Comparison Of Ear Notch Immunohistochemistry Against E2 (Gp53) Protein And Antigen-Capture Elisa In Sera, For The Detection Of Calves Persistently Infected With Bovine Diarrhea Virus

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Citation

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Abstract

The objective of this study was to compare two methods of detection of calves persistently infected (PI) with bovine viral diarrhea virus (BVDV): immunohistochemistry (IHC), using ear skin biopsies, and antigen capture enzyme-linked immunosorbent assay (ELISA$_{Ag}$) using sera. We also aimed to determine the site of immunopositivity. Samples were taken from 80 Holstein calves of up to 3 months of age. For IHC, the streptavidin-biotin-peroxidase complex method was used, along with a monoclonal antibody binding to E2 (gp53) protein of BVDV type 1 and 2. For ELISA$_{Ag}$, a commercial kit was used based on the E^{rns} (gp44–48) protein of BVDV type 1 and 2. Twelve of the 80 skin biopsies (15%) were IHC positive. Immunopositivity was observed in the epidermis, hair follicles and dermis mononuclear cells being similar to the reported in previous studies. This study confirms that the anti-BVDV monoclonal antibody types 1 and 2, E2 (gp53), were able to detect viral antigens in the skin biopsies, previously fixed in 10% formalin. All sera were negative in ELISA$_{Ag}$. Statistical analysis showed a significant difference between the two methods in their capacity to detect PI animals, P (<0.01), indicating that IHC was more sensitive than ELISA$_{Ag}$, identifying 15% of infected animals.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is classified in the Pestivirus genus within the Flaviviridae family. It is an important pathogen of cattle that can cause reproductive failure, weak born or persistently infected (PI) calves and mucosal disease. BVDV also contributes to the bovine respiratory disease complex (Fray et al., 2000; Liebler-Tenorio et al., 2000; Cornish et al., 2005). BVDV has two genotypes, BVDV 1a 1b and BVDV 2, with each genotype presenting two biotypes, cytopathic (cp) and non-cytopathic (ncp), based on whether or not they cause cellular alterations. Persistent infection results from cows being exposed to the ncp variant of the virus before day 125 of gestation, allowing the fetus to develop immunotolerance to the virus and letting the virus persist after birth (Fulton et al., 2000; Brock, 2004; Bolin and Grooms, 2004; Zimmer et al., 2004). PI animals continuously shed great quantities of the virus even when they are clinically healthy and are therefore considered an important source of viral dissemination among the herd. One of the main strategies that has been used to eliminate BVDV in herds is to identify and remove PI calves (Fray et al., 2000; Cornish et al., 2005). For this, several diagnostic tests have been used which involve viral isolation (VI), inverse transcription, polymerase chain reaction (PCR) and real time (RT)-PCR, antigen capture enzyme-linked immunosorbent assay (ELISA$_{Ag}$), viral genome identification in peripheral blood leucocytes (PBL) and immunohistochemistry (IHC) (Liebler-Tenorio et al., 2000; Njaa et al., 2000; Malhum et al., 2002; Brodersen, 2004; Luzzago et al., 2006; Hilbe et al., 2007). In order to opportunely detect PI calves, a sensitive, economical, easy to perform diagnostic test is required, to facilitate prevention and control strategies of this infection in herds. The IHC test is based on viral antigen detection in infected animal skins. This test has been effective for PI animal detection since only a small portion of the skin, generally obtained from the ear, is required, numerous samples can be analyzed simultaneously and the results have been satisfactory. The aim of this study was to detect PI calves of up to 3 months old, by IHC in skin biopsies and ELISA$_{Ag}$ in serum samples,

compare the results of both tests and determine immunopositive localization in skin biopsies.

MATERIAL AND METHODS

SAMPLE COLLECTION

Ear skin biopsies and sera were taken simultaneously from 80 Holstein calves of up to 3 months old, from herds with a BVDV infection background in the Complejo Agropecuario Industrial de Tizayuca, Sociedad Anonima (CAITSA), located on the Mexico-Pachuca highway in the state of Hidalgo, Mexico.

IMMUNOHISTOCHEMISTRY

For skin biopsies, ear notches were used to obtain a tissue fragment of $1.0 \times 0.8 \times 0.4$ cm approximately. Biopsies were fixed in 10% formalin, at pH of 7.4 for 24 h. Tissues were processed by routine histological technique and cut to a thickness of 3 mm to be examined by IHC using the streptavidin-biotin-peroxidase complex method (Haines et al., 1992). Cut tissue samples were mounted on slides with poly-L-lysine, deparaffined for 1 h at 60ºC and rehydrated in decreasing concentrations of ethanol.

Then, endogenous peroxidase inhibition was carried out using a 3% H_2O_2 in methanol solution, followed by antigenic retrieval using Target Retrieval Solution (Dako Corporation), according to the manufacturer's instructions, along with heat treatment for 3 min and 30 sec in a microwave oven. Endogenous avidin and biotin were blocked using an Avidin/Biotin Blocking Kit (Zymed Laboratories Inc.), according to the manufacturer's instructions, and the samples were then ready for antibody treatment. A monoclonal primary antibody that binds to E2 (gp53) of BVDV (genotypes 1 and 2) (VMRD, Inc.), was diluted 1:150 in phosphate-buffered saline (PBS) and incubated with the tissue sample for 1 h in a humid chamber (HC) at ambient temperature (AT). After a short rinse with PBS, a biotin conjugated protein G (Rockland Immunochemicals Inc.) was then applied as a secondary antibody, diluted at 1:100 in PBS, and incubated for 1 h in a HC at AT. After washing the slides three times for 5 min in PBS, streptavidin (Zymed Laboratories Inc.) was then applied for a further 30 min incubation in a HC at AT. Finally, the reaction was developed using aminoethylcarbazole (AEC) and the Histostain SP Kit (Zymed Laboratories Inc.), to contrast with Mayer's hematoxylin. Positive and negative tissue controls were included.

Antigen capture enzyme-linked immunosorbent assay (ELISA$_{Ag}$)

Blood samples were obtained from calves by coccygeus vein puncture and obtained sera were treated with antigen capture enzyme-linked immunosorbent assay (ELISA$_{Ag}$) using the IDEXX HerdChek BVDV Antigen/Serum Plus Test Kit (IDEXX Laboratories Inc.), following the manufacturer's instructions. This assay is based on the detection of protein E^{ms} (gp44–48) of BVDV genotypes 1 and 2.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the chi-square test of homogeneity and the SAS program.

RESULTS

Twelve of the 80 ear skin biopsies (15%) gave positive IHC results. Immunopositivity was observed as a red color which was distinctive in the cytoplasm of the epidermis and the keratinocytes (Figure 1), hair follicles and mononuclear cells of the dermis (Figures 2 and 3). The 80 sera were negative in the ELISA$_{Ag}$ test. Statistical analysis found that the capacity to detect PI animals by means of ELISA$_{Ag}$ and IHC was significantly different (P <0.01), with 15% of animals diagnosed as positives by IHC.

Figure 1

Figure 1. Ear skin biopsy, treated with the Streptavidin-Biotin-Peroxidase Complex with anti-BVDV antiserum against E2 (gp53) protein from genotypes 1 and 2, from a persistently infected (PI) calf with bovine viral diarrhea virus (BVDV). Viral antigens in keratinocytes are evident. Bar = 107 µm.
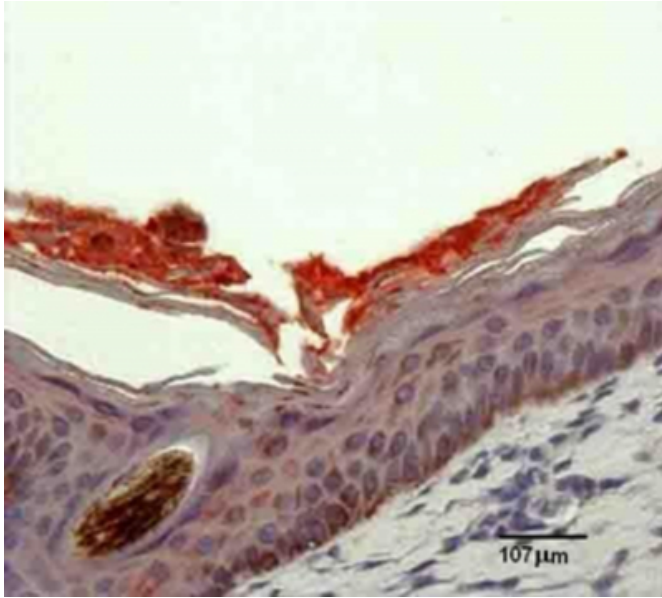

Figure 2

Figure 2. Ear skin biopsy, treated with the Streptavidin-Biotin-Peroxidase Complex with anti-BVDV antiserum against E2 (gp53) protein from genotypes 1 and 2, from a persistently infected (PI) calf with bovine viral diarrhea virus (BVDV). Viral antigens on the epidermis are evident. Bar = 107 µm.
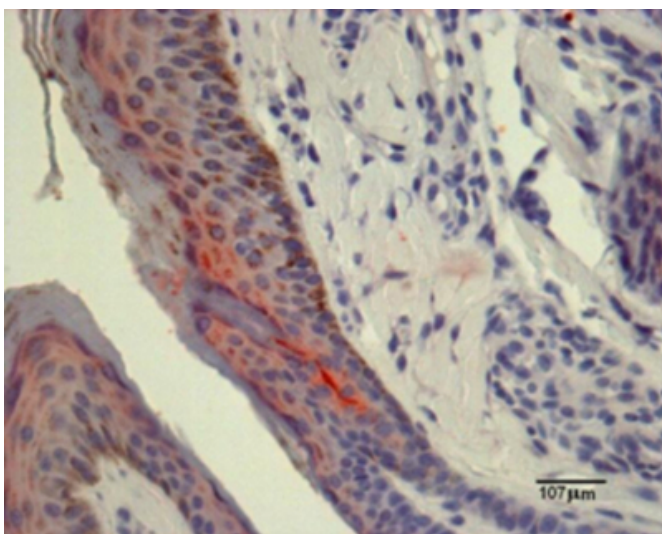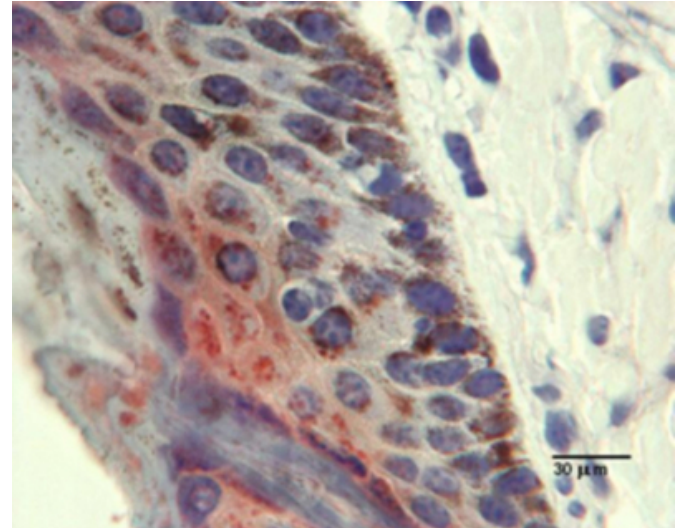

Figure 3

Figure 3. Ear skin biopsy, treated with the Streptavidin-Biotin-Peroxidase Complex with anti-BVDV antiserum against E2 (gp53) protein from genotypes 1 and 2, from a persistently infected (PI) calf with bovine viral diarrhea virus (BVDV). This image is a close-up of the image in Figure 1. Bar = 30 µm.

DISCUSSION

The results of this study indicate that IHC on ear tissue samples from calves is a sensitive diagnostic method for the detection of PI animals. It has been reported that not all monoclonal antibodies detect BVDV antigens in formalin-fixed, paraffin-embedded tissues (Haines et al., 1992; Brodersen, 2004). However, several studies have used monoclonal antibody (15C5) which reacts with BVDV EO (gp48) (E^{rns}/gp48) protein and gives good results in biopsies of skin and other tissues (Haines et al., 1992; Bazler et al., 1995; Cornish et al., 2005; Luzzago et al., 2006; Montgomery, 2007). In this work, the anti-BVDV monoclonal antibody types 1 and 2, E2 (gp53), were able to detect viral antigens in the skin biopsies, previously fixed in 10% formalin. Although it is known that formol fixation affects protein antigens, this effect could possibly be reverted during treatment with citrate buffer and heat, helping antigen retrieval.

The results of this study are similar to the results carried out by Njaa et al. (2000), who found that out of 42 animals BVDV-positive by VI, 41 (97.6%) were also positive by IHC in skin biopsies from different parts of the body. In another study carried out by Grooms and Keilen (2002), using blood samples and skin biopsies from 332 calves between 1 and 4 months old, six (1.8%) animals were

BVDV-positive by VI and IHC. In these studies it was also concluded that IHC was a sensitive method, comparable with VI, for the detection of PI animals by BVDV. However, the results of our study differ from those of Cornish et al. (2005), who found that $ELISA_{Ag}$ and IHC detected 100% of the PI animals. The fact that both diagnostic methods had the same sensitivity in their study could be due to the fact that ear skin biopsies were used in $ELISA_{Ag}$ rather than serum. On the other hand, the results of our study also differ from those of Luzzago et al. (2006) who found that of 31 PI animals, 29 of these were positive by $ELISA_{Ag}$ and IHC, and 2 animals were positive by $ELISA_{Ag}$ but negative by IHC. The difference between the results of both studies could be due the fact that they were used different monoclonal antibodies for IHC. According to another study carried out by Kuhne et al. (2005) in which $ELISA_{Ag}$ was carried out on sera and ear biopsy samples of 11 calves PI by BVDV, no viral antigens were detected in sera after colostrum ingestion, as was found in our study, but viral antigens were detected in ear biopsy samples. It was concluded that $ELISA_{Ag}$ can be a reliable and economic method for the diagnosis of BVDV in skin samples. A possible explanation for the lack of BVDV antigen detection in sera by $ELISA_{Ag}$ could be that anti-BVDV antibodies are circulating in calves as a result of a humoral or acquired passive immunity response. In this way, the antigen sites of circulating viral particles could be camouflaged by antibodies present in serum, resulting in inhibition of virus-binding during $ELISA_{Ag}$ (Palfi et al., 1993; Grooms and Keilen, 2002; Brodersen, 2004; Zimmer et al., 2004; Sandvik, 2005; Hilbe et al., 2007). For this reason, it is not recommended that detection methods targeting viral antigens in serum are used in PI animals less than 3 months old, due to the presence of colostrum antibodies.

CONCLUSIONS

It is concluded that the immunopositive reaction to anti-BVDV antibodies in ear skin biopsies of PI calves was similar to the reported in previous studies (Njaa et al., 2000; Grooms and Keilen, 2002; Brodersen, 2004; Cornish et al., 2005; Luzzago et al., 2006), confirming the presence of the virus at this site. This study also confirms that IHC is an efficacious method for the early detection of PI animals, being more sensitive than $ELISA_{Ag}$, in sera. IHC can be used as a routine technique to detect BVDV and can be applied to retrospective studies using stored tissues. The identification and timely elimination of PI calves decreases economic losses, dissemination of the virus in the herd and therefore avoids the development of complete generations of PI animals.

References

1. Baszler TV, Evermann JF, Kaylor PS, Byington TC, Dilbeck PM: Diagnosis of naturally occurring bovine viral diarrhea virus infections in ruminants using monoclonal antibody-based immunohistochemistry. Vet Pathol; 1995; 32: 609-618.
2. Bolin SR, Grooms DL: Origination and consequences of bovine viral diarrhea virus diversity. Vet Clin North Am Food Anim Pract; 2004; 20: 51-68.
3. Brock KV: The many faces of bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract; 2004; 20: 1-3.
4. Brodersen BW: 2004. Immunohistochemistry used as a screening method for persistent bovine viral diarrhea virus infection. Vet Clin North Am Food Anim Pract; 2004; 20: 85-93.
5. Cornish TE, van Olphen AL, Cavender JL, Edwards JM, Jaeger PT, Vieyra LL, Woodard LF, Miller DR, O'Toole D: Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine diarrhea virus. J Vet Diagn Invest; 2005; 17: 110-117.
6. Fray MD, Paton DJ, Alenius S: The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. Anim Reprod Sci; 2000; 60-61: 615-627.
7. Fulton RW, Saliki JT, Confer AW, Burge LJ, d'Offay JM, Helman RG, Bolin SR, Ridpath JF, Payton ME: Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. J Vet Diagn Invest; 2000; 12: 33-38.
8. Grooms DL, Keilen ED: Sceening of neonatal calves for persistent infection with bovine viral diarrhea virus by immunohistochemistry on skin biopsy samples. Clin Diagn Lab Immunol; 2002; 9: 898-900.
9. Haines DM, Clark EG, Dubovi EJ: Monoclonal antibody-based immunohistochemical detection of bovine viral diarrhea virus in formalin-fixed, paraffin-embedded tissues. Vet Pathol; 1992; 29: 27-32.
10. Hilbe M, Stalder H, Peterhans E, Haessig M, Nussbaumer M, Egli C, Schelp C, Zlinszky K, Ehrensperger F: Comparison of five diagnostic methods for detecting bovine viral diarrhea virus infection in calves. J Vet Diagn Invest; 2007; 19: 28-34.
11. Kuhne S, Schroeder C, Holmquist G, Wolf G, Horner S, Brem G, Ballagi A: Detection of bovine viral diarrhoea virus infected cattle-testing tissue samples derived from ear tagging using an Erns capture ELISA. J Vet Med B Infect Dis Vet Public Health; 2005; 52: 272-277.
12. Liebler-Tenorioa EM, Lanwehra A, Greiser-Wilkeb I, Loehrb BI, Pohlenza J: Comparative investigation of tissue alterations and distribution of BVD-viral antigen in cattle with early onset versus late onset mucosal disease. Vet Microbiol; 2000; 77: 163-174.
13. Luzzago C, Frigerio M, Tolari F, Mazzei M, Salvadori C, Del Piero F, Arispici M: Indirect immunohistochemistry on skin biopsy for the detection of persistently infected cattle with bovine viral diarrhoea virus in Italian dairy herds. New Microbiol; 2006; 29: 127-131.
14. Mahlum CE, Haugerud S, Shivers JL, Rossow KD, Goyal SM, Collins JE, Faaberg KS: Detection of bovine viral diarrhea virus by TaqMan reverse transcription polymerase chain reaction. J Vet Diagn Invest; 2002; 14: 120-125.

15. Montgomery DL: Distribution and cellular heterogeneity of bovine viral diarrhea viral antigen expression in the brain of persistently infected calves: a new perspective. Vet Pathol; 2007; 44: 643-654.

16. Njaa BL, Clark EG, Janzen E, Ellis JA, Haines DM: Diagnosis of persistent bovine viral diarrhea virus infection by immunohistochemical staining of formalin-fixed skin biopsy specimens. J Vet Diagn Invest; 2000; 12: 393-399.

17. Palfi V, Houe H, Philipsen J: 1993. Studies on the decline of bovine virus diarrhoea virus (BVDV) maternal antibodies and detectability of BVDV in persistently infected calves. Acta Vet Scand; 1993; 34: 105-107.

18. Sandvik T: Selection and use of laboratory diagnostic assays in BVD control programmes. Prev Vet Med; 2005; 72: 3-16.

19. Zimmer GM, Van Maanen C, De Goey I, Brinkhof J, Wentink GH: The effect of maternal antibodies on the detection of bovine virus diarrhoea virus in peripheral blood samples. Vet Microbiol; 2004; 100: 145-149.

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