Turk J Vet Anim Sci
26 (2002) 245-248

Research Article

The Prevalence of Bovine Viral Diarrhoea Virus (BVDV) Infections in Cattle and Existence of Persistently Infected Cattle in the Trakya Region

Seyyal AK
Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University, Avcılar, İstanbul - TURKEY

İbrahim FIRAT
Department of Pathology, Faculty of Veterinary Medicine, Istanbul University, Avcılar, İstanbul - TURKEY

H. Hakan BOZKURT
Department of Histology and Embryology, Faculty of Veterinary Medicine, Istanbul University, Avcılar, İstanbul - TURKEY

Veli GÜLYAZ
Veterinary Research Institute, Pendik, İstanbul - TURKEY

Kemal AK
Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University, Avcılar, İstanbul - TURKEY

Received: 10.10.2000

Abstract: The aim of this study was to determine the prevalence of BVDV infection and existence of persistently infected (PI) cattle in the Trakya region.

In total, 260 samples of leukocytes were isolated from dairy cows (65 samples) and breeding bulls (65 samples) and also from cows and bulls in slaughterhouses (65 samples from each). After two blind passages in foetal bovine kidney (FBK) cell culture, they were screened for BVDV antigen with indirect immunoperoxidase labelling. For detecting PI cattle among BVDV positive live animals, new samples were taken and labelled, approximately two months after the first sampling.

It was found that a total of 35 (13.46%) cattle (16 live and 19 slaughtered) were positive for the BVDV antigen. BVDV antigens were also detected in 4 of the 16 leukocyte samples, which were taken from 16 BVDV positive animals.

Key Words: Bovine Viral Diarrhoea Virus, immunoperoxidase, prevalence

Trakya Yöresindeki Sığırlarda Bovine Viral Diarrhoea Virus (BVDV) İnfeksiyonlarının Prevalansı ve Persiste İnfekte (PI) Hayvanların Saptanması Üzerinde Çalışmalar

Özet: Bu araştırmada BVDV infeksiyonunun Trakya yöresindeki prevalansı ve persiste infekte (PI) hayvanların saptanması amaçlanmıştır.

Bu amaçla, Trakya yöresinde özel sektör, kamuya ait ve halk elinde bulunan süt sığırı ve damızlık hayvanlardan (65 inek, 65 boğa) ayrıca bu yöredeki mezbahalara gelen dişi ve erkek hayvanlardan (65'er örnek) alınan toplam 260 lökosit örneği fötal dana böbrek (FDB) hücre kültüründe iki kör pasajdan sonra BVDV antijenleri yönünden indirekt immunoperoksidaz testi ile incelenmiştir. BVDV antijeni yönünden pozitif canlı sığırlardan PI hayvanları saptamak amacıyla ilk örnekleme tarihinden yaklaşık iki ay sonra alınan lökosit örnekleri yeniden test edilmiştir.

BVDV antijeni yönünden 260 lökosit örneğinin indirekt immunoperoksidaz testi ile incelenmesi sonucunda 19'u mezbahadan, 16'sı canlı hayvanlardan olmak üzere 35 (% 13,46) örneğin pozitif olduğu saptanmıştır. Pozitif sonuç alınan 16 canlı hayvandan 2. kez alınan lökosit örneklerinin 4'ünde (4/16) tekrar BVDV antijeni saptanmıştır.

Anahtar Sözcükler: Bovine Viral Diarrhoea Virus, immunoperoxidase, prevalans

* This project was supported by the Research Fund of the University of Istanbul. Project number:1126/010598

Introduction

Bovine viral diarrhoea virus (BVDV), which belongs to the genus Pestivirus in the family Flaviviridae, is common all over the world (1). BVDV is a pathogen of cattle associated with reproductive problems, ranging from abortion to congenital defects (2-5). Two pathogenic biotypes of BVDV, cytopathic (CP) and noncytopathic (NCP), have been described based on the presence or absence of visible cytopathic effects in vitro when susceptible monolayers are infected (2,5). Only NCP BVDV has been reliably shown to cross the placenta, invade the foetus and set up the persistent infection so crucial for successful virus spread. NCP BVDV is the cause of a wide range of congenital, enteric and other diseases. CP virus is usually associated only with mucosal disease (MD), a severe and invariably fatal disease now known to occur only in persistent infection (1,6,7). Virus persistence in all organs and the birth of a lifelong infected calf is the outcome of foetal infection before 100 days of gestation. Persistently infected (PI) animals shed the virus to the environment, infecting their neighbours; thus, a PI bovine introduced into a herd infects susceptible pregnant cows, inducing abortions and general reproductive failure (2,5,7,8). Pregnancy of a PI cow results in the birth of a PI calf (6,9). PI heifers reaching breeding age may have reduced fertility but those that do calve will always produce PI offspring and this has been shown to be an important cause of persistent high levels of infectious virus in some herds (10). To date, the seemingly healthy PI virus carrier is the most important source of pestivirus infection. Virtually all body excretions and secretions contain infectious virus, with nasal discharge and saliva being the most potent sources. The spread of virus from PI to susceptible animals will increase under farming conditions involving close physical contact among the animals (9,11). Theoretically, transmission of undetected NCP BVDV in association with bovine in-vitro-produced embryos might result in infection of embryo recipients, early embryonic death, abortion, or birth of persistently infected offspring (6).

Many studies have been carried out on BVDV infections in Turkey and their prevalence has been reported (12-15). Investigations have also been carried out to determine PI animals (16,17).

The aim of this investigation was to determine the BVDV infection prevalence and PI animals in the Trakya region.

Materials and Methods

Blood samples: The blood samples of 260 cattle were collected. One hundred and thirty (65 samples from dairy cows, 65 samples from bulls) of them were from the slaughterhouse and 130 samples (65 samples from dairy cows, 65 samples from breeding bulls) were from the field.

The age of the bulls from which samples were taken in the slaughterhouse ranged from 10 to 18 months. These animals were generally food animals with no apparent problem. The ages of the cows ranged from 4 to 6 years. In general these animals were brought to the slaughterhouse due to infertility problems.

The age range for privately owned animals was 1-6 years in males and 4-7 years in females. With the exception of 2 animals, none of the cows from which samples were taken had any health problem. One of these 2 animals was 6 years old and had been clinically diagnosed with metritis. The cow had been inseminated twice but had not become pregnant. The other cow was 4 years old and had also been diagnosed with metritis. This cow was being treated with antibiotics. The male animals had no health problems.

Immunoperoxidase staining kit: The peroxidase test was performed using a commercial kit (IPEX-BVD kit, Central Veterinary Laboratory, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom).

Virus: Non-cytopathic BVDV strain, which was obtained from Pendik Veterinary Control and Research Institute, originated from the Department of Virology, Faculty of Veterinary Medicine, University of Ankara.

Cell culture: Primary culture of foetal bovine kidney (FBK) was prepared for BVDV isolation, using Glasgow-essential medium (GMEM, Sigma) and gamma-irradiated foetal calf serum.

Methods

Preparation of buffy coat: Buffy coat cells were prepared from blood samples with EDTA by centrifugation at 2000 rpm for 10 min. Plasma was removed and the leukocyte-rich layer was recovered. The cells were washed three times in PBS, and resuspended in foetal calf serum with10 % dimethylsulfoxide and stored in a deep-freeze.

Cell culture and virus isolation: Cell cultures were prepared as described elsewhere (18). The cultures were

routinely checked for BVDV with indirect immunoperoxidase before use. Primary monolayer FBK cell cultures were resuspended and 100 ml amounts of a suspension of FBK cells (approx. 1.5 x 10^5 cells ml^{-1} in Eagle's MEM with 5% BVDV antibody-free calf serum and 50 µg gentamycin ml^{-1}) were dispensed into the wells of six well plates in which coverslips were placed.

Buffy coat cells were added to primary FBK monolyer cell cultures in the flasks. After two blind passages, the culture fluids harvested from frozen FBK cell cultures were inoculated in monolyer cells on the coverslips. The cells were then tested for BVDV with indirect immunoperoxidase.

Indirect Imunoperoxidase test: The procedure was employed as described in the IPEX-BVDV kit. The test was performed using monoclonal antibodies (Mab mix). The cells on the indirect immunoperoxidase were acetone fixed and the coverslips were washed with wash fluid for 3x5 minutes (0.01 M PBS, pH 7.6, 25% Tween 80). Mab mix was added at an optimal dilution of 1:100 in dilution fluid. After 15 min of incubation at 25ºC , the coverslips were washed as before. The final step was the addition of a freshly prepared solution of substrate (18 mg of DAB in 30 ml of 0.001 M PBS, pH 7.6, supplemented with 12 mg of sodium perborate tetrahydrate) for 15 minutes. The coverslips were washed and counterstained with Harris' haemotoxylin.

New buffy coat samples were obtained from live cattle two months after the initial sampling and BVDV antigens were examined by immunoperoxidase tests.

Results

Results obtained from buffy coats taken from the slaughterhouse: BVDV antigens were found in 15 of 65 cows and 4 of 65 bulls by indirect immunoperoxidase test in cell cultures. The BVDV antigen was found in 19 of a total of 130 animals (Table 1).

Results obtained from buffy coats taken from live animals: BVDV antigens were determined in 8 of 65 cows and 8 of 65 bulls owned privately in large and small institutions. Sixteen of the 130 live animals were found to be positive with respect to the BVDV antigen (Table 1).

Persistently Infected Animals: In 4 of the positive 8 female and 8 male live animals, the BVDV antigen was detected again. Two of these animals were female and 2 were male (Table 2).

Discussion

BVDV is a highly successful and important pathogen which infects ruminant species world-wide. The presence and prevalence of disease has been determined in various studies in Turkey (12-17). In the investigation carried out by Gelfert (13), the prevalence in Turkey was found to be 60%. Özkul et al. (15) determined the seroprevalence to be 68.8% and virus isolation to be 2.42% in 538 ruminants with genital tract disorders. While finding 79.5% seropositivity in 142 ruminants, Şimşek and Öztürk (16) also determined 1.41% viraemic animals.

Table 1. The results of Immunoperoxidase test in the buffy coats of 260 cattle.

	Sex	No. of samples	Positive	%	Negative	Total Positive	%
Slaughterhouse	female	65	15	23.07	50		
	male	65	4	6.15	61		
						35	13.46
Live Animal	female	65	8	12.31	57		
	male	65	8	12.31	57		

Table 2. Leukocyte sample results taken 2 months later from positive live animals in which the BVDV antigen was found.

Sex	Positivity in First Sampling	Positivity in Second Sampling	Proportion of PI Animals	Total Proportion
female	8	2*	2/8	4 /130
male	8	2	2/8	3.07%

* Animals with clinically diagnosed disorders.

In this study, 13.46% viraemic animals were found in 260 samples. Of the 130 samples that were obtained from a slaughterhouse, 65 were cows above 4 years of age which had infertility problems. The ratio of 23.07% in the results shows that infection plays a significant role in animals with these problems. Results obtained from samples belonging to healthy male specimens taken from the slaughterhouse are lower than those of females at a rate of 6.15%. However, this is a high value when compared to the findings of other researchers. The fact that these samples were taken from the slaughterhouse and that a second sampling could not be done has led to inadequate information as to whether or not this infection is persistent or acute. The infection prevalence was seen to be high at 12.31% also in animals with no apparent health problem (except 2 females).

Persistently infected ruminants have a constant viraemia and excrete virus continuously, especially in nasal secretions and saliva (9,11). Studies have been carried out to determine persistently infected animals in Turkey. Şimşek and Öztürk (16) determined BVDV antigens in 2 of 142 animals. Second leucocyte samples were obtained from 2 ruminants 2 months after the initial sampling and BVDV antigens were not detected. Özkul et al. (17) determined animals persistently infected with pestivirus at rates of 0.5%, 0.12% and 0.12% respectively in 3 of 5 dairy institutions.

In this study, 16 positives were found in 130 animals in the first sampling, of which 8 were cows and 8 were bulls. In the leukocyte samples taken from these animals 2 months later, only 4 were positive (3.07%). Two of these 4 animals were female and had clinical genital system disorders. This 3.07% PI animal presence is also high when compared to the findings of other researchers.

The fact that the results obtained both from samples from the slaughterhouse and from live animals were higher than those reported by other researchers reveals the necessity of studying BVDV infection in the Trakya region.

References

1. Wengler, G.: Flaviviridae. In Classification and Nomenclature of Viruses. Fifth report of the International Committee On Taxonomy of Viruses, eds. R.I.B. Francki, C.M. Fauget, D.L. Knutson, F. Brown. Archives of Virology (SupplE. 2) 1991, 223-233.
2. Baker, J.C.: Clinical aspects of bovine virus diarrhoea virus infection. Rev. Sci. Tech. off. Int. Epi., 1990, 9, 25-41.
3. Kirkbride, C.A.: Etiologic agents detected in a 10-year study of bovine abortions and stillbirths. J. Vet. Diagn. Invest. 1992, 4: 175-180.
4. Munoz, D.P., Lager, I.A., Mersich, S., Zabal, O., Ulloas, E., Schudel, A.A., Weber, E.L.: Foetal infections with Bovine Viral Diarrhoea Virus in Argentina. Br. Vet. J. 1996, 152, 175-182.
5. Nettleton, P.F., Entrican, G.: Ruminant Pestiviruses. Br. Vet. J. 1995, 151, 615-641.
6. Givens, M.D., Galik, P.K., Riddell, K.D., Stringfellow, D.A.: Uterine tubal cells remain uninfected after culture with in vitro produced embryos exposed to Bovine Viral Diarrhea Virus. Vet. Microbiol., 1999, 70, 7-20.
7. Brownlie, J.: The pathogenesis of bovine virus diarrhoea virus infections. Rev. Sci. Tech. off. Int. Epi., 1990, 9, 43-59.
8. McGowan, M.R., Kirkland, P.D.: Early reproductive loss due to Bovine Pestivirus Infection. Br. Vet. J. ,1995, 151, 263-270.
9. Meyling, A., Houe, H., Jensen, A.M.: Epidemiology of bovine virus diarrhoea virus. Rev. Sci. Tech. off. Int. Epi., 1990, 9, 75-93.
10. Staver, P.J., Journee, D.L. H., Binkhorst, G.J.: Neurological disorders, virus persistence and hypomyelination in calves due to intrauterine infections with bovine virus diarrhoea virus II. Virology and epizootiology. Vet. Quart., 1983, 5,156-164.
11. Kobrak, A., Weber, E. L.: Bovine diarrhea virus: an update. Rev. Argent. Microbiol., 1997, 29, 47-61.
12. Alkan, F.: Arthrogrippothyphosa ve Hydranencephaly' li Buzağı Doğumlarında Bovine Viral Diarrhea-Mucosal Disease (BVD-MD) 'in insidensi üzerinde araştırmalar. A. Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi, 1989, Ankara.
13. Gelfert, C.C. : Epidemiological investigation of the distribution of BVD virus among cattle in Turkey. Inavgural- Dissertation, Tieraerztliche Hochschule, 1991, Hannover.
14. Burgu, İ., Özkul, A.: Detection by cultural isolation of bovine virus diarrhoea (BVD) virus following field infections in cattle and their fetuses in Turkey. Dtsch. Tierärztl. Wschr., 1993, 100,361-363.
15. Özkul, A., Çabalar, M., Bilge, S., Akça, Y., Burgu, İ.: Süt sığırcılığı işletmelerinde rastlanan IBR/IPV ve BVD virus enfeksiyonlarının infertilite olgularındaki rolü. Ankara Üniv., Vet. Fak. Derg., 1995, 42, 381-387.
16. Şimşek, A., Öztürk, F.: Klinik olarak sağlıklı sığır sürülerinde persiste Bovine Viral Diarrhea virus enfeksiyonlarının araştırılması ve epizootiyolojik önemi. Vet. Bil. Derg., 1997,13, 113-119.
17. Özkul, A., Alkan, F., Akça, Y., Burgu, İ.: Süt sığırcılığı işletmelerinde pestivirus infeksiyonlarının epidemiyolojisi: Viral Persistens. III.Ulusal Veteriner Mikrobiyoloji Kongresi, 1998, 115-116.
18. Cunningham, C. H.: A laboratory guide in virology. Sixth ed., United States, 1966.