BOVINE VIRAL DIARRHOEA VIRUS (BVDV) INFECTION IN PREGNANT COWS AND THEIR FOETUSES

ORHAN YAPKIÇ, SİBEL YAVRU, OYA BULUT, MEHMET KALE\textsuperscript{1} AND AYHAN ATA\textsuperscript{2}

Department of Virology, Faculty of Veterinary Medicine, Selcuk University, 42075, Campus, Konya, Turkey
\textsuperscript{1}Department of Virology, \textsuperscript{2}Department of Theriogenology and Artificial Insemination, Faculty of Veterinary Medicine, Akdeniz University, 15100, Burdur, Turkey
e-mail:oyapkic@selcuk.edu.tr

Received for publication March 31, 2005.

Abstract

The aim of the study was to characterize bovine viral diarrhoea virus (BVDV) infection in pregnant cows and their foetuses. Blood samples from 64 clinically healthy cows, determined as pregnant after slaughter, and from their foetuses were collected into tubes with and without EDTA. In total 128 blood samples were examined for BVDV antibodies and 128 leukocyte samples for BVDV antigens by direct and indirect ELISA. Thirty-six out of 64 (56.25\%) sera from adult cows were detected as seropositive while only one (1.56\%) serum from a foetus was seropositive. Leukocyte samples from pregnant cows were negative for BVDV antigen while BVDV antigen was detected in only one (1.56\%) leukocyte sample from a foetus. A foetus, 4-month-old, belonging to these of seropositive cows, was detected as BVDV antigen negative and antibody positive. The other one, 2-months-old, was found as antigen positive and antibody negative.

Key word: cows, foetus, BVDV, ELISA.

Bovine viral diarrhea virus (BVDV), an enveloped positive –strand RNA virus, is a common pathogen of cattle. The virus causes a wide range of clinical syndromes depending on age and immune status of the animal at the time of infection. BVDV is currently classified in the pestivirus genus of the \textit{Flaviviridae} family, which also includes hog cholera and border disease viruses (12). Field isolates of BVDV can be divided into two biotypes according to their ability to induce cytopathic effect in bovine cell cultures: cytopathic or non-cytopathic. When a seronegative pregnant cow is infected with a non-cytopathic BVDV biotype, the virus can be easily transferred to the foetus. Infections during the foetal life lead to abortion, mummification, teratogenesis or persistent infection of a calf (1). Persistently infected calves show immunological tolerance to the virus and may be born as apparently healthy animals. They shed the virus during their life being responsible for perpetuation of BVDV infection in cattle population. Fatal mucosal disease occurs only in persistently infected animals and is induced by the cytopathic biotype of BVDV. This event may take place by mutation to the cytopathic biotype or superinfection with a cytopathic biotype of BVDV (3, 8).

Material and Methods

Animals. A total of 64 clinically healthy cows, determined as pregnant after slaughter, and their 64 foetuses were used in the study. The age of foetuses were calculated according to the method by Richardson \textit{et al.} (22).

Serum samples. In total 128 blood samples, which were taken from cows and foetuses into normal tubes during slaughter at slaughterhouse, were centrifuged at 1 500 - 2 000 rpm/15-20 min to obtain sera. The serum samples were kept in deep-freezer under -25°C and inactivated at 56 °C for 30 min before use.

Leukocyte samples. Leukocytes were obtained from blood samples, taken after slaughter into tubes with EDTA (ethylenediamine tetraacetic acid), by centrifugation at 2 000-2 500 rpm/15-20 min and stored in deep freezer at -25°C till use.

ELISA kits. Commercial direct and indirect ELISA kits (Institute POURQUIER) were used for the detection of BVDV antigens in leukocyte samples and BVDV antibodies in serum samples as described in the test procedure.

Results

In 34 cows out of 64 (56.25 \%) examined showed the presence of BVDV antibodies, while only one 4-month-old foetus was seropositive. All leukocyte samples from pregnant cows and all from foetuses,
except for one 2-month-foetus (1.56%), were detected as negative for BVDV antigen.

Discussion

Bovine viral diarrhoea (BVD) is a common world-wide infection causing economical losses in cattle breeding (16). When the BVDV infection affects the pregnant cow the virus can cross via placenta to the foetus and depending on the period of pregnancy the foetus can be persistently infected or can develop immune response.

When non-cytopathic biotype of BVDV infects the dam before the 120th d of pregnancy the foetus is not able to develop a sufficient immune response and the virus produces the persistent infection. The animal develops immunotolerance and excretes the virus throughout the life being a source of infection for other animals in a herd (12). When the infection occurs in foetuses older than 120 d they are able to develop immune response and then BVDV antibodies can be detected after birth.

To detect the BVDV persistently infected animals the following tests are used: direct immunofluorescence (DIF) (10), peroxidase linked assay (PLA) (10), enzyme linked immunosorbent assay (ELISA) (3), polymerase chain reaction (PCR) (11), plaque test (26), interference (23), hybridization (4), synthetic oligonucleotids (13) and flow cytometry (21).

To detect the antibodies against BVDV the following tests are used: serum neutralization (SN) (2), indirect immunofluorescence (IF) (14), ELISA (16, 20), agar gel immunodiffusion (AGID) (7), neutralization peroxidase linked assay (NPLA) (18) and complement fixation (CF) (9).

We found 34 dams of 64 tested (56.25%) to be BVDV antibodies positive. Bock et al. (1) studied 886 blood sera from cows and blood sera from 6 calves experimentally infected with BVDV, using serum neutralization test (SN) and ELISA, and found that ELISA was an efficient method of the detection of BVDV antibodies during large scale screening of cattle for BVDV infection. Obando et al. (17) applied ELISA for 615 blood sera and reported that they detected seropositive results at a rate of 36.7%. Chu et al. (6) studied 50 blood sera for BVDV antibodies with ELISA and SN and found that ELISA was much more sensitive method than SN. Stokstad et al. (24) experimentally infected with BVDV 19 pregnant cows between day 74 and 81 of pregnancy. Cows became infected and developed antibodies against BVDV. They reported that 16 of 19 calves were born as persistently infected. All the cows had antibodies against BVDV but the cows giving birth to persistently infected calves had higher levels of the antibodies (24).

As mentioned above the foetuses become immunocompetent approximately on the 120th d of gestation. In the present study, one foetus (4-month-old) and its dam were detected as seropositive to BVDV. Based on the results presented it is impossible to determine when the cow was infected, although the antibodies were detected in the foetus as early as in the 4th month of gestation.

One foetus (2-month-old) was detected as BVDV antigen positive. This may indicate that the dam was infected with non-cytopathic BVDV for the first time during the early period of pregnancy.

Thur et al. (25) studied for BVDV 213 foetuses aborted and calves died after calving in 1992-1994. They detected antibodies against BVDV in 6% of foetuses by indirect ELISA. They reported that 9 of the foetuses were positive for BVDV by direct ELISA, immunohistochemical staining and virus isolation in cell culture.

Brownlie et al. (5) have infected 46 pregnant cows with BVDV by intrauterine route after 120 d of pregnancy. Nine cows aborted, 4 cows have delivered but the calves died, 12 calves were born healthy, 4 calves had clinical symptoms of the disease and 17 calves had viraemia.

Özkul (19) studied 353 blood samples and various organs of 50 pregnant cows and their foetuses for BVDV antigen. The antigen was found only in one leukocyte sample of seronegative cow and its foetus.

In conclusion, although the prevalence of persistently infected cattle in the population is usually less than 1%, BVDV infection causes the economic losses in cattle breeding. Persistently infected cattle, often clinically normal, act as a reservoir of BVDV. For this reason, control and eradication programs of BVDV infection should be implemented as obligatory.

References