velvetting stags is likely to be of little consequence provided they were kept separate from breeding stags at all times, and were not themselves used for breeding at any time. Thus the management of an infected group of stags is likely to vary depending upon whether they are breeding or velvetting stags, and the ability to keep groups of stags separate.

Infection of hinds appears to be of little consequence to the hind, although the hind can act as a mechanical vector in the transmission of infection between stags. The possibility of long-term survival of the organism in the vagina of some hinds suggests that they may be able to act as a reservoir of infection from year to year although the consequences of this (whether a large enough concentration of *B. ovis* is present to infect stags in the following year) is unknown.

7. Control of *B. ovis* in the New Zealand farmed deer industry

The prevalence of *B. ovis* in the New Zealand farmed deer population is low. In addition, the infection appears to be self-limiting in the majority of stags, which contrasts with the infection in rams. Infection in hinds appears to be of little consequence. Thus the disease is not considered a significant enough risk to deer farming operations to warrant a market assurance programme or a formal national control scheme. Good management, involving keeping rams and stags separate, should ensure that *B. ovis* remains a rare disease of stags.

It appears that stags are most likely to become infected from rams and it is strongly recommended not to run rams and stags together in the same paddock. Deer farmers involved in breeding stags for sale may wish to ensure that any introduced stags are free from *B. ovis* using serology. If deer farmers wish to check the *B. ovis* status of their breeding stags, then serology would be a simple option. Veterinarians should also be aware that *B. ovis* infection can result in poor reproductive performance of hinds, and should consider this possibility when investigating poor pregnancy rates.

8. Acknowledgments

This research was funded by:

The New Zealand Deer Farmer's Association
The New Zealand Game Industry Board
The New Zealand Ministry of Agriculture and Forestry
The Massey University Research Fund
The Massey University IVABS Postgraduate Research Fund

AgriQuality Serology off-set some of the serology costs.

A number of people, particularly Geoff Purchas and Graham McCool, provided valuable technical assistance.

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