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Does the *Bluetongue virus* circulates in cattle population of Mat district, Albania?

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Abstract

Bluetongue is a viral, infectious, non-contiguous, vector transmitted disease of ruminants animals, caused by an *Orbivurus*. Despite the disease is not zoonoses, it is with high economic importance and as other OIE listed disease, significantly interfere with animal health and trade. Clinically, most affected species are sheep, however cattle serve as reservoir of infection and play major role on epidemiology of disease. Presence of Blue tongue disease proved only when it is based on laboratory tests.

Key words: bluetongue, competitive ELISA, infectious, surveillance, non-contiguous

Introduction

Bluetongue is an non-contagious viral disease of domestic and wild ruminants characterized by vascular injury that produces widespread oedema and tissue necrosis [2 ,4, 5]. The first clinical descriptions of bluetongue disease was published in 1905, and for more than 100 years its distributions map is expanded [2, 3]. Bluetongue virus (BTV), is a virus of the genus *Orbivirus* in the family *Reoviridae* [1, 4]. Three out five viruses of *Orbivirus* genus (Bluetongue virus, African horse sickness and Epizootic haemorrhagic disease virus) causes OIE listed disease [5]. Economic impact on the livestock of bluetongue disease is significant, and it is related mainly with high mortality, which could reach 30% or even more, trade restrictions etc. Addition cost are: direct costs associated with morbidity of sick animals include weight loss, reduced milk yield, abortion and associated veterinary costs. It is estimated that during a major BTV-8 outbreak the milk product lost in Germany was estimated to be 30506 million tonnes [2]. The cost of preventive measures in UK to prevent the BV-8 outbreak was 10 million British steeliness which contributed to protect British farmers from a potential £485m loss in their annual income, as well as to protect 10,000 jobs throughout the UK's economy that would otherwise be lost [1, 3].The Bluetongue virus as other orbiviruses are classified as a double-stranded ARN genome which contains 10 segments surrounded by three layers of structural proteins, respectively, subcore, outer core and outer capsid. The segmented genome encode different proteins of outer capsid and variation on proteins VP2 and VP5, determine the serotypes and explain the clinical severity, within species and between ruminant species [4, 5]. BTV occurs throughout temperate and tropical areas of the world and reflect the distribution of vector *Culicoides* spp. Different midge species transmit different constellations of BTV. Up to date, they are described 26 BTV serotypes and their distribution of BTV has recently been altered from classically range of geographic latitude [3]. It is assumed that climate change have significant contributed in expansion of BTV, particularly in Europe. The *Culicoides* spp are not able to flight in long distances, the wind could transport them from endemic areas to free BTV areas and initiate the disease outbreak in susceptible animals. Bluetongue disease (BTD) outbreak occur in certain season of the year and is related with vector activity. Despite that BTV, as other members of Reoviridae family, are moderately resistant to heat, organic solvent and non-ionic detergents, they are not able to survive for long time, however the disease outbreak overwinter in susceptible animals occur. The *Culicoides* spp acquired and transmit BT infection. The persistence of BTV infection is believed that is related with 1) vertical transmission in vector, 2) survival of infected adult vector, 3) carrier infected animals in which viraemia last for long time in particular infected animals, and 4) vertical transmission in infected ruminants. which certain occur [5, 6]. Current studies suggest existence of alternative transmission route, such as digestive, and

maybe both hard and soft ticks [1]. Risk factors for BTV are related with host (species, age and breed), pathogen agent (expression of VP2/VP5 according virus types), environment and climatic condition (temperature, humidity, exposure to sunlight), presence of *Culicoides* spp, management factors [5]. Clinical BT disease is variable, started from completely sub-clinical cases, moderate which are the common pattern for different serotype in infected ruminant, especially in endemic areas, up to severe clinical cases. Among ruminant animals, sheep and particular deer breeds are highly susceptible and severe clinical cases with significant morbidity and mortality have been recorded. Cattle are moderately susceptible to BTV, the infection is usually subclinical, and viraemia last for long time. The range of clinical signs is very wide, and they include temperature, depression, oedema (lips, face, ears, eyelids) erosions, ulcers, salivations and discharge, lameness, abortion etc [5]. Confirmation of bluetongue disease could be achieved by different laboratory techniques such as virus isolation, molecular methods, complement fixation test, indirect immunofluorescence and ELISA. There are developed two different ELISA assays; antigen detection ELISA and antibody detection by competitive ELISA. In order to detect the virus serotype, neutralisation assays are performed usually by reference laboratories [1, 5]. Clinical signs are indicative, and only in some cases cyanosis of tongue could be recorded, from which the disease was named. Control of BT disease is based on strictly applying biosecurity and management measures, vaccination, control of animal movement and vector control. Vaccination, currently is based in live modified vaccine types, and is a successful approach if the vaccine strain match the field strain [5].

The aim of this study was to investigate presence of Bluetongue disease in cattle in Mat district by evidencing specific antibody against blue tongue virus. We follow up the positive cases and we did competitive ELISA. The sera positive samples we sent to reference laboratory Teramo, Italy, in order to detect the virus serotype by virus neutralisation and consequently to predict potential BTD outbreaks in Albania ruminant animals.

Materials and Methods.

Study area

Mat district is one of the thirty-six districts of Albania, part of Dibër region. Mat district has a surface from 997 km^2, 60.000 habitans 10 communes and 2 municipalities. The majority of human population live in rural, 73% and 23% live in urban areas. The main livestock population in Mat constitutes by 15640 cattle and 73875 sheep and goats, which represent the major sources of the income to rural communities and the national economy as well. Ruminant. The Mat district is located at 41^o 26' N and 19^o 52`S surrounded by Mirdita, Dibra, Bulqiza, Kurbin, Kruja and Tirana districts. The management of ruminant animals is based on mixed system where the outdoor is superior compare to indoor management. Geographic location and animal management system facilitate exposure and distribution of BTV infection. The Mat valley, where the river with the same name runs, the ruminant animals are in largest number. We selected samples from Burrel, Gurrë, Klos, Komsi, Lis, Suç and Ulëz (Figure 1).

The blood samples

A total of 180 blood samples were selected from sera bank of cattle collected in Mat district. Serum samples were kept frozen at -20°C until used for detection of BTV-specific IgG antibodies using competitive enzyme-linked immuno-sorbent assay (cELISA). At the Infectious Disease Laboratory of Veterinary Medicine Faculty the samples were tested for the presence of BTV antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA) (bluetongue antibody test kit, IZSA&M, Teramo, Italy)

Competitive Enzyme-Linked Immunosorbent Assay (cELISA)

The sera were screened for BTV-specific IgG antibodies basically as described by the manufacturer's specifications. cELISA was performed in 96-well antigen-coated microplates. Briefly, aliquots of 50 µl test sera as well as positive and negative controls sera were transferred undiluted to the BTV antigen coated plates, using multi-channel pipette. After incubation, for 60 min at temperature 37°C in a wet camber, the plates were washed, and 50 µl of antibody-peroxidase conjugate were added to each well. The plate was then incubated at 30 min at room temperature in the darkness. The plates were then washed and 100 µl the substrate solution was added to each well. The reaction was stopped using 50 µl of the stopping solution. The results were read either visually or by using ELISA reader set at 450 nm. The test validation procedure was applied as follow: the mean of negative control produced an Abs greater than positive-negative Abs threshold; the mean of positive control was less than positive minus negative Abs threshold; and the MAb control produced Abs greater than 0.80. The obtained results were analyzed

and were interpreted as positive or negative samples. The first step was calculate the mean value of MAb Control Abs, secondly define the positive -negative Abs threshold, by multiplying the MAb control Abs with 0.35; and finally the samples were screened as positive all samples that produced a values of Abs less or equal than *positive - negative* Abs control. Remained samples that produced a values of Abs greater than *positive - negative* Abs control were considered as negative samples.

Figure 1 Map of Dibra District, sampling area

Results.

Table 1 shows the origin of samples, number of samples tested in each village and competitive ELISA results (positive and negative results) According the ELISA test, 28 out of 180 (15.56%) sera samples were positive for specific antibody against bluetongue virus (Figure 2). The positive results were detected in three out of 9 sampled areas. The positive animals were adult and belong both local and crossbreed. Interestingly the positive results were located alongside of Mat River and all positive animals are managed according a mix system, outdoor and indoor system as well.

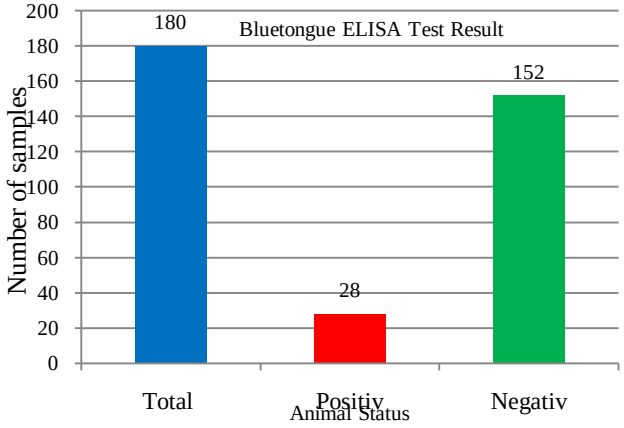

Figure 2. Serological results for specific IgG antibodies against Bluetongue virus, based on competitive ELISA test.

Table 1 Number of samples testet and ELISA positive and negative results.

Sampled Village	*Total samples*	*Positive*	*Percentage (%)*	*Negative*
Lagjia e Re	34	12	35.2	22
Kolaj	18	7	38.8	11
Zenisht	16	0	0	16
Klos	15	0	0	15
Gurrë	14	0	0	14
Komsi	20	0	0	20
Lis	16	0	0	16
Suç	27	9	33.3	18
Ulëz	20	0	0	20
Total	**180**	**28**	**15.5%**	**152**

Despite the risk factors are not analyzed, we presume that they could have a significant impact on serological prevalence of BTD. The ELISA test is useful as a screening test (5). We follow up the positive animals (not all animals we were able to find in the farm), the blood was collected from tail vein and second ELISA test was performed. The strongest positive sera were sent to International Reference Laboratory, Teramo, Italy in order to carry out sera neutralisation test for virus serotype determining (data not published yet). In total the proportion of positive animals was variable, ranged from 0 to 38,8%. The positive animals tend to be in cluster and the environment conditions, location and management system was quite similar.

Conclusion.

The bluetongue virus is circulating in cattle population in Albania. Further study are underway in order to estimate prevalence of disease at level of national cattle herd and determining the specific Bluetongue serotypes which are circulating in the cattle population.

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