Serological Investigation for Bovine Viral Diarrhea and Bovine Herpesvirus Type-1 Viruses in Precolostral Calves and Their Dams**

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Abstract: In this study, BVDV and BHV1 infections were examined serologically in dams and their calves before colostrum intake to determine the status of the infections during pregnancy. For this purpose, total of 162 cows and their calves from 3 organised dairy herds and 7 family type small enterprises were sampled. Obtained 324 sera samples was tested using standard Virus Neutralisation test (VNT) to determine antibody presence and titration values. BVD seropositivity in dams was varied between 77.7% and 100% in organised herds, while lower proportion (57.1%) was detected in small farms. The rate of the seropositivity in neonatal precolostral calves were determined as 10% (3/30) and 14.6% (17/116) in herd 2 and 3, respectively. Highest value was detected in family type enterprises as 28.5% (2/7) for BVDV. In total, out of 162, 155 dams (95.6%) and out of same number of calves, 22 (13.5%) was found to be BVDV seropositive. BHV1 proportions was 70.9% in organised herds while 42.8% in small farms, in total 69.7% (113/162) in dams. Only one calf was found to be positive for BHV1 with 1/32 titer while its dam has 1/64. It was concluded that, precolostral antibody examination could be a usefull tool for detection of circulated viruses and incidence of the infections in herds due to express of immuncompetent period of the foetuses. Additionally, natural intra-uterine BVDV infection was determined by many reports but BHV1 infection was revealed for the first time with this study.

Keywords: *Bovine herpesvirus type 1, bovine viral dirrhea virus, cattle, precolostral antibody, vertical transmission*

Prekolostral Danalarda ve Onların Annelerinde Bovine Viral Diarrhea ve Bovine Herpesvirus Type-1 Viruslarının Serolojik Olarak Araştırılması

Özet: Bu araştırmada inekler ve onların henüz kolostrum almamış yavruları, gebelik dönemindeki BVDV ve BHV1 enfeksiyon durumlarını belirlemek için serolojik olarak incelendi. Bu amaçla, süt sığırcılığı yapan üç adet organize ve yedi adet aile tipi küçük işletmeden toplam 162 inek ve yavruları örneklendi. Elde edilen 324 serum örneği antikor varlığı ve titre değerlerinin belirlenebilmesi için standart Virus Nötralizasyon (VN) kullanılarak test edildi. Annelerdeki BVD seropozitiflik oranı organize sürülerde %77.7 ile %100 arasında değişirken, küçük işletmelerde daha düşük bir oran (%57.1) belirlendi. Yenidoğan prekolostral buzağılarda ise sürü 2 ve 3'te oranlar %10 (3/30) ve %14.6 (17/116) olarak bulundu. Buzağılarda en yüksek BVDV oranı, %28.5 (2/7) ile aile tipi işletmelerde tespit edildi. Toplam olarak, annelerin %95.7'sinin (155/162) ve aynı sayıda 22'sinin (%13.6) BVDV seropozitif olduğu belirlendi. BHV1 oranları organize işletmelerde %70.9 iken küçük çiftliklerde %42.8, toplamda ise %69.7 (113/162) idi. Sadece tek bir yavru 1/32 titre ile BHV1 için antikor pozitif olarak bulunurken, annesi ise 1/64 değerine sahip idi. Sonuç olarak, prekolostral antikor muayenesi fötüslerin immunkompetent dönemlerini ortaya koyduğu için, sirkülasyonda olan virusların ve sürülerde enfeksiyonların insidenslerini ortaya konması için yararlı bir araç olabileceği sonucuna varılmıştır. Ek olarak, her ne kadar doğal intra-uterin BVD enfeksiyonu birçok çalışmayla bildirilmiş ise de, BHV1 enfeksiyonu ilk kez bu çalışma ile ortaya konulmuştur.

Anahtar Kelimeler: *Bovine herpesvirus tip 1, bovine viral dirrhea, sığır, prekolostral antikor, vertikal bulaşma*

Introduction

Bovine Viral Diarrhoea/Mucosal Disease (BVDV/MD) complex diseases (Olafson et al., 1946; Ramsey and Chivers, 1953) were caused by a pestiviruses in *Flaviviridae* family. The agents are enveloped and include a positive sense single stranded RNA genome with a length 12.3kbp (Horzinek 1990). Pathological feature of Bovine Viral Diarrhoea is mainly bound to interactions of cyto-pathogenic (cp) and non-cytopathogenic (ncp) biotypes and stage of pregnancy. The virus can penetrate placenta and infect the foetus. The infection with cp strain in first trimester is generally results with abort or foetal resorbtion while the foetal development continues in the ncp strain BVDV infections. These animals may be born clinically normal but they will be persistently infected (PI) in the rest of their life (Malmquist 1968; Coria and McClurkin, 1978). PI individuals may be born with low birth weight, and they have growth retardation and lower level of immunity. Therefore, they are vulnerable to other infectious diseases (Reggiardo and Kaeberle 1981; Potgieter et al. 1984). Clinical picture of BVDV infection is mostly silent or mild in adults. Common findings

are leucopenia, salivation, cough, depression, infertility, anorexia, diarrhoea and ulserations in mouth (Grahn et al., 1984; Brownlie, 1990; Reuter et al., 1991).

Infectious Bovine Rhinotracheitis is an Alphaherpesvirus named bovine herpesvirus type 1 (BHV1) in subfamily, *Varicellovirus* genus. Based on genome analysis and viral polypeptide patterns, five subtypes were determined until now. Type 1 and 2a are responsible for respiratory form of the infection, where as 2b mainly causes many infections such as infectious pustular vulvovaginitis (IPV) and infectious pustular balanopostitis (IPB). Subtype 3a and 3b causes encephalitis (Wentink et al. 1993). BHV1 infection is cause to variety of disorders in respiratory, reproductive, gastrointestinal and nervous systems (Kendrick 1973; Narita et al., 1982; Jericho 1983). Abortion mostly occurs in the second half of gestation period, foetal death and absorption in the early pregnancy may be evaluated as infertility (Kendrick 1973; Miller et al., 1978). Antibody (Ab) fractions may not pass to the foetuses due to syndesmochorial placenta feature in cattle. Immun system of the cattle foetuses develops among 90 and 120. days of the gestation (Schultz 1973). In the case of in-utero infectios of foetuses after immun system formated, the non-fatal infections generally result with recovery and birth of seropositive calves (Kendrick, 1971; Scott et all., 1973). Both BVDV and BHV1 infections are quite common in Turkey like many countries in the world.

The objective of this study was to determine the profile of BVDV and BHV1 infections in organised dairy herds and small enterprises in Afyonkarahisar province, Aegean region, Turkey.

Materials and Methods

Sampled animals

In this study, blood serum samples were obtained from 162 clinically normal cows and their calves, which were sampled at the precolostral time. 155 samples were collected in three organised dairy cow enterprises and 7 cow and their precolostral calves were obtained from family type small farms in Afyonkarahisar province, Central Anatolia. Total of 324 blood serum samples from precolostral calves and their dams were collected. The sampling was performed just after the birth from dams and their foetuses by veinpuncture of Vena Jugularis before colostrum intake. All of the sampled animals were clinically normal during sampling. First two organised herds were medium scale herds and the third herd was a big dairy enterprise. There was no detailed record of the herds 1 and 2 but mastitis and high insemination number, prolonged service period and three abort cases was reported by the owners of the herds. Total of 1580 cattle has been breeding in herd three, 464 of them were male animals with different ages and has been keeping in a separate barn in the farm. Rest of the female 1.116 animals has been reared in a two group as young and fertiles. Detailed heath records were obtained from this farm.

The animals were vaccinated with multivalent vaccines including BVDV and BHV1 two years ago. According to the farm records, diarrhoea and pneumonia in calf and different reproductive problems were increased especially in last one year. Few numbers of the samples (7) was obtained from seven family type small farms which have among 1 and 4 cattle in each of them. Total of 324 serum samples was used in the study. All animals had no history of diseases that could be attributed to a natural infection in the time of sampling. Obtained blood samples were centrifuged at 3000g for 10 min. and separated into the stock tubes, and stored at -20^{o}C until to the test.

Cell Culture, Medium and Test Viruses

Madine Darby Bovine Kidney (MDBK) Cell Line was used for propogation of BHV1 ve BVDV, titration and Virus Neutralization tests. As a medium, Dulbecco's Modified Minimal Essential Medium (DMEM) and Foetal Calf Serum (FCS) (5-10%) were used in the study. For the detection of specific antibodies for BVDV and BHV1, reference virus strains "NADL" and "Colorado" were employed. Tissue culture infective dose of the viruses (100DKID$_{50}$/0,05ml) were determined as $10^{-3.5}$ and $10^{-4.5}$ for BHV1 and BVDV, respectively.

Virus propagation and Titration Test

BHV1 and BVDV viruses were inoculated to the cell culture. Later on flasks were freezed at -80^{o}C, thawed and centrifuged in 4000 rpm for 20min. Virus suspensions were portioned and frozen. One tube was diluted in Log 10 basis up to the 10^{-8} point and carried into the four wells of tissue culture plates as 0.1ml and cell culture suspension (300.000cells/ml) was added as 50µl. Plates left to the incubator with 5% CO_2. Every day wells were controlled using inverted microscobe and results were evaluated according to Spearman-Karber (1964) method.

Serologic examination

Sera samples were examined for the presence for BVDV and BHV1 specific antibodies using standard Virus Neutralisation test (VNT) (Frey and

Liess, 1971). For this purpose, serum samples were diluted with medium as 1/5 for BVDV, and pure serum used for BHV1. Every sample was putted into two wells of the tissue culture plates (50µl) with same volume of virus suspension containing approximately 100$TCID_{50}$ into per 50 µl. After incubation (1h for BVDV and 2h for BHV1), 50µl cell suspension (300.000cells/ml) were added and incubated at 37°C, 5% CO_2 for 24-72h. Test results were evaluated on the basis of micromorphology of cells using inverted microscope. Later on, all Ab positive sera were diluted in a series as 1/5, 1/10….1/640 for BVDV and 1/1, 1/2….1/512 for BHV1 and VNT test applied to determine Ab titer values (Serum Neutralisation$_{50}$, SN_{50}).

Results

Total of 324 blood serum samples from precolostral calves and their dams were controlled using VNT, 1/5 and higher serum dilutions were accepted as positive for BVDV and 1/1 and higher dilutions for IBR. BVDV infection was found to be more prevalent than IBR. Seropositivity for BVDV was detected in herd 1, 2 and 3, 77.7%, 100 and 98.2%, respectively. The lowest proportion was observed in family type enterprises as 57.1%. , 155 of 162 cow samples (95.6%) were found to be positive. All of the calves in herd 1 were negative. Proportion of calves were determined as %10 (3/30) and %14.6 (17/116) in herd 2 and 3, respectively. The highest rate in calves was observed in family type enterprises as 28.5% (2/7). BHV1 specific antibodies were also detected in all of the studied herds. Family type farms showed lowest seropositivity rate with 42.8% (3/7). Proportion of IBR positive cows was among 66.6% and 73.3% in organised herds. In total, out of 162 cows, 113 (69.7%) was found to be positive. Unlike BVDV, the number of IBR positive calves was very low, only one showed seropositivity with 1/32 titer in herd 3, the mother of this calf has 1/64 value (Table 1).

Table 1. Serologic test results for BVDV and BHV1 in precolostral calves and their dams.

	No of sampled animals	Herd Size	BVDV				BHV1			
			Cows		Calves		Cows		Calves	
			Ab(+)	(%)	Ab(+)	(%)	Ab(+)	(%)	Ab(+)	(%)
1	9	108	7	77.7	-		6	66.6	-	-
2	30	145	30	100	3	10	22	73.3	-	-
3	116	1.116	114	98.2	17	14.6	82	70.6	1	0.8
4*	7	1-4	4	57.1	2	28.5	3	42.8	-	-
Total	162		155	95.6	22	13.5	113	69.7	1	0.6

**; Family type small private enterprises*

The total of 155 cows was found to be positive for BVDV, Ab titer values was among 1/5 and 1/320, peak value was observed in 1/40 point (Figure 1). Ab titers was varied between 1/10 and 1/80 of BVDV seropositive 22 calves. The highest rate of the calves was found in the serum Ab titer 1/40. As could be seen in Figure 2, titer was slightly higher in dams and all other seropositive cows. IBR specific Ab titer distribution in the cows were among 1/1 and 1/64 but most of the animals have titers among 1/2 and 1/32 (Figure 3).

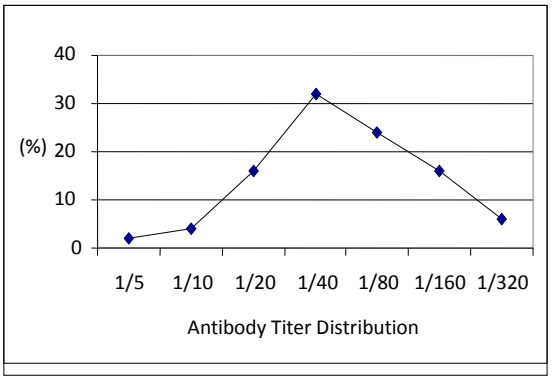

Figure 1. The antibody titer values of the BVDV positive cows.

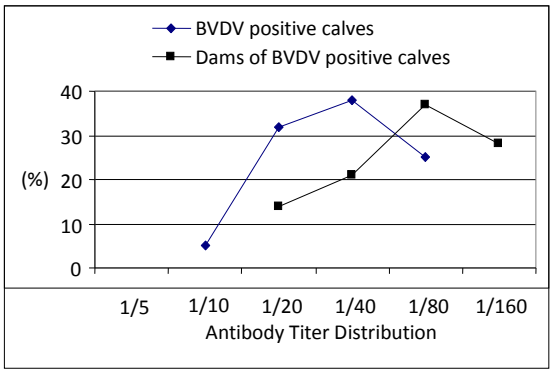

Figure 2. Antibody titers for BVDV of the calves and their dams.

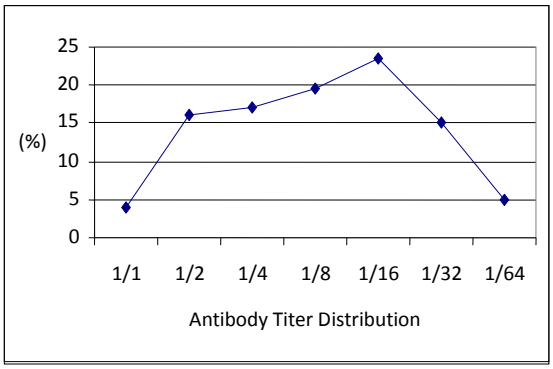

Figure 3. Antibody titer values of the BHV1 positive cows.

Discussion

BVDV and BHV1 infection are quite common infections in many parts of the world. Due to latent-persistent characters, effects of the both infections on various organ systems could be life-long and may cause to economic loss as direct and indirectly. Virus infections in the pregnant cows generally results with foetal infections (Brown et al., 1979; Bielefeldt-Ohmann, 1983; Straver et al., 1983; Brownlie, 1990). BVDV is one of the most studied infections in terms of pathogenesis. Outcomes of the fetal infection are mainly bound to biotype of the virus and stage of the pregnancy. Immune competence status of the individuals is directly determines the pathogenetic pathway. Syndesmochorial placenta type in cows does not allow transfer any king of gamma globulins to the foetus. Examination the precolostral calves as serologically gives important data for recent infections status in herds (Classick and Fernelius, 1970).

In this study, Precolostral calves and their dams were serologically investigated for the presence of antibodies against Bovine Herpesvirus 1 and Bovine Viral Diarrhoea using virus neutralisation test.

There was 108 animal in herd 1 in the time of sampling. There was no detailed health record. According to obtained information of the farm owner, as a general complaint, there were mastitis events and increased number of insemination problems in herd 1. In herd 1, Out of 9 cows, BVDV and BHV1 specific antibodies were found in 7 (77.7%) and 6 (66.6%), respectively. All of the 9 calves were detected as seronegative for both infections. The number of breeding cattle was 145 in herd 2, mostly in lactating period. In anamnesis, 3 abort event and prolonged service periods in some animals was reported. In this herd, all of the cows (30) and their 3 precolostral calves were detected as positive for BVDV. In the light of obtained data, it is obvious that virus in the circulation in the herd. BHV1 Ab was found to be only in cattle as 73.3% (22/30). Most of the samples were collected from herd 3, 116 cows and their calves have been sampled in 5 month period.

In the time of sampling, there was 1.580 cattle in the herd 3, 1.116 of them was cows and calves. Rest of the animals were young males and they have been keeping in a separate barn in the farm. The animals were vaccinated before using multivalan vaccines including studied infections but no vaccination was performed more than last two years period. There was regular health record in this herd. Enteric and respiratory disorders have been seen before in the herd. Infertility and prolonged service period were recorded in some adults.

In he herd 3, 976 cattle were controlled for BVD Ag and Ab presence nearly 8 month before this study. As a result of this examination, Ab rate was 82.2%, while ncp virus was detected in 5 animals in the ages between 6 month old and 1 year old (Gür et al, 2008). After three week, Ag(+) animals were re-controlled to confirm PI and sent to slaughter. In this study, as a result of the VNT, out of 116 samples from herd 3, 114 cows (98.2%) and 17 calves (14.6%) were detected as Ab positive for BVDV in this study. Immun-protection duration is about 1 year of the used vaccine for BVDV. Considering last vaccination time, anamnesis and laboratory findings, it is possible to say that obtained seropositivity was belongs to natural infection and circulation could have been continued. BVDV Ab titer values were higher in dams of seropositive calves as can be expected. (Figure 1 and 2). Presence of persistently infected calves is a probability among seronegative calves. Out 116 cows, BHV1 Ab was determined 70.6% (82) and in only one calf.

Immune system develops among 90 to 120. days of gestation in cattle (Schultz 1973). Earliest detectable maternal Ab formations had detected in foetus in 79. day (Scott et al., 1973). In an experimental study (Kendrick, 1971), cows were infected with BVDV in 40–235. days of the gestation and 93. day was reported as earliest Ab determined time in foetus. According to these data, it can said that Ab detection in precolostral calves shows the natural infection during nearly last 5 month of gestation. Fetal exposure to slight or mild infections, seroconvertion can be expected and proved for many infections. Orban and co-workers (1983) were vaccinated BVDV seronegative dams between 190 and 265. Days of gestation using live vaccine and out of 36, 34 precolostral calves was detected as positive among 1/80 and 1/216 titer values. Researchers had used

same vaccine in 48 serpositive dams and BVDV specific antibodies were determined in only 4 calves between 1/20 and 1/80 values. Schefers et al. (2008) were detected precolostral BVDV Ab as 7.4% (33/446) and 6.2% (32/515) in two state in USA. In this study, it is obvious that 22 (13.5%) calves have been infected starting 79. days of the gestation to the birth. Ab titer values of the dams of precolostral positive calves (average 1/80) were also higher than other cows (1/40) (Figure 1-2). In addition, it is possible to say that 22 seropositive calves would not be PI but persistence is a probability for other Ab negative calves. However, high PI proportion could not be expected due to high Ab proportions of the dams.

Yapkıç (1999) were controlled 62 precolostral calves and its dams, all dams and 7 calves were detected as seropositive for BVDV, titer values were among 1/20 and 1/120 in calves. Orban and collequaes (1983) were reported Ab titer between 1/20 and 1/180. Obtained titer interval was was lower than previous reports. Prevalance studies shows that the infection could be quite prevalent up to 96.8% (Çabalar ve Karaoglu, 1999). Obtained data for BVDV in this study (95.6) was one of the highest even for Turkey.

Because of latent structure on BHV1 infection, serologic examinations have been preferred to determine the infection status of the herds. Even though many European countries have been implementing eradication studies, the infection is still common in many pars of the world. BHV1 Ag was first detected by Burgu and Akça (1987) in Turkey but serologic evidence has been known since 1971 (Erhan et al., 1971). Subsequent reports revealed commonness of the BHV1 infection all over the Turkey. The serological distribution of the BHV1 infection were reported 54% by Gurturk et al. (1974), 68% by Cabalar and Akca (1994) and 74% by Bilge (1998) in various parts of Turkey. In this study, seropositivity rates of 66.6%, 70.6% and 73.3% were determined in herds 1, 3 and 2, respectively. Lowest value was observed in family type small enterprises. Transmission rate is directly determines by number of breeding animals and management practises in herds. The obtained seropositivity rates for BHV1 (69.7%) were similar with previous reports. Only one positive calf was in herd 3 with 1/32 titer, dam has 1/64 titer. Effect of BHV1 on embriyos and foetuses was described by many researchers (Bowen et al. 1985; Miller and Van der Maaten, 1986) but contrary to BVDV, there were no many studies for precolosral examination for BHV1. Miller et al. (1978) were infected the cows intranasally in last trimester of gestation, congenital disorders like abort and stillborne was detected and 8 calves borned alive. Bosc et al. (2000) were detected many BHV1 Ab positive precolostral calves in very low titers. They are interpreted as oral intake of maternal blood by the calf at birth or leakage of maternal antibodies during pregnancy. Detected Ab titer (1/32) in a calf and in its dam (1/64) was not low, in-utero infection is point at issue. Experimental studies show that calves of immun dams may be protected but infection of seronegative dam results with foetal infection and postnatally ill calves. Pospisil et al. (1996) was reported 40% abortion. We know that intensive breeding are facilitating risk of transmission. Proportion of both infections in small farms was lower than organised herds, as expected. As a result, circulation of BVDV and BHV1 were detected in nearly all studied herds. Considering long term effects of the both infections on various organ systems and indirect effects like immun system depression, eradication seem as a necessity for sustainable and profitable herd management.

At the beginning of herd constitutions, the cows were generally chooses based on race and body condition score criterias. Same situation is valid for studied organised herds in this study. Multi-local animal collection comprises great risk especially in the aspect of persistent and latent infections. Expected economic accomplishments would not come true in subsequents years in the absence of whole herd examines. Other remarkable point is difficulties in clinical diagnosis. Many viral infections have no specific or patognomonic clinical findings including studied viruses. Laboratory verification of the all individuals is an obligation for long-term prosperous herd management, particularly for non-acute infections.

Obtained result showes natural infection for both infections in this study. As conclusion, both infection was found to be very prevalent and currently has been circulated in most studied enterprises. Precolostral BVDV Ab presence was not low. Natural in-utero BHV1 detection is also important data of this study. Ab screening in the cattle is not adequate to make definite herd profile, however precolostral examines would reveal directly recent infections. By the way, it can be preferred as a usefull tool on design of control/eradication programmes.

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