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Prevalence of Bovine Viral Diarrhea Virus Antibodies among the Industrial Dairy Cattle Herds in Suburb of Shiraz, Iran

Khalil Badiei, Mohsen Ghane and Khodadad Mostaghni

Department of Clinical Sciences, School of Veterinary Medicine,
Shiraz University, Shiraz, Iran

Abstract: A cross-sectional study was made to investigate the prevalence of bovine viral diarrhea (BVD) virus using an indirect enzyme-linked immunosorbent assay (ELISA) test in industrial dairy cattle herds in suburb of Shiraz (Iran). Blood samples were collected from 994 dairy cows of different parities in 36 herds. None of the herds were vaccinated against BVDV. Cows were divided into different groups according to theherd geographical location (North, West, East and South of Shiraz city), the herd size (small, medium and large), parity and production level (low, average and high yielding cows). Results revealed that 512 (51.51%) cows were ELISA seropositive. However, the true BVDV seroprevalence was 52.43%. All of the herds were antibody positive against BVDV. The prevalence ranged from 11.8 to 100% within the herds. There were no significant differences between the presence of antibodies to BVDV and the herd size. The proportion of seropositive cows increased with their parity ($P<0.05$), BVDV seroprevalence of cows in different production groups were significantly different ($p<0.05$) but there was no difference among four geographical regions. According to these results, it was inferred that the presence of persistently infection (PI) animals within the herds in suburb of Shiraz-Iran, is responsible for the presence of antibody.

Key words: BVDV · Prevalence · Industrial dairy cattle · ELISA · Shiraz · Iran

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is considered as a worldwide pathogen with moderate to high prevalence of both exposed herds and seropositive animals within herds [1]. This virus (BVDV) is a single-stranded RNA virus in the genus *Pestivirus* within the Flaviviridae family [1]. The virus have two genotypes, categorised as genotypes I and II on the basis of genetic differences [2]. Estimations of the economic impact of the infection have been made in high milk producing countries such as England and Denmark, with annual national losses calculated at between 10 and 40 million US$ per calvings [3, 4].

Infection with BVDV results in a clinical spectrum ranging from subclinical to the highly fatal form known as ‘‘mucosal disease’’ [5]. Infection with BVDV can cause diseases of the alimentary and respiratory tracts and reproductive problems. Most primary infections in seronegative immunocompetent cattle are subclinical, but they can cause outbreaks of fever, loss of appetite, diarrhea, salivation, leucopenia and changes in platelet function [6]. The virus has been shown to have an immunosuppressive effect, it may enhance secondary bacterial and viral infections and it can cause reproductive problems such as embryonic death, mummification, abortion, stillbirth and a reduced rate of conception [6-10]. The measurement of antibody responses of animals exposed to BVDV either through a natural exposure or an immunization protocol is still a standard procedure. For BVDV, The test formats have been largely limited to enzyme-linked immunosorbent assays (ELISAs) [11].

ELISAs are versatile diagnostic methods for prevalence studies which can be used to detect almost any immunoreactive molecule. For BVDV serology have become popular for several reasons; independent of cell culture, easily applied for mass screening and test results can be read in a few hours [12, 13].

The purpose of this study was to investigate seroprevalence of BVDV in industrial dairy cattle herds in suburb of Shiraz-Iran (one of the major cattle-breeding areas of Iran) and to estimate the possible influence of

Corresponding Author: Khalil Badiei, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Tel: +98-711-2286940.

geographical location, herd size, different parity and milk production level on BVDV prevalence in these dairy cattle herds.

MATERIALS AND METHODS

The cross-sectional study was conducted on the industrial dairy herds in the Shiraz Suburb of Fars province, southern Iran. Fars province is one of the most important parts of the country contributing to dairy industry. Two dairy cattle production systems are described in this area. One is a system of small independent farms which make up about 38% of all the cows in suburb of Shiraz. The herd density is about 5-50 animals per farm with a low technology level and milk production (average milk yield about 3575 Kg/cow/year). The second system is the commercial industrial dairy herds with an approximate herd size of more than 50 cows, with more advanced technology and average milk production of about 193 Kg/cow/year. In this study, we included only commercial industrial dairy farms. The population of the tested industrial dairy cattle herd complexes was between 50 and 100. The total dairy cow population under the study was about 10000. All of the cows were Holstein breed. Cows had never been vaccinated against BVD. Most of the farmers used artificial insemination in their herds. The herds were stratified to small (50-100 cows), medium (101-200 cows) and large size (> 200 cows). The study was carried out with a random cluster sampling design, and herd selection was based on the geographical location and density of cattle in the region. Samples were collected from approximately 10 percent of herd population in 4 geographical regions: North, West, East and South of Shiraz City. Totally, 994 blood samples were tested from 36 industrial dairy cattle herds. In order to study the effect of cows' age on distribution of BVDV antibodies, cows were divided into different age groups (under 2 years old, 1 parity, 2 parity, 3 parity, 4 parity and over cows). The effect of level of milk production on distribution of BVDV antibodies was determined via grouping the cattle into low (<27 kg, based on daily milk production at day 50 postpartum), average (27-42 Kg) and high (>42 kg). Blood samples were taken from the tail vein into a plain vacutainer tube. The samples were allowed to clot at room temperature for 40 minutes and then centrifuged at 3000 X g for 10 minutes. The serum was collected and stored at - 20°C until analysis. Undiluted serum samples were tested for antibodies to BVDV using a commercially available indirect ELISA (SVANOVIR BVDV-Ab ELISA; Svanova Biotech) according to the manufacturer's instructions. Relative to the serum neutralization test, as in the manual, this assay has a sensitivity of 100 percent and a specificity of 98.2 percent. The plates were read in an automatic plate reader (Immunoskan Plus) at 450 nm and the results were expressed as optical density (OD). Samples with a corrected OD value below 0.20 were considered negative.

The Rogan and Gladen's [1978] correction of apparent prevalence were used for estimation of the true prevalence for seropositive of the samples. It was equated the true prevalence= (apparent prevalence +Sp-1)/ (Se+Sp-1) [1 4]. Differences in prevalence between the herds and group were tested using Chi-square statistical method. P value less than 0.05 considered statistically significant.

RESULTS

Results showed that 512 (apparent prevalence 51.51%) out of 944 samples were BVD-antibody positive (Table 1). However, 482 (48.89%) cows had corrected OD value below 0.20 and BVD-antibody Negative. However, the true prevalence of BVDV antibody-positive was 52.43%. All of the herds had antibody against BVDV. The prevalence ranged from 100-11.8% within the herds. The number of seropositive animals increased with the age. The infection rate in animals in different age and parity was shown in table 2. The number of seropositive animals in 1 and <2 years old cattle was significantly lower than the number of animals in other parity groups ($P<0.05$). The prevalence of seropositive animals in parity groups of 2, 3 and ≥4 were not significantly different (Table 2). No significant difference ($p>0.05$) was observed among BVDV prevalence in four different geographical regions; North, West, East and South of Shiraz city (Table 3). The herd size (small, medium and large) had no effect ($p>0.05$) on the BVDV seroprevalence (Table 4). There were significant differences among the prevalence of BVDV in different milk producing groups ($p<0.05$) and more seropositive animals were seen in average milk producting group but highest prevalence were seen in high producing cows (Table 5).

Table 1: Prevalence of BVDV-seropositive and seronegative cows in industrial dairy cattle herds in suburb of Shiraz-Fars

	Number of Herds	Number of Samples	Number (%) Seropositive	Number (%) Seronegative
Total	36	994	512 (51.51)	482 (48.49)

Table 2: Number and percentage of BVDV-seropositive and seronegative cows of different parities

Parity and Age	Number of samples	Number of seropositive	Number of seronegative
1 and <2 years old	312 (31.4%)	97 (18. 9%)*	215 (44. 6%)
2	186 (18.7%)	95 (18.6%)*	91 (18.9%)
3	195 (19.6%)	114 (22.3%)*	81(16.8%)
≥ 4	301 (30.3%)	206 (40.2%)*	95 (19.7%)
Total	994 (100%)	512 (51.51%)	482 (49.89%)

*Significant differences ($p<0.05$)

Table 3: Number and percentage of BVDV-seropositive and seronegative cows in different geographical regions in industrial dairy cattle herds in suburb of Shiraz-Iran

Location	Number of Samples	Number of Seropositive	Number of Seronegative
North	332 (33.4%)	167 (32.6%)	165 (34.2%)
West	268 (27.0%)	136 (26.6%)	132 (27.4%)
East	119 (12.0%)	71 (13.9%)	48 (10.0%)
South	275(27.7%)	138 (27%)	137 (28.4%)
Total	994 (100%)	512 (51.51%)	482 (49.89%)

Table 4: The seroprevalence of BVDV in small, medium and large herds in industrial dairy cattle herds in suburb of Shiraz-Fars (Iran)

Herd size	No. of herds examined	Positive	Negative		Positive	Negative
Small	3	3	Small	35(3.5%)	12(2.3%)	23(4.8%)
Medium	18	18	Medium	334(33.6%)	169(33%)	165(34.2%)
Large	15	15	Large	625(62.9%)	331(64.6%)	294(61.0%)
Total		36	Total	994(100%)	512(51.51%)	482(49.89%)

Table 5: Number and percentage of BVDV-seropositive and seronegative cows in No and low, average and high yielding dairy cattle

Production Levels	Number of Samples	Numbe of Seropositive	Number of Seronegative
No and Low	438(44.1%)	201(39.3%)*	237(49.2%)
Average	417(42.0%)	227(44.3%) *	190(39.4%)
High	139(14.0%)	84(16.4%)*	55(11.4%)
Total	994 (100%)	512 (51.51%)	482 (49. 89%)

DISCUSSION

The present results showed a relative high exposure to BVDV in dairy cattle herds in suburb of Shiraz-Iran. Using the indirect ELISA test, the true prevalence of antibody positive herds was 52.43% which had no much difference with apparent prevalence. Since vaccination against BVDV was not practiced in the herds in suburb of Shiraz, serological response reflected natural infection. Research based on the detection of BVDV antibodies, either in individual animals or in bulk milk, have been shown that the prevalence of BVDV within the herds is mostly in the range of 70% up to 100% [3, 4, 15, 16]. The BVD virus infection was determined in Iran according to a SN test by Mirshamsy *et al*. [17] which was estimated 16–69% positive in that study. However, in another study it increased up to 100% using a serum neutralization test (SNT) in dairy cattle herds of Tehran [16]. The relative high prevalence of antibodies to BVD virus found in this study indicates that the infection has an important presence in the bovine population of southern Iran. It was shown that the prevalence of seropositive animals in herds with one or more persistently infected (PI) animals was 87%; however, it was 43% in herds without PI animals [6]. Therefore, the high prevalence of antibodies to BVDV may be due to presence of PI animals in these herds. These results clearly demonstrated that the prevalence of antibodies to BVDV in dairy cattle in Shiraz suburbs does not differ greatly from the other reported surveys carried out in other part of the world. Prevalence of seropositive

animals of different age groups differed in our study and showed a tendency to higher risk of infection among older cows (>2 years old) compared to younger animals (aged <2 years). Mockelinien *et al.* [18] reported that seroinfection in animals older than 4 years was lower and younger cows (aged 1–4 years) were subject to a greater risk of infection. Also, the present results differed from that obtained by Houe and Meyling [15] who mentioned that the risk of BVDV infection was approximately similar in all age group in Danish dairy herds. BVDV antibodies in most cases are life long and the probability of infection in older animals during their life is more. Differing in the number of seropositive animals between the younger and older animals in other studies may be due to differences in herd size, cow rearing systems and animal keeping conditions. In the present study BVD virus antibody prevalence was not significantly different among four geographical regions. This might be explained by the fact that the animals were kept in intensive similar systems and minimum interchange of animals between herds occurred. Another factor could be the preponderance of artificial insemination rather than natural mating in the area. Results also showed that the herd size didn't affect the number of seropositive animals. It is probably due to the similar intensive management system in industrial dairy cattle herds around Shiraz- Iran. However, Houe and Meyling [15] showed that herds with higher cattle population density had higher prevalence of infection. The present results also showed that the BVD virus affected different classes of milk producing dairy cows to the same level. It is probable that the housing conditions of these different classes were similar and no action was taken to control the disease in herds. It has been reported that BVD infection in adult dairy cows can cause reduced milk yield [3, 4]. Due to high prevalence of seropositive animals in the region, control measures should be taken against BVD to optimize the milk production, especially in high producing dairy cows. Houe and Meyling [15] indicated that the high prevalence of seropositive BVDV animals in a herd is due to a recent or an ongoing infection most likely due to the presence of PI animals. This may also be true in our study.

Based on our results, it was concluded that BVDV seroinfection is widely present in industrial dairy cattle herds in suburb of Shiraz-Iran. It seems that more detailed studies should be undertaken to clarify different epidemiological aspects of BVDV in Shiraz as one of the important poles of dairy production in Iran.

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