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A study regarding bovine enterovirus type 1 infection in domestic animals and humans: An evaluation from the zoonotic aspect

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ABSTRACT. Bovine enteroviruses (BEV) are members of *Enterovirus* genus of the family *Picornaviridae*. BEV1 has a broad host spectrum, including humans. The virus usually causes subclinical infection, but fatal/severe cases have also been reported in different animal species. There is quite limited data regarding BEV1 in humans. The purpose of this study is to investigate human infection and to identify possible risk factors for viral exposure. For this purpose, blood serum samples (n=1,526) were collected from a city center and nearby villagers simultaneously from humans and farm animals in Elazig province in Eastern Anatolia. As a result of serum neutralisation test, BEV1 specific antibody presence detected in cattle was 85.3% (163/191), 73.5% in donkeys (64/87), 71.8% in goats (115/160), 46.5% in sheep (93/200), 43.9% in horses (40/91), 41.3% in dogs (19/46) and 33% in humans (248/751). Although a high contamination potential was mentioned for people living in rural areas, it was determined that infection rates in rural areas (31.6%) and urban centers (32.2%) were very close. There was no difference according to sex. Viral exposure is higher in the 40 to 70 age range. In addition, the serological evidence of the infection in donkeys was identified for the first time with this study.

KEY WORDS: bovine enterovirus type 1, human, seroprevalence, zoonosis

The *Picornaviridae* is a non-enveloped positive sense single stranded RNA virus family. *Enterovirus* genus is one of the subfamilies comprising more than 80 serotypes that can infect cattle and other animal species. Most are known as human pathogens that can cause a wide variety of clinical disorders [27]. The human enteroviruses were classified into five species: poliovirus and human enterovirus (HEV) A–D [14]. Some serotypes causes subclinical infection, non-specific febrile illness or mild upper respiratory symptoms but some of them cause mild or heavy symptoms like aseptic meningitis, myocarditis, acute haemorrhagic conjunctivitis, undifferentiated rashes, acute flaccid paralysis (AFP), and neonatal sepsis-like disease [27].

Enteroviruses are known to have a high mutation potential [26, 28, 30]. All bovine enterovirus (BEV) field isolates are classified in two serotypes [14, 16]. Zell *et al.* [33] re-analyzed capsid proteins 1C (VP3) and 1D (VP1) as a sequence and phylogenetic level and confirmed two BEV clusters as in previous studies. In the same study, serogroup A, 8 and 9 subtype were determined in A1 and A2, respectively. In cluster B, there were nine serotypes in three subgroups. The host spectrum of BEV2 is restricted to domestic cattle contrary to type 1 [17]. BEV1 specific antibodies (Abs) are detected in various species such as water buffalo [8], African buffalo and impala [10], sheep and goats [12], humans, cattle, dogs, horses and monkeys [22, 24, 32], dolphins [25], alpaca [20], possums [34].

BEVs are very stable in environmental conditions due to pH, temperature, salinity and disinfectant resistance [19]. In addition, the ease of transmission, high amounts of virus scattered in every gram of feces, the role of subclinical infected animals and the survival of virions in water and soil for a long time, ensure that this virus is a contaminant in the environment [3, 4, 29]. BEVs are in circulation all over the world. However, the pathogenesis and virulence of BEV1 in many species is almost unknown. Acute BEV infection in adult cattle is usually subclinical. After oral transmission, the agent passes into the intestines without affecting from the low gastric pH. During viral proliferation in the intestines, the agent is taken to the lymph nodes, and seroconversion develops in a short time. Clinical disorders are usually seen in newborns and young animals. Mainly, alimentary, reproductive and nervous systems are affected [5, 21, 23, 35].

The research articles related to BEVs have been carried out mostly in the 1960s. After a couple of reports in the mid-1980s, the

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infection remained nearly unnoticed until the last decade. Lately, acute infection cases with poor prognosis have been reported from different parts of the world such as fatal enteric disease in a heifer in U.S.A. [2], respiratory disorders in cattle in China [33], severe diarrheal disease in a yak in Qinghai-Tibetan Plateau [11] and severe respiratory infection in an Alpaca in U.S.A. [20]. Furthermore, virus isolates from feces samples of healthy and diarrheic animals have been examined on a genomic level [18, 29, 31].

Total of 3.020 samples from eight species were evaluated for BEV1 specific Abs in an extensive study carried out in Turkey in 2008 [9]. Seropositivity was determined in horse, goat, sheep, cattle, dog and human samples obtained from different parts of the country, mostly from provinces in the Central Anatolia region, while no positivity was detected in camels and gazelles [9].

The aim of this study was to reveal potential risks of BEV1 infection in human health.

For this purpose, blood samples were collected from the same limited location simultaneously from humans and all farm animals, namely cattle, sheep, goats, horses, donkeys and dogs in Elazığ province in the Eastern Anatolia Region of Turkey.

MATERIAL AND METHODS

Sample collection

Blood serum samples were obtained from cattle (n=191), donkeys (n=87), goats (n=160), sheep (n=200), horses (n=91), dogs (n=46) and humans (n=751) (M: 443, F: 308). A total of 1,526 samples were obtained from seven species (Table 1). The samples were collected from animals and humans over six months and above to avoid the possibility of maternal ab presence. Animal specimens were collected from the villages around Elazığ city center (38°40’29.3”N- 39°13’21.0”E). All the animals used in the study were from small-scale family type farms. The number of cattle was less than 10 in every farm. Horses and donkeys were standard bred draft animals averaging 1–3 per farm. Samples from dogs were obtained from the dogs on the farms. No more than 10 samples were collected from every studied sheep and goat flocks. Mix-breed (sheep-goats and cattle) breeding is rare in the province. The management practice was mainly free grazing by sharing the same pasture for 7–8 month during the year depending on climatic conditions. Closed system breeding is preferred for the rest of the year.

All animals were clinically normal during sampling. Their sex was not taken into consideration. As a result of their breeding preferences, the majority of the cattle, sheep, goats and donkeys were female. The sex of horses and dogs was almost equal. Human samples were obtained from the city hospital from patients with different complaints. The patient’s age, sex and residential area were noted. Blood samples of all animal species were taken from Vena Jugularis into vacutainer tubes and human samples were taken by hospital staff using standard method (All procedures were approved by the ethics committee of Faculty of Medicine, Fırat University in 2010 (permission No. 13)). Blood samples were centrifuged at 3,000 rpm for 8–10 min. Serum fraction was separated and inactivated by keeping them at 56°C for 30 min., and then stored at −20°C until testing.

Cell culture

Madin Darby Bovine Kidney (MDBK) Cell Culture (ATCC, CCL-22) was utilized for virus propagation, titration and serological tests. Foetal calf serum (2–10%) (FCS) added Eagles’s Minimal Essential Medium (EMEM) was preferred as a medium.

Test virus

BEV1, [Enteric Cytopathogenic Bovine Orphan (ECBO)], strain LC-R4 (ATCC VR-248) (10–3.5 $TCID_{50}$/0.1 m*l*) was used. The LC-R4 strain isolated in Michigan in 1957, is one the first isolates [15] and it was classified as a group 1 in cluster A by Zell *et al.* [31]. Tissue culture infective dose 50% ($TCID_{50}$) of the virus was calculated by using the standard Spearman and Kaerber method.

Serum neutralisation test

The serum samples were tested for BEV1 specific abs using the serum neutralisation test as described by Egbertson and Mayo [6]. Blood samples were centrifuged at 3,000 rpm for 10 min. Separation of sera was followed by inactivation at 56°C for 30 min, subsequently, all the serum samples were diluted at a rate of 1/5 with the cell culture medium. Aliquot’s of 50 μl from the dilution was placed into two wells of the tissue culture plates with the same volume of virus suspension containing approximately 100$TCID_{50}$ per 50 μl.

Table 1. Bovine enterovirus type 1 antibody (Ab) data of the human and animal species

No	Species	Sample No.	BEV1		GMAb+
			Ab (+)	(%)	
1	Cattle	191	163	85.3	1:43
2	Donkey	87	64	73.5	1:25
3	Goat	160	115	71.8	1:24
4	Sheep	200	93	46.5	1:19
5	Horse	91	40	43.9	1:16
6	Dog	46	19	41.3	1:13
7	Human	751	248	33.0	1:13
	Total	1,526	742	48.6	

GMAb+, Geometric mean of Ab positive samples.

At the end of 1 hr of incubation, the cell suspension of 50 *µl* (300,000 cells/m*l*) was added and incubated at 37°C with 5% CO_2 for one day. Test results were determined as the basis of micromorphology of the cells by using the tissue culture microscope. All ab positive serum samples were serially diluted into 1/5, 1/10, 1/20, 1/40, 1/80, 1/160 and re-tested as above to determine the antibody titer values.

RESULTS

Serum neutralization test

Samples with no virus propagation observed at a 1/5 dilution were considered positive. The highest proportion was detected in cattle as expected as 85.3% (163/191). Second highest value was observed in donkeys, out of 87, 64 (73.5%) was positive for BEV1. A near value was seen in goat [71.8%, (115/160)]. Sheep and horse samples have very close rates, 46.5% (93/200) and 43.9% (40/91), respectively (Table 1). Minimum positivity was determined in human samples with 33% (248/751) (Table 1).

Among all species, the highest ab titer was detected in cattle. The ab means of all species are given in Table 1, and ab titer distributions can be seen in Fig. 1.

Out of 443 female samples, 149 (33.6%) was found to be positive. Proportion in center originated samples 32.3% while 30.8% in rural samples. Data of male was exhibited close proportions in all groups as 32% (Table 2). In total, seropositivity in central and rural areas was very close to each other (32.2 and 31.6%).

Ab positivity according to age showed regular distribution (Fig. 2). Percentage of viral exposure is higher between 40 and 70 years old.

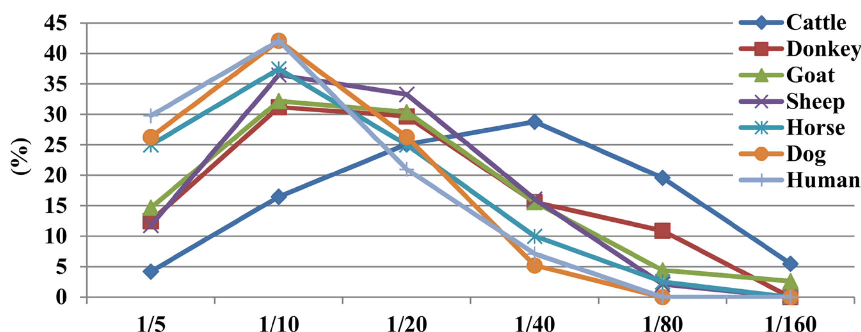

Fig. 1. Bovine enterovirus type 1 antibody titer distribution of farm animals and human samples (%).

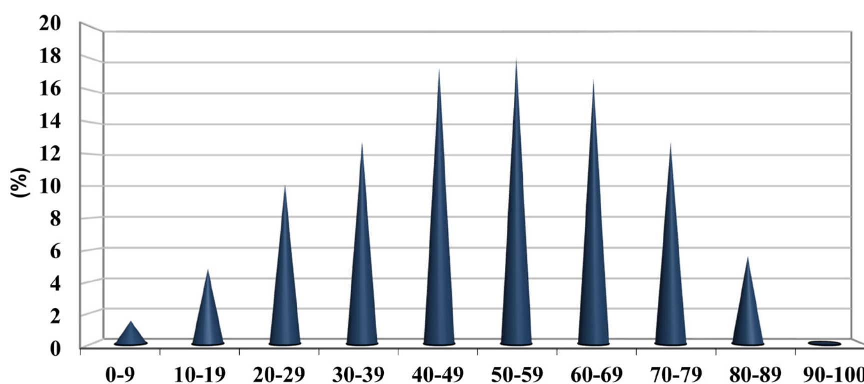

Fig. 2. Bovine enterovirus type 1 seropositivity distribution in human according to age (%).

Table 2. Bovine enterovirus type 1 antibody (Ab) data of human according to gender and settlement

Settlement	Sample No.	Sex of the samples				Sex of the BEV1 Ab (+)				Total	
		F	(%)	M	(%)	F	(%)	M	(%)	Ab (+)	(%)
Center	487	300	61.6	187	38.3	97	32.3	60	32	157	32.2
Rural	139	81	58.3	58	41.7	25	30.8	19	32.7	44	31.6
ND	125	62	49.6	63	50.4	27	43.5	20	31.7	47	37.6
Total	751	443	59	308	41	149	33.6	99	32.1	248	33

ND: None defined.

DISCUSSION

Considering a zoonotic or vice versa transmission possibility, blood samples were collected from the same limited area during the same time interval. In additions to humans, all domestic animal species sharing the same farm environments were included in the study. The protection duration of maternal BEV1 abs is still unknown in most species. Therefore, the mean duration of abs protection was taken into account and six months or older individuals were sampled from all the studied species.

Among the determined abs rates and titer values of all sampled species, once again cattle was found to be the most sensitive specie [7, 9]. The rate (85.3%) was the highest detected in Turkey so far. It was maximum 64.8% (986/1,520) in the former study [9].

One of the significant and interesting data revealed in the study was that the second highest ratio was determined in donkeys which was 73.5% (64/87). In the only study carried out in donkeys so far, Jiménez-Clavero *et al.* [13] tested fecal samples of sheep, goats, horse and donkeys. Positivity was noted in all of these species except donkeys. Serological evidence related to BEV1 in donkeys has been unveiled for the first time with this study (Table 1).

The following percentage belonged to goats with 71.8%, which was higher than the only study on the subject in Turkey with 53.3% (736/1,380) [1]. Sheep data was also higher (46.5%) than previously (32.8%) [9]. However, there was a significant difference in horses (43.9%) and dogs (41.3%) compared to the previous study conducted in Central Anatolia which reported 12.8 and 3.2%, respectively [9].

One of the main focuses of the study was to evaluate human samples that have detailed information. A total of 751 human samples were collected from patients who went to the local hospital with various complaints. The human samples were grouped according to the permanent addresses that people gave for hospital records. According to the test results, there were no differences in terms of viral exposure among people from urban (32.2%) and rural areas (31.6%). There was also no significant difference between men (32.1%) and women (33.6%). The distribution of the presence of abs by age was also evaluated. We found that the incidence of infection was the highest between the 40 and 70 age groups (Fig. 2). The determined proportion in the total of human samples (33%, 248/751) was close to the report of the study carried out in Central Anatolia (30.3%, 74/144). In the mentioned study [9], there were no exposure-related interpretations due to an absence of detailed data on the origin of the samples and patient information including age, sex and residential area.

There is no data on the virus excretion time interval in acutely infected individuals in the sensitive species. In the only experimental infection with another BEV type which has close properties with LCR-4, the viral excretion continued in calves from between 2 days and more than 6 months [22]. Neither the duration of protection of the formed Abs after acute infection nor the duration of the presence of detectable abs is known in either cattle or any other species. Nevertheless, it is estimated that prolonged viral excretion and the resistance nature of the agent may cause repeated viral exposure in all species. This study revealed that one-third of the individuals had been exposed to the virus regardless of where they live. It would be expected that the seropositivity rate would be higher among those living in rural areas, but data did not indicate any difference in people living in urban areas.

The animal samples were obtained from family type privately owned traditional small-scale farms. Ruminants and equine species graze in the villages and nearby grasslands throughout most of the year. Dogs with owners are guard dogs and associate freely in the outdoor conditions with stray dogs. Drinking water sources for animals were generally open and are shared by all the farm animals. Under these circumstances, transmission between human and domestic livestock is almost inevitable, despite the availability of acceptable sanitary conditions.

Clinical outlooks, the duration of viral excretion and immunity are not known to a large extent in the majority of sero-conversion detected species. There is no clear information in literature except for some case reports with poor prognosis in different species [2, 11, 19, 33]. The geometric mean of antibodies (GMAT) data may give insight about the susceptibility of species in this unknown area. It is noteworthy that the GMAT titers showed direct parallelism with the determined ab ratios as can be observed in Table 1. The highest mean value is determined in cattle (1:43) as expected. It was interesting to see that the highest second ratio (73.5%) and average titer (1:25) was found in donkeys determined sero-reactive for the first time by this study. Although GMAT in goats (1:24) is approximately the same as donkeys, the value in horses (1:16) comes after the sheep (1:19). According to the test results, humans and dogs were the least reactive species to the agent (1:13). This study suggests that there may be differences in exposure to the infection between people living in cities who have no direct contact with animals and people living constantly in close contact with domestic livestock in rural areas, however the fact that such a difference was not observed may be due to the resistant character of the virus. It is understood that one in three persons are exposed to the virus regardless of close contact with animals.

Although there is no association between BEV1 and any clinical disorder in humans, it is a matter of concern for pathogenesis in infants and immune depressive individuals. The fact that clinicians or researchers have not shown an interest in the subject may also have a role. Lack of research on the subject continues the existence of questions. BEV strains have not been isolated in Turkey so far. However, new studies are needed to determine circulating serotypes and their possible contribution to respiratory, enteric and reproductive disorders in humans and animal species.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

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