

A serologic investigation of blue tongue virus (BTV) in cattle, sheep and gazella subgutturosa subgutturosa in southeastern turkey

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Abstract Bluetongue virus (BTV) is a vector-borne disease of ruminants disseminated in the tropic and sub-tropic zone of the world. It is also an important problem in the Middle East. Three serotypes (4–9–16) of the virus have been isolated so far in Turkey. *Gazella subgutturosa subgutturosa* (Goitred Gazella) is native species of Anatolia. Due to risk of extinction, captured Gazelles have been taken under protection in a restricted area at Ceylanpinar state farm. In this study, the presence of Bluetongue virus (BTV) was investigated serologically in 82 Goitred Gazella, 684 sheep and 100 cattle. Seropositivity rates in Gazella, sheep and cattle were detected as 40.2%, 29.5% and 88%, respectively.

Keywords Bluetongue virus (BTV) ·
Gazella subgutturosa · Sheep · Cattle · Serology

Abbreviations

BTV Bluetongue virus
IUCN International Union for the Conservation of
Nature and Natural Resources
UNDP United Nations Development Programme

GAP Southeast Anatolia Project
G.s.s. Gazella subgutturosa subgutturosa
Ha hectar

Introduction

Bluetongue is an infectious but non-contagious disease, characterized mainly with fever, hyperemia and ulceration of oral mucosa, coronitis, lameness, abortion and death. Bluetongue virus is the type species of the Orbivirus genus, family Reoviridae, having double stranded RNA genome which consists of ten segments. Twenty four serotypes were recognised so far (Roy 2002).

BTV was observed in 19th century in Africa but described in 1902 by Spreull and one of the first reports out of Africa was from Cyprus in 1943. The first outbreak seen in Turkey was in Hatay in 1944–1947. This outbreak was deflated by strict measures. The second appearance was in Aegean region in 1977, later on Marmara and Mediterranean regions have been affected. The virus was identified as BTV Type-4 (Urman et al. 1979; Yonguc et al. 1982). The disease was detected serologically and virologically in many studies so far and type 4, 9 and 16 were identified in Turkey.

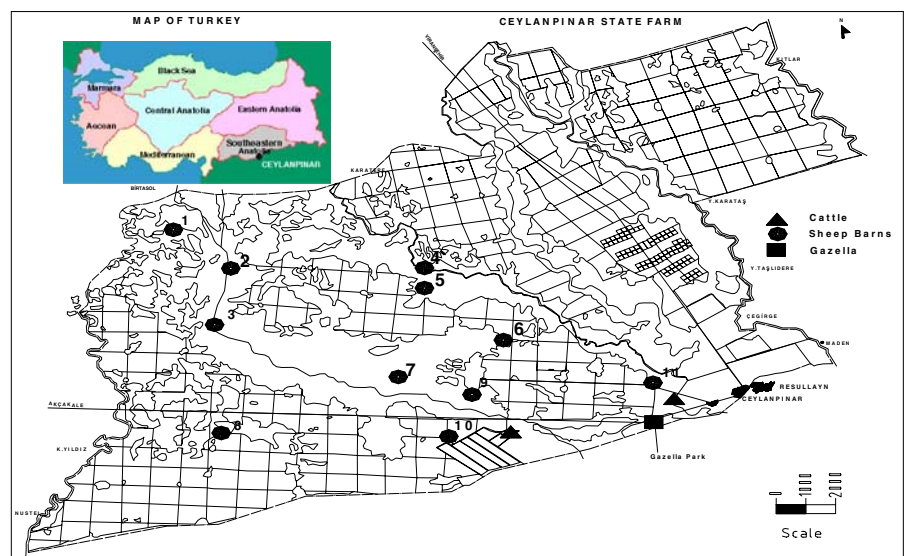
BTV has a very wide host range including sheep, cattle, goat, gazella spp., bighorn sheep, antelope and various types of artiodactiles (Hourrigan and Klingsporn 1975; Hoff and Hoff 1976; Bender et al. 2003).

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It was reported by Guldenstaedt (1780) that the species of Goitred Gazella was native to Anatolia. It is commonly known as Persian or goitered gazella, ranges from Turkey through Iran to central Asia. They have been living in a wide open area in Turkey. Although, big herds were observed in the wildlife 30 years ago around Viransehir, Akcakale and Karacadag territory (Southeast Anatolia), a survey performed between 2001–2002, United Nations Development Programme (UNDP), GAP (Southeast Anatolia Project) Biodiversity Research Project indicated that there is no viable gazelle population in the wildlife in around region (Can and Lise 2004). Goitred gazelles were listed in the Red Book the International Union for the Conservation Nature and Natural Resources (IUCN) as a rare and endangered species. Currently, only population held in captivity is in Ceylanpinar state farm by the Turkish Ministry of Agriculture. Ceylanpinar (36°51'N–40°03'E, Altitude is 398 m), one of the biggest farms in the world, established on 17.610 km area in a territory in Sanliurfa province, Southeast Anatolia, Turkey.

Captive Gazellas were put under protection by Turkish Ministry of Forestry in 1978 and the management of farm was given to the Ministry of Agriculture in 1982. Gazellas have been breeding without any medical treatment and vaccination in 8.5 km restricted areas. Animals were fed with only dry grass in winter and summer. The map of the farm was shown in Fig. 1, every square is scaled as a 4 kmsq.

Fig. 1 The map of the Ceylanpinar state farm



Epidemiology of BTV infection is vector dependent, especially *Culicoides spp.* (Mellor 2000). Main species carrying the virus are *C. imicola* and *C. obsolotus* (Dik 1989; Yilmaz 1994) but *C. imicola* is the more important species in Turkey (Mellor et al. 1995). Distribution of *Culicoides spp.* lie in a band of latitudes between 35°S and 40°N, this could be extended up to 50°N (Mellor et al. 2000).

Optimal conditions for flying activities for *Culicoides spp.* are 60% humidity and 13–14°C temperature. Average life expectancy for this species is about two months. Infected vectors carry the virus during their lifespan. According to meteorological data (Fig. 2), *Culicoides spp.* is active in this region from April to October but the highest activity are seen in July and August (Uslu and Dik 2004).

The purpose of this study was then to determine the Bluetongue infection using serologically techniques in *Gazella subgutturosa subgutturosa*, and to compare data with the other sensitive species; sheep and cattle were also examined.

Materials and methods

Sampled animals

Total of 82 blood serum samples were collected from the captured Gazellas around 4–6 week-old after the birth in late months of July and August. Gazellas were trapped in a metal cage (2×2 m) containing grass.

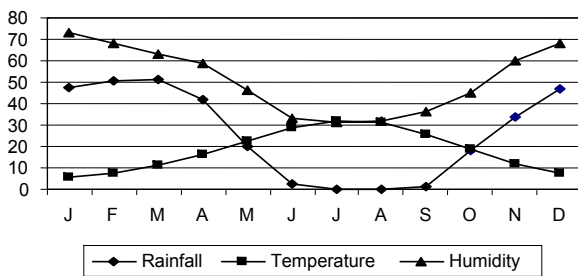


Fig. 2 Average values of rainfall (mm), temperature (°C) and humidity (%) of last 10 years in Ceylanpinar

Captured animals were immediately sampled and released. Age of the sampled animals was not determined but all of them were adult.

Nearly 50,000 ivesi sheep breeds have been breeding in 11 separate groups in the farm and approximately equal number of sheep were sampled in 5 different sheep barns (Barn 4, 6, 7, 9 and 11) (Fig. 1). Total of 684 blood samples were collected. The Gazelle and Cattle units close to the sheep barns were selected for sampling. Since there were no clinical symptoms in the sheep herd, the sampling was performed arbitrarily. The sheep were feeding with grass in the field.

Intensive breeding have been applying in cattle in two barns. Approximately 2000 cattle have been rearing in two separate groups: first one is very close to the Gazella (Fig. 1), but less than 100 cattle have been breeding in this location. All cattle samples were obtained from second unit and all the sampled animals were dairy cows.

According to the farm records, clinical symptoms related to BTV were not observed in the sheep but some of the cattle showed slight symptoms like facial oedema and salivation. There was no information for Gazella due to observing difficulties.

Samples were taken from vena jugularis, centrifugated at 3000 g for 10 min. and than samples were transferred to the stock tubes and freezed at -20°C until the test.

Competitive ELISA test

ELISA test was preferred for its high sensitivity (100%) and specificity (99%) in comparison to the other serological tests including Virus Neutralisation and AGID (Pearson et al. 1992). A competitive ELISA kit was used to detect BTV specific antibodies in ruminant sera produced by Veterinary Medical

Research and Development (VMRD, Inc., USA). This test was performed according to the producer's description and plates were read with an ELISA reader at 630 nm and results were calculated. Test sera were taken as positive according to produced optical density less than 50% to the negative control.

Results

Test results

The BTV specific antibodies were detected in 33 (40.3%) out of 82 Gazella. The existence of clinical symptoms has not been known due to monitoring difficulties. Sheep have been breeding in 11 different points in the farm and samples were collected from 5 of them. Although no viable clinical findings were reported, seropositivity rate was found as 29.5% and there were not any statistically significant differences between sheep barns.

The highest proportion (88%) was detected in cattle. Total of 866 animals were tested, 323 (37.3%) of them were found to be positive for BTV (Table 1).

Discussion

Many studies have been performed in different parts of Turkey for BTV. The BTV infection have been intensively seen in the South East Anatolia, Mediterranean coast, West (Aegean Coast) and North-West (Marmara Region) of Anatolia. Burgu et al. (1992) performed a serosurvey among sheep and cattle in the South, South-East and Aegean region, the proportion found as 25% and 15.5%, respectively. Bolat (1986) found 7.29–16.23% seropositivity in the East and Southeast and Erturk (1994) detected 0–42% in sheep

Table 1 The prevalence of BTV (Ab) positives

Species	Number of samples	BTV Ab (+)	Proportion (%)	CI
Gazella s.s.	82	33	40.2	0.29–0.51
Sheep	684	202	29.5	0.26–0.33
Cattle	100	88	88	0.82–0.94
Total	866	323	37.2	

CI: Confidence interval

in Southeast Anatolia. BTV was studied within another serosurvey in cattle of nine South-East cities and average value was reported as 52.58% and the positivity rate was detected as 66.6% in a family type small cattle farm in Sanliurfa province (Ozgunluk 2003).

In this study, sampling was performed in late July and August, 2005. Seropositivity status in Gazella was found as 40.2% (33/82). To compare the data, other sensitive species were studied in the same farm.

Obtained values in sheep (29.5%) were found close to the recorded previously but the seropositivity in cattle (88%) was found to be higher than that of expected. A few clinical events related to BTV were only reported in cattle. The close observation in Gazella is not possible because of their breeding conditions. Therefore, there was no information about clinical status of Gazella.

Central cattle unit and 11th sheep barn are very close to the Gazella's park, the distance between them is nearly 3–6 km (Fig. 1). Since most of the cattle have been breeding in other cattle unit and clinical symptoms were shown in dairy cows in this place, sampling was performed in the second cattle unit. According to the test results, 88 out of 100 cows were positive for BTV, this is the highest detected value in this region so far and it is verifying an outbreak in the farm.

When BTV was introduced to Turkey for the first time, disease symptoms were very heavy and mortality was high, but currently, clinical pictures are generally mild or implicit. It is known that, some sheep breeds are more sensitive to the infection, but ivesi is a native breeds for this region and has been breeding for centuries. According to the farm records, there has not been any clinical finding in sheep. This situation can be explained by the sub-clinical infection or missing mild symptoms in a large number of animals. According to Mellor (1994) sheep, goat and cattle are generally resistant against the clinic infection and majority of the outbreaks are totally silent.

In this search, the samples were collected from 5 out of 11 sheep barn selected as its proximity close to the cattle and gazelle units. Test results were not given as separately in sheep barn basis, because there were no statistically important differences between them. Distance among the sampled sheep barns was maximum 16 km to each other, but BTV is a vector-borne disease and direct contact is not necessary for the contagion.

The number of Gazella spp. in the wild declined dramatically in all over the world. To prevent them from extinction, captured animals are kept in the restricted areas in countries like Turkey, Iran and Saudi-Arabia. Their wild nature does not allow for treatment or vaccination. Some of them die during capture. Bender et al. (2003) were performed a serologic study in Oryx Gazella, ninety-six percent (48/50) of them were found to be seropositive for BTV in New Mexico, USA. Frölich et al. (2005) studied Arabian Oryx, 15.9% (31/194) and 39.3% (37/94) seropositivity rates were detected in Unites Arab Emirates and Saudi Arabia, respectively. The determined positivity in Gazelle in this study (40.2%) was found to be very close to the reported value in Saudi-Arabia (39.3%).

GAP (Southeast Anatolia Project) is one of the biggest regional development projects in the world, aiming to increase the use of water in farming in 1.764.372 ha area with 22 dams and large water canal net. Eight dams and 10 watering projects were completed and watering started in 1995. To date, it has reached nearly 12% of the aimed area. The average value of rainfall, temperature and humidity of Ceylanpinar in last 30 years are given in the Fig. 2. The climate of Ceylanpinar is suitable for *Culicoides* spp. With the putting into practising of the envisaged projects, ecological changes, increase in the fly population and prevalence of vector-born diseases like BTV are expecting in next decades. BTV seroprevalance studies in the region show that the process has already started. According to reports, seropositivity value in cattle was found as 15.5% and 52.58% in 1992 and 2003 respectively (Burgu et al. 1992; Ozgunluk 2003). In this study, the rate was detected as 88% in cattle.

Reservoir-vector-climate trio was very important at the epidemiology for the BTV infection. Viremia period for infected animals is critical for continuing virus circulation in the field. Maximum viremia period in sheep have been detected as 54 days (Koumbati et al. 1999) and 112 days in cattle (Du Toit 1962). Cattle acts as silent reservoirs for virus for a long time (McLachlan et al. 1992), it is clear that, their breeding can lead to increase the incidence of BTV in other ruminant species in same region.

Beside other symptoms, BTV causes reproductive disorders (Luedke 1985). Clinical course of the infection was unknown in Gazella but as a race face

to extinction, birth complications could be effect their reproduction. Considering medication is not feasible in Gazella, vaccination of domestic ruminant species will help to take under control of BTV in them, as well. Hence, it is need to further investigations to determine the circulated serotypes in the region.

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