AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

STANDING COMMITTEE ON AGRICULTURE AND RESOURCE MANAGEMENT

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Ovine Campylobacteriosis

Pathology and Bacteriology

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Contents

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1. Introduction

Campylobacteriosis (Vibriosis) is associated with late ovine abortion, usually in the last trimester of pregnancy, and with perinatal mortality. The aetiological agents include *Campylobacter fetus* and *C. jejuni*. Infection occurs through ingestion of the organism. The incubation period is one to three weeks. Most abortions occur in the last month of pregnancy. Morbidity may exceed 50%. Some ewes may develop metritis and die.

Sexual transmission and infertility are not features of campylobacter infections of sheep. Acquired immunity develops in aborted ewes and among other sheep in the infected flock. Although vaccination is available, campylobacter abortion can occur in vaccinated animals. This may occur because of strain differences between infecting organisms and those used in the vaccine. In addition, heavy challenge may overwhelm protection afforded by vaccination. Heavy stocking rates, as in block grazing, predispose sheep to campylobacter abortion.

Campylobacter abortion has also been diagnosed in goats.

2. Pathology

2.1. Gross Pathology

Aborted foeti may show only subcutaneous oedema but blood stained fluid occasionally may be present in body cavities giving a pot-bellied appearance. When present, gross lesions are observed in the placenta and/or the aborted foetus. Placental lesions are minimal but the foetal cotyledons may be covered with yellow or dark brown exudate and necrotic plaques and oedema may occur in the intercotyledonary areas. By comparison, *Toxoplasma*-induced placentitis is characterised by white foci in the foetal cotyledons.

About 10–30% of the foeti or dead neonate lambs show gross liver lesions. These consist of large yellow–brown necrotic lesions up to 2–3 cm in diameter with irregular margins but more commonly the liver is enlarged, friable and diffusely necrotic.

Liver lesions caused by *Fusobacterium necrophorum, Listeria monocytogenes,* and *Yersinia pseudotuberculosis* need to be distinguished from those caused by *Campylobacter fetus* and *C. jejuni*. *F. necrophorum* produces necrocaseous lesions in the livers of neonate and older lambs, mostly two to four weeks old, but not aborted lambs. *F. necrophorum* is pleomorphic and smears taken from lesions may reveal large filamentous Gram negative rods or short coccoid forms. In listerial or yersinia abortion or post natal septicaemic listeriosis or yersiniosis, multiple small focal abscesses may occur in the liver and sometimes in other organs.

2.2. Histopathology

The placental lesions consist of extensive necrosis of the chorionic epithelium with arteriolitis and infiltration by polymorphonuclear leukocytes. Masses of the *Campylobacter* organisms may be found in terminal vessels in the chorionic villi. A foetal purulent broncho-pnenumonia may also be detected. The discrete focal necrosis seen in *Toxoplasma* placentitis does not occur in ovine camplyobacteriosis.

The liver lesions present as areas of necrosis. Small zones of normal liver tissue may be seen within the necrotic zones. Polymorphonuclear and mononuclear leukocyte (mainly histiocytic) infiltration is prominent. Bacteria are not easily seen in the liver lesions. The liver lesions of listeriosis are much smaller and consist of dense cellular infiltration rather than necrosis.

Gross and microscopic lesions in aborted lambs, indistinguishable from those produced in camplyobacter abortion, have been found in abortions caused in the United States of America by a non-classified *Flexispira roppini*, an anaerobic flagellated bacterium (Kirkbride et al., 1986; Bryner *et al.*, 1987; Paster *et al.*, 1991; Vandamme *et al.*, 1991).

3. Bacteriology

3.1. Description

Campylobacter are Gram negative curved or spiral rods measuring 0.2–0.8 x 0.5–5 µm. Coccoid forms may occur on culture media. They have a characteristic darting motility, are oxidase positive and neither ferment nor oxidise carbohydrates.

Both *Campylobacter fetus* subsp. *fetus* (formerly *C. fetus* subsp. *intestinalis*) and *C. jejuni* have been implicated in ovine abortion in Australia (Clark and Monsbrough, 1974, 1979). The diagnosis of ovine campylobacteriosis is most easily made by demonstrating the presence of *Campylobacter* spp. in either the foetus or placenta. Because of the characteristic 'seagull' shape of this Gram-negative organism, campylobacteriosis is frequently diagnosed from Gram-stained abomasal smears or in abomasal content or body fluid or cotyledonary scrapings viewed by dark field or phase contrast microscopy (x40 objective). Using this latter method the bacteria can usually be detected as rapidly motile organisms.

3.2. Samples for Culture

The foetus and placenta should be submitted.

3.2.1. Placenta

It is preferable to culture from cotyledons that have a yellow–brown discoloration, indicating antemortem necrosis. A normal cotyledon appears dark plum–red with post mortem autolysis. Avoid culturing placental tissue that is highly contaminated with soil or faeces.

However, as some contamination is unavoidable it may be necessary to use enrichment or selective media when culturing from placentas.

3.2.2. Aborted Foetus

C. fetus may be isolated from the lung, liver or abomasal contents. It can usually be isolated by inoculating material onto 5–10% sheep agar and incubating the plates at 37°C for up to five days in an atmosphere of 10% carbon dioxide in air.

However, when culturing for *C. jejuni* it is recommended to inoculate onto a BPNA (Dufty and McEntee, 1969), (see 6.2.), and incubating for 48 hours at 42°C using a mixture of 90% nitrogen, 5% oxygen and 5% carbon dioxide. Commercial gas generating kits are available.

Alternatively enrichment and selective media can be used (see 3.3. and 3.4.).

Material from lambs or cotyledons may be inoculated directly onto selective media although single colonies can easily be missed and the enrichment technique is advised.

3.3. Enrichment Medium (Clark et al., *1974)*

The procedure to be adopted for both abomasal contents and cotyledon is as follows. Add about 1 mL of abomasal contents or about 1 g of cotyledon to 5–10 mL of enrichment medium in a screw-capped bottle (see 6.1.). Incubation at 37°C for five days is recommended.

3.4. Selective Media

Plate one drop of enrichment medium onto a BPNA plate. It has been shown to be beneficial to add a drop of the enrichment medium to the surface of a 0.45 µm filter placed on a BPNA plate. Remove the filter once the material has passed through. As an alternative to BPNA plates, growth and selective supplements are available commercially and recommended for the isolation of *C. fetus*/*C. jejuni* (see 6.3.). These are particularly useful to smaller laboratories since one vial is sufficient to supplement 500 mL of blood agar base. Incubate in an atmosphere of 10% carbon dioxide in air at 37°C. Growth may take from two to five days. A duplicate selective plate should be incubated at 42°C for 48 hours in a gas mixture of 90% nitrogen, 5% oxygen and 5% carbon dioxide. A cylinder of this special mixture is most useful in *Campylobacter* bacteriology since the same mixture is used in the preparation and post inoculation of the enrichment medium. Commercial gas generating kits are available.

Table 1. Differentiation of *Campylobacter fetus* subsp. *fetus* from *C. jejuni*

Test	C. fetus *subsp.* fetus	C. jejuni
Growth temp. (°C)		
42	–	+
37	+	+
25	+	–
Sensitivity to[1]:		
Naladixic acid	R[2]	S
Cephalothin	S[2]	R
Hippurate hydrolysis	–	+

[1] Using 30 µg discs (Bergey, 1984)
[2] R = resistant; S = susceptible.

3.5. Identification

Colonies on selective media are often pink and are typically effuse and may tend to spread in the streak line or spread on wet plates. Colonies on 10% sheep blood agar form discrete domed colonies. Colonies may be fully identified using the same scheme which is applied to bovine strains, however, the following tests are necessary at this time:

(a) Growth on 5–10% sheep blood agar at 42°C, 37°C and 25°C. Plates are incubated in a gas mixture of 90% nitrogen, 5% oxygen and 5% carbon dioxide.
(b) Differentiation of *Campylobacter fetus* subsp. *fetus* from *Campylobacter jejuni* (Table 1).

4. Summary

Ovine campylobacteriosis may be characterised by gross lesions in the placenta and/or the foetal liver.

The aetiological agents include *C. jejuni* and *C. fetus*.

Dark field or phase contrast microscopy on fresh foetal abomasal content and placental scrapings is a fast and efficient method of diagnosing ovine campylobacteriosis.

C. fetus and *C. jejuni* may be isolated and identified using appropriate media, incubation temperatures, and microaerophilic conditions.

5. References

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6. Appendixes

6.1. Appendix 1 — Campylobacter fetus *spp. Enrichment Medium*

5-Fluoro-uracil (Roche)	30 mg
Polymyxin B sulfate 'Aerosporin'	10 000 units
Brilliant green (BDH)	5 mg
Cycloheximide (Upjohn)	10 mg
Foetal calf serum	100 mL

Inactivated, sterile bovine serum may be used.

Dispense in 15 mL quantities in screw-capped bottles and gas with mixture of 90% nitrogen, 5% oxygen and 5% carbon dioxide.

Refrigerate for five days before use. At storage temperature of 4°C life is three to four weeks.

6.2. Appendix 2 — BPNA Selective Medium (Clark et al., *1974)*

Defibrinated bovine blood agar	10%
Bacitracin (Wellcome)	15 units/mL
Polymyxin B sulfate 'Aerosporin'	1 unit/mL
Novobiocin (Upjohn)	5 µg/mL
Cycloheximide (Upjohn)	20 µg/mL

6.3. Appendix 3 — Isolation Media

Oxoid Campylobacter Selective Supplement Codes SR69, SR98 and SR85.

Oxoid Campylobacter Growth Supplement Code SR84.

6.4. Appendix 4 — Suppliers

Calbiochem–Novabiochem. PO Box 140, Alexandria, NSW 2015. Tel. (02) 318 0322, (008) 023 956; Fax (02) 319 2440. 5-Fluoro-uracil and polymixin B sulfate.

CSL. 45 Poplar Rd, Parkville, Vic. 3052. Tel. (03) 389 1911; Fax (03) 389 1434. Inactivated, sterile bovine serum.

FSE. 47–49 Overseas Drive, Noble Park, Vic. 3174. Tel. (03) 795 0077; Fax (03) 790 1900. Brilliant Green (BDH).

Oxoid Australia. PO Box 220, Heidelberg West, Vic. 3081. Tel. (03) 458 1311, (008) 331 163; Fax (03) 458 4759. Oxoid products.

Upjohn. 55–73 Kirby St, Rydalmere, NSW 2116. Tel. (02) 638 0531; Fax (02) 684 2130. Cycloheximide and Novobiocin.

Wellcome Diagnostics. Wellcome Australia. 63 Wadham Pde, Mt Waverley, Vic. 3149. Tel. (03) 807 6111, (008) 225 171; Fax (03) 807 5085. Bacitracin.