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Bovine viral diarrhoea: a seven year old persistently infected cow - a case report

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ABSTRACT

In this report a persistently infected cow was under surveillance for three years. In that period the level of antibodies and level of viremia were determined. Persistent infection was confirmed in blood serum samples taken at 3 week intervals and tested on the virus antigen by antigen enzyme-linked immunosorbent assay and by inoculation in cell culture, which after incubation was stained by an imunoperoxidase technique. In order to determine the changes in antibody level and virus titer levels, blood samples were collected and tested each six months. Also, data on milk production were collected. The level of viremia was $10^{4.5}$ throughout the period of the study, and antibodies were not detected by VNT. Milk production was above-average at all times. Average daily milk production over 305 days of lactation in 2009 and 2010 was 25 and 21 litres per day respectively. In the first five months of 2011 daily milk production was 15 litres per day. The cow was taken out of herd at the age of seven years.

Key words: bovine viral diarrhoea, persistent infection, cow, milk production

Introduction

Bovine viral diarrhoea (BVD) is one of the most common diseases in cattle (HOUE, 1999). BVD is caused by two species of the positive single stranded RNA virus (BVDV1 and BVDV2) from the genus *Pestivirus* within the *Flaviviridae* family (SIMMONDS et al., 2012). Both BVDV species may occur in cytopathic (cp) and non-cytopathic (ncp) biotypes according to whether or not they produce a visible change in cell cultures.

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Vertical transmission of the non-cytopathic biotype during the first 120 days of pregnancy may cause the birth of persistently infected (PI) calves (ROEDER et al., 1986).

PI cattle are a permanent source of the virus among and between herds, and play a key role in the epidemiology of the disease (HOUE, 1999). It is generally accepted that PI calves do not live long. In the first year of life, an up to 50% higher death rate was found in PI calves compared to those not infected (DUFFEL and HARKNESS, 1985). Even apparently normal calves have a higher death rate than uninfected calves, because they are predisposed to illness (BARBER et al., 1985; WERDIN et al., 1989; MUNOZ-ZANZI et al., 2003). The reasons for this may be due to functional defects of the immune system, causing immune suppression (ROTH et al., 1981 and 1986). Also, diabetes mellitus has repeatedly been reported in PI animals (MURONDOTI et al., 1999). Furthermore, due to mutation of the ncp biotype to cp biotype, mucosal disease may be developed. Mortality in cases of mucosal disease is nearly 100% (EVERMANN and BARRINGTON, 2005).

Despite the high mortality of PI cattle, cases of PI cattle that live several years have been documented (HOUE, 1993; BROCK et al., 1998). In one report, the oldest PI cow was 5.5 years old (BROCK et al, 1998) and in another, 3 years old (HOUE, 1993). According to our knowledge the oldest PI cow was recorded in Norway. This cow was seven-years old when she was demonstrated to be PI, and was still healthy when subsequently killed. (LØKEN, personal communication).

In this report, milk production in PI cow was monitored for the first time for three years.

Materials and methods

Persistent infection was confirmed in blood serum samples taken at 3 week intervals and tested on the virus antigen by antigen enzyme-linked immunosorbent assay (Ag ELISA) and by inoculation in the cell culture, which was stained after incubation by the immunoperoxidase technique. Ag ELISA (Herdchek BVDV Ag/Serum Plus, Idexx, Liebefeld-Bern, Switzerland) was performed according to the manufacturer's instructions and the immunoperoxidase test according to the Manual for terrestrial animals (ANONYMOUS, 2008).

After persistent infection was confirmed, the cow was monitored until she was moved from the herd (10 June 2011). At the time of sampling, there were 50 Simmental cattle in the herd. All the cattle were located strictly on the farm without the use of common pasture. Over the past six years no new animals have entered the herd.

In order to establish the antibody titre, the virus neutralization test (VNT) was performed according to the Manual for terrestrial animals (ANONYMOUS, 2008). Briefly, sera were inactivated at 56 °C for 30 min. Two-fold serial dilutions of sera ranging from 1:2 to 1:256 were made in microtitre plates. For each reference strain, two wells were

used. Fifty µL of BVDV 1 and BVDV 2 reference strains (NADL and 8644-supplied by Friedrich Loeffler Institute, Insel Riems, Germany) prepared in order to obtain 100 $TCID_{50}$ were added to duplicate columns. After 1 hour of incubation at 37 °C, 100 µL Madin Darby bovine kidney cells (MDBK) were added at a density of 150 000/mL. Plates were incubated for four days at 37 °C in the incubator at 85% humidity. After incubation, the presence of viruses were confirmed using BVDV specific monoclonal antibodies (VLA, Weibridge, UK) and anti-mouse rabbit antibodies, conjugated with fluorescein isothiocionate (Sigma-Aldrich Inc., St. Louis, SAD).

Levels of viremia were determined by making serial 10-fold dilutions of serum. 50 µL of serum and 50 µL of MDBK cells, at 300 000/mL density were added to a well. For each dilution four wells were used. After four days the incubation cells were stained using the immunofluorescence technique.

In order to determine the changes in levels of viremia, as well as specific antibodies, sera from the PI cow was tested every six months.

In order to estimate the influence of infection on production, data about milk production were recorded.

Results

Persistent infection was confirmed in the cow (Fig. 1). Sera samples were positive by Ag ELISA and by isolation of the virus. Also, in 2011 persistent infection was confirmed by Ag ELISA and by virus isolation in one calf that originated from the infected cow (Fig. 2).

The isolated virus belongs to the BVDV 1 non-cytopathic strain (BEDEKOVIC et al., 2012). During the study there was no reported change in virus titer and specific antibodies were not detected (Table 1).

Table 1. BVD virus levels and specific antibodies titres in serum from PI cow

		SN antibody titer	
Date of sampling	virus titre (50 µL)	NADL	CS 8644
03-11-2008	$10^{4.5}$	< 1:2	< 1:2
01-12-2008	$10^{4.5}$	< 1:2	< 1:2
07-06-2009	$10^{4.5}$	< 1:2	< 1:2
18-12-2009	$10^{4.5}$	< 1:2	< 1:2
05-06-2010	$10^{4.5}$	< 1:2	< 1:2
15-12-2010	$10^{4.5}$	< 1:2	< 1:2
08-06-2011	$10^{4.5}$	< 1:2	< 1:2

Average daily milk production over 305 days of lactation in 2009 and 2010 was 25 and 21 litres per day respectively. During the first five months of 2011, milk production was 15 litres per day.

Fig. 1. Persistently infected cow. The cow was five years old. The cow has not shown any symptoms of BVD infection.

Fig. 2. Two week old female calf from the PI cow. After PI was confirmed the calf was moved out of the herd. During that period (three weeks) the calf did not show any symptoms of BVD infection.

Discussion

Numerous studies about PI animals have been made, however, according to our findings, cases of PI cows with data about milk production have not been reported. Also, we could not find any case in which PI dairy cow were moved out of production because of a decline in milk production caused by age. According to LØKEN (personal communication) the oldest recorded PI cow was seven-years old when she demonstrated PI, and was still healthy when subsequently killed.

It is usually stated that PI cattle die before they reach two years of age. In most cases the age of PI animals was less than three years (HOUE, 1993). In some cases PI animals probably could have stayed alive for many months, even years. However, in most cases PI animals have problems with production. They have weaker growth and are more susceptible to other diseases (HOUE, 1993; LARSSON et al., 1994). As a result many PI female cattle are moved out from herds before they reach reproductive age. Furthermore, PI animals may develop mucosal disease. Mortality in cases of mucosal disease is almost 100% (EVERMANN and BARRINGTON, 2005).

According to our findings this is the first time that the case of a PI cow with above-average milk production has been described. According to the veterinarian and owner, no abnormalities or diseases were recorded in the herd over the past five years. Furthermore, the cow has not shown any symptoms of disease and has calved four times. One of the reasons why no abnormalities were recorded within the herd may be that over the last six years no new animals have been brought into the herd. With that kind of management, the possibility of the introduction of new microorganisms to the herd is minimal. This is important because of the influence of microorganisms on the immunity system of the animals. Also, this strongly suggests that infection was present within the herd for at least six years. Despite the fact that the cow was PI, her milk production was above-average and owner decided to keep her within the herd.

Finally, the cow was moved out from the herd after full seven years of life because of her age and due to a decline in her milk production. It is very indicative that all four calvings were difficult. Each time the veterinarian needed to help by correction of the foetus position. However, all animals survived and have not shown any symptoms of disease. Three male calves were moved out of the herd because of management. A female calf was moved out of the herd when PI infection was established.

PI animals have great importance in BVD epidemiology. They act as a reservoir of the virus and support virus maintenance between and among the herd. According to the study by BROCK et al. (1998) the virus level changes over time in PI animals. Also according to the same study, antibodies against BVDV may be developed. In our case over three years of surveillance, no changes in virus titre were recorded. Also, no antibodies were recorded by ELISA or VNT. The reasons for this may be found in the different approaches to the

study. In other study (BROCK et al, 1998) PI animals were housed separately which may have influenced their stress level. Cattle are herd animals and individual accommodation may have a negative influence on their health. In our case, the animals were placed in realistic field conditions and the influence of stress was minimal. This implies the need for more study under field conditions for a better understanding of the epidemiology of the disease.

This case shows that PI animals may be infected with the BVD virus for their entire life without any clinical disorder and with above average milk production.

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SAŽETAK

U ovom radu praćene su promjene u razini protutijela i promjene u titru virusa tijekom tri godine u perzistentno zaražene krave. Perzistentna zaraza je dokazana imunoenzimnim testom i izdvajanjem virusa u uzorcima krvnog seruma uzorkovanima dvaput u razmaku od 3 tjedna. Radi dokazivanja promjene u razini protutijela i titru virusa krv je od spomenute krave uzorkovana svakih šest mjeseci. Također su prikupljeni i praćeni podaci o mliječnosti krave. Titar virusa bio je $10^{4,5}$ tijekom cijelog istraživanja pri čemu nije zabilježena pojava specifičnih protutijela. Mliječnost je bila iznad prosjeka tijekom cijeloga istraživanja. Prosječna dnevna proizvodnja mlijeka tijekom 305 dana laktacije 2009. i 2010. godine iznosila je 21 odnosno 25 L, a tijekom prvih 5 mjeseci 2011. godine 15 L. Krava je izlučena iz stada u dobi od sedam godina.

Ključne riječi: virusni proljev goveda, perzistentna infekcija, krava, mliječnost