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SEROLOGICAL SURVEY OF BOVINE VIRAL DIARRHOEA VIRUS BY ANTIGEN CAPTURE ELISA IN DAIRY HERDS IN FARS PROVINCE, IRAN

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Summary

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Bovine viral diarrhoea virus (BVDV), a pestivirus from the Flaviviridae family occurs worldwide in cattle populations and causes considerable economic losses. The purpose of this study was to detect BVDV by enzyme linked immunosorbent assay in dairy herds. From blood samples routinely received for bovine viral diarrhoea diagnosis, 400 sera samples (from 12 industrial dairy herds) were examined in an antigen capture ELISA (ACE) using a commercial kit. BVDV antigen was detected in 16 cattle out of 400 tested (4%) and 8 of 12 farms (66.6%) were infected with BVD virus. Our study reveals that BVDV infection is widely present in industrial dairy herds in Fars province. Further investigations are needed for detection and eradication of the BVDV infection source (calves with persistent infection) in this region.

Key words: antigen capture ELISA, bovine viral diarrhoea virus, dairy herds, pestivirus

INTRODUCTION

Bovine viral diarrhoea (BVD) occurs worldwide in cattle populations and cause considerable economic losses. The causative agent is a pestivirus, family Flaviviridae (Simmonds *et al*., 2012). Two antigenically distinct genotypes by monoclonal antibodies directed against the E2 protein, or by genetic analysis (Pellerin *et al.*, 1994; Paton *et al.*, 1995; Baroth *et al.*, 2000; Ridapth *et al*., 2010) are known. BVD interferes with reproductive and immunological functions and causes subsequent losses due to reproductive disorders and impaired herd performance (Wentink *et al*., 1990). The ability of the virus to cross the placenta during the first trimester of pregnancy can result in the birth of immunotolerant and persistently infected (PI) calves which shed the virus during their entire lifespan (Kampa *et al.*, 2007).

Several diagnostic tests exist to detect infected cattle herds. Virus isolation, serological and molecular techniques have been used for the detection and identification of genotypes and subgenotypes of BVDV. The serological methods such as enzyme-linked immunosorbent assay (ELISA) are usually employed for diagnosis of BVDV in clinical samples (Fenton *et al*., 1991; Entrican *et al.*, 1995). ELISA has good sensitivity, specificity and repeatability for detecting antibodies against BVDV; it is easy to transfer, economical,

and easy to perform (Bleak & Ballagi-Pordawy, 1993; Pacheco & Lager, 2003). Antigen capture ELISA (ACE) is a relatively new assay available as a commercial test kit, using monoclonal antibodies to capture viral antigen *Erns* (gp44–48) and to detect antigen-antibody complexes (Brinkhof *et al.*, 1996; Saliki & Dubovi, 2004). The new generation of antigen-capture ELISA is able to detect BVD antigen in blood, plasma and serum samples.

Fars province is one of the major dairy cattle-breeding areas of Iran with at least 100 industrial dairy herds. The purpose of this study was detection of BVD infected cattle by ACE in dairy herds in Fars province, Iran.

MATERIALS AND METHODS

Animals and study design

The study was conducted on the industrial dairy herds in Fars province, Southern Iran. The population of the tested industrial dairy cattle herd complexes was between 200 to 600 cattle. All cows were of the Holstein breed, and all were housed in intensive systems. Samples were collected from 5-month to 5-year-old cattle (male and female) originating from 12 industrial dairy herds.

Blood collection

In order to detect bovine viral diarrhoea infection, 400 randomised blood samples of industrial dairy cattle herds were collected from the jugular vein into plastic test tubes and transported to the laboratory. In the laboratory, blood was centrifuged at 1800 rpm for 15 min and the serum collected. Two mL of serum was poured into the microtubes. The serum was then stored at –20 °C until testing.

Antigen capture enzyme-linked immunosorbent assay

All samples were tested using commercial BVDV Antigen Test Kit/Serum Plus (HerdChek, IDEXX Laboratories, IDEXX Switzerland AG), in which microliter plates were coated with anti-*Erns* monoclonal antibodies. The kit is based on the detection of the *Erns* (gp44–48) glycoprotein of the BVD virus. The sensitivity and specificity of the test as per manufacturer's instruction were 96.3% and 99.5%, respectively. Fifty μL of the detection antibodies were added to each well. Then, 50 μL of negative and positive controls were added into the appropriate wells. In the next step, 50 μL of sample was loaded into remaining wells and incubated for 2 hours at 37 °C and washed (by wash solution five times). Following the final washing, the plate was slapped vigorously down on a bench top covered with paper towels. After washing, 100 μL of conjugate was added and incubated for 30 min at room temperature. Then 100 μL of TMB substrate was added and the plates were incubated in the dark for 10 min. The reaction was terminated by addition of 100 μL of stop solution to each well. Finally, the absorbance was monitored in ELISA reader (BDSL Immunoscan Plus) at 450 nm. Optical density (OD) readings were determined, and the presence or absence of BVDV antigen in each sample was determined by calculating the sample to OD value (S-N) for each sample according to the manufacturer's directions.

RESULTS

The presence or absence of BVDV antigen in the sample was determined by the corrected OD value (S–N) for each sample as followed: S−N = Sample A_{450} – NCx. Samples with S–N values less or

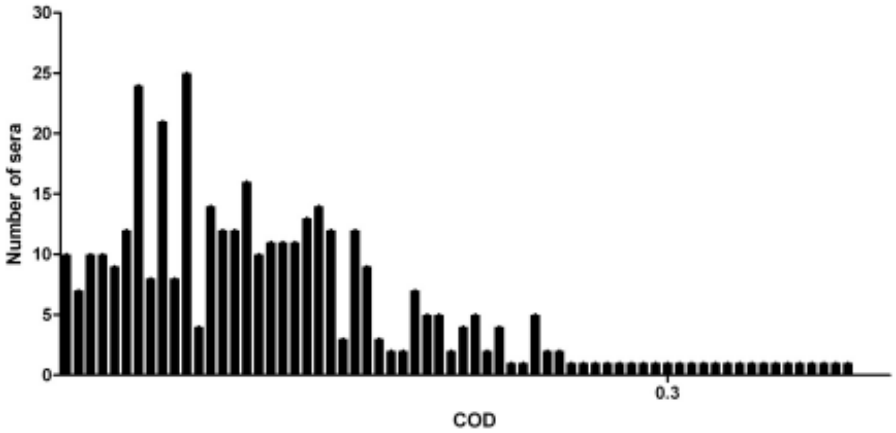

Fig. 1. Frequency distribution diagramme of corrected optical density (COD) values (S–N values) as measured by a commercial *Erns*-capture ELISA (HerdCheck BVDV antigen/Serum Plus) in 400 bovine sera.

equal to 0.3 were classified as negative and samples with S–N values higher than 0.3 – positive for BVDV antigen. NCx was the negative control.

BVDV antigen was detected in 16 out of 400 tested samples (4%) and 8 of 12 dairy herds (66.6%) (Fig. 1).

Most of the positive samples (10 cases) were from animals aged less than 2 years, four cases were from cattle between 2 to 3 years of age and 2 cases – from cattle more than 3 years old. Three of infected cattle were male and 13 – female.

DISCUSSION

Recent studies suggest that immunohistochemistry and ACE results correlate with virus isolation (Cornish *et al.*, 2005; Kuhne *et al*., 2005). An obvious advantage of an Ag ELISA using MAbs for both capture and detection of antigen is the avoidance of immune sera, which may cause variations in performance of the assay (Gottschalk *et al.*, 1992).

Bock *et al.* (1986) studied 886 blood sera from cows and blood sera from 6 calves experimentally infected with BVDV and found that ELISA was an efficient method for detection of BVDV during large scale screening of cattle for BVDV infection. Shannon *et al.* (1991) examined 418 animals in their research by different methods for detecting BVDV infection; at the end of the study they detected 108 animals as BVD antigen positive by ELISA. They declared antigen ELISA a suitable method for routine diagnostics on field samples.

Previously, BVD diagnostic work has usually been complicated by contamination of cell cultures and reagents with the virus or specific antibodies, necessitating careful monitoring of the test system. A major advantage of the ACE is the independence of cell cultures, making a rapid diagnosis from a local laboratory possible apparently without reduced fidelity (Sandvik & Korgsrud, 1995). Kampa *et al.* (2004) compared ACE and immunoperoxidase tests. They concluded that the

sensitivity and specificity of the ELISA was 100% in relation to immunoperoxidase, and that the agreement between the tests was perfect.

In our study, cattle (n=400) were sampled from 12 herds, from 5 months to 5 years of age, and most of the animals (n=220) were <2 years of age. Serological studies on BVDV in Iran indicated seroprevalences of 51.75% in Ahvaz (Haji Hajikolaei *et al.*, 2007), 23.32% in Shahrekord (Hemmatzadeh *et al*., 2001), 29.8% in Tabriz (Rezaeisaber *et al.*, 2011), 11.1% in Mashhad (Talebkhan Garoussi *et al*., 2007) and 51.58% in Tehran (Kargar *et al*., 1995).

Badiei *et al*. (2010) reported seroprevalence 59.46% for BVDV infection in dairy cattle herds and all of the herds were antibody positive against BVDV among industrial dairy cattle herds in a suburb of Shiraz. The prevalence rate of BVDV in aborted foetuses of Iran was estimated 18.49% (402 positive from 2,173 samples) by antigen capture ELISA (Safarpoor Dehkordi, 2011). In another study, 19 samples (7.4%) were positive for BVDV infection from 375 tested cattle by ELISA around Tehran (Kargar & Hemmatzadeh, 2004).

Out of a total of 157 cows within 18 dairy cattle herds in suburb of Mashhad – Iran, 57 (36.3%) were calves, 36 (22.9%) were heifers and 64 (40.8%) were adult dairy cows. Five (3.18%) animals from these herds were positive for BVDV by ACE (Talebkhan Garoussi *et al*., 2011).

According to a study performed in 2010 by a veterinary organisation in suburb of Shiraz (the center of Fars province) among dairy herds, the BVDV infection were detected in 2 dairy herds. We have obtained samples from these herds and established that the infection was detected again in both of them.

There is no programme for control and eradication of BVDV in dairy herds in Iran. Vaccination may be a useful tool to reduce losses by protecting native cattle against BVDV infection. For disease control, it is necessary to cull the animals with persistent infection and to prevent new entry of BVDV. Hashemi Tabar *et al*. (2010) reported 1.67% BVDV infection (from 120 samples) positive by ACE among the aborted dairy cows in industrial dairy cattle herds. According to our study, we can claim that cows with persistent infection may be the source of BVDV infection in dairy cattle in this region.

In conclusion, the results of this study indicate that BVDV infection is widely present among industrial cattle herds in Fars province, Iran. The results presented in this study confirmed previous reports of high BVDV infection incidence in this area of Iran. Further investigations are needed for detection of the source of BVDV infection (persistent infection) in this region,

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