Serologic investigation for bluetongue virus type 4, 9 and 16 in Anatolian water buffaloes

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Abstract

Bluetongue has emerged as a threat for ruminant species especially in European countries; three serotypes of Bluetongue virus have been described in Turkey. In this study, a total of 380 sera were obtained from healthy adult buffaloes in 8 different provinces in Central and Northern Anatolia. Sera were tested using a Virus Neutralisation test for types 4, 9 and 16. Seropositivity rates of 16.5% (63/380), 7.1% (27/380), and 8.4% (32/380) were detected for types 4, 9, and 16, respectively. No positives were found in Northest province (41°17'). This is the first report of Bluetongue virus in buffaloes in Turkey.

BTV, infection, serotype

Bluetongue virus (BTV) infection is a vector-borne disease of various ruminant species. It has a double stranded RNA genome which consists of 10 segments, and is placed in the *Orbivirus* genus in the family *Reoviridae*. Twenty four serotypes have been recognised (Roy 2002). Bluetongue infection has a wide distribution in the tropical and subtropical zones of Africa, America, Australia, Indian subcontinent, Southeast Asia and the Middle East (Mellor and Boorman 1995; Mellor and Wittmann 2002). It is an infectious but non-contagious disease, characterized mainly by fever, hyperaemia and ulceration of oral mucosae, coronitis, lameness and death.

BT was included in the former OIE list A diseases. The BT outbreak in Cyprus in 1943 caused 60-70% mortality and important economic losses in sheep (Gambles 1949). In 1944, a severe outbreak was seen in the Hatay province in Turkey. BT reappeared in the Aegean region of Turkey in 1977. The outbreak then spread to the regions of Marmara and Mediterranean. Three serotypes (4, 9 and 16) have been identified to date in Turkey (Urman et al. 1979; Yonguç et al. 1982).

BT has a wide host range including sheep, water buffalo, cattle, goat, *gazella* spp., bighorn sheep, antelope and various types of artiodactyles. Most studies have been carried out in cattle and sheep. There have been few reports on BTV in buffaloes. In Turkey, the buffalo population was over one million until 1980, but today there are less than 90.000. The main reason is that the buffaloes are no more profitable due to a longer production cycle and low milk yield; the improvement efforts for genotypes were only practised on cattle. Production of buffalo meat and milk products like soujouk and kaimak on the national and regional levels has slowed down the decrease of the buffalo population, however, the decline is continuing. Buffalo breeding occurs mostly in family type small enterprises generally with other domestic ruminant species in Central Anatolia and the Black Sea Coastal Region in Turkey.

The purpose of this study was to investigate the BTV infection in Anatolian buffaloes using Virus Neutralisation test (VNT) to determine serotype differences.

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Materials and Methods

Study design and sample collection

Blood samples were collected from 380 healthy adult (1-8 years old) buffaloes from 8 provinces in the Central and East Anatolia and the Black Sea Region of Turkey. The numbers of tested animals from provinces; Afyon, Amasya, Samsun, Ankara and Sivas were as follows Tokat, Konya, Elazığ respectively (Fig. 1).

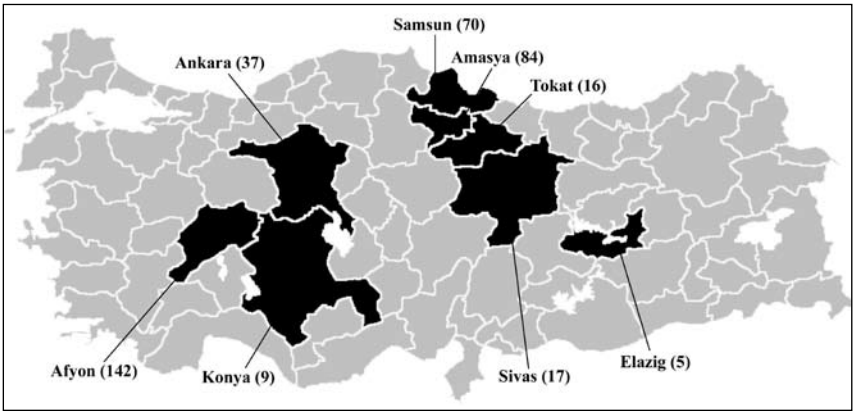

Fig. 1. The locations and numbers of buffalo samples collected from selected provinces in Turkey.

The numbers of breeding buffaloes were 2–10 of the sampled enterprises, and they were housed together with cattle and/or sheep in most of the farms. The only organised buffalo herd used in this study was a farm in the Afyon province in Central Anatolia and 94 samples were obtained from this herd. The rest of the samples were collected from small private herds. Sampling was performed regardless of sex. Blood samples were collected from the jugular vein into sterile tubes with silicon. After centrifugation at 3000 × *g* for 10 min, the sera were decanted and inactivated.

Cell culture

VERO cell culture was used for virus propagation, titration and microneutralisation tests. Dulbecco's Minimal Essential Medium (DMEM) containing 10% Foetal Calf Serum (FCS) was used as a cell culture medium.

Bluetongue virus

Bluetongue virus types 4, 9 and 16 were used as test viruses in the study. Tissue culture infective dose of 50% ($TCID_{50}$) of the virus suspension was calculated as 10^5/0.1 ml, $10^{4.5}$/0.1 ml, and $10^{5.2}$/0.1ml for type 4, 9, and 16, respectively.

Virus neutralisation technique

For the detection of BTV type specific antibodies, the microneutralization test was used. For this purpose, the blood samples were centrifuged at 3000 *g* for 10 min. Serum was separated, inactivated at 56 °C for 30 min and kept at -20 °C until testing. Initially, all serum samples were diluted 1/10. Aliquots of 50 µl from each dilution were put into duplicate wells of the tissue culture plates with the same volume of virus suspension containing 100 TCID. After 1 h of incubation, aliquots of 50 µl cell suspension (300.000 cells/ml) were added and incubated at 37 °C with 5% CO_2 and checked every day. Test results were determined based on cytopathology of cells using an inverted microscope.

After these tests, a Serum Neutralisation$_{50}$ (SN_{50}) test was performed on antibody positive samples to determine the antibody titers. For this purpose, a series of serum dilutions were prepared (1/5, 1/10....1/320), and the test was repeated as described above. In the virus neutralisation test, 1/10 and higher was accepted as positive.

Results

According to the test results (Table 1), all serotypes were detected in buffaloes in the Afyon and Tokat provinces whereas all buffaloes were negative for any of the serotypes in the Samsun province. The most prevalent serotype was type 4, it was detected in 6 out of 8 provinces with a 5.9%–68.7% prevalence. Each of the BTV types 9 and 16 was found in 4 provinces with a prevalence of 2.7%–31.2% for type 9 and 5.9%–56.2% for type 16. Total

seroprevalence was 16.5% (63/380), 7.1% (27/380), and 8.4% (32/380) for type 4, 9, and 16, respectively.

Table 1. Virus neutralisation test for Bluetongue virus types 4, 9, and 16 in buffaloes in Turkey.

Provinces	No of samples	Type 4		Type 9		Type 16	
		Ab (+)	(%)	Ab (+)	(%)	Ab (+)	(%)
Afyon	142	46	32.3	20	14	17	11.9
Amasya	84	-	-	-	-	5	5.9
Samsun	70	-	-	-	-	-	-
Ankara	37	3	8.1	1	2.7	-	-
Sivas	17	1	5.9	-	-	1	5.9
Konya	9	1	11.1	1	11.1	-	-
Elazığ	5	1	20	-	-	-	-
Tokat	16	11	68.7	5	31.2	9	56.2
Total	380	63	16.5	27	7.1	32	8.4

The numbers of positive animals for only one serotype were 56, 36, and 10 for only type 4, 9 and 16, respectively. Dual positivity was determined in 27 buffaloes and only 4 were positive for all types (Table 2), while 22.9% (87/380) were negative for all serotypes.

Table 2. Multiple infections for the Bluetongue Virus types 4, 9, and 16 in buffaloes in Turkey.

No	Provinces	No of samples	Type 4-9		Type 4-16		Type 9-16		Type 4-9-16	
			Ab (+)	(%)	Ab (+)	(%)	Ab (+)	(%)	Ab (+)	(%)
1	Afyon	142	6	4.2	7	4.9	2	1.4	3	2.1
2	Amasya	84	-	-	-	-	1	1.2	-	-
3	Samsun	70	-	-	-	-	-	-	-	-
4	Ankara	37	-	-	-	-	-	-	-	-
5	Sivas	17	-	-	-	-	-	-	-	-
6	Tokat	16	2	12.2	7	43.7	1	6.2	1	6.2
7	Konya	9	1	11.1	-	-	-	-	-	-
8	Elazığ	5	-	-	-	-	-	-	-	-
	Total	380	9	2.3	14	3.7	4	1.05	4	1.05

As a result of the SN_{50} test, antibody titre distributions are given in Fig. 2. Although serum dilutions were prepared as 1/5, 1/10....1/320, no positives were detected in the dilution 1/320. As shown in Fig. 2, a bell-shaped curve was seen only for type 4, and the highest value was obtained at a 1/40 dilution. The highest titres of types 9 and 16 were determined at the dilution of 1/10 and a gradual decrease was also observed.

Discussion

Many seroepidemiological studies have been performed especially in the last two decades in different parts of Turkey. Seropositivity in sheep and catttle was detected as 1–36% and 2.3–34.4%, respectively in different surveys in Turkey (Bolat 1986; Ertürk 1994). Burgu et al. (1992) have reported 52.6% seropositivity in 890 cattle from 9 provinces in the Southeast Anatolia with prevalence ranging from 22.4% to 75.2%. The highest prevalence of 88% was determined in cattle in Southeast Anatolia (Gür 2008).

In this study, 380 clinically healthy adult buffaloes in 8 different provinces were tested for BTV 4, 9, and 16 serotypes and type 4 was found to be the most prevalent

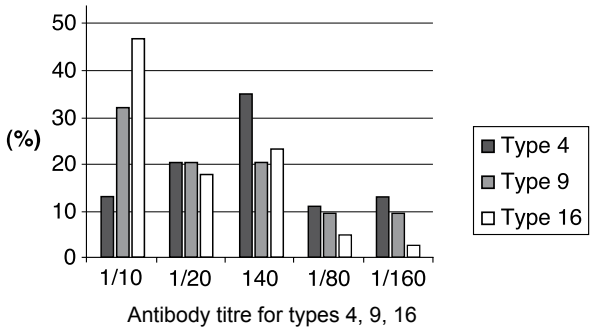

Fig. 2. Proportional antibody titre distribution for bluetongue virus types 4, 9 and 16 in buffaloes in Turkey

serotype (16.5%). Types 16 and 9 was detected in four province as 8.4% and 7.1% values, respectively.

The data in this study suggest that the occurrence of the BTV infection is lower than in cattle. However, most of the cattle seroprevalence surveys were performed in the Southeast and Aegean regions. This study was conducted in Central and Northern Anatolia. Average winter and summer temperatures were 9 and 29 °C, respectively, in the Mediterranean Region (annual 18–20 °C) compared to 1–2 and 23 °C in Central Anatolia (annual 10–12 °C). Therefore, the differences in climate can explain the different prevalences in different regions.

The epidemiology of BTV infection is vector dependent with the main vectors of *Culicoides* spp. (Mellor 2000). In a recent outbreak in the European countries, *C. obselotus* complex was detected as more abundant than *C. pulicaris* complex; the remaining subspecies have lesser importance in Northern countries (Meiswinkel et al. 2008; Cagienard et al. 2009), *C. imicola* is also among the dominant species in Southern Europe (Calvete et al. 2009). *C. imicola* (principal afro-asiatic vector) and *C. obsoletus* are the most important species for BTV in Turkey (Dik 1989; Mellor et al. 2008). A total of 57 different *Culicoides spp.* have been found in Turkey. They are active from mid-April to mid-October, the highest activity was observed in the months of July and August (Uslu and Dik 2004). Mands et al. (2004) investigated the attractiveness of animal odour (deer, sheep, pony, calf and water buffalo) to vectors. They used hair extract in a serial dilution and found that water buffalo odour was significantly attractive to *C. impunctatus* and *C. pulicaris.*

Adult vector and host entity are the main factors responsible for virus circulation in the field. Duration of viraemia depends on the host species. Maximum viraemia periods in sheep and goats have been determined for 54 and 47 days, respectively (Koumbati et al. 1999) and 112 days in cattle (Du Toit 1962). Pathogenesis of viral infections seems similar in cattle and water buffaloes, although there are no reports of the viraemia period of BTV in buffaloes.

There are limited data concerning the incidence of BTV exposure in buffaloes, especially those being raised in close proximity or together with cattle. Genetic selection to increase the milk yield has not been applied to buffaloes except for one herd in the Afyon province. Buffalo breeding styles vary from region to region. Tokat, Amasya and Samsun provinces are located in the Black Sea region, where buffaloes are mostly reared freely on grasslands with natural small ponds and puddles. The buffaloes can have mud bath in these wetlands; the described conditions are ideal for vectors.

The distribution of *Culicoides* spp. was thought to be between the latitudes 35°S and

40°N, however, BTV infection has undergone a huge expansion in a large geographic area since 1998, the latest outbreaks in Northern Europe after 2006 showed that the virus had reached 58°N (Wilson and Mellor 2009). The expansion seems to continue, probably due to global warming.

In this study, the sampled provinces lie in the band between 37°41'N and 41°17'N. The Samsun province is located in the Black Sea border and no seropositivity was detected in this study for all types of virus; Amasya is located to the south of Samsun and only type 16 was found in this province with a prevalence of 5.9%. However, all three serotypes were found in the Tokat province at a high level. Afyon is one of the southernmost provinces in the study, 32.3%, 14% and 11.9% percentages were found for type 4, 9, and 16, respectively. The values obtained in the Afyon province were higher compared to the other central Anatolian provinces. Population dynamic and quantity of the vector species have not been well investigated on a regional basis in Turkey. Additionally, climatic features of the studied regions are considerably variable. These factors may affect the virus profile.

In conclusion, Bluetongue virus infection was investigated for the first time in the buffaloes in Turkey and type 4 was found to be the most prevalent type compared to types 9 and 16. Buffalo were bred together with other farm animals such as cattle, sheep and goats on small family type farms. This rearing style could create a problem for BTV infection. In addition, the climatic conditions of Turkey are suitable for *Culicoides* spp. Latest reports particularly in European countries show that the latitude of distribution continues to expand and BTV becomes an emerging infection (Purse et al. 2005; Melhorn et al. 2007; Cagienarn et al. 2009; Wilson and Mellor 2009). Climatic alterations have a consequential effect on vector dynamics, so studies of serotype distribution will continue. Applicable control methods like vaccination, restriction and more controlled transport of live animals may help to reduce the economic impact of the BTV infection; international cooperative efforts are a necessity.

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