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Review

Laboratory diagnosis of ruminant abortion in Europe

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ABSTRACT

Abortion in ruminants is a major cause of economic loss worldwide, and the management and control of outbreaks is important in limiting their spread, and in preventing zoonotic infections. Given that rapid and accurate laboratory diagnosis is central to controlling abortion outbreaks, the submission of tissue samples to laboratories offering the most appropriate tests is essential. Direct antigen and/or DNA detection methods are the currently preferred methods of reaching an aetiological diagnosis, and ideally these results are confirmed by the demonstration of corresponding macroscopic and/or histopathological lesions in the fetus and/or the placenta. However, the costs of laboratory examinations may be considerable and, even under optimal conditions, the percentage of aetiological diagnoses reached can be relatively low. This review focuses on the most commonly occurring and important abortifacient pathogens of ruminant species in Europe highlighting their epizootic and zoonotic potential. The performance characteristics of the various diagnostic methods used, including their specific advantages and limitations, are discussed.

Introduction

An increase in the number of spontaneous abortions in a herd or flock is a dramatic event for the farmer involved, and a range of epizootic and/or zoonotic diseases, or even emerging diseases, may be the cause. Farmers, their veterinary practitioners and, where appropriate, State veterinarians require rapid and reliable results from diagnostic veterinary laboratories, a process that is not always easily achieved. While a plethora of pathogens can cause abortion in ruminants, there is no single diagnostic procedure that can be used to identify these, and in some circumstances the infectious event triggering an abortion may precede it by some weeks or even months and evidence of the presence of the pathogen may have become obliterated by autolysis. By this time it may no longer be possible to demonstrate a rise in maternal antibody indicative of recent infection. Since attempting to rule out all the possible causes of abortion can prove costly, diagnostic laboratories primarily focus on the most likely aetiologies and those with zoonotic potential.

This review assesses the most important viral, bacterial, fungal and protozoal causes of abortion in cattle, sheep and goats in Switzerland, focusing on the methods used to reach a diagnosis and highlighting protocols that optimise pathogen detection. The information presented will be of interest to laboratory diagnosticians, as well as veterinary practitioners and State veterinarians. An overview of the infectious abortifacients discussed is given in Table 1.

Viral causes

Bovine herpesvirus type I

Bovine herpesvirus 1 (BoHV-1) infections remain a major cause of abortion, venereal and respiratory disease in ruminants in countries where the pathogen has not been eradicated (Kirkbride, 1992). Latency with recurrent infection is typical of infection with these viruses: during latency the virus survives within cells without causing clinical signs, and upon reactivation, repeated abortion may occur (Nandi et al., 2009). Given that virus is shed during reactivation, an infected animal remains a source of infection for in-contact herd mates. For this reason, Switzerland and other European countries such as Austria, Denmark, Finland, Sweden, Italy and Norway have now eradicated this economically significant infection.

BoHV-1 abortion can be diagnosed by demonstrating the presence of the virus in the aborted fetus and, in countries free of the virus, specific antibodies in maternal sera. PCR is currently the most sensitive method for identifying the virus in fetal tissues, particularly the liver (Crook et al., 2012). In endemic regions, serology is of little value in establishing a diagnosis of BoHV-1 abortion, as maternal infection may precede abortion for up to 2 months (Kennedy and Richards, 1964). Thus by the time abortion occurs, maternal antibody levels may have already peaked and demonstrating a rise in

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Table 1
Overview of infectious causes of abortion in cattle, sheep and goats in Europe.

Infectious agent		Cattle	Sheep	Goats
Viruses	Bovine herpesvirus type-1	++, dt, epi	–	-
	Pestiviruses	++[a], dt, epi and vt	+[b], dt, epi and vt	-
	Teratogenic viruses:			
	Bluetongue virus	+, vb, enz	+, vb, enz	+, vb, enz
	Schmallenberg virus	++, vb, enz	++, vb, enz	++, vb, enz
Bacteria	*Brucella* spp.	++, dt, epi, zoo	++, dt, epi, zoo	++, dt, epi, zoo
	Chlamydia abortus	+, dt, epi, zoo	++, dt, epi, zoo	++, dt, epi, zoo
	Coxiella burnetii	++, dt, epi, zoo	++, dt, epi, zoo	++, dt, epi, zoo
	Salmonella Abortusovis	–	++, dt, epi	-
	Miscellaneous bacteria	+	+	+
Parasites	*Neospora caninum*	++, ih and vt	+, ih	+, ih
	Toxoplasma gondii	-	++, ih	++, ih
	Tritrichomonas fetus	+	-	-
Fungi	*Aspergillus fumigatus*	+	+	+

++, important in this species; +, occasional cause in this species; –, of unknown significance in this species; epi, epizootic; enz, enzootic; zoo, zoonotic; vb, vector borne; dt, direct transmission; ih, intermediate host; vt, vertical transmission.
[a] Bovine viral diarrhoea virus.
[b] Border disease virus.

specific antibody levels may no longer be possible. The only grossly visible evidence of BoHV-1 infection in the fetus is subtle multifocal necrosis, particularly of the liver. The fetus is typically autolysed on expulsion with haemoglobin-tinged fluid in its body cavities. Microscopic examination of the liver and adrenal glands may facilitate the identification of necrotic foci with attendant leucocyte infiltration (Schlafer and Miller, 2007). When such lesions are observed, further tests for BoHV-1 infection are recommended, even in regions free of infection.

Pestiviruses

Bovine viral diarrhoea virus (BVDV) and Border disease virus (BDV) belong to the genus pestivirus of the family *Flaviviridae*. They are single-stranded RNA viruses, and exist as both non-cytopathic and cytopathic biotypes, respectively. An animal may remain persistently infected with a non-cytopathic biotype if exposed during the first trimester of pregnancy (Bachofen et al., 2008; Hilbe et al., 2009). In a retrospective study of bovine abortion in Switzerland between 1986 and 1995, 22/223 (9.9%) were positive on immunohistochemistry for BVDV infection, the second most common cause of infectious abortion after *Neospora caninum* infection (Reitt et al., 2007). Macroscopically visible brain malformations such as porencephaly, hydranencephaly and cerebellar hypoplasia may result from fetal infection (Moening, 1990; Nettleton and Entrican, 1995; Grooms, 2004). However, since such lesions may also result from infection with other viruses, or exposure to toxic compounds or genetic disorders, and since fetal infection with BVDV does not necessarily produce morphological alterations, demonstration of the presence of virus is required to confirm the diagnosis.

BDV can cause infertility, abortion, stillbirth and the birth of 'hairy-shaker' lambs depending on the time of fetal infection. The name 'Border disease' was coined as the disease was first reported in the border region between England and Wales (Sawyer, 1992). In persistently infected sheep the non-cytopathic virus induces histologically visible myelin deficiency in the central nervous system (CNS), resulting in tremor and an increase or enlargement in the number of primary hair follicles. These 'hairy-shaker' animals are persistently infected with virus (Nettleton, 1987; Sawyer, 1992; Nettleton and Entrican, 1995; Nettleton et al., 1998), and antigen can be found in smooth muscle cells (e.g. of blood vessels), epithelial cells (e.g. of hair root sheath), lymphocytes, neurons and glial cells using immunohistochemistry (Brodersen, 2004; Saliki and Dubovi, 2004; Sandvik, 2005; Hilbe et al., 2007a, 2007b).

Persistent infection with either BVDV or BDV is best confirmed by immunohistochemistry and RT-PCR on skin. Samples from the ear are typically used for this purpose and no fixation or pretreatment is required. When a whole aborted fetus is available, snap frozen samples of skin, tongue and thyroid gland are used for immunohistochemistry (Brodersen, 2004; Sandvik, 2005; Hilbe et al., 2007a, 2007b). Carrying out an ELISA on skin or tissue such as thyroid gland from aborted bovine fetuses gives false-positive results, perhaps due to the effects of autolysis.

BVDV or BDV may cross the placenta in persistently infected animals or during the viraemic phase in an acutely infected animal. Infection may go unnoticed as clinical signs may be absent or very mild during acute infection with BVDV and the impact on the fetus depends largely on the stage of fetal development at the time of infection: fetal resorption, abortion, mummification or malformation such as cerebellar hypoplasia or porencephaly may result (Brownlie et al., 1987; Moening, 1990; Nettleton and Entrican, 1995; Grooms, 2004). Infection with non-cytopathic BVDV at approximately 40–120 days of gestation can induce immunotolerance in the fetus to the infecting virus strain (Brownlie et al., 1987; Brock, 2003; Grooms, 2004; Bachofen et al., 2008), ultimately resulting in persistent viraemia in these animals. By approximately 150 days of gestation the fetus is sufficiently immunocompetent to eliminate the infection (Nettleton and Entrican, 1995; Brock, 2003; Grooms, 2004). With BDV ovine fetuses become persistently infected approximately between days 60 and 80 of gestation (Nettleton, 1987; Sawyer, 1992; Braun et al., 2002).

As abortion due to BVDV infection is often the result of one or more persistently infected animals in the herd, control measures must be directed at identifying and eliminating such individuals (Presi and Heim, 2010; Presi et al., 2011). Since pestiviruses are not strictly species specific, sheep may infect cattle and vice versa (Carlsson, 1991; Sawyer, 1992; Braun et al., 2002). This factor should therefore be considered in herds where no obvious source of infection can be detected. Zoonotic infections with pestiviruses have not been reported.

Teratogenic viruses

When an increased number of abortions with attendant malformations of the CNS and musculoskeletal systems occur, infection with members of the Orthobunyavirus group or Bluetongue virus (BTV) must be considered. Since these viruses are vector borne their geographic range is limited by the habitat of competent vectors. Nevertheless, both Schmallenberg (SBV) and Bluetongue viruses have both recently caused epizooties with consequent massive economic loss in Europe, an area where infections with these viruses had