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Clinical responses and reproductive outcomes in pregnant ewes experimentally infected with bovine viral diarrhoea virus (type-1c) between days 59 and 69 of gestation

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ABSTRACT

Low lambing rates and the birth of persistently infected (PI) lambs have previously been recorded in sheep infected with BVDV. However, there is little information available in regards to the clinical profile of acute BVDV infections in sheep. As a result the aim of this study was to investigate the clinical, haematological and reproductive responses in pregnant ewes infected with the predominant Australian BVDV strain, BVDV-1c.

Twenty-two pregnant ewes were experimentally inoculated with serum derived from a BVDV PI cattle serum between 59 and 69 days gestation. A further 11 pregnant ewes were left uninfected. No clinical changes were observed in the inoculated ewe group although a mild leukopaenia and a prolonged decrease in eosinophil counts was detected. Severe foetal losses, physical and neurological abnormalities in lambs and the birth of a persistently infected lamb was also recorded in the inoculated ewe group.

Results from this study suggest that acute BVDV-1c infections in sheep are clinically in-apparent, unless infection occurs in a pregnant flock, where severe reproductive losses can be seen at lambing. To eliminate the reproductive losses associated with BVDV infection close contact between sheep and cattle, of unknown BVDV status, should be avoided during the joining and pregnancy periods.

1. Introduction

Expressions of acute bovine viral diarrhoea (BVDV) infections in cattle can range from subclinical to severe illness, depending on the type and strain of virus present (Baker, 1995; Saliki and Dubovi, 2004). Pyrexia, mild leukopaenia, immunosuppression and a wide array of reproductive losses are commonly observed following acute BVDV outbreaks (Wellenberg et al., 2002). Reproductive dysfunction results from the ability of BVDV to cross the placenta and establish an infection within the developing foetus (Nettleton and Entrican, 1995; Niskanen and Lindberg, 2003), with the outcomes dependent of the stage of gestation at which infection took place (Grooms, 2004). A variety of reproductive outcomes including; abortion, foetal absorption, animals born with congenital or neurological abnormalities as well as animals born with persistent BVDV infections commonly occur following infection of pregnant cattle (Grooms, 2004; Broaddus et al., 2009).

In susceptible cattle populations, persistently BVDV infected (PI) animals are the main source of infection. These PI animals shed high quantities of virus in their excretions and secretions (Niskanen and Lindberg, 2003) and are capable of infecting BVDV naïve animals. Permanently infected animals arise when infection of the dam occurs prior to the foetus developing immunocompetence, during early to mid-gestation (18–125 days) (Grooms, 2004).

Previous studies have shown that BVDV-specific antibodies occur in a variety of non-bovine species, including sheep (Scherer et al., 2001), deer (Nettleton, 1990), goats (Bachofen et al., 2013), camels (Gao et al., 2013), pigs (Tao et al., 2013) and alpaca (Goyal et al., 2002). The clinical manifestations and reproductive losses associated with BVDV infection in sheep are of particular interest due to their frequent proximity to cattle in many countries and the risk of cross-species transmission. Several studies have been undertaken to determine the reproductive losses associated with maternal infection with BVDV. Results from these studies indicate high abortion rates, ranging from 52 to 100%, in ewes infected with BVDV between 25 and 100 days gestation (Snowdon et al., 1975;

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Parsonson et al., 1979; Scherer et al., 2001; Evans et al., 2015). It has also been demonstrated that the birth of persistently BVDV infected lambs may occur when infection takes place early in gestation (Snowdon et al., 1975; Scherer et al., 2001; Evans et al., 2015). However, the majority of these studies have used types or strains of virus not typically found in Australia, where infection appears to be predominated by a single type-1 strain in cattle (Ridpath et al., 2010). The clinical profile of acute infections of BVD Type 1-c in adult sheep is not known.

The aim of the study was to investigate the clinical, haematological and reproductive responses in pregnant ewes infected with the predominant Australian BVDV strain, BVDV-1c.

2. Materials and methods

2.1. Experimental animals

The study was approved by the University of Adelaide's Animal Ethics Committee prior to commencement of this project (S-2014-111A).

The Merino ewes used in this study were sourced from the University of Adelaide's commercial flock, resident at its Roseworthy Campus in South Australia. Eighty two ewes were tested for both BVDV-specific antibodies by IDEXX BVDV/MD/BDV p80 Protein Antibody Test Kit (IDEXX Laboratories Inc, Rydalmere, NSW), and BVDV-specific antigen by IDEXX Bovine Viral Diarrhoea Virus (BVDV) Antigen Test Kit/Serum Plus ELISA (IDEXX Laboratories Inc, Rydalmere, NSW). All animals were found to be negative for BVDV antibodies and antigen according to the cut-off value established for the antibody ELISA by Evans et al. (in press), and the manufacturer's cut-off for the antigen ELISA.

2.2. Breeding management of the experimental ewes

During April 2015, the oestrus cycles of the experimental sheep were synchronised using a commercially available progesterone releasing intra-vaginal device (EAZI-BREED CIDR sheep and goat device, Animal Health, Pfizer Australia Pty Ltd). The CIDR's were removed sequentially from groups of 20 ewes on days 14, 15 and 16 and from 22 ewes on day 17 post CIDR insertion. Upon CIDR removal, each group of ewes were randomly allocated to one of four paddocks. Each of these paddocks housed two or three Border Leicester or Merino rams. The ewes and rams were separated at 25 days post CIDR insertion following an 8–11 day mating period. The ewes were then left undisturbed until transabdominal ultrasound scanning at 59 days following the first CIDR removal.

2.3. Source of BVDV Type1-c inoculum

In September 2014 blood was taken from a persistently BVDV-1c infected heifer from a farm in Meningie, South Australia. This heifer was produced in an earlier study at the University of Adelaide's Roseworthy Campus, South Australia, and was confirmed to be persistently infected with BVDV-1c (Lanyon et al., 2014). Blood was collected into 10 ml collection tubes and then centrifuged at 2400 × g for 10 min. Serum was decanted off and stored at −80 °C in 10 ml tubes. The serum was tested for the presence of BVDV-specific antibodies and BVDV-specific antigen by ELISAs (ibid) was thawed (at 2–8 °C overnight) once only prior to use.

2.4. The experimental groups

Ewes confirmed pregnant by ultra-sound scanning were randomly assigned to one of two treatment groups. The control group consisted of 11 ewes, pregnant with 23 foetuses while the inoculated group, consisted of 22 ewes, pregnant with 42 foetuses. Ewes in the inoculated group were subcutaneously infected with 2 ml freeze-thawed BVDV PI cattle serum, between 59 and 69 days of gestation. Each 2 ml dose of BVDV PI serum contained, equal to, 1.3×10^7 of viral genome copy numbers, based on the absolute quantification in a real-time quantitative reverse transcriptase PCR method.

Animals from both control and inoculated treatment groups were maintained in two paddocks separated by a 1.5 m gap and were fed *ad lib* feed and water. All animals, ewes and lambs from both treatment groups, were systematically observed on a daily basis using a defined checklist of clinical signs as indicators of overall health and wellbeing. Lambs were also weighed weekly from day of birth (DOB) until eight weeks of age.

Ewes had rectal temperatures taken at each blood sampling time point and were scanned for pregnancy by ultrasound on days 14, 21, 35, 49, 56 and 98 post-inoculation. Foetal losses were recorded when a ewe was found not pregnant at scanning. All ewes that carried their pregnancies to term were allowed to lamb naturally.

2.5. Sampling protocols

Blood samples were taken from all the experimental ewes on the following days where day 0 is the day on which the 'inoculated group' was inoculated: 0, 3, 5, 7, 10, 12, 14, 17 and then weekly until a week prior to lambing. Blood samples were taken into plain vacutainer serum tubes and EDTA collection tubes by venepuncture of the jugular vein.

Lambs from both treatment groups had DOB and weekly weights recorded, until 8 weeks of age. Ear notch and blood samples (plain vacutainer serum tubes and EDTA collection tubes) were also collected on DOB from all lambs born in the inoculated group. Blood samples were then collected from all live lambs weekly until eight weeks of age. Aborted foetuses, stillborn lambs and deceased lambs from the inoculated treatment group were submitted to the Veterinary Diagnostic Laboratory of the University of Adelaide for post-mortem examination and sample collection.

Serum was obtained from the blood samples by centrifuging the plain vacutainer serum tubes at 2400 × *g* for 10 min and the serum decanted in to 1 ml storage tubes before being stored at −80 °C.

2.6. Serum and tissue analysis

Thawed serum samples from all ewes were tested for BVDV-specific antibodies using the IDEXX BVDV Ab P80 ELISA, where signal-to-noise ratios (S/N) of <63.5% were considered positive as per Evans et al. (in press). Thawed serum samples were also tested for BVD viral antigen using the IDEXX BVDV Antigen ELISA (IDEXX Laboratories, Rydalmere, NSW) whereby a sample was considered positive if it had an S-N value of <0.3, as *per* manufacturer's instructions.

All serum samples from live, inoculated treatment group lambs were tested for BVDV-specific antibodies using a competitive ELISA, the IDEXX BVDV Ab P80 protein test kit. Either serum, peritoneal fluid or ear tissue samples from all live, aborted or dead lambs, from the inoculated treatment group, were tested for BVD viral antigen using IDEXX BVDV Serum/Ag PLUS ELISA (IDEXX Laboratories Inc. Rydalmere, NSW), as *per* manufacturer's instructions.

EDTA blood samples from all ewes, on days 0–21 post inoculation, were analysed using a CellDyn Machine (model: 3700). Blood samples collected into EDTA tubes were analysed within 48 h of the samples being taken to determine the haematological parameters: total and differential leukocyte counts, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, and platelet counts.