Transmission of *Brucella ovis* from rams to red deer stags

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**Abstract**

**Aim:** To determine whether *B. ovis* will transmit from infected rams to non-infected red deer stags (*Cervus elaphus*) grazing together in the same paddock.

**Methods:** Six rams artificially infected with *B. ovis* were grazed with six non-infected 14-month-old red deer stags for a four and a half month period from March 4 to July 20, 1999. Stags were blood sampled at one- to six-weekly intervals to test for *B. ovis* antibodies using a complement fixation test. Stags that seroconverted were semen sampled to test for *B. ovis* infection by bacteriological culture.

**Results:** Between day 92 and day 124 of grazing together (June 4 and July 6), sera from five of the six stags became positive in the *B. ovis* complement fixation test. *B. ovis* was cultured from semen samples from four of the seropositive stags.

**Conclusions:** *Brucella ovis* can be transmitted from infected rams to non-infected stags grazing in the same paddock, suggesting that *B. ovis* infection in farmed deer in New Zealand initially came from infected rams. Whether transmission occurs from direct contact between rams or stags, or indirectly by environmental contamination needs to be established.

**Key words:** *Brucella ovis*, deer, stags, rams, transmission.

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**Introduction**

*Brucella ovis* was first recognised in 1953 as an important cause of epididymitis in rams (Buddle and Boyes, 1953). While *B. ovis* primarily produces infection in sheep, artificial infection of a range of laboratory animals produced serological titres and pathological changes in the epididymes only of gerbils (Cuba-Caparo and Myers, 1973). Burgess *et al.*, (1985) demonstrated serological and semen culture evidence of infection in experimentally-infected male goats but there are no reports of natural infection occurring in goats.

Random sampling of frozen sera from wild white-tailed deer bucks in Oklahoma revealed that several were positive to a *B. ovis* slide agglutination test (Barron, 1984). The source of this infection was not established. Barron *et al.*, (1985), showed that white-tailed deer bucks could be experimentally infected with *B. ovis* by the conjunctival route and that epididymitis resulted. Bucks remained infected up to 14 months post-inoculation.

In June 1996, *B. ovis* was cultured from the semen of a rising three-year-old New Zealand-bred stud red deer stag with inferior semen quality (Bailey, 1997). Trace-back studies failed to reveal the source of the infection. Subsequently, *B. ovis* has been recognised as an emerging cause of epididymitis in young stags sent for slaughter in New Zealand (Scott, 1998), although the source of infection in these stags has not been determined.

West *et al.*, (1999) demonstrated the rapid transmission of *B. ovis* from experimentally infected 14-month-old stags to non-infected stags grazing together during the rut. Non-infected rams grazing with the infected stags did not become infected suggesting that rams grazed in conjunction with *B. ovis*-infected stags were at low risk of becoming infected.

There is a need for a better understanding of the epidemiology of *B. ovis* infections in deer to underpin control and eradication programmes. The objective of this study was to establish whether *B. ovis* will transmit from infected rams to non-infected stags grazing in the same paddock.

**Materials and Methods**

**Animals**

On January 14 1999 (day -50), six three-year-old Perendale rams to be used as the challenge source of infection were artificially infected with *B. ovis* by intravenous injection of 2 ml of an inoculum containing 1.82 x 10^5 colony forming units/ml. At days -36, -23, -7, 92 and 124 all rams were blood sampled by jugular venepuncture for *B. ovis* antibody testing. At days -23, -7 and 92 all rams were semen sampled by electroejaculation for bacteriological culture using techniques adapted from Bailey (1986). At day 92, a preputial swab (Difco Laboratories culture swab) was taken from inside the prepuce of each ram for bacteriological culture.

On March 4 1999 (day 0), the rams were shifted onto a 2 hectare paddock containing a gully and a swamp, with six seronegative 14-month-old red deer stags. Rams and stags were grazed together in this paddock for 62 days. The animals were moved to a 0.6 hectare paddock from days 62 to 92, then to a 0.5 hectare paddock from days 92 to 123, and finally to another 0.5 hectare paddock from day 123 until slaughter at day 138 on July 20, 1999. The three paddocks grazed between days 62 and 138 did not contain any swamps or wallows.

**Results:**

Stags were blood sampled on days 20, 55, 92, 124 and 131. Stags that seroconverted were semen sampled by electroejaculation and semen was cultured for *B. ovis* using techniques adapted from Bailey (1986).
Serology
Blood was collected into plain vacuated blood tubes and the serum removed and tested for \textit{B. ovis} antibodies using the Complement Fixation Test (CFT) conducted at AgriQuality Serology, Upper Hutt, New Zealand. The \textit{B. ovis} CFT was performed at serial dilutions of 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048. Titres are expressed as fractions with the numerator denoting the strength of the reaction up to a maximum value of 4. For ease of presentation the CFT titres have been converted into scores 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).

Results
All six experimentally infected rams became seropositive to the \textit{B. ovis} CFT within 14 days of inoculation and titres were maintained throughout the trial period. \textit{Brucella ovis} was cultured from semen of five of the six rams semen sampled on days -23, -7 and 92. \textit{Brucella ovis} was not cultured from the semen of the sixth ram at any stage. \textit{Brucella ovis} was cultured from the preputial swabs of four of the six rams taken on day 92 (Table I).

<table>
<thead>
<tr>
<th>Ram number</th>
<th>Day -23</th>
<th>Day -7</th>
<th>Day 92</th>
<th>Day 92 Prepuce</th>
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<tr>
<td>88</td>
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<tr>
<td>97</td>
<td>+</td>
<td>+</td>
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\anye = culture positive
\ane = culture negative

Bacteriological culture results from semen and preputial swabs of rams experimentally infected with \textit{B. ovis}.

Blood samples from stags on days 20, 55 and 92 (March 24, April 29, June 4) were \textit{B. ovis} CFT negative. On days 124 and 131 (July 6 and July 13) sera from five of the six stags were positive in the \textit{B. ovis} CFT (Figure 1). On day 131, four of the five seropositive stags were successfully semen sampled and \textit{B. ovis} was cultured from all four semen samples. \textit{Brucella ovis} was not cultured from the semen sample collected from the seronegative stag.

Discussion
These results confirm that \textit{B. ovis} can be readily transmitted from infected rams to non-infected stags grazing together in the same paddock. This suggests that the initial source of \textit{B. ovis} infection in deer was likely to have been from rams and may explain why the infection in deer has been found on unrelated properties in differing geographical locations (Scott, 1999). Once the infection is present in a group of stags it can spread from stag to stag in the absence of infected rams (West \textit{et al.}, 1999). Thus natural infection of stags on a property could be either an isolated event occurring by transmission from rams or by the introduction of infected deer onto the farm.

It is intriguing that no seroconversion occurred during the first 92 days of contact with infected rams yet during the next 32 days five of the six stags seroconverted. While the means of transmission was not determined during this study, it is possible that one stag contracted infection by direct contact with an infected ram and then spread infection to the other stags by direct contact. However, following experimental infection of rams via mucous membranes it took a minimum of 2 weeks for a CFT titre to develop and a minimum of 4 weeks for infection to localise in the epididymes and accessory sex glands and thus \textit{B. ovis} to be shed in the semen. (Laws \textit{et al.}, 1972; Webb \textit{et al.}, 1980; Plant \textit{et al.}, 1986). If the pathogenesis of infection in stags is similar to rams, the time frame of 32 days is not long enough for one stag to become infected, shed \textit{B. ovis} in the semen, infect the other stags, and for those stags to seroconvert. Thus it seems likely that all five stags became infected at about the same time, possibly from a point source of infection.

It is most likely that all five stags contracted infection by direct contact with infected rams. Hartley \textit{et al.}, (1955) suggested that \textit{B. ovis} transmits from ram to ram by rectal copulation, although the evidence for this is largely circumstantial. The size difference between rams and stags is such that rectal copulation between the species would be unlikely. If transmission were by direct contact, it may be by stags licking or sniffing the preputial area of rams. \textit{Brucella ovis} was cultured from the prepuce of four of the six infected rams. Alternatively, rams engaging in homosexual activity with one another commonly ejaculate onto the perineal region of their mate. Stags subsequently licking or sniffing the semen deposited onto this site may be a means of transmission. Plant \textit{et al.}, (1986) demonstrated that \textit{B. ovis} can be transmitted by the intra-nasal route. Transmission can occur following oral inoculation (Buddle, 1955).

Alternatively it is possible that transmission occurred from environmental contamination, possibly by semen or less likely urine of the infected rams, with the stags subsequently ingesting contaminated grass or water, or sniffing or licking semen or urine deposited on pasture. There is no evidence of this means of transmission occurring in sheep (Buddle, 1955; Hartley \textit{et al.}, 1955).

A knowledge of how \textit{B. ovis} is transmitted is vital for development of \textit{B. ovis} control or risk management strategies. This study highlights the need for rams infected with \textit{B. ovis}, or of uncertain \textit{B. ovis} status, to be kept separate from deer. It also confirms that sheep grazing on

Figure 1. The \textit{B. ovis} CFT scores of stags measured on days 0, 20, 55, 92, 124 and 131 after introduction to infected rams.
farms where deer contract *B. ovis* infection should be investigated as the potential source of the organism. Research is continuing to determine whether *B. ovis* is transmitted from infected rams or stags to non-infected stags by direct contact or indirectly by environmental contamination.

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