Journal of Animal and Veterinary Advances 8 (12): 2613-2616, 2009
ISSN: 1680-5593

A Serologic Investigation of Blue Tongue Virus Serotypes (BTV-9, BTV-16) in Cattle in The Southeastern Anatolia Project Area in Turkey

Irfan Ozgunluk
Department of Virology, Faculty of Veterinary Medicine,
University of Harran, Yenisehir Campus, 63300 Sanliurfa, Turkey

Abstract: In this research, seroprevalence of BTV serotypes (BTV-9, BTV-16) were investigated in 740 cattle sera collected from 9 provinces in Southeast Anatolia Project Area in Turkey. Virus neutralization test was used to discriminate antibody response against BTV serotypes. The overall seroprevalence of BTV was recorded as 31.76%, comprising individual percentages for BTV-9 and BTV-16 were 20.00 and 22.03%, respectively. In the study, the seroprevalence of blue tongue virus infection in GAP varied between 20.90 and 52.17% on the province basis, whilst antibody percentage for BTV-9 and BTV-16 were found in GAP varied between 8.60 and 32.39%, 11.94 and 49.28%, respectively.

Key words: Bluetongue, cattle, seroprevalence, GAP, project area

INTRODUCTION

Bluetongue (BT) is an arthropod-borne disease, which is characterized by fever, congestion, oedema, haemorrhages, hyperemia and ulceration of oral mucosa, coronitis and lameness in domestic and wild ruminants (Roy, 2002). In addition, this virus is responsible for fetal death, congenital defects and reproductive failure in cattle (Murphy *et al*., 1999). Bluetongue Virus (BTV) is classified in Reoviridae family genus Orbivirus, having double stranded RNA genome, which consists of ten segments. At present, 24 internationally recognized serotypes of the virus have been reported (Gibbs and Greiner, 1994; Mertens, 1999; Roy, 2002).

BTV was observed in 19th century in Africa but described in 1902 (Herald, 1954) 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.

The Southeastern Anatolia Project (GAP) is a multi-sector and integrated regional development effort approached in the context of sustainable development. The project area covers nine administrative provinces (Adiyaman, Batman, Diyarbakir, Gaziantep, Kilis, Mardin, Siirt, Sanliurfa and Sirnak) in the basins of the Euphrates and Tigris and in Upper Mesopotamia. The water resources development component of the program envisages the construction of 22 dams and 19 hydraulic power plants and irrigation of 1.82 million hectares of land. The one of basic objective of GAP is development stockbreeding in many parts of region that is unsuitable for agriculture and irrigation. From this point of view, investigate viral diseases are more helpful to be successful in this purpose (Ozgunluk, 2003).

BTV is called as non-infectious arthropod-borne disease and blood sucking midges play role in transmission between infected and susceptible animals. Main objective of this study is to detect the seroprevalence the local serotypes (serotype 9 and 16) of Bluetongue Virus in the Southeastern Anatolia Project (GAP) area.

MATERIALS AND METHODS

Serum samples used in this survey were collected between May 2001 and July 2002 from 9 provinces namely Adiyaman, Batman, Diyarbakir, Gaziantep, Mardin, Kilis, Siirt, Sanliurfa and Sirnak located in Southeastern Anatolia Project area. There were a total of 740 samples from cattle analyzed (Table 1). All the sampled animals were older than 1 year old and randomly selected from privately owned small capacity family farms. There was no clinical disorder recorded during the sampling and no vaccination program had been applied against viruses examined in this study. Serum samples were heat inactivated at 56°C for 30 min and stored at -20°C, until testing.

Table 1: Seropositivity rates of BTV-9 and BTV-16 in cattle populations detected according to provinces

Provinces	No. of animals	Seropositivity rate: BTV-9 (+) No.	BTV-9 (+) %	BTV-16 (+) No	BTV-16 (+) %	Only BTV-9 (+) No	Only BTV-9 (+) %	Two serotypes (+) No	Two serotypes (+) %	Only BTV-16 (+) No	Only BTV-16 (+) %	Total (+) No	Total (+) %	Total (-) No	Total (-) %
Adiyaman	75	14	18.67	15	20.00	10	13.33	4	5.33	11	14.67	25	33.33	50	66.67
Batman	117	18	15.38	16	13.68	10	8.55	8	6.84	8	6.84	26	22.22	91	77.78
Diyarbakir	94	21	22.34	16	17.02	7	7.45	14	14.89	2	2.13	23	24.47	71	75.53
Gaziantep	93	8	8.60	20	21.51	2	2.15	6	6.45	14	15.05	22	23.67	71	76.34
Mardin	85	21	24.71	16	18.82	16	18.82	5	5.88	11	12.94	32	37.65	53	62.35
Kilis	69	17	24.64	25	36.23	6	8.70	11	15.94	14	20.29	31	44.93	38	55.07
Siirt	71	23	32.39	13	18.31	13	18.31	10	14.08	3	4.23	26	36.62	45	63.38
Sanliurfa	69	18	26.09	34	49.28	2	2.90	16	23.19	18	26.09	36	52.17	33	47.83
Sirnak	67	8	11.94	8	11.94	6	8.96	2	2.99	6	8.96	14	20.90	53	79.10
Total	740	148	20.00	163	22.03	72	9.73	76	10.27	87	11.76	235	31.76	505	68.24

Bluetongue Virus serotypes (BTV-9, BTV-16) were used in Virus Neutralization Test (VNT). BTV-9 and BTV-16 serotypes were originated from Department of Virology at Ankara University, Faculty of Veterinary Medicine, Ankara, Turkey. Vero cell line was employed for propagation and neutralization steps of viruses. Cell cultures were grown in Dulbecco's MEM supplemented with 10% Foetal Calf Serum (FCS).

Serological screening was performed using a VNT as previously described (Karaoglu *et al*., 2007). This assay is serotype specific and sensitive for serological screening of viral infections. Serum samples were used in 1: 10 dilutions in order to detect antibodies against BTV-9 and BTV-16. Two subsets of sera were tested for their neutralizing activity against BTV serotypes. The 50 μL serum samples in duplicate were mixed with equal volume of 100 TCID50 diluted BTV suspensions in 96-well plates. After neutralization at 37°C for 1 h, 50 μL of Vero cell suspension was added all wells used of the 96-well plate. Test results were evaluated by inverted light microscope after 4-5 days of incubation in 5% CO_2 atmosphere at 37°C. All the viruses used in present study are in cytopathogenic nature thus, inhibition of virus growth indicated by non-destructed monolayers of cell cultures was evaluated as indicator of virus neutralization. For scoring a sample as positive for the investigated antibodies, both wells used for the same sample were asked to be free of cytopathogenic effect.

RESULTS AND DISCUSSION

Among 740 animals, 505 (68.24%) were free of antibodies to both of tested viruses, while only 235 (31.76%) samples were positive for antibodies to two serotypes. Commonly, antiviral antibodies were detected against two viruses in the same animal 76 (10.27%). Total of 72 (9.73%) animals were found seropositive for BTV-9, while 87 (11.76%) samples were positive for antibodies to BTV-16 (Table 1).

According to the results of this study, average rate of BTV-16 in cattle have been found higher than BTV-9, but higher seropositivity for two serotypes was varied between provinces.

Prevalence of BTV-9 antibodies in Southeastern Anatolia Project area varied between 8.60-32.39% in province basis. The highest BTV-9 prevalence detected was 32.39% in Siirt province, which is followed by Sanliurfa (26.09%), Mardin (24.71%), Kilis (24.64%), Diyarbakir (22.34%), Adiyaman (18.67%), Batman (15.38%), Sirnak (11.94%) and Gaziantep (8.60%) provinces. The seroprevalence of BTV-16 was found between 11.94-49.28% in the study. The highest seroprevalence of BTV-16 was in Sanliurfa (49.28%) province, which was followed by Kilis, Gaziantep, Adiyaman, Mardin, Siirt, Diyarbakir, Batman and Sirnak provinces, with 36.23, 21.51, 20.00, 18.82, 18.31, 17.02, 13.68 and 11.94%, respectively (Table 1). Probably, it depends on presence of serotype and sensitive animals. This situation indicated that the GAP area suitable for midges activity and have risk for other midges-borne infections.

Many studies have been performed in different parts of Turkey for BTV-4. This study indicated that the BTV infection have been intensively seen in the South East Anatolia, Mediterranean region, Aegean Coast and Marmara Region in Turkey. Burgu *et al*. (1992) performed a serosurvey among sheep and cattle in the South, Southeast and Aegean region, the proportion found as 25 and 15.5%, respectively. Bolat (1986) examined 1290 blood serum samples collected from sheep in Eastern and Southeastern Anatolia regions. They found 7.29-16.23% seropositivity in the East and Southeast. BTV-4 was studied within another serosurvey in cattle of eight Northeast Anatolia provinces and seropositivity rate was reported as 36-72% (Yildirim and Burgu, 2005).

Karaoglu *et al*. (2007) performed serosurvey in cattle in Trace, the proportion found as 69,04, 71,38 and 80,20% for BTV-4, BTV-9 and BTV-16, respectively. In this study, seropositivity rates BTV-9 and BTV-16 (20.00 and 22.03%)

were found much less than the seropositivity rates (71.38 and 80.20%) as detected by Karaoglu *et al*. (2007).

Ozgunluk (2003) determining seropositivity rates of BTV-4 infections in cattle in Southeastern Anatolia project area detected 52.58% seropositivity by VNT. This study was performed in same region and found seropositivity rates for other serotypes (BTV-9 and BTV-16) lower then BTV-4. This finding addressed that BTV-4 is widespread in GAP area but other serotypes must take under consideration to preventive measures for BTV infection.

Bluetongue is an economically important infection of ruminants that causes serious decreases in reproductive ability, abortions and congenital malformations (Murphy *et al*., 1999). There are 3 major components recognized in the epidemiology of the infection such as reservoir animal, vector and climatic conditions. The most important reservoir is cattle and they can be infected rarely (Gibbs and Greiner, 1994; Gorman, 1990). The viraemia lasts about 100 days in cattle (Luedke *et al*., 1977), while only 30 days in sheep (Goldsmith *et al*., 1975). Thus, it is reported that cattle play an active role either in transmitting the virus or in passaging the virus to appropriate climatic condition over winter, in which the vector midges are biologically in-effective (Goldsmith *et al*., 1975; Luedke *et al*., 1977).

It is known that the vector midges (*Culicoides* sp.) has approximately, 1000 sub-species in tropical and sub-tropical regions, in which proper ecologic situations have been provided for their life cycle. However, only 17 of them have directly been related to transmission of the BTV. Bluetongue virus infection of ruminants are commonly seen in most of the tropical and subtropical regions between 35° South and 40° North latitudes, in which *Culicoides* midges are extremely effective (Gibbs and Greiner, 1994; Hawkes, 1996). *Culicoides* sp. responsible of transmitting project disease have been investigated in Turkey and 57 species of them have previously been identified (Karaoglu *et al*., 2007). Mellor *et al*. (1995) have identified 13 *Culicoides* sp. in Southern, Southeastern and Western Turkey, in which congenitally.

GAP 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 climate of GAP area is suitable for *Culicoides* sp. With the putting into practicing of the envisaged projects, ecological changes, increase in the fly population and prevalence of arthropod-borne diseases.

BTV-4 was reported previously, by means of this study, valuable data were obtained on BTV-9 and BTV-16 GAP area. These data leaded to figure out the occurrence of the two serotypes province basis of GAP. Results indicated that high prevalence of BTV infection may reflect the presence of novel serotypes of BTV in the region. The well-known features of the disease, such as plurality and persistency in cattle may complicate local attempts to eradicate and/or control the disease.

Preventive approaches based on the fight with vector midges are accepted as to be non-practical and unsuccessful. Because of the region, which located next to Syria and Iraq, in addition to near to Iran has an adequate geographic position and climatic feature, various vector-borne exotic diseases able to invade the country from its neighboring countries.

The most valuable protective and control measurement against BT is effective vaccination. However, presence of several serotypes in country should take into consider in both preparation and administration of the vaccine as there is no close antigenic relation between BTV serotypes. According to this study, at least three serotypes of BTV exist in the region. Therefore, successful protection against BT can be achieved by using polyvalent vaccines included of local BTV serotypes.

CONCLUSION

Antibody against BTV-9 and BTV-16 were detected in all provinces located in the region. Ecologic alternation caused by the project would cause an increase in the population of bitting midges and their increased flying activity. Under these circumstances, if necessary control measures would not be taken, this infection transmitted by bitting midges would raise and cause economical losses.

REFERENCES

Bolat, Y., 1986. Serologic studies on incidence of bluetongue disease of sheep in elazig, Diyarbakir and sanliurfa. S.U. Vet. Fak. Derg., 2: 103-112. http://veteriner.selcuk.edu.tr/veteriner/8498BIB.htm.

Burgu, I., H.K. Urman, Y. Akca, A. Yonguc, P.S. Mellor and C. Hambling, 1992. Serologic Survey and Vector Surveillance for Bluetongue in Southern Turkey. CRC. 1st Edn. In: Walton, T.E. and B.I. Osburn (Eds.). Bluetongue, African Horse Sickness and Related Orbivirusus. Crc. Press Inc, Boca Raton, Fladelphia, USA, pp: 168-174. ISBN: 10: 0849351693, 13: 978-0849351693.

Gibbs, E.P. and E.C. Greiner, 1994. The epidemiology of bluetongue. Comp. Immunol. Microbiol. Infect. Dis., 17(3-4): 207-220. DOI: 10.1016/0147-9571(94)90044-2, PMID: 8001346.

Goldsmit, L., E. Barzilai and A. Tarmor, 1975. The comparative sensitivity of sheep and chicken embrios to bluetongue virus and observations on viremia in experimentally infected sheep. Aust. Vet. J., 51: 190-196. DOI: 10.1111/j.1751-0812.1975.tb0053. PMID: 169790.

Gorman, B.M., 1990. The bluetongue viruses. Curr. Top. Microbiol. Immunol., 162: 1-19. PMID: 2166641.

Hawkes, R.A., 1996. The Global Distribution of Bluetongue. In: St. Geore, T.D. and Peng Kegao (Eds.). Bluetongue Disease in Southeast Asia and the Pacific. ACIAR Proc. No. 66: 6-14. ISBN: 1863201874, 9781863201872.

Herald, R.C., 1954. Bluetongue. Bacteriol. Rev. Dec., 18 (4): 239-253. PMID: PMC440987.

Karaoglu, T., I. Ozgunluk, B. Demir, A. Ozkul and I. Burgu, 2007. Seroprevalence of culicoides-borne disease in cattle in European Turkey. Ankara Univ. Vet. Fak. Derg., 54: 121-125. DOI: 10.1501/Vetfak_0000000266 http://dergiler.ankara.edu.tr/dergiler/11/237/2201.pdf.

Luedke, A.J., M.M. Jochim and R.H. Jones, 1977. Bluetongue in cattle: Effects of Culicoides variipennis transmitted bluetongue virus on pregnant heifers and their calves. Am. J. Vet. Res., 38 (11): 1687-1695. PMID: 201192.

Mellor, P.S., D.M. Jennings, C. Hambling, I. Burgu, H.K. Urman, Y. Akca, R. Haziroglu, F. Alkan, A.D. Yonguc, A. Ozkul, H. Eren, 1995. Control of akabane disease and surveillance of bluetongue and ephemeral fever. 24. United Nations Development Programme, Food and Agriculture Organization of the United Nations, Rome, pp: 1-14. http://www4.fao.org/cgi-bin/faobib. exe?rec_id=356695&database=faobib&search_type=link&table=mona&back_path=/faobib/mona&lang=eng&format_name=EFMON.

Mertens, P.P.C., 1999. Orbiviruses and Coltiviruses. 2nd Edn. In: Granoff, A. and R.G. Webster (Eds.). Encylopedia of Virology, Harcourt Sience and Technology Comp, Academic Press, San Diego. California, USA, 2: 1043-1061. ISBN-10: 0122270304, 13: 978-0122270307.

Murphy, F.A., J.E.P. Gibbs, C.M. Horzineck and M.J. Studdent, 1999. Reoviridae. 3rd Edn. Veterinary Virology, Academic Press, New York, USA, pp: 391-404. ISBN: 10-0-12-511340-4, 13-978-0-12-511340-3.

Ozgunluk, I., 2003. Seroepidemiological Investigation of Bluetongue (BT), Akabane (AKA) and Ibaraki (IBA) Disease in Cattle in Southeastern Anatolia Project (GAP) Area. Ph.D Thesis. The Graduate School of Health Sciences of Ankara University, Ankara, pp: 2-10, 32-35. http://sagbilens.ankara.edu.tr/ithesis.php?id=1105.

Roy, P., 2002. Orbivirus. 1st Edn. In: Tidona, C.D. and G. Darai (Eds.). The Springer Index of viruses. Springer-Verlag, Berlin, Germany, pp: 957-963. ISBN: 978-3-540-67167-1.

Urman, H.K., U. Milli, N. Mert, S. Berkin, M.M. Kahraman, H. Yuce and H. Avvuran, 1979. Congenital Bovine epizootic arthrogryposis and hydranencephaly in Turkey. Ankara Univ. Vet. Fak. Derg., 26: 287-292. DOI: 10.1501/Vetfak_0000001027.

Yildirim, Y. and I. Burgu, 2005. The seroprevalence of Bluetongue (BT), IBR, PI-3, EBL and BVD infections in cattle in Northeastern Anatolia. Ankara Univ. Vet. Fak. Derg., 52: 113-117. DOI: 10.1501/Vetfak_0000000036.

Yonguc, A.D., W.P. Taylor, L. Csonton and E. Worrall, 1982. Bluetongue in Western Turkey. Vet. Rec., 111: 144-146. PMID: 6289510.