

Serological and Virological Investigations of Bovine Viral Diarrhoea Virus (BVDV) Infection in Dairy Cattle Herds in Aydın Province

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Abstract: A total of 288 dairy cattle from 4 different closed barns were sampled in order to investigate the presence of bovine viral diarrhoea virus (BVDV) infection.

Based on the data gathered from neutralisation peroxidase-linked antibody (NPLA) tests, which were conducted to detect BVDV antibodies, all of the barns monitored previously had the infection; the rates of seropositive animals were between 44% and 100%. Overall, the results obtained from Aydın province showed that 248 of the 288 dairy cows (86%) monitored were seropositive.

BVDV antigen ELISA kits (Bio-X Diagnostics- Belgium) were used to detect BVDV antigen (NS3 protein) in blood samples. The cattle found to be positive in the first trial were subjected to subsequent sampling 45 days later. Fourteen animals (4.9%) that were BVDV Ag positive during both trials were diagnosed as persistently infected with the virus.

Key Words: BVDV, epidemiology, ELISA, persistent infection

Aydın İli ve Çevresindeki Sığırcılık İşletmelerinde Bovine Viral Diarrhoea Virus (BVDV) Enfeksiyonunun Serolojik ve Virolojik Olarak Araştırılması

Özet: Bu araştırmada, Aydın İli ve çevresinde bulunan, özel sektöre ait 4 farklı kapalı süt inekçiliği işletmesindeki toplam 288 hayvan örneklendi.

Örneklenen hayvanlarda, BVD antikorlarının tespit edilmesi için yapılan Neutralization Peroxidase Linked Antibody (NPLA) testi sonucunda, kontrol edilen işletmelerin tamamına (% 100) enfeksiyonun girdiği ve işletmeler bazında % 44 ile % 100 arasında değişen seropozitiflik saptandı. Yöre genelinde ise, örneklenen toplam 288 hayvandan 248 (% 86) adedinde seropozitiflik saptandı.

Araştırmanın virolojik kısmında, toplanan kan örneklerinde BVDV antijeninin (NS3 proteinin) tespit edilmesi amacıyla, Bio-X Diagnostics, Belçika, tarafından üretilen BVDV antijen ELISA kiti kullanıldı. Antikoagulanlı tüplere alınan kan örnekleri ELISA ile BVDV antijenleri yönünden incelendi, pozitif bulunan hayvanlar 45 gün sonra tekrar örneklenecek test edildi. Her iki örneklemede de BVD antijen yönünden pozitif olarak saptanan toplam 14 (% 4,9) hayvan persiste enfekte olarak değerlendirildi.

Anahtar Sözcükler: BVDV, epidemiyoloji, ELISA, persiste enfeksiyon

Introduction

Bovine viral diarrhoea (BVD), which was first reported in 1946, is one of the most serious gastrointestinal, respiratory, and reproductive infections leading to intrauterine infections and serious consequences in cattle.

Infection can result in entities ranging from subclinical infection to mucosal disease (1-3). The causative agent is bovine viral diarrhoea virus (BVDV), which is classified as a member of the Pestivirus family. The infection of pregnant cows during the first 80-120 days of gestation

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could cause births of immunotolerant calves persistently infected (PI) with BVDV (4). Intrauterine BVDV infections are serious problems, which cause high rates of abortion, still births, foetal resorption, mummification, congenital malformations, weak calf births, and growth retardation (4,5). BVDV has been implicated in the bovine respiratory disease complex and has often been associated with bovine herpes virus type 1 (BHV-1), bovine respiratory syncytial virus (BRSV), and parainfluenza virus type 3 (PI-3) (6).

Field isolates of BVDV have 2 biotypes, cytopathic (cp) and noncytopathic (non-cp), according to their abilities to form cytopathologic effects in cell cultures. The non-cp biotype of the virus is responsible for producing immunotolerated calves if the infection takes place within the first trimester of pregnancy (7,8). Superinfection of persistent infected viraemic animals with a cytopathic strain that is antigenically related generally causes a fatal mucosal disease (9). Persistent infected animals are the main source of epidemics and they continuously spread large quantities of virus (10). Acutely infected animals spread lesser quantities of virus and the clinical period lasts for only a few days, without detectable symptoms (11). Removal of persistently infected animals from the herd is the most effective method of control and prevention.

The prevalence of the infection in Turkey was reported to be between 62% and 80% by previous investigations (12-14).

The aim of this study was to investigate the serological status of BVDV infection and to detect the

prevalence of PI animals in randomly selected dairy herds of Aydin province.

Materials and Methods

Cell Culture and Virus

Primary foetal calf kidney (FCK) cell cultures were used for neutralisation peroxidase-linked antibody (NPLA) tests. The cells were tested for the presence of intrinsic BVDV using antigen capture enzyme-linked immunosorbent assay (Ag-cELISA) before the application. A non-cp isolate (0712/Hannover) of BVDV was used either in NPLA or in ELISA as a positive control. The titre of the virus was calculated by the method reported by Frey and Liess (15).

Animals and Sampling

The animals used in the study were selected from 4 private dairy farms in Aydin province. All animals of various ages reared in the herds were sampled during the study. A total of 288 animals were sampled from the 4 herds and the distribution of the samples of each herd is given in Table 1.

Animals that were found to be viraemic (n = 37) by the first Ag-cELISA were re-sampled 45 days later in order to verify the status of viral persistence.

Meanwhile, a standard questionnaire for collecting data including first calving age, number of inseminations for the last pregnancy, service period, and average milk yields of the animals was used to evaluate the reproductive performance of each herd.

Table 1. Seroprevalence of BVDV according to the herd and animals sampled.

| Herd Code | Number of Sampled Animals | Number of BVDV Antibody Carrier Animals | Seroprevalence (%) |
|-----------|---------------------------|---|--------------------|
| I | 41 | 41 | 100 |
| II | 61 | 54 | 89 |
| III | 69 | 67 | 97 |
| IV | 117 | 86 | 74 |
| TOTAL | 288 | 248 | 86 |

Blood Samples

Blood samples were collected from jugular veins for monitoring the serological and virological status of the animals against BVDV. For serological purposes, the blood samples were taken into tubes with coagulant (Kaolin) and were centrifuged in the laboratory at 3000 rpm for 10 min to remove the sera, which were then heat-inactivated at 56 °C for 30 min prior to testing.

The blood samples collected for virological purposes were collected in tubes with anticoagulant (EDTA). As recommended in the procedure of Ag-cELISA, fresh whole blood was used.

Antigen Capture Enzyme-Linked Immunosorbent Assay (Ag-cELISA)

The commercially available BVDV Ag-cELISA kit (Bio-X Diagnostics, Belgium) was used to diagnose the presence of BVDV antigen (protein NS3) in the blood samples. The test was performed according to the manufacturer's directions and evaluated spectrophotometrically with a 450 nm filter on an ELISA-reader.

Neutralisation Peroxidase-Linked Antibody (NPLA) Test

Tests were performed according to the method described by Holm Jensen (16). Briefly, a 1/5 dilution of each serum sample was mixed with an equal volume of the noncytopathogenic biotype of BVDV (0712/Hannover) in duplicate wells of the 96-well microplates and were incubated in a chamber containing a 5% CO₂ atmosphere for 1 h at 37 °C. Following the neutralisation, FCK cells resuspended in Dulbecco's minimum essential medium (DMEM) supported with 5% foetal calf serum (FCS) was added to the system. The 3-day incubation of the test plates under the same conditions was followed by heat fixation at 80 °C for 2-4 h. The cells were incubated with heterotypic polyclonal α -BVDV antibody conjugated with horseradish peroxidase (HRPO) for 1 h at room temperature. The reaction was visualised by adding colorigenic substrate (dimethyl formamide + aminoethyl carbazole) containing H₂O₂ (3%) and evaluated by microscopic examination on the basis of the presence of reddish-brown aggregates within the cells.

Results

Herd Data

Evaluation of the questionnaires revealed that the first calving age, number of inseminations for the last pregnancy, service period, and average milk yield of PI animals were close to the herd averages. The average age of the herds was noted as more than 5 years of age, while the eldest PI animal detected was 4.6 (mean: 3.1) years old. The remaining reproductive performance criteria of the PI animals were also within the herd ranges (Table 2).

Table 2. The averages of some reproductive parameters of PI animals and herds.

| Parameter | Average | |
|--|---------|----------|
| | of PI | of Herds |
| First calving age (months) | 32.3 | 28-35 |
| Number of inseminations for the last pregnancy | 1.9 | 1.4-2.2 |
| Service period (days) | 168 | 108-189 |
| Age (years) | 3.1 | > 5 |

NPLA Test

The seroconverted animals were detected in all farms used in the study. In all, 248 animals out of 288 (86%) were seropositive for BVDV. The seroprevalence of the disease was 100%, 89%, 97%, and 74% in farms I, II, III, and IV, respectively (Table 1).

Ag-cELISA

A total of 288 whole blood samples were tested by Ag-cELISA for BVDV Ag in order to identify viraemic animals. The first round of tests revealed that 37 animals were BVDV Ag positive. In the subsequent tests that were performed to define viraemic status, 14 animals were found to be persistent BVDV carriers. The distribution of the PI animals for individual herds is summarised in Table 3.

Discussion

Worldwide, there are many studies analysing the prevalence of BVDV infection. Even though different seroconversion rates are reported in these studies, most

Table 3. Number of persistently infected animals in each herd.

| Herd Code | Number of Blood Samples | Number of BVDV Ag Positives | |
|-----------|-------------------------|-----------------------------|---------------------------|
| | | 1 st Sampling | 2 nd Sampling* |
| 1 | 41 | 11 | 3 (7.3%) |
| 2 | 61 | 14 | 4 (6.6%) |
| 3 | 69 | 11 | 6 (8.7%) |
| 4 | 117 | 1 | 1 (0.8%) |
| Total | 288 | 37 | 14 (4.9%) |

* The second sampling was performed only to animals found to be viraemic at the end of the first sampling. Thus, results indicate number of PI animals.

of them report a rate between 60% and 85% (14,17-21). Likewise, the prevalence of PI animals varies and the majority of these studies report a prevalence between 0.5% and 4% (22-27).

Previous studies in Turkey revealed a rate of BVDV seropositivity between 62% and 80% (12-14). According to the records of the herd managers, the animals involved in this study were not vaccinated against BVDV and the serological cases were thought to have been the result of natural BVDV infections; therefore, the seroprevalence of the infection was 86% in the region. The seroprevalence of the infection among the herds varied between 44% and 100%. The prevalence of PI animals was 4.9%.

The rates of transient and/or persistent BVDV infections in the herds studied were higher than those reported elsewhere (22-25). This was thought to be caused by disregarding the proper use of preventive control methods. As a free-ranging type of management was utilised in the herds, viraemic animals that were in close contact with the other animals may have effectively exposed susceptible animals to the virus. This situation seems to be the best model of virus circulation between animals in this type of herd management.

The failure to apply the proper preventive and control measurements was most likely due to a lack of knowledge and economic difficulties. Apart from the primary losses the infection caused, the farmers were not aware of the fact that BVDV infection, by suppressing the immune system, could lead to predisposition to other infections, which are difficult to determine quantitatively. The calculation of the economic losses that the infection causes is quite complex; economic losses after epidemics

vary in terms of the immune state of the herd, the pregnancy of the cows throughout the infection period, and the strain of the virus that infected the animals. Due to ignorance and economic burden, the eradication of PI animals and vaccination cannot regularly be performed. It is known that PI animals are the main source of circulating BVDV within a herd. It is reported that especially in dairy herds, in which the animals are reared in close contact to each other, a new-born PI calf would be able to spread the disease to 90% of the other animals in the herd within the 3 months (28).

It is known that differences in the structure of cow populations, raising systems, and management techniques affect the prevalence of BVDV infection. In studies conducted in countries other than Turkey, it is reported that there is a correlation between population density and the seroprevalence of the infection (5). According to the results of this study, the high seroprevalence of the infection was thought to be due to the herds being large and the animals being in close contact. When the results of this study were examined, 37 animals were BVDV Ag positive in the first round of testing. Fourteen of them were found to be positive for BVDV Ag in the second sampling and accepted as PI animals (Table 3). Therefore, it was determined that 23 (8%) animals were transiently viraemic and the virus was eliminated thereafter. This indicates that the infection was highly prevalent within herds.

Reproductive performance data gained from the present study's questionnaires revealed that there was no significant variation between PI and the remaining animals in the herds. This situation may be explained by the short amount of time the PI animals were in the

herds. When the average ages (3.1) of these animals is taken into consideration, the time they have been in the herd may not have been sufficient for spreading the virus to susceptible animals. Furthering this possibility is another supporting datum: the fairly low number of susceptible females prior to their first insemination in comparison to rest of the herd.

Infectious diseases caused by certain viruses may have a negative influence on herd performances in dairy cattle farms. BVDV infection, as a significant one of those infections, should be routinely scrutinised in herds with the simplest diagnostic system. The results of this study

have emphasised once more that the number of PI animals may reach about 5% of the herd in traditional farms and they should be immediately removed from the herds, and heifers should be seroconverted by vaccination before their first insemination. Thus, some dramatic impacts of the presence of PI animals may be prevented.

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