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## Attempted transmission Of *Brucella ovis* between stags and from stags To rams

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### ABSTRACT

In August 1997 three 9-month-old Red x Wapiti stags were inoculated intravenously with *Brucella ovis*. Between October 1997 and June 1998 these three stags were grazed with 8 control stags of a similar age and 10 one-year-old Perendale rams, which had been confirmed free from *B. ovis* infection before mixing. At monthly intervals the 11 stags and 10 rams were blood sampled for *B. ovis* serology and when infection was suspected, this was confirmed by bacterial culture of a semen sample. Two of the three artificially infected stags became persistently infected with *B. ovis* and shed the organism in their semen. They remained positive to the *B. ovis* complement fixation (CF) serological test throughout the trial. The CF titre fell below the positive cut-off level 4 months after inoculation in the other artificially infected stag which did not develop persistent *B. ovis* infection.

During April and May 1998, 7 months after mixing with the artificially infected animals, *B. ovis* infection was identified in 4 of the 8 control stags. There was no evidence of *B. ovis* transmission to the control rams. The trial demonstrated the rapid transmission of *B. ovis* infection between young stags during the rut. This would suggest that sexual activity is important for *B. ovis* to spread, although the exact means of transmission could not be determined. Rams grazed in conjunction with *B. ovis* – infected stags have a lower risk of becoming infected.

**Keywords:** stags; rams; *Brucella ovis*.

### INTRODUCTION

*Brucella ovis* is an important cause of epididymitis in rams and a national voluntary control scheme is used in New Zealand to ensure that sale rams are free of infection (Bruere and West 1987). In 1996 *B. ovis* infection was identified in a Red deer stag in the South Island (Bailey 1997). The stag had epididymitis, abnormal semen and a serum sample reacted positively in the complement fixation (CF) test. It was not determined how the stag became infected.

By June 1997 it had become apparent that *B. ovis* infection of stags was more serious than first thought as up to 50% of some groups of 18 month old stags sent for slaughter had evidence of *B. ovis* infection. These stags had epididymitis and sera from many of them reacted positively in the CF test. This high prevalence of infection immediately after the rut, suggested that, as in rams, sexual activity is an important means by which *B. ovis* is spread. Sheep to sheep transmission is believed to occur by sexual contact but has been the subject of some debate. Hartley *et al.*, (1955) were able to demonstrate transmission to rams by ewes previously mated during the same oestrous period to infected rams. Buddle (1955) demonstrated transmission of infection to 5 of 13 rams when they were grazed with 30 infected rams for 8 months. Whilst the tupping period provides opportunities for infection of rams, spread of infection between rams occurs in the absence of ewes. The common practice of rams mounting other rams and the observation by Hartley *et al.*, (1955) that some rams have faecal material in the prepuccial cavity, suggest that infection may be transmitted by rectal copulation.

Experimental infection of rams has been achieved by instillation of infected semen or cultured organisms onto

mucous surfaces such as the penis, prepuce, conjunctiva, nasal and rectal mucosae as well as by the oral route. Rams and ewes grazing pasture contaminated with *B. ovis* – infected placentae failed to develop brucellosis (Hartley *et al.*, 1955, Buddle 1955).

Sheep are the only species considered to sustain natural infection with *B. ovis* and attempts to infect rabbits, guinea pigs, rats, hamsters, gerbils and mice have been largely unsuccessful (Burgess 1982). Male goats inoculated with *B. ovis* developed only a transient infection (Rahaley and Dennis 1982). Experimental infection of white-tailed deer in America produced pathological signs virtually identical with those in sheep (Barron *et al.*, 1985) and buck to buck transmission occurred with the infection persisting for over 14 months.

This study investigated the rate of transmission of *B. ovis* infection between stags and the ease of transmission from stags to rams.

### MATERIALS AND METHODS

During August 1997, three 9-month-old Red x Wapiti stags previously confirmed as free of *B. ovis* infection were inoculated intravenously with  $2.3 \times 10^9$  colony forming units of *B. ovis* which had been isolated previously from a stag in the South Island. Following confirmation that *B. ovis* was present in the semen of two of the artificially infected stags, the three inoculated stags were grazed with 8 control stags of a similar age and 10-one-year old Perendale rams between October 1997 and June 1998. The control stags and rams had been confirmed free from *B. ovis* infection using the CF test before mixing. The 2.1 ha paddock contained a gully with wet areas used by stags to wallow.

At monthly intervals the 11 stags and 10 rams were

blood sampled from the jugular vein for *B. ovis* serology and when infection was suspected, a semen sample was obtained by electro-ejaculation for bacterial culture. Additional semen samples were obtained from all the stags during May and June and from all the rams during May, June and July. The three artificially infected stags were electro-ejaculated monthly.

Samples of semen were cultured on sheep blood agar and selective agar plates (modified Thayer-Martin medium with nitrofurantoin) and *B. ovis* was identified using standard bacteriological techniques.

The CF test was conducted at the Central Animal Health Laboratory as described previously (Ris 1974). Sera were tested at 6 doubling dilutions from 1/4 to 1/128 and at each dilution the strength of the reaction was graded from 1 to 4 for 25 to 100% fixation. CF titres were given a numerical value (score) between 0 and 24 as described previously (Bailey *et al.*, 1987). The interpretation parameters used for the CF test were: score 0–4 negative, score 5–7 suspicious and score 8–24 positive.

## RESULTS

### Artificially infected stags

Two of the three artificially infected stags became persistently infected with *B. ovis*. Serum from both these stags remained positive in the CF test throughout the trial (Figure 1). Fourteen days after intravenous inoculation, *B. ovis* was isolated from the semen of both stags. Between September and December, during the non-breeding season, the testes of the stags were small and soft and semen samples could not be easily obtained from the stags by electro-ejaculation. By January, testes size and tone had increased, and thereafter *B. ovis* was cultured from the semen at each monthly collection until June when the trial ceased.

The other artificially infected stag did not develop a persistent *B. ovis* infection; *B. ovis* was not cultured from its semen and the CF test fell below the positive cut-off level 4 months after inoculation (Figure 1).

### Control stags

The eight control stags remained negative to the CF test until April when serum from four of the eight control stags returned strong positive reactions in the CF test (Figure 1). *Brucella ovis* was subsequently cultured from the semen of all these four stags. The remaining four control stags showed no serological evidence of *B. ovis* infection and the organism was not cultured from their semen in May or June.

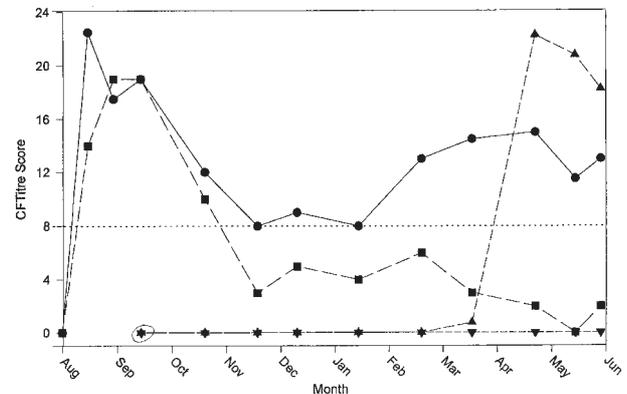
### Control rams

There was no serological evidence of *B. ovis* infection of the 10 control rams. *Brucella ovis* was not cultured from the semen of the 10 rams when sampled on three separate occasions during May, June and July.

## DISCUSSION

The rapid rise in CF titre score and the isolation of *B. ovis* from the semen of artificially infected stags 14 days

**FIGURE 1:** *Brucella ovis* complement fixation titre score of two artificially infected —●— stags persistently infected with *Brucella ovis*, one artificially infected stag —■— that did not develop a persistent infection, four control stags that became infected with *Brucella ovis* —▲— and four control stags that remained uninfected —▼—. A CF titre score of 8 is considered positive (dotted line).



after intravenous inoculation of live organisms, indicates how quickly the infection is established in deer. Subsequently the mean CF titre score declined and 3 to 4 months after inoculation was just above the positive cut-off level for the CF test. This contrasts with studies in rams where the CF titre score persisted at a high level for over 12 months (Bailey 1986). However the CF titre scores of the two stags with persistent *B. ovis* infection remained above the positive cut-off level for the 10 month duration of this trial which gives some reassurance that the CF test will be useful in the control of brucellosis in deer.

The trial has confirmed field reports (Noel Beatson pers.comm.) that *B. ovis* infection can spread rapidly between young stags when they are grazed with infected stags. The rapid rise in mean CF titre scores in 4 control stags in late April and the subsequent confirmation of infection by semen culture suggests that transmission occurred sometime in early April which is the time of peak sexual activity for Red and Wapiti deer. The means by which infection is transmitted from stag to stag is unknown and may be through direct physical contact or indirectly through environmental contamination. Direct ram to ram transmission is well documented and is believed to occur through homosexual activity, although compelling evidence for this is lacking. Further studies of the sexual behaviour of stags may elucidate the means of transmission between stags. In addition, the role of environmental contamination in stag to stag transmission should be determined because of its potential impact on the control of brucellosis within infected herds.

Despite *B. ovis* infection transmitting between stags, rams grazing with them were not infected. This would suggest that the risk of rams becoming infected from stags is small but cannot be discounted. It would seem prudent to recommend that rams should not be grazed with stags whose brucellosis status is unknown, especially in flocks that wish to retain *B. ovis* accredited – free status. In addition, until more is known about the transmission of *B. ovis* from rams to stags, it would be prudent not to graze stags in paddocks grazed by infected rams.

## CONCLUSIONS

The trial demonstrated rapid transmission of *B. ovis* infection between young stags during the rut which suggests that some aspect of sexual activity is important for *B. ovis* to spread between stags, although the exact means of transmission could not be determined. Rams grazed in conjunction with *B. ovis* infected stags have a lower risk of becoming infected.

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