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< Short Communication >

Identification of Korean native cattle persistently infected with BVDV using Ear-notch method

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Abstract

Bovine viral diarrhea Virus (BVDV) infections cause respiratory, gastrointestinal, and reproductive problems, such as infertility, abortion, stillbirth, and sickly offspring. Many countries have reduced the economic damage through the application of different control programmes, and some have successfully eradicated BVD. Detection and elimination of cattle persistently infected (PI) with BVDV is important for BVD eradication because PI cattle are a main source of BVD transmission. In this study, the prevalence of Korean native cattle persistently infected (PI) with BVDV was investigated and determined in 49 farms with 3,050 cattle. The all samples were collected by ear notch sampling. Korean native cattle with initial positives on antigen-ELISA (Ag-ELISA) were sampled again after 3～4 weeks and cattle with second positives in both Ag-ELISA and immunohistochemistry (IHC) were identified as PI cattle. Among the 49 farms, 14 (28.6%) farms had at least more than one PI cow and 21 (0.69%) of 3,050 cattle were determined as PI cattle. As a result of this work, it is suggested that national BVD eradication program is required to reduce economic losses by BVDV infection in Korean cattle industries.

Key words : Bovine viral diarrhea virus, Cattle, Persistently infected

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is an important cattle pathogen that causes considerable economic losses worldwide (Houe, 1999; Brodersen 2004; Bachofen et al, 2010). In cattle industries, BVDV infections cause respiratory, gastrointestinal, and reproductive problems, such as infertility, abortion, stillbirth, and sickly offspring. Many countries have reduced the economic damage through the application of different control programmes, and some have successfully eradicated bovine viral diarrhea (BVD) (Rossmanith et al, 2005; Presi et al, 2011; Loken and Nyberg, 2013).

Detection and elimination of cattle persistently infected (PI) with BVDV is important for BVD eradication because PI cattle are a main source of BVD transmission (Brodersen, 2004; Houe et al, 2006). In Korea, there is no official BVDV control programme, and only a limited study on the prevalence of BVDV has been reported (Park et al, 2004; Lee et al, 2008; Oem et al, 2009; Oem et al, 2010). Although BVDV vaccination has been performed on some cattle farms, reproductive problems, such as stillbirths and malformations in neonatal calves, caused by BVDV infections are sometimes reported in Korea (Oem et al, 2009; Oem et al, 2010). In addition, there is little information available on the status of BVDV infections, such as the prevalence of PI cattle with BVDV. The purpose of this

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study is to investigate and determine the prevalence of Korean native cattle PI with BVDV before performing BVD national eradication program.

MATERIALS AND METHODS

In 2013, ear-notch skin samples measuring 6 mm in diameter were collected from 3,050 Korean native cattle on 49 farms. According to the manufacturer's recommendations for BVDV Ag/Serum Plus Test kit (IDEXX Laboratories, Inc., Liebefeld-Bern, Switzerland) for the detection of BVDV antigen in PI cattle, samples were collected using pliers designed for ear tagging and skin notch sampling, covered with the soaking buffer provided in the kit, and stored at −80℃ until further testing; samples were then processed as instructed by the manufacturer. Cattle with an initial positive Ag-ELISA result were resampled at ear-notch skin after 3～4 weeks and retested with Ag-ELISA (Cornish et al, 2005).

Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded blocks of ears. Briefly, paraffin sections of 5 μm thickness were deparaffinized in xylene and hydrated through a graded alcohol series, and then washed in distilled water. To enhance antigen retrieval, the tissue sections were soaked in heat-induced sodium citrate buffer (pH 6.0) for 30 min, cooled to room temperature, and then placed in 3% H_2O_2 in methanol for 10 min to block endogenous peroxidase activity. After blocking, the primary monoclonal anti-BVDV Ab 15C5 (1:500, Syracuse Bioanalytical, Ithaca, NY, USA) was incubated with the tissue sections in 37℃ overnight. The next day, tissue sections were stained with biotinylated anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA, USA) for 1 h at room temperature, washed, and incubated with VECTASTAIN® ABC Reagent (Vector Laboratories, inc., Burlingame, CA, USA) 37℃ for 30 min. After washing, The RedMap Kit (Ventana Medical Systems) served as a chromogen substrate, rinsed, counterstained with hematoxylin and bluing reagent (Ventana), mounted, and photographed. Negative control slides were prepared using isotype matched IgG at the same dilution as the primary antibody.

RESULTS AND DISCUSSION

BVDV antigens were found mainly in cytoplasm of keratinocytes of the epidermis, hair follicles, smooth muscle of vessels, and cytoplasm of histiocytes in the dermis of the ear (Fig. 1). Cattle with positive results in both tests were regarded as PI cattle. There was no discrepancy between results of two tests. Table 1 shows the results for the detection of BVDV antigen in the 3,050 ear-notch samples.

Overall, 21 of 3,050 (0.69%) cows and 14 of 49 (28.6%) farms were PI. The prevalence of PI cattle ranged from 0.62～0.88%, depending on the region examined

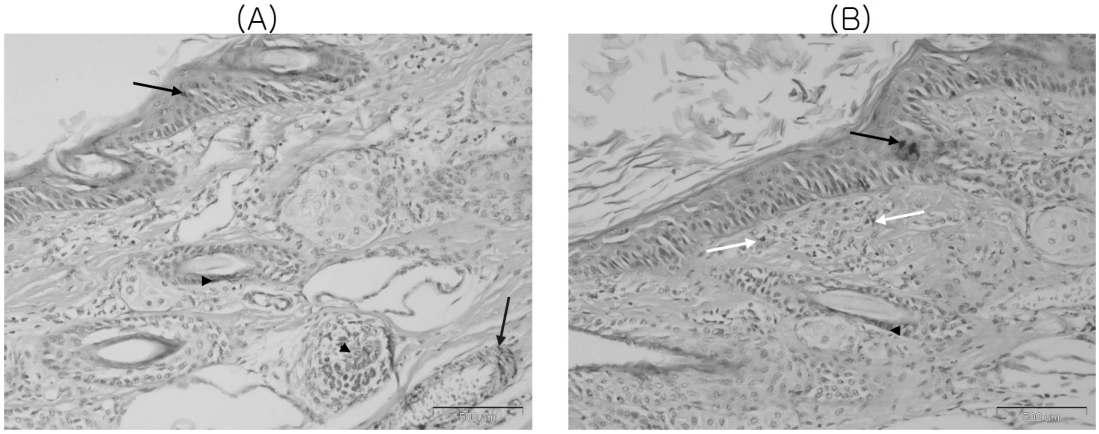

Fig. 1. Skin from the ear of Korean native cattle persistently infected with bovine viral diarrhoea virus (BVDV) Immunohistochemistry. BVDV Viral antigen was detected in the epidermal keratinocytes (black arrow head) hair follicles (black arrow head) (A), and infiltrating histiocytes (white arrow) in the dermis (B). ABC method, counterstain with Mayer's hematoxylin, ×400. Bar=100.

Table 1. The prevalence of persistently infected (PI) Korean native cattle

Region*	Number of Farms Tested	Number (%) of PI Farms	Number of Cattle Tested	Number (%) of PI Cattle
Northern	18	3 (16.7)	433	3 (0.69)
Middle	23	8 (34.8)	1933	12 (0.62)
Southern	8	3 (37.5)	684	6 (0.88)
Total	49	14 (28.6)	3050	21 (0.69)

*Northern, Gyeonggi and Gangwon provinces; Middle, Chungchong, Jeonbuk, and Gyeongbuk provinces; Southern, Gyeongnam and Jeonnam provinces.

(Table 1).

Our results are consistent with reports in the literature from other countries, which have reported that 0.1～2.0% of cattle were PI with BVDV (Letellier et al, 2005; Houe et al, 2006; O'Connor et al, 2007; Fulton et al, 2009). The farm prevalence of the Northern region (16.7%) was quite lower than those of the Middle and Southern regions (34.8% and 37.5%, respectively) (Table 1).

Current diagnostic methods used to detect PI cattle include virus isolation (VI), reverse transcription (RT)-PCR, Ag-ELISA, and IHC (Cornish et al, 2005). The Ag-ELISA was originally developed for use with serum samples but was modified by the manufacturer for use with ear-notch samples. IHC and Ag-ELISA using ear notches both had a high sensitivity (100%) for detecting BVDV infection in calves (Cornish et al, 2005; Hilbe et al, 2007). However, Ag-ELISA using serum samples may result in false-negatives because of interference from maternal antibodies (Hilbe et al, 2007).

Ear-notch sampling is recognised as a preferred method to detect PI cattle because it is easy to perform and does not require sophisticated equipment (Al-Khaliyfa et al, 2010). In particular, the diagnostic advantage of using skin samples instead of serum or plasma is that there is less interference from maternal antibodies at a very early stage of age when detecting PI animals (Cornish et al, 2005; Hilbe et al, 2007). Consequently, the current study adopted ear-notch sampling and Ag-ELISA and/or IHC methods to detect PI cattle in Korea.

Only a vaccination policy has been implemented to control BVDV infection in Korea. Despite vaccination, BVDV continues to cause significant economic losses to the cattle industry. Therefore, a programme for detecting PI cattle and removing them from the herds should be introduced to control BVDV infection and to reduce the economic losses resulting from BVDV infections in Korea.

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ETHICAL STANDARDS

The research performed complies with the current laws of South Korea.

CONFLICT OF INTEREST

None.

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