

Investigations into the epidemiology of *Brucella ovis* in stags

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Abstract

Brucella ovis was previously thought to be a pathogen primarily of sheep but since 1996 it has been recognised in deer. The initial source of infection in deer was not determined from trace-back studies, but a trial undertaken in 1999 showed that *B. ovis* transmitted from infected rams to 5 of 6 stags grazing in the same paddock. Further trials failed to demonstrate transmission between infected and non-infected stags by successive grazing (swapping paddocks) or by grazing in adjacent paddocks. This is similar to findings in rams and suggests that for transmission of *B. ovis* to occur, animals must be in direct contact with one another.

A trial in 1998 demonstrated that *B. ovis* transmitted between stags grazing in the same paddock over the rut period. While transmission of *B. ovis* between rams is thought to occur by rectal copulation, behavioural studies of stags in bachelor groups undertaken during the rut suggest that groups of stags engage in less sexual activity than groups of rams. It is probable that transmission of *B. ovis* from rams to stags and from stags to stags is by sniffing or licking of infected semen.

Introduction

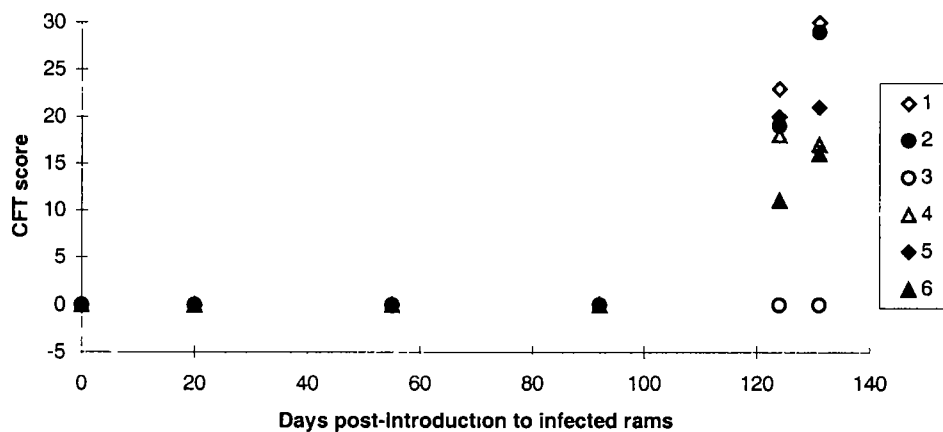
In 1996 *B. ovis* was cultured from the semen of a New Zealand born stud stag (Bailey, 1997) and since then epididymitis due to *B. ovis* infection has been detected in several lines of young stags sent for slaughter (Scott 1998, Scott, 1999). Since 1998 research has been undertaken at Massey University to establish the epidemiology, effects and control of this disease in deer and to determine whether it will be a significant disease to the industry. This paper discusses various experiments designed to establish the transmission of *B. ovis* from ram to stag and stag to stag, with a view to controlling the disease on deer farms.

Transmission from rams to stags

Brucella ovis is an obligate pathogen most commonly associated with sheep and the initial source of infection in deer was not identified by trace-back studies (Scott, 1999). To determine whether *B. ovis* can be transmitted from infected rams to stags, 6 rams were artificially infected with *B. ovis* and 5 of these were subsequently demonstrated to be shedding the organism in semen. From March to July 1999, these six rams were grazed in the same paddock as 6 rising-2-year old stags that were free from brucellosis at the start of the trial. The stags were blood sampled every 2 to 6 weeks for *B. ovis* antibody testing using the complement fixation test (CFT). Reproductive organs from the stags were collected at slaughter in July for bacteriological culture to confirm infection.

All 6 stags remained seronegative until day 92 (June 4) after mixing with the infected rams. Tests carried out on day 124 (July 6) found that 5 of the 6 stags were seropositive (Fig 1). After autopsy, *B. ovis* was cultured from the seminal vesicles of all 5 seropositive stags but was not cultured from the seronegative stag.

Figure 1. Individual stag *B. ovis* CFT scores measured over 131 days post-introduction (March 4 - July 20) to infected rams



Note that for ease of presentation the CFT titres have been converted into scores. The *B. ovis* CFT was performed at serial dilutions of 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048. Titres were expressed as fractions with the numerator denoting the strength of the reaction up to a maximum value of 4. The CFT score is calculated such that a titre of 0 = a score of 0, 1/4 = 1, 2/4 = 2, 3/4 = 3, 4/4 = 4, 1/8 = 5, etc up to a maximum titre of 4/2048 = 40. The cut-off value for positive reactions was 4/8 = 8 as adopted by Bailey (1986).

These results confirm that *B. ovis* can be transmitted from infected rams to stags grazing in the same paddock. Trial work from 1998 established that *B. ovis* can be transmitted from stag to stag when in the same paddock (West *et al.*, 1999) and thus for any property where *B. ovis* infection is found in stags the source of the organism could have come from rams on the property, or from the introduction of infected stags. Rams of unknown or infected *B. ovis* status should not be grazed with stags, particularly breeding stags.

Transmission from stag to stag when not in direct contact

West *et al.* (1999) demonstrated transmission of *B. ovis* between stags during the rut period. It was not determined at this trial how the transmission occurred - whether by direct sexual contact or by grazing pasture or wallowing in water contaminated with the organism. For control of *B. ovis* it is important to establish whether transmission occurs only when animals are in direct contact (as is the case in sheep) or whether it can occur by infected and non-infected stags swapping paddocks or grazing in adjacent paddocks.

In January 1999, six rising-2-year old stags were artificially infected with *B. ovis* and 5 of these were subsequently demonstrated to be shedding the organism in semen. These infected stags swapped paddocks with six non-infected rising-2-year old stags on a once to twice weekly basis from March to mid-August 1999. In total the 2 groups swapped paddocks 32 times. Non-infected stags were blood sampled every 2 to 6 weeks and none of the stags seroconverted.

At the same time, six non-infected rising-2-year old stags were grazed in an adjacent paddock to the infected stags so that the 2 groups were separated by a standard deer fence at all times. These stags were blood sampled every 2 to 6 weeks and none of the stags seroconverted.

In sheep, transmission has not been demonstrated to occur by swapping grazing (Buddle, 1955; Hartley *et al.*, 1955; Keogh *et al.*, 1958) suggesting that for transmission of *B. ovis* to occur the animals must be in the same paddock. During the control phase on a *B. ovis* infected property, keeping infected and non-infected animals separate should be sufficient to prevent transmission occurring.

Routes of transmission for *B. ovis* - behavioural studies

Experimentally, rams have been successfully infected with *B. ovis* by inoculation of the nasal, rectal, preputial, conjunctival and oral mucus membranes (Plant *et al.*, 1986; Brown *et al.*, 1973, Buddle, 1955; Simmons and Hall, 1953). The organism localises in the epididymes and accessory sex glands and is subsequently shed in semen, seminal fluid and possibly urine. The environmental viability of *B. ovis* is unknown.

In the field, *B. ovis* in rams is thought to be transmitted by rectal copulation although the evidence for this is largely circumstantial (Hartley, 1955).

To investigate possible routes of transmission for *B. ovis* in stags, a number of behavioural observational studies of groups of stags were carried out during the rut in 1999 and 2000. The 1999 trials were conducted from mid-April to the end of April and the 2000 trials were conducted from mid-March to mid-May. Thirteen groups of 6 to 133 stags of varying ages were observed for periods of 1 to 9 hours on 6 Southern North Island properties. Most of the observations were carried out during the day but one was conducted at night. There was a total of 125 observation hours.

All incidents of mounting (high and low) and preputial sniffing or licking were recorded. Individual animals were not identified. The data have been collated and analysed to give the average time between the observation of the different behaviours per group of 20 animals (Table I). For example, in 1999 mounting would be seen every 4.5 hours on average in a group of 20 stags. Most of the activity in the 2000 trial was seen between mid-March to mid-April which may help explain the differences in frequency of observed behaviours between the 2 years.

Among the stags, 207 mounts were observed (197 low, 10 high). It could not be determined if rectal copulation occurred during the high mounts, but this did not occur during low mounts. It was noticed that during the low mount the mounting stag extruded the penis but it was not possible to determine if ejaculation onto the perineum of the stag being mounted occurred.

In a similar study in 1999, the behaviour of 2 groups of rams was also observed for a total of 49 hours. Data is included in Table I.

Table I. Average time between observation of behaviours per 20 animals.

	Mounting	Preputial sniffing or licking
Stags - 1999	4.5 hours	11 hours
Stags - 2000	1 hour	4.5 hours
Rams - 1999	25 minutes	10 minutes

In conclusion, groups of stags show less sexual behaviour than groups of rams but behaviours that are considered high-risk for transmission of *B. ovis* do occur. In stags it is possible that sniffing or licking infected semen, either from the preputial area or deposited on the perineum after one stag has been mounted by another, may be a more important route of transmission than rectal copulation. Sniffing or licking infected semen may also be an important route of transmission in rams (Plant *et al.*, 1986).

Transmission of *B. ovis* - implications

In 1998, tentative proposals for controlling *B. ovis* in deer were suggested (West *et al.*, 1998). These proposals assumed that the epidemiology of the disease in stags is similar to that in rams.

To prevent a herd becoming infected, stags should not be grazed with rams of unknown *B. ovis* status and new introductions to the herd should be confirmed *B. ovis* negative before mixing. Ideally, all stags should be blood tested annually, but as infection is most important in breeding stags, testing these at a convenient time such as velvet harvesting or TB testing and keeping them separate from velvet stags may be the most practical option. Devising a management plan for an infected herd should therefore be on a case-by-case basis.

Diagnosis of *B. ovis* in stags

In rams, scrotal palpation and serology (most commonly with the complement fixation test - CFT) are the main methods used for diagnosing *B. ovis* infection. In questionable cases, use of the other serological tests (ELISA and Gel Diffusion) and bacteriological culture of semen can be utilised. In stags, scrotal palpation is difficult not only because of the danger involved but also because the epididymal lesions are less easily identified than those of rams.

From experimental studies involving over 70 deer, the CFT appears to be highly sensitive at detecting acute infections. It also appears to have a high specificity - of 183 sera tested from non-infected animals there has been one positive and one suspicious result. In both of these cases the sera were negative in the ELISA and negative in the CFT at re-testing one week later. We have observed that in some deer the CFT and ELISA titres decreased more rapidly over time than in rams. This is being investigated further this year.

Conclusions

- *B. ovis* can be transmitted from ram to stag and stag to stag when animals are confined together
- it is unlikely that *B. ovis* can be transmitted via pasture or through fences
- groups of stags demonstrate behaviours which are considered high-risk for transmission of *B. ovis*, although they show less sexual activity than groups of rams
- it is probable that *B. ovis* in stags can be controlled on a property using similar methods to those in rams
- due to the difficulty in palpating epididymal lesions in stags, serodiagnosis is a more useful diagnostic method for deer

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