Brucella ovis investigation in an accredited ram stud

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Introduction

Brucella ovis (*B. ovis*) is a venereally transmitted bacterium that causes epididymitis in rams, leading to infertility and economic loss. Chronic infection results in spermatic granulomas and fibrosis, especially in the epididymal tail (Foster *et al*. 1987; Burgess 1982). In New Zealand, accredited flocks - mainly studs - undergo annual testing to maintain *B. ovis*-free status, while commercial flocks rely on purchasing *B. ovis* free rams from studs and good biosecurity. Diagnosis combines flock history, scrotal palpation, and serology, primarily using the Complement Fixation Test (CFT) and ELISA. Despite robust protocols, false positives or undetected infections may occur. If serology is inconclusive, further diagnostics such as semen culture or, if needed, post-mortem culture of reproductive tissues are used (Hilbink *et al*. 1993). Final decisions on re-accreditation rest with the attending veterinarian.

Case history

A *B. ovis* free accredited stud consisting of 170 rising two-tooth Romney rams was visited to evaluate ram soundness and perform re-accreditation prior to ram sales in one month. Prior to this visit, all sire rams tested negative for *B. ovis* on the CFT in March 2023. The stud had no previous history of *B. ovis*, and the risk of incursion was assessed as low.

Clinical findings

The sale rams were palpated and five rams with epididymitis were identified. The five rams were tested for *B. ovis* using the CFT and one of the five rams tested positive. The *B. ovis* ELISA was then performed on the five samples and the CFT positive ram also tested weak positive. The four negative rams were culled. Seven days later the positive ram was re-tested and was negative on CFT and a weak positive on ELISA. Semen was submitted for microbiological culture which found heavy growth of *Arcanobacter pluranimalium*. A third re-test was performed seven days later, and he returned positive results again on both the CFT and the ELISA. Differentials included *A. pluranimalium*, *B. ovis*, gram-negative pleomorphs, and trauma. *B. ovis* was considered less likely due to the flock's accreditation, low incursion risk, and absence of prior infection. Negative CFT results in the other four rams further supported a likely false positive. However, as *B. ovis* is slow-growing and may be outcompeted in culture, euthanasia was recommended to enable tissue culture and localise potential infection within the reproductive tract.

Post-mortem exam

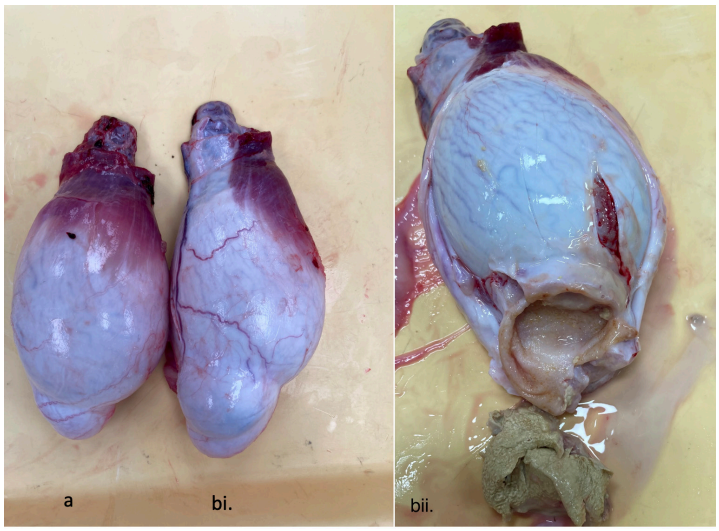
The ram was euthanized for post-mortem examination and reproductive tract culture. A 3cm encapsulated lesion with purulent, caseous necrosis was found near the atrophied left testicle, but it did not communicate with surrounding tissue. Both epididymides appeared grossly normal. The seminal vesicles were firm with nodular changes, indicating chronic inflammation.

Microbiological culture results

Culture of reproductive tissues revealed scant mixed growth in the epididymides and lesion, and heavy growth of *A. pluranimalium* in the seminal vesicles, ampullae, and bladder. *B. ovis* was not detected. As a precaution, nine

sale rams were CFT-tested while awaiting results: all tested negative. The ram was classified as a *B. ovis* false positive, prompting the flock's re-accreditation as *B. ovis*-free.

Figure 1. Atrophied left testicle (bi) containing a 3cm diameter lesion (red arrow) near the left epididymal tail. The right testicle (a) is normal. Bii shows cut surface of left testicular lesion. Image from post-mortem examination, courtesy of Dr Anne Ridler.



Discussion

In the present case, a risk-averse approach was adopted to ensure the flock remained free from *B. ovis*, which was critical due to the potential liability for the veterinarian if a false negative result led to an outbreak. Additionally, the emotional and financial burden on the farmer through loss of accreditation and culling of sale rams was an important consideration. Despite a low pre-test probability of infection based on the flock's history and prior negative serology, an initial positive result warranted further investigation to clarify the ram's *B. ovis* status, particularly with rams intended for sale within the month.

Serological testing, supported by clinical findings and flock history, helps differentiate true positives (infected animals) from false positives (uninfected animals with reactive titres). Test accuracy is influenced by host immune response, sample collection, thresholds for positivity, and result interpretation. In New Zealand, the complement fixation test (CFT) is the primary screening tool due to its slightly higher specificity compared to ELISA, reducing false positives (Worthington and Cordes, 1981; Worthington *et al*. 1984). High-sensitivity tests are typically used to rule out disease due to their low threshold for detection, which reduces false negatives. However, interpreting results also depends on pre-test probability. In low-risk, accredited flocks, the CFT is preferred for its high specificity. A negative CFT result - supported by clinical and historical data strengthens confidence in disease absence and avoids unnecessary additional testing. If positive reactors are identified on the CFT, ELISA is used for re-testing. While ELISA has high sensitivity early in infection, it is more prone to false positives due to lower positivity thresholds (Elderbrook *et al*. 2020). True positives typically arise from introduction of infected animals, inadequate isolation, or contact with neighbouring infected rams (Bruère and West 1987). In such cases, infection can spread rapidly and affected rams often exhibit high antibody titres (Hilbink *et al*. 1993). Therefore, if the CFT-positive ram were truly infected, it would be expected that more rams would have tested positive, yet this was not observed. The isolated positive may reflect cross-reactivity with antibodies from other antigenically similar pathogens.

Culture remains the gold standard for confirming B. ovis infection where serological results are equivocal (Ridler *et al*. 2014). While semen culture can be performed ante-mortem, false negatives are possible due to intermittent shedding, extra-reproductive tract localisation, or overgrowth by competing flora (Buddle 1955; Ridler *et al*. 2014). Furthermore, semen collected via electroejaculation can contain contaminants from multiple urogenital organs. In contrast, post-mortem culture of the epididymides, ampullae, and seminal vesicles offers greater specificity. In this case, the negative culture results from these organs and the absence of communication between the lesion and the epididymis supported the conclusion that the ram was a false positive. Interestingly, *Arcanobacterium pluranimalium* was cultured from the ampullae, seminal vesicles, and urinary bladder, but not

from the lesion itself. Originally classified under *A. haemolyticum* (previously *Corynebacterium haemolyticum*), *A. pluranimalium* is a facultative anaerobic gram-positive bacterium, occasionally implicated in reproductive disease in sheep (Foster and Hunt 2011). However, its absence from the lesion suggests it was unlikely to be involved, and the lesion may have been incidental. Further research is needed to clarify its role in ovine epididymitis.

In conclusion, this case illustrates the value of a systematic approach to investigating suspected false positives in *B. ovis*-accredited studs. This strategy is particularly important for veterinarians working within accreditation schemes, where false positives carry significant consequences.

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