Ovine Campylobacteriosis

S Hum
M Hornitzky
T Berg

Elizabeth Macarthur Agricultural Institute
PMB 8
Camden, NSW 2570
Australia
steven.hum@dpi.nsw.gov.au

Part 1. Diagnostic Overview

Summary

Ovine Campylobacteriosis (vibriosis, epidemic abortion of sheep) is caused by *Campylobacter fetus* subsp *fetus* or *Campylobacter jejuni*. Both organisms can cause epidemics of abortion characterised by gross lesions in the placenta and/or foetal tissues. Delivery of weak lambs at term is also a feature. Abortion causes heavy contamination of pasture with subsequent widespread infection in the flock.[1,2]

C fetus remains the major cause of campylobacter-associated ovine abortions in Australia and New Zealand, compared with the United States where it has changed from *C fetus* to *C jejuni*.[3]

Examination by dark field or phase contrast microscopy of fresh abomasal content of foetuses and of placental scrapings is a fast and efficient method of diagnosing the diseases. *C fetus* and *C jejuni* may be isolated using appropriate media, incubation temperatures and microaerophilic conditions, and identified by standard methods.

Acquired immunity develops in aborted ewes. The disease occurs in Australia and New Zealand. Vaccine is available in both countries, however it may not prevent abortion in all outbreaks. *Campylobacter* infection has also been diagnosed in goats.

1. Aetiology

Campylobacter fetus and *Campylobacter jejuni* are microaerophilic, gram-negative bacteria. The organisms can be found in the intestinal tract of some sheep and other wild and domesticated animals that show no sign of disease.[4] There is limited information available on the intestinal carrier stage of *C fetus*. In sheep and cattle, the occurrence of *C jejuni* varies but rarely exceeds 50%.[5] There appears to be a greater incidence in young animals than in older animals, and in animals at higher stocking densities (for example, in feed lots, saleyards) compared with those on pastures.

2. Clinical Signs

The typical syndrome includes abortion during the last 8 weeks of pregnancy or, in some instances, delivery of weak lambs at term. Usually there is no indication of impending abortion, but some ewes may show a prior vaginal discharge. Occasionally abortion is followed by metritis and subsequent death of the ewe.

The timing of infection may be important in determining the magnitude of an abortion outbreak in a flock. In an experimental model almost all ewes aborted if infected at 105 days of gestation, whereas after infection at 126 days only 20% aborted. This may reflect increasing immuno-competence of the foetus.[6]

3. Pathology

3.1 Gross pathology

Aborted foetuses may show only subcutaneous oedema but blood-stained fluid occasionally may distend body cavities, giving a pot-bellied appearance. In such cases, 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 foetuses or dead neonatal lambs show gross liver lesions consisting of extensive yellow-brown multifocal necrosis up to 2-3 cm in diameter with irregular margins. 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 *C 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. Abortion and post-natal septicaemia caused by *L monocytogenes* or *Y pseudotuberculosis* are characterised by multiple small focal abscesses occurring in the liver and sometimes other organs.

3.2 Histopathology

The placental lesion consists of extensive necrosis of the chorionic epithelium with arteriolitis and infiltration by polymorphonuclear leucocytes. Masses of *Campylobacter* organisms may be found in terminal vessels in the chorionic villi. A foetal purulent broncho-pneumonia may also be seen. The discrete focal necrosis seen in *Toxoplasma* placentitis does not occur in ovine campylobacteriosis.

The liver lesions are areas of necrosis, with, sometimes, small zones of normal liver tissue within the necrotic areas. Polymorphonuclear and mononuclear leucocyte infiltration is prominent. Bacteria are not easily seen in the liver lesions. By comparison, the liver lesions of listeriosis are much smaller and consist of dense cellular infiltration rather than necrosis.

Aborted lambs in the United States of America have exhibited gross and microscopic lesions indistinguishable from those produced in *Campylobacter* abortions, but in these cases the abortions were attributed to *Flexispira rappini,* an anaerobic flagellated bacterium that has not been classified.[7,8] This organism has not been isolated in Australia or New Zealand.

4. Epidemiology and Transmission

The organisms are part of the normal gut flora in some species including sheep, cattle and birds and as such are ubiquitous in the environment Carrier sheep are thought to be largely responsible for disease outbreaks usually under intensive management practices. Transmission is by ingestion of contaminated feed, water and aborted material; sexual transmission and infertility do not occur. Abortion occurs 7-25 days after infection. Usually, the incidence of abortion is 10-20%, but in some outbreaks half the ewes abort their lambs. Acquired immunity develops in aborted ewes and among other sheep in the infected flock. Most ewes recover promptly and fertility in subsequent years is not affected, however carrier sheep persist and can be the source of infection for newly introduced susceptible animals

5. Occurrence and Distribution

The disease is of worldwide distribution and has been reported in all States and Territories in Australia and in New Zealand. The disease tends to be associated with certain management practices, such as heavy stocking, strip/block grazing or grain feeding.[9] Ovine camplyobacteriosis occurs more frequently in Tasmania than in mainland Australia and is the leading cause of diagnosed sheep abortions in New Zealand.[10]

6. Diagnostic Tests and Specimens

Ovine campylobacteriosis can be diagnosed by demonstrating or isolating *Campylobacter* organisms either from the foetus or the placenta. *C* fetus and *C jejuni* have similar morphology and motility, therefore the diagnosis is most easily made by demonstrating the presence of curved, S-shaped or spiral rods measuring 0.2-0.9 µm x 0.5-5.0 µm in abomasal smears, or by the observation of rapidly motile organisms with characteristic darting or corkscrew motion in abomasal content, body fluid or cotyledonary scrapings using dark-field or phase contrast microscopy.

For definitive diagnosis, however, isolation and identification of *C fetus* or *C jejuni* are required.

7. Treatment, Prevention and Control

Aborted tissues, foetal membranes and discharges are all infectious and should be collected and removed to prevent the spread of infection within the flock. It is important to protect the water supply from contamination. Once infection spreads through the flock, little can be done to reduce losses.

Effective killed single or multiple strains of *C fetus* vaccines and a vaccine containing *C* fetus as well as *C jejuni* are available and widely used for prevention and control in New Zealand. Vaccines that contain *C fetus* do not protect against abortions caused by *C jejuni* or certain field strains of *C fetus* with different antigenic make up of the vaccine strain. In addition, heavy challenge may overwhelm protection afforded by vaccination. Nevertheless, vaccination of yearly replacement ewes and of ewes that have never been exposed to the bacteria is recommended in endemic areas to prevent later losses, since few vaccine breakdowns have been reported in New Zealand.[11]

A vaccine has recently been introduced to Australia. It contains a single strain of *C fetus* that has shown cross-protection against several field strains.

If vaccine is used during an outbreak, early diagnosis is important and vaccination must start as soon as the diagnosis is established.

Part 2. Tests Methods

1. Bacteriology

1.1 Collection of Specimens for the Isolation of *C fetus* and *C jejuni*

1.1.1 Transport of Specimens

As campylobacters are sensitive to environmental conditions transport to the laboratory and sampling processing should be carried out as soon as practicable. Ideally whole foetus and placenta should be submitted. If specimens are collected they should be sent in sterile containers in an insulated container containing an ice brick(s). Swabs need to be submitted in transport tubes containing media to protect the organism from drying and the toxic effect of atmospheric oxygen. Same day processing is ideal but a delay of no longer than 2 days is acceptable. No recommendation on the ideal temperature for transportation can be made. However, temperatures above 20^{o}C and below 0^{o}C are not recommended, Specimens should be stored at 4^{o}C where delays in processing are anticipated.[12]

1.1.2 Samples for Culture

The foetus and placenta should be submitted.

1.1.2.1 Placenta

It is preferable to culture from cotyledons that have a yellow-brown discoloration, indicative of ante-mortem necrosis. A normal cotyledon appears dark plum-red with post-mortem autolysis.

Culturing placental tissue that is grossly contaminated with soil and faeces should be avoided. However, as some contamination is unavoidable, it may be necessary to use selective media when culturing from placentas.

1.1.2.2 Aborted Foetus

C fetus and *C jejuni* may be isolated from the lung, liver or abomasal contents of aborted foetuses. They can usually be isolated by inoculating material onto 5-10% sheep blood agar or Skirrow's agar (see Part 3).

1.2 Isolation of *Campylobacter* spp

1.2.1 Selective Media

There is a range of media described for the isolation of *Campylobacter* spp, but due to the presence of specific antimicrobials (for example, those containing cephalosporins) some are not suitable for the culture of *C fetus*.[13] The recommended culture medium for *C fetus* and *C jejuni* is Skirrow's medium.

1.2.2 Incubation Conditions

Incubate plates at 37°C in an atmosphere of 90% nitrogen, 5% oxygen and 5% carbon dioxide. Commercial gas-generating kits and incubators can provide this specified gaseous environment. Cultures for *C fetus* should be incubated for up to three days and cultures of *C jejuni* should be incubated for two days.

1.2.3 Passive Filtration

Passive filtration is useful when samples for culture are likely to be contaminated. It obviates the need for selective media. Macerate, mix or homogenise samples of placenta, lung, liver or abomasal contents in an approximately 1/10 dilution of phosphate buffered saline (PBS) to produce a suspension. A 0.45 µm or 0.65 µm filter is placed on the surface of a non-selective blood agar plate and 100 µL of the suspension is carefully placed onto the filter. Incubation at 37°C or room temperature for 30-40 min allows the bacteria to migrate through the filter, which is then removed. The fluid that has passed through the filter is then spread with a sterile glass or plastic spreader and the plate is incubated as described previously.[12,14]

1.3 Identification

1.3.1. Microscopy

Dark field or phase contrast microscopy on fresh foetal abomasal content and placental scrapings is a fast and efficient method of diagnosing Campylobacteriosis.
The detection of spiral or curved slender rods measuring 0.2-0.9 µm x 0.5-5.0 µm with a rapid and corkscrew-like motility is indicative of *Campylobacter* spp. This method is also useful for identification of suspect *Campylobacter* colonies that are suspended in saline prior to examination, however, coccoid forms may occur in culture media. Gram stain of the fresh abomasal contents is also useful for the detection of *Campylobacter*-like forms.

1.3.2 Colony Morphology

C fetus has small (1 mm), round, slightly raised, smooth, translucent colonies said to have a 'dew drop' appearance with a regular edge. The colonies of *C jejuni* are usually flat, grey, and larger than those of *C fetus,* and can be spreading and watery on moist plates.

1.3.3 Differentiation of *C fetus* from *C jejuni*

The *Campylobacter* species are thin, curved, gram negative rods with a corkscrew-like motility. They are non-fermentative, oxidase positive and catalase variable. Most are microaerophilic. *C fetus* can be differentiated from *C jejuni* based on capability to grow at 25°C and 42°C, sensitivity to nalidixic acid and cephalothin, and hippurate hydrolysis (Table 1).

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

Test	*C fetus* subsp *fetus*	*C jejuni*
Growth temp		
42°C	V*	+
37°C	+	+
25°C	+	-
Sensitivity to**		
Nalidixic acid	R***	V*
Cephalothin	S***	R
Hippurate hydrolysis	-	+

* Variable ** 30 µg discs; *** R= resistant, S = sensitive

2. Molecular Biology

A number of PCR-based methodologies have been developed for the identification of *C fetus* and *C jejuni* cultures. The multiplex assay described by Hum et al[15] is widely used for the identification and differentiation of *C fetus* subsp *fetus* and *C fetus* subsp *venerealis* isolates, and has been useful for the direct detection of *C fetus* subsp *fetus* in abomasal samples from aborted foetuses. This assay, targeting the *cstA* gene, was adapted in the development of the multiplex assay[16], which can differentiate between cultures of *C fetus* and *C jejuni*, as well as other *Campylobacter* spp. Multiplex assays targeting the *cdt* genes are reported to discriminate between *C fetus*, *C jejuni* and *C coli*.[17] However, none of these PCR assays has been evaluated and approved by SCAHLS.

References

1. Mearns R. Other infectious causes of abortion. In: Aitken ID, editor. *Diseases of Sheep*. Blackwell, Oxford, 2007:131-132.

2. Peel B, Mason R. Ovine Campylobacteriosis. In *Australian Standard Diagnostic Techniques For Animal Diseases,* edited by Corner LA and Bagust TJ, CSIRO for the Standing Committee on Agriculture and Resource Management, 1993, East Melbourne.

3. Sahin O, Plummer PJ, Jordan DM et al. Emergence of a tetracycline-resistant *Campylobacter jejuni* clone associated with outbreaks of ovine abortion in the United States. *J Clin Microbiol,* 2008;46:1663-1671.

4. Dennis SM. The possible role of the raven in the transmission of ovine vibriosis. *Aust Vet J* 1967;43:45-48.

5. Manser PA, Dalziel RW. A survey of *Campylobacter* in animals. *J Hyg Camb* 1985;95:15-21.

6. Grogono-Thomas R, Dworkin J, Blaser MJ, Newell DG. Roles of the surface layer proteins of *Campylobacter fetus* subsp. *fetus* in ovine abortion. *Infect Immun* 2000;68:1687-1691.

7. Kirkbride CH, Gates CE, Collins JE. Abortion in sheep caused by a nonclassified anaerobic, flagellated bacterium. *Am J Vet Res* 1986;47:259-262.

8. Dewhirst FE, Fox GJ, Mendes EN et al. '*Flexispira rappini'* strains represent at least 10 *Helicobacter* taxa. *Int J Syst Evol Microbiol* 2000;50:1781-1787.

9. Plant JW, Beh KJ, Acland HM. Laboratory findings from ovine abortion and perinatal mortality. *Aust Vet J* 1972;48:558-561.

10. West DM. Ovine abortion in New Zealand. *NZ Vet J* 2002;50 (supplement), 93-95.

11. Gumbrell RC, Saville DJ, Graham CF. Tactical control of ovine *Campylobacter* abortion outbreaks with a bacterin. *NZ Vet J* 1996; 44:61-63.

12. Offices International des Epizooties. *Terrestrial Animal Health Code 2008. Chapter 2.9.3. Campylobacter jejun*i *and Campylobacter coli* www.oie.int/eng/normes/mmanual/2008/pdf/2.09.03_CAMPYLO.pdf

13. Offices International des Epizooties. *Terrestrial Animal Health Code 2008. Chapter 2.4.5 Bovine genital campylobacteriosis* www.oie.int/eng/normes/Mcode/en_chapitre_1.11.5.htm

14. Steele TW, McDermott SN. The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from faeces. *Pathology* 1984;16:263-265.

15. Hum S, Quinn K, Brunner J, On SLW. Evaluation of a PCR assay for identification and differentiation of *Campylobacter fetus* subspecies. *Aust Vet J* 1997;75:827-831.

16. Yamzaki-Matsune W, Taguchi M, Seto K et al. Development of a multiplex PCR assay for identification of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis. J Med Microbiol* 2007;56:1467-1473.

17. Asakura M, Samosornsuk W, Hinenoya A et al. Development of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the detection and identification of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. FEMS *Immunol Med Microbiol* 2008;52:260-266.

Part 3. Reagents and Test Kits

1. Skirrow's Campylobacter Agar

Composition per litre

Campylobacter agar base	990 mL
Antibiotic supplement	10 mL

***Campylobacter* agar base**

Proteose peptone	15.0 g
Agar	12.0 g
NaCl	5.0 g
Yeast extract	5.0 g
Liver digest	2.5 g

Preparation of base

Add components to base distilled/deionised water and bring volume to 900 mL. Gently heat and bring to boiling. Mix thoroughly. Gently heat and bring to boiling. Autoclave 121^{o}C for 15 min. Cool to 50^{o}C

Antibiotic supplement

Composition per 10 mL

Vancomycin	10 mg
Trimethoprim	5 mg
Polymixin B	2,500 Units

Preparation of medium

Prepare 990 mL of *Campylobacter* agar base. Autoclave and cool to 45-50^{o}C. Aseptically add 10 mL of the antibiotic supplement. Mix thoroughly. Pour into sterile Petri dishes.

Commercially made alternatives may be used.