AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

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Ovine Epididymitis

Pathology, Bacteriology and Serology

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1. Introduction

Ovine epididymitis is most commonly caused by infection with *Brucella ovis* (*B. ovis*), *Actinobacillus seminis* (*A. seminis*) and *Histophilus ovis* (*H. ovis*).

A variety of other organisms have also been isolated from cases of ovine epididymitis. These include: *Actinobacillus pyogenes, Corynebacterium pseudotuberculosis, Escherichia coli, Streptococcus* spp., *Staphylococcus* spp., *Pasteurella haemolytica, Pasteurella multocida, Brucella abortus* (S19), *Pseudomonas maltophilia, Actinobacillus actinomycetem-comitans, Actinobacillus lignieresi,* and *Yersinia pseudotuberculosis.* (Simmons and Hall, 1953; Dodd and Hartley, 1955; Galloway, 1966; Ekdahl *et al.*, 1968; Laws and Elder, 1969; Ivanov and Masalski, 1974; Zamora *et al.*, 1977; De Long *et al.*, 1979; Burgess, 1982).

Infection with *B. ovis* results in a bacteraemia with subsequent localisation in the tail of the epididymides and accessory sex glands. Hyperplasia of the tubular epithelium in the tail of the epididymides may result in the formation of spermatic granulomata which are largely responsible for the palpable lesions which occur. The spermatic granulomata caused by *B. ovis* are almost always found in the tail of the epididymides. Occasionally lesions in the head and body of the epididymides are seen, often in addition to the caudal damage.

The many other organisms which can infect the epididymides are capable of causing lesions and epididymitis indistinguishable from those of infection with *B. ovis*. Jansen (1980) suggested organisms could migrate from the preputial cavity along the urethral lumen and eventually to the epididymides. His data show this occurs most readily when levels of luteinising hormone (LH) and follicle stimulating hormone (FSH) are high, i.e. in young, sexually active rams on a high plane of nutrition.

Infection with organisms does not always result in the formation of an epididymitis. *B. ovis* may produce an epididymitis or it may simply produce a serological response. A similar situation occurs with *A. seminis* — cases of epididymitis may resolve leaving only interstitial fibrosis of the epididymides and adhesions.

Diagnosis of ovine epididymitis is based on:

(a) clinical examination;
(b) gross pathology;
(c) histopathology;
(d) microscopic examination of semen and/or tissues;
(e) microbiology on semen and/or tissues.

Aetiology is determined by:

(a) microscopic examination and culture of semen;
(b) serological examination;
(c) isolation of organisms from tissue samples;
(d) examination of flock history; and
(e) histopathology and immunohistochemical staining.

2. Clinical Examination

Ovine brucellosis is usually not associated with any generalised clinical signs. Infection with *A. seminis* may range from subclinical to a severe acute febrile condition with swollen painful testes. Other organisms may produce a variety of clinical conditions. *H. ovis* can cause acute orchitis or epididymitis.

Palpation of scrotal contents is best done with the ram held in a sitting position and the testes held to the bottom of the scrotum. However, palpation may be carried out from the rear with the ram in a standing position. Features examined should include: pampiniform plexes, testes, as well as head, body and tail of the epididymides. The position and severity of the lesions as well as resilience of testes and epididymides should be noted for future reference.

The most commonly observed palpable lesion is a thickening and enlargement of the tail of the epididymides. Lesions confined to the body or head are more likely to be the result of congenital malformations.

Other abnormalities may include atrophy or lack of tone of testes, scrotal abscesses and severe adhesions.

3. Microbiological Examination and Culture of Semen and Tissues

3.1. Collection and Transportation

Semen may be collected by electroejaculation into sterile bottles. Exteriorisation of the penis is recommended to minimise introduction of contaminant organisms. Care should be taken to prevent cross contamination between rams. The penis should be held by a sterile cloth or tissue. New disposable gloves, for each ram, should be worn by the operators and the ejaculator probe cleaned and sterilised between the ejaculation procedures.

Samples should be sent to the laboratory packed on ice by the quickest method available (preferably within 24 hours of collection). Where circumstance permit, it is preferable to collect semen samples at the diagnostic laboratory.

3.2. Smears

3.2.1. Modified Ziehl–Neelsen stain

3.2.1.1. Reagents

(a) Dissolve 1 g of basic fuchsin in 10 mL of absolute ethanol (C_2H_5OH) and add to 90 mL of 5% aqueous phenol (C_6H_6O) solution. Dilute 1/5 for use.
(b) 1% Aqueous methylene blue or 0.5% malachite green.
(c) 0.5% Aqueous acetic acid (CH_3CO_2H).

3.2.1.2. Method

Stain smears for five minutes with modified Ziehl-Neelsen stain. Decolourise for 20 s with 0.5% aqueous acid. Wash in tap water for 30 s. Counterstain for 90 s with 1% methylene blue or 0.5% malachite green. Wash in tap water. Allow to dry in air.

3.2.1.3. Interpretation

B. ovis usually stains red against a blue background, i.e. positive for modified acid fast stain. Other organisms, e.g. *E. coli* may also be stained red but can be differentiated from *Brucella* by their morphology. *A. seminis* stains blue with this technique, i.e. negative for modified acid fast stain.

Gram stain is useful in demonstrating organisms such as *Pasteurella* sp., *Corynebacterium* sp., *A. seminis* or *H. ovis*. With the exception of *Corynebacterium* these bacteria stain Gram negative and may be difficult to find if present in small numbers.

Excretion of organisms can be intermittent; hence repeated testing may be necessary.

Microscopic examination of semen and/or tissues (Searson, 1986) for *B. ovis* using the modified acid fast stain is not as reliable as culture. However, under field conditions, the time elapsing from collection of samples to receipt in the laboratory may partly cancel the advantages of culture. In addition, the presence of polymorphs should be recorded when microscopic examination is carried out. It is preferable to make semen smears in the laboratory rather than the field to minimise the risk of contamination.

Dried, fixed smears should also be stained using a gram stain. A gram stain is of most value in detecting the gram positive organisms. The presence of neutrophils in a semen or tissue smear is a very useful indicator of infection in the male genital tract. They are not specific for any particular organism.

3.3. Culture

B. ovis and *A. seminis* may be isolated from the tail of the epididymides, ampullae, ductus deferens and seminal vesicles. Less commonly, they may be isolated from testes and occasionally, from bulbourethral glands (Searson, 1986). If tissue samples are collected in the field, they should be transported on ice rapidly to the laboratory.

Most organisms which are capable of producing epididymitis grow well on 10% sheep blood agar incubated at 37°C in a 10% carbon dioxide atmosphere. A selective medium assists in the suppression of contaminant organisms which are sometimes a problem in the isolation of *B. ovis* from field material. Organisms such as *A. seminis* and *H. ovis* will not grow on the selective medium.

3.3.1. Modified Thayer Martin Medium — Selective Medium for Isolation of *Brucella ovis*

Preparation of selective medium based on the method described by Brown *et al.* (1971).

Base — Trypticase soy agar (BBL)	40 g
Distilled water	1 L

Autoclave at 121°C for 15 min. Cool.

VCN supplement (Oxoid SR 101) which contains

Table 1. Biochemical differentiation of *Brucella ovis*, *Histophilus ovis and Actinobacillus seminis*

Biochemical reactions	B. ovis	H. ovis	A. seminis
Catalase	+	−	+
Oxidase	−	+	−
Glucose fermentation	−	+	−
Sucrose	−	−	−
Lactose	−	−	−
Mannitol	−	+	−
Urea hydrolysis	−	−	−
Nitrate reduction	−	+	−
Indole reduction	−	+	−
H_2S production[1]	−	−	−
Modified ZN[2]	+	−	−
Growth on nutrient agar	−	−	+
Ornithine decarboxylase		+	+
Lysinede carboxylase		−	−
Arginine decarboxylase		−	−
ONPG[3]		−	−

[1] H_2S = hydrogen sulfide; [2] Modified Ziehl–Neelsen; [3] o-Nitrophenyl-O-galactopyranoside.

Formula (per vial)	
Vancomycin	1500 µg
Colistin sulphate	3750 µg
Nystatin	6250 units

VCN supplement is available from Oxoid Australia (PO Box 220, Heidelberg West, Vic. 3081. Tel. (03) 458 1311, (008) 331 163; Fax (03) 458 4759]. Dissolve two vials in 100 mL of sterile distilled water. Add to base [BBL products are available from Becton Dickinson, 2A, 15 Orion Road, Lane Cove, NSW 2066. Tel. (02) 418 6166; Fax (02) 418 6881]. Add 100 mL of sheep blood. Dispense.

3.3.2. Blood agar — for Routine Culture

Base — Trypticase soy agar and 10% sheep blood.

3.3.3. Primary Culture

Samples are plated onto selective medium and blood agar. The plates are incubated at 37°C in an atmosphere of 10% carbon dioxide, examined for growth for up to seven days.

3.3.4. Identification

B. ovis is a small, non-motile, gram-negative modified acid fast coccobacillus, usually occurring singly. On blood agar the colonies are about 1 mm in diameter, grey, smooth and round. Growth may not be visible until the forth or fifth day.

A. seminis is a non-motile, pleomorphic Gram-negative non-acid fast bacillus (1 x 4 µm). At 24 hours colonies are pinpoints. At 48 hours they are grey, convex, round and 1–2 mm in diameter.

H. ovis is a small pleomorphic non-motile Gram-negative non-acid fast bacillus. On blood agar the colonies are smooth, grey and 1 mm in diameter by 72 hours incubation and have a 'fried egg' appearance by 96 hours.

Carbohydrate fermentation may be carried out in Andrade's broth with 1% of the relevant sugar (e.g. sucrose or lactose) and 10% bovine serum. The biochemical reactions depend on the

Table 2. Identification of *Actinophilus semini* and *Histophilus ovis* using API–Zym

Test cupule	A. seminis	H. ovis
1	0	0
2	5	1/2
3	1	1/0
4	1/0	1/0
5	0	0
6	3/4	2
7	1/0	1
8	0	0
9	0	0
10	0	0
11	4/5	2
12	1	1
13	0	0
14	0	0
15	4	5
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0/2

composition of the base medium. Some typical reactions for the more commonly isolated organisms causing epididymitis are listed in Table 1.

Identification of *A. seminis* and *H. ovis* may also be confirmed using the API-Zym (Cousins and Lloyd, 1988) [Api-Zym is available through various agents, e.g. Bacto Laboratories, PO Box 295, Liverpool, NSW 2170. Tel. (02) 602 5499; Fax (02) 601 8293]. The following reaction strengths are observed (Table 2).

4. Serology

The present standard method for *B. ovis* infection is a warm complement fixation test (Searson, 1982) which is described in the standard procedure for ovine brucellosis. An enzyme-linked immunosorbent assay (ELISA) technique has been described in *Ovine Brucellosis* (see this Volume).

Serological diagnosis of *A. seminis* infection is based on a gel diffusion technique or a complement fixation test (Baynes and Simmons, 1960; Livingstone and Hardy, 1964; Simmons *et al.*, 1966; Rahaley, 1978). Six serotypes have been described in South Africa. Therefore, a local strain or mixture of local strains should be used for antigen (Van Tonder, 1973). No reports of cross-reactions with *B. ovis* in serum samples from infected sheep have been published. Cross reactions have been encountered between *H. ovis* and *A. seminis*.

Titres to *B. ovis* are maximal about two to three months after infection. By 6–12 months post infection, titres may have fallen to a level below the threshold of the complement fixation test. Animals with epididymitis usually retain a titre of eight or more for several years.

Large numbers of rams infected with *A. seminis* will revert to negative status within 6–12 months post infection. This includes many of the rams with epididymitis.

Serology should not be relied upon as the sole method of diagnosis of *A. seminis* and in flocks where *B. ovis* infection persists despite culling of serologically positive animals, culture of semen should be employed.

5. Pathology

5.1. Gross Pathology

Acute epididymitis results in adhesions between the epididymides and parietal layer of the tunica vaginalis.

Spermatic granulomata caused by infections with bacteria are usually found in the tail of the epididymides although they may also occur in the body and head. In addition secondary testicular lesions such as atrophy, fibrosis and calcification occur.

5.2. Histopathology

The histopathology of *B. ovis* epididymitis has been described by Kennedy *et al.* (1956) and Biberstein *et al.* (1964).

In a review of bacterial infections of the genitalia of rams by organisms other than *B. ovis*, Jansen (1980) noted that it is impossible to associate particular histopathological lesions with a single species of bacteria because in many instances the same type of lesion is caused by different bacteria. In addition, more than one type of bacteria may be isolated from the affected organ.

Interstitial reactions in the epididymides includes oedema, fibrosis and infiltration with mononuclear cells. Epithelial reactions include hyperplasia, metaplasia and intraepithelial cyst formation. These cysts may contain neutrophils and cell debris and there may be migration of neutrophils into the tubular lumen.

Chronic inflammatory lesions may also be observed in vas deferens, ampullae, ductus deferens and seminal vesicles.

The epithelial lesions in the epididymides may result in extravasation of sperm with resultant spermatic granuloma formation. The appearance of the spermatic granuloma will be the same irrespective of the bacterial aetiology.

Abscesses must be differentiated from spermatic granulomata. Abscesses do not contain sperm and frequently contain large numbers of neutrophils.

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