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Seroprevalance of Bovine Enterovirus Type 1 (BEV1) in Goats in Turkey

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Abstract: Host spectrum of bovine enterovirus type 1 infection is wide range including in goats. In this study, BEV1 infection was investigated as serologically in clinically healthy and sick goat herds with respiratory and reproductive problems. Total of 1380 goat serum samples were obtained from 6 points in Afyonkarahisar, Eskisehir and Nevsehir provinces. In herd basis, positivity proportion was detected between 17.6 and 80%. Out of 727 samples from Afyonkarahisar, 304 (41.8%) goat was found to be seropositive for BEV1. Determined proportions in Nevsehir and Eskisehir provinces were 53.5 and 67.7%, respectively.

Key words: Bovine enterovirus type 1, goat, serologic investigation, Afyon, Turkey

INTRODUCTION

Bovine Enteroviruses (BEV) was classified in *Enterovirus genus* in *Picornaviridae* family. Genom composes of positive single stranded RNA. Many field isolates were obtained so far and isolates were collected in 2 groups (La Placa *et al*., 1965; Knowles and Barnett, 1985). According to the recent study, isolates were re-classified using phylogenetic analysis (Zell *et al*., 2006), again 2 BEV clusters revealed (A and B or type 1 and 2). Total of 17 serotypes (8A1 and 9A2) were located in type 1. There were 3 sub-groups in type 2. In this study, one of the first isolates, which was used for test virus (Kunin and Minuse, 1958), located in A1 (Zell *et al*., 2006).

BEV has worldwide distribution (Kurogi *et al*., 1976; Hamblin *et al*., 1985). The agents can be isolated from both clinically healthy and sick animals. Pathogenesis of BEV infection is only described in cattle. Alimentary (McFerran, 1962), respiratory (Moll and Ulrich, 1963; McClurkin, 1977) and reproductive systems are effected preliminary (Moll and Finlayson, 1957; Dunne *et al*., 1974). Another unusual cases with high mortality was also reported (Blas-Machado *et al*., 2007). Host spectrum is quite different for type 1 and 2. Type 1 was determined as serologically in many species like humans, horses, dogs, cattle, pigs, rabbits, fowl, deer, impalas, buffaloes, llamas, sheep and goats (Moscovici *et al*., 1961; McFerran, 1962; Yamada, 1965; Mehrota, 1973; Hamblin *et al*., 1985; Sharma *et al*., 1986; Puntel *et al*., 1999). Host spectrum of BEV 2 was restricted by only domestic cattle.

Bovine enteroviruses excessively resistant to heat, salinity and disinfectants (Mahnel, 1974; Ley *et al*., 2002). In addition, post-mortem acidity is not enough for inactivation of virus in tissues. The agent may survive for many years in environmental conditions. Up to now only one seroprevalence was carried out in goats in Turkey and 27.6% (132/477) proportion was determined in clinically normal animals (Gur *et al*., 2008).

Pathogenesis of the BEV1 infection was not studied clearly in many sensitive species except cattle so far. According to the further informations, the clinical course of BEV infection is generally progress silently, especially in adult cattle. Same things probably could be valid in small ruminants and other species. Due to high morbidity and stability of the virus under environmental conditions, incidence may be increase in a short time in the herds (Taylor *et al*., 1974).

In this research, seroprevalance of bovine enterovirus type 1 was investigated in goats both healthy and respiratory-reproductive system disorders determined herds in Turkey.

MATERIALS AND METHODS

Study animals: In this study, 1380 goats was sampled between late 2005 and mid 2007. Almost, all of the goats were adult, youngest animals were above 6 months old. Sex of the animals was ignored. Blood samples used in this study were collected from 3 province in central Anatolia and Aegean Region in Turkey. Sampling were performed in 4 different points in Afyonkarahisar

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Table 1: The number of sampled animals and BEV1 test results in goat

Herd No.	Provinces	Clinical findings	No. samples	BEV1 Ab (+)	BEV1 (%)
1	Afyon-Olukpinar	Respiratory disorders	65	52	80.0
2	Afyon-Emirdag	Clinically normal	17	3	17.6
3	Afyon-Anitkaya	Abort (30-80%)	252	102	40.4
4	Afyon-Peribacalari	Respiratory disorders	393	147	37.4
5	Nevsehir	CAEV and pseudotuberculosis	71	38	53.5
6	Eskisehir	Abort and respiratory disorders	582	394	67.7
	Total		1380	736	53.3

Serum Neutralisation$_{50}$ (SN_{50}) test was applied to all qualitatively positive samples (n = 736). Antibody titter values were determined between 1/5 and 1/160 as may be shown in Fig 1. Proportion of BEV1 titters showed a ordinary distribution, highest point was found to be in 1/20 point

province; Olukpinar (n = 65), Peribacalari (n = 393), Emirdag, Catalli (n = 17) and Anitkaya (n = 252) villages. Widespread respiratory disorders were determined in sampled herds in Olukpinar and Peribacalari. In Anitkaya village, samples was obtained from 3 different herds with have abortion among 30 and 80% proportions. There was a clinically healthy sampled herd from Emirdag. Total of 727 Hair Goats were sampled from Afyonkarahisar province. A Zaanen race goat herd (n = 71) were used in Nevsehir province. Newborn deaths, respiratory and alimentary system disorder and arthritis had been recorded in this herd, as result of detailed laboratory investigation Caprine Arthritis Encephalitis Virus (CAEV) and Paratuberculosis infections were detected. In another studied herd was Angora race big herd that have respiratory disorders and abort events in Eskisehir province (n = 582) (Table 1).

Blood samples were obtained from the Juguler wein and pulled into tubes and centrifuged at 3000 g for 8-10 min. Sera fraction was separated to the stock tubes and inactivated at 56°C for 30 min and stored at -20°C until to the test.

Cell culture: In virus, propagation and neutralisation test, Madin Darby Bovine Kidney (MDBK) cell culture (ATCC, CCL-22) was utilised. As medium, Eagle's Minimal Essential Medium (EMEM) and 5-10% Foetal Calf Serum (FCS) was used.

Virus: Bovine enterovirus type 1, LC-R4 (ATCC VR-248) strain ($10^{-4.5}$ $TCID_{50}$/0.1 mL) was utilised in the serologic test. Tissue Culture Infective Dose 50% ($TCID_{50}$) of the virus was calculated according to Spearman and Kaerber method.

Virus neutralisation test: Standard Virus Neutralisation Test (VNT) was reliable test to detect and discriminate the BEV1 specific antibody presence (Egbertson and Mayo, 1986). Every sample were diluted with medium as 1/5 and putted to the 2 well in the plate with same amount of 50 µL virus suspension. After incubation for 1 h, cell

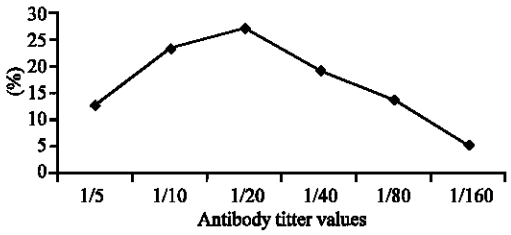

Fig. 1: Distribution of BEV1 antibody titter values

suspension (300.000 cell mL^{-1}) was added to the wells. Later on plates were incubated at 37°C with 5% CO_2 in air for 1-2 days. Wells were evaluated on the visibility of micromorphology of the cells by using tissue culture microscope.

Later on, all BEV1 seropositive samples were diluted as 1/5, 1/10…1/320 in the wells and same procedure were applied for determining antibody titter values (Fig. 1).

RESULTS

Microneutralisation test: As a result of VNT, at least 1/5 sera dilutions were accepted as seropositive. BEV1 specific antibody presence was found to be among 17.6 and 80% proportion in herd basis. Out of tested 1380 sample, 736 (53.3%) was determined as seropositive. Results were given in Table 1. Out of 727 samples from Afyonkarahisar province, 304 (41.8%) was positive for BEV1. In Nevsehir and Eskisehir provinces, 53.5 and 67.7% values was determined, respectively.

Infection value in respiratory disorders determined herds (1, 4, 6) was found to be 57% (593/1040). In the herds, which have abort problem (3, 6), detected proportion value was 59.4% (496/834), while 17.6% proportion observed in clinically normal herd in Emirdag, Afyon.

Statistical analysis: Test results of BEV1 positive animals in clinically healthy herd (2) and Respiratory disorders (1, 4, 6) and reproductive system disorders (3, 6) was compared statistically using P test. A difference was

determined in between both healthy and respiratory disorders detected herds and healthy and reproductive disorders observed herds ($p \leq 0.05$).

DISCUSSION

In this study, BEV1 infection was investigated serologically in healthy and sick goat herds. Out of 4 sampling performed villages in Afyonkarahisar province, widespread typical respiratory disorders like coughing, nasal discharge, weight and condition loss were detected in herds 1 and 4, positivity values were varied between 37.4 and 80% values.

According to obtained information from local veterinary Authorities, increased abort events was observed in last couple of years in Anitkaya village in both goats and sheep. Goats having mostly been breeding together with sheep in this village and abort proportion in the whole herds were among 30-80%. Percentage of BEV1 infection was found to be 40.4%. In herd 2, there were no clinical disorders in goats and seropositivity was found to be only 17.6%.

Arthritis, respiratory disorders, long term enteritis, condition loss, newborns deaths were observed in herd 5. As a result of previous detailed laboratory investigation, Caprine Arthritis and Encephalitis Virus (CAEV) and Pseudotuberculosis infections were revealed in this herd. BEV1 specific antibodies were found to be 53.5% (38/71) in this herd.

Total of 582 specimens was collected from a big Angora race goat herd from Eskisehir. According to obtained information from the farm's practitioner's information, low percentage of aborts and respiratory disorders especially, in newborns and youngs has observed in the herd for 3 years before sampling and healing has not seen in all of the animals as a result of drug therapy. Test result showed that out of 582 goats 394 of them (67.7%) was found to be positive for BEV1.

Studies for BEV 1 infection were mainly focused on cattle both in the world and Turkey. Earlier studies have demonstrated that BEV1 infection is quite prevalent, incidence shows variation in region and farm basis. Previously Entero and reovirus were isolated from calves with pyrexia and diarrhea (Kurogi *et al*., 1976). In Spain, researchers have been reported that 78% of the cattle fecal samples were BEV1 positive (Jimenez-Clavero *et al*., 2005). Firstly, presence of BEV1 and 2 infections was reported and 53.5% value was detected in cattle for type 1 (Alkan *et al*., 1997). Later on, 13.1% (38/288) proportion was reported in cattle in Aydin province (Gur *et al*., 2004) and 3.9% (14/355) value was reported in buffaloes (Gur *et al*., 2006). BEV1 was isolated from goats (*Capra hircus*) in 1985 (Jain and Batra, 1985). Specific antibodies were also determined (Moscovici *et al*., 1961). Virus isolation was not reported so far in Turkey in any species but the infection was investigated serologically in 7 animal species and human, detected seroproportion in clinically normal goats was among 0 and 23% in 5 goat herds in Afyonkarahisar province (Gur *et al*., 2008), the value is similar with obtained proportion in healthy goats in this study. BEV1 specific antibodies were found to be quite higher in respiratory (57%) and reproductive (59.4%) disorders diagnosed herds than healthy animals (17.6%). Statistical analysis shows that there was a difference among healthy and sick herds.

Enteric viruses like enteroviruses are transmitted via the fecal-oral way and infect and replicate in the gastrointestinal tract of the host. Resistant character of the enteroviruses to pH allows survive along digestive tract and the agent shed in extremely high titters (10^5-10^{11}) in g^{-1} of feces from the infected animals (Taylor *et al*., 1974; Farthing, 1989). Long term survival in the field conditions (Ley *et al*., 2002) and wide host spectrum are an explanation for high prevalence. As a general concession, BEV infection does not cause serious health problems and it is usually asymptomatic, pathogenesis was not introduced clearly particularly in the species except cattle. Some BEV strains may cause abortion in guinea pigs (Moll, 1964). A typical facts and high mortality reported cases become the infection disputable in the alone or mix infection (Blas-Machado *et al*., 2007).

CONCLUSION

Herd and regional differences, number of the breeding animal in the herds, age distribution, breeding conditions, multi-species breeding are main factors effecting incidence. Reproductive, respiratory and alimentary system disorders have multifactoral aetiology. Presence of mix infection with other bacterial and/or viral infections in studied herds was a strong probability, some of them had been proved but statistical analysis shows a connection between stated problems and BEV1 infection in goats. Further, investigations are necessary to lightening the pathogenesis of the Bovine enterovirus type 1 infection in the sensitive species.

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