Brucella ovis infection of deer - current and future implications in sheep

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Introduction

In New Zealand a voluntary national scheme aimed at controlling the spread of *Brucella ovis* in sheep was designed by representatives of the Society of the Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association, the Animal Health Division of the Ministry of Agriculture and Sheep Breed Societies. Before this scheme was officially adopted, veterinarians had already eradicated brucellosis from a considerable number of ram flocks using ram palpation and the complement fixation test (CFT) as the main tools for diagnosing infected rams. The CFT, along with additional serological tests such as the enzyme-linked immunosorbent assay, gel diffusion and immunoblot were developed and validated at the Central Animal Health Laboratory, Wallaceville. This scheme involves a cooperative effort between veterinarians, sheep breed societies and farmers, as well as the Ministry of Agriculture (Bruere 1987, Bruere and West 1987).

In 1977 *B. ovis* infection was estimated, via a postal survey, to affect 4% of stud rams and 12% of commercial rams with an overall national prevalence of 11%. Fourteen percent of stud flocks and 15% of commercial flocks were estimated to be infected. Testing during the initial phase of the accreditation scheme supported the survey figures with a seroprevalence of 4.6% for stud rams and 14% for commercial rams. In recent years the proportion of stud rams that return a positive CFT has been relatively stable at 1% and over 85% of the registered ram breeding flocks are accredited free from ovine brucellosis (Reichel and West 1997). The remaining ram breeding flocks are most likely free of brucellosis also, but have failed to reaccredit for a variety of reasons.

The accreditation scheme has provided a pool of accredited flocks with rams reliably free of *B. ovis* infection and has largely prevented the spread of *B. ovis* infection from ram breeding flocks to commercial flocks. In its present form it will not eradicate the disease, although on a regional basis many veterinary practices have largely achieved that within both stud and commercial flocks.

Sheep are the only species reported to sustain natural infection with *B. ovis* and there are few published reports of experimental infection of other animal species. Attempts to infect rabbits, guinea-pigs, rats, hamsters, gerbils and mice were 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 manifestations virtually identical with those in sheep (Barron 1984). Buck to buck transmission occurred and the infection was still present in one buck at slaughter, 429 days after inoculation into the conjunctival sac (Barron *et al* 1985). These studies followed the discovery that a number of samples of frozen-stored sera from wild, white-tailed deer gave a positive reaction to a *B. ovis* slide agglutination test. This raised the possibility of sustaining a natural infection of *B ovis* within a wild population of deer (Barron 1984).

Implications for the sheep industry

A naturally acquired *B. ovis* infection in a stag was first identified in the South Island, in June 1996 (Bailey 1997). The stag had epididymitis, abnormal semen and returned a positive serological reaction in the CFT.

This unexpected finding did not elicit much concern other than speculation as to how the stag may have become infected in the first place. No infected rams were found on the farm but the stag was purchased in February/March 1996 and may have been infected prior to introduction. Trace back studies have not been completed to elucidate the source.

In June 1997 it became apparent that *B. ovis* infection in stags was more serious than first thought. A relatively high prevalence of infection was identified in stags at slaughter by astute veterinary observation. In one group of 34 stags, epididymitis was identified at postmortem inspection in 16 stags and *B. ovis* was isolated from 11 of these 16. In addition, of the 34 sera tested by CFT for *B. ovis*, 28 were positive, 2 were suspicious and 4 were negative. This suggests that *B. ovis* infection can spread rapidly between young stags. The fact that the high prevalence was identified immediately after the rut, suggests, as in rams, that sexual activity between males is an important means of spread.

The issue of whether rams could become infected from stags is also of concern. Sheep to sheep transmission is believed to occur by close 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 oestrus period to infected rams. Buddle (1955) demonstrated transmission of infection to 5 of 13 rams when they were run 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) at the time of semen collection, that some rams have faecal material in the preputial 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. Despite the success of experimental oral infection and the observation that *B. ovis* can survive in infected material for some months on pasture (Buddle 1955) it has not been possible to demonstrate transmission via pasture. Rams and ewes grazing pasture contaminated with infected placentae failed to develop brucellosis (Hartley *et al* 1955, Buddle 1955).

The ease with which *B. ovis* infection can be spread via mucous membranes suggests a possible route of transmission between stags and between stags and rams. Rectal copulation may not be required. The aggressive sexual behaviour exhibited between stags confined together in the absence of hinds during the rut, poses a risk to other stags and possibly to rams, if fluid containing *B ovis* contacts mucosal surfaces. However, outside of the breeding season, stags demonstrate little sexual activity and it is possible that the period of transmission may be more defined in stags than in rams.

Recommendations for Sheep Farmers

Until more is known, or until *B. ovis* infection is eradicated from deer, it would be prudent to recommend that rams should not be run with stags, especially in flocks that wish to retain *B. ovis* accredited-free status. However it is likely that the risk is small, especially during the non breeding season. A study involving infected stags running with non-infected rams and stags is currently underway at Massey University.

Recommendations for Deer Farmers

Suggestions on the control of *B ovis* infection in deer have been made to the deer industry (West unpublished data) and summarised in Cervus (Scott and West 1997).

Details of these recommendations are given below.

The initial investigations on the outbreak of *B ovis* infection on a small number of deer farms in the South Island are still in progress. However a number of suggestions can be made already and it is appropriate to initiate action now and make tentative recommendations even though the information is incomplete.

What we know

1 *Brucella ovis* is infecting deer and causing epididymitis in stags.

2 It appears that the infection can spread rapidly in some stags, probably during the mating period. Stags classified as rising 3 year old showed a high prevalence of infection when slaughtered in late May and June following the rut.

3 Serological tests appear to work satisfactorily in deer and can be used to aid in the investigation and control.

What we might speculate on

1 Fertility of infected stags is likely to be reduced in a similar way to infected rams, given the similarity, and severity of the lesions in the epididymis.

2 Infected stags are likely to carry the infection for years. A study in America on a single white-tailed stag demonstrated that when a mature stag was inoculated at 17 months of age, it was still infected when euthanased 14 months later. A second stag inoculated at 18 months of age was still infected when killed 43 months later. In the South Island case a stag was identified as infected 5/6/96 and was still infected 24/7/96. Samples were collected at slaughter (19/2/97) but have not been processed to my knowledge.

3 It is likely that stags were initially infected from rams because there are a number of infected ram flocks in New Zealand.

4 Young stags under 14 months of age are unlikely to be infected because they have not reached puberty.

5 Infection is likely to be transmitted between stags by sexual activity during the rut. The infection rate has been high in male-only groups.

6 Infection may not spread as rapidly between some stags and during some periods. For example the stag originally identified as infected was run with 4 other stags between mid February until March 1996. The stag was then mated to 43 hinds. Following mating (June 1996) and until slaughter (February 1997) the stag was again run with the 4 other stags. Although this infected stag was demonstrated to be infected in June 1996 the other 4 stags did not show any evidence of infection when tested on 24/7/96 and 1/8/97. This would suggest that the risk of transmission between stags is lower outside the period of the rut.

7 The effect of *B ovis* infection in hinds may be important for two major reasons.

a Does it affect the fertility or cause abortion in hinds? If so there will be an overwhelming need to control the infection. Limited testing to date does not shed much light on this. Of the 43 hinds mated to the stag (red 320) found to be infected on 5 June 1996 after the rut, only one hind had a positive complement fixation blood test (4/16) which later was reported to be suspicious (1/8).

b If hinds can be infected following mating with an infected stag, can they transmit the infection to other stags if they are mated, either in that same oestrus, or at the next oestrus, or in the following rut? In sheep, ewes develop transient infections and do not transmit infection from one season to the next. The ewe may act as a mechanical means of spread if a non-infected ram mates the ewe after an infected ram during the same oestrus. *B ovis* can also be cultured from a proportion of ewes in the following oestrus (17 days later) and thus the ewe acts as a potential source of infection only during the mating season when infected and non-infected rams are joined with ewes.

8 There is no evidence that *B ovis* infection is spread between sheep via pasture contamination. The role of pasture or wallow contamination in transmission between deer remains unknown.

Tentative proposals for the control of *B. ovis* in Deer

Recommendations for deer farmers:

1 Prevent the introduction of *B. ovis* into your herd

a Source stags from herds tested free of *B ovis* or isolate and blood test stags upon arrival.
b Do not introduce hinds to be mated to your stags if they have already been mated to stags on other farms in the same rut period.
c Avoid lending or leasing stags to other farmers. If you do, hold them in isolation and blood test them before reintroduction to your herd.
d Do not run stags with rams and/or maintain *B ovis* accredited free rams.

2 Prevent *B. ovis* infection affecting your valuable breeding stags

a Prevent the introduction of *B. ovis* into your herd as above.
b Maintain your breeding stags separate from other stags or rams.
c Blood test your breeding stags annually.

3 Determine if your herd is infected with *B. ovis*

a Blood sample all sexually mature stags (over 15 months) for the *B ovis* blood test.

Note 1: - this assumes that, as in sheep, the male is the maintenance host and the female is only infected for a short period.

Note 2: - palpation of the scrotal contents in rams is a useful supporting diagnostic test but the relative teste size makes it easier to detect epididymitis in rams than stags. It is also more dangerous to examine stags. However sire stags should be palpated and other stags palpated when the opportunity arises (e.g. velvet harvest).

b Deer farms with a high proportion of velvet stags may find it costly and impractical to blood sample all the stags. The alternatives are:

i blood sample all the sire stags (kept in isolation) and only a proportion of the velveting stags.

Note: Sampling 60 animals in any sized herd gives a 95% chance of detecting a 5% prevalence of infection.

or

ii Blood sample all the sire stags (kept in isolation) and maintain other stags in age groups so that older stags cannot infect younger stags.

Note: This assumes that, as in sheep, *B. ovis* requires direct (sexual) contact to spread and it is not spread via the pasture.

4 Eradication of *B. ovis* from infected herds.

a Blood sample all sexually mature (over 15 months) stags to determine which groups are infected.
b For valuable animals a test and cull programme at approximately 1 to 2 month intervals should be the best option. However if the prevalence of infection is high in a single group of stags of lesser value, then slaughter of the group should be considered.
c Two clear tests at least 60 days apart are recommended to ensure eradication in sheep flocks.
d Maintain each age group of stags in isolation from other age groups.
e Ensure rams are free of *B ovis* infection.

5 Deer *B. ovis* herd accreditation scheme

Accreditation schemes are quality assurance programmes to ensure deer farmers can purchase stags with confidence that they are free of *B ovis*.

The scheme used in the sheep industry has the following features.

a Blood sample and palpation of breeding sires.
b Palpation only of young rams before sale and blood sample of any with lesions suggestive of *B. ovis*.

The aim is to reduce blood testing to a minimum and to avoid large scale blood testing of sale rams.

Most ram breeding flocks also have a commercial ewe flock mated to commercial rams. The commercial rams can be a source of infection and they must also be examined by palpation and up to 20 should be blood sampled.

Ram breeding flocks tend to have a uniform management system. Breeding sires are single-sire mated to separate groups of ewes (to identify parentage). The resulting male progeny born in spring are maintained as a separate flock from weaning (or soon after) until sale the following November/December at 15 months of age

To design an accreditation scheme for deer herds selling stags, it would be important to know the uniformity of management between herds. If stags of varying ages are sold at various times of the year then it will be more difficult to design a simple scheme. An alternative is to blood test stags that are to be sold. While this may be seen as a simple option, it has the major disadvantage that almost total reliance is then placed on a serological test, which, when used in this way will result in occasional false positive and false negative reactions.

It may be desirable to refrain from recommending a formal accreditation scheme until more factual information on both the prevalence of infection and the epidemiology of the disease in deer is available. However veterinarians and stag breeders may agree to adapt the principles of the sheep

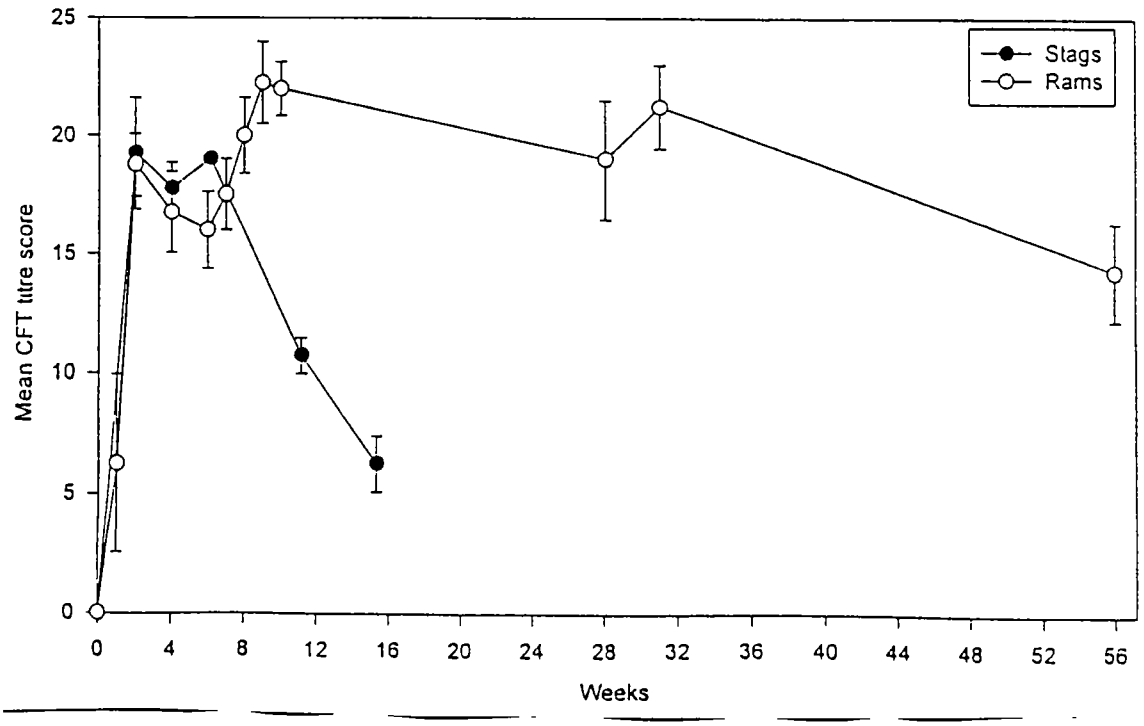
scheme, that is, to annually monitor the breeding sires and to examine and blood sample all, or a proportion, of the sale stags. The veterinarian can then certify what has been done and the results of any tests.

Serology

It appears from limited data that serological tests are accurate and useful in deer. Nevertheless false positive reactions will occur. In sheep, the proportion of false positives is about 0.3% (99.7% specificity). When false positives are suspected it is important to use additional and repeat testing. Additional blood tests include the enzyme-linked immunosorbent assay, gel diffusion and immunoblot. In some situations a definitive diagnosis such as culturing *B. ovis* from semen or genitalia may be necessary.

It appears from preliminary observations that the serological response might be of shorter duration in deer compared with sheep. Monitoring of small numbers of naturally infected deer has demonstrated a rapid reduction of titre levels in some instances (Bailey 1997). Similarly, 4 stags infected by intravenous inoculation of *B ovis* have shown a marked fall in titre compared with rams infected by the same means (Figure 1).

Figure 1. Serology response of stags and rams infected with *Brucella ovis*



Ram serology adapted from Bailey (1986)

It should be noted that the tentative proposals outlined earlier assume that the serological tests are reliable in deer. Veterinarians and farmers should apply caution when interpreting blood tests.

Conclusions

Brucella ovis infection of deer is unlikely to pose a major threat to the voluntary accreditation scheme in sheep and farmers can take simple precautions to ensure rams are separated from stags. The importance and impact of *B. ovis* infection on the deer industry has yet to be clearly defined. The affect on stag fertility and the sale of stags is a major concern especially in view of the rapid spread of infection between stags and the widespread movement of stags within the industry. Unless positive steps are taken to identify infected deer herds and apply control procedures, there is the potential for this infection to spread rapidly and disrupt the sale of stags as breeding sires. Rapid eradication of *B. ovis* infection from deer should be a priority for the deer industry.

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