EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 11/ February 2017

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Serological Evidence of Blutongue Virus Serotype 9 Circulation in Albania Cattle during 2012-2013

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Abstract:

First outbreak of bluetongue disease in Albania was recorded during late summer beginning of autumn 1998. The infection in cattle generally is mild and only certain strain of bluetongue virus results clinical disease, however they serve as reservoir of infection.

Materials and methods. *In total, 1348 bovine sera blood samples from serum bank were tested by serological competitive ELISA method. Identified cluster positive farms were identified and were follow on at farm level. In follow on survey 42 animals, located in five target different districts were bleed and tested by using cELISA tested. The five representative cELISA positive samples were sent for serotyping at reference bluetongue laboratory Teramo, Italy.*

Results. *In 1390 sera blood samples 104 were positive for presence of total IgG against bluetongue virus. The VN test confirmed the c-ELISA results revealing the presence of antibodies against BTV serotype 9.*

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Conclusion. *The bluetongue virus serotype 9 circulate in cattle population in Albania. The prevalence based on competitive ELISA test results and titer based on serum neutralization test low.*

Key words: Bluetongue virus serotype 9, weak positive, serum neutralization test, follow up

INTRODUCTION

Bluetongue is a viral non-contagious disease, transmitted by arthropods and affect domestic and wild ruminants in tropical and temperate regions of the world (Willson, 2008, Barratt and Maclachlan NJ, 1995). Historically, there were identified 24 bluetongue virus (BTV) serotypes, whereas in last eight years at list three additional serotypes are identified (BTV-25, BVT-26, BVT-27). Epidemiological studies indicate that in Europe circulate nine BTV serotypes (Sanchez *et al*., 2008, Mellor *et al.,* 2008, Purse *et al*., 2005). Bluetongue disease (BTD) has a great economic impact and significantly interfere with international trade. All breeds and ages of sheep are susceptible to BTV, and both morbidity and mortality are much higher than cattle (Barratt and Maclachlan NJ, 1995).

The outcome of bluetongue is highly variable and it range from unapparent to a fatal case depending on species, breed and age of the infected animal and the serotype and strain of the virus (Elbers *et al*., 2008). Goats are less susceptible, very rarely they develop clinical disease, however recent studies indicate that goats play an important role as reservoir of infection (Zientara *et al.,* 2014).

Among the domestic ruminants, clinical signs are recorded in sheep, particularly in fine wool breeds and include fever, facial oedema, ulceration on the oral mucosa and coronitis. BTV could be isolated from clinically healthy cattle and they could be serologically positive. However, during

epizootic outbreaks, infected cattle develop clinical disease depending the BTV serotypes. Clinical signs include fever, appetite lost, erosive lesions in oral mucosa, feet etc (Trejedor, 2004). In pregnant animals BT infection, could causes abortion, hydraencephaly, congenital deformity, and birth of viraemic calves which may or may not develop BT antibody. Cattle are the main mammalian reservoir of Bluetongue virus (Maan *et al.*, 2012, Sanchez *et al.*, 2008, Willson *et al.*, 2008, Nevill, 1971). Serologic tests detect total immunoglobulins, but are not able to serotype the BTV. Until last decade, most reference laboratories used traditional identification methods such as: virus isolation, serum or virus neutralization tests (SNT or VNT). Today, there are available advanced molecular methods, which offer several advantages compare to classical methods, however classical method is valid, useful and are using in parallel with modern techniques. Identification of BTV serotype is important for vaccination programmes and for BTV epidemiology studies (Zienara *et al,* 2014, Sanchez *et al.*, 2008).
In our survey we demonstrate presence of total IgG antibodies to BTV, follow on the cluster positive farms based on competitive enzyme-linked immunosorbent assay (c-ELISA). In addition, representative strongest positive samples were analyzed by serum neutralization test, which confirmed circulation of BTV serotype 9 (Ventura *et al.*, 2004, Trejedor, 2014).

MATERIALS AND METHODS

Time framework. Sera blood samples were collected during end of 2012 beginning 2013 in framework of bovine brucellosis survey. The bovine serum bank is established at veterinary faculty. The cELISA test was done at June 2013, and follow up survey, including the cELISA test was done during July – August. The selected serological positive samples were sent to

the BT reference laboratory at beginning September and both cELISA and serum neutralization tests results were obtained by end of September 2013.

National survey

From bovine sera bank were randomly selected 1348 bovine sera blood samples that belong to 16 Albanian districts and were tested by using cELISA produced by the IZSA&M in Teramo[2].

Follow up survey

Based on cELISA results, 42 animals from 20 farms were sampled. The blood samples were collected from coccygeal vein into plain vacutainer tubes. From blood samples, sera were separated and stored at −20°C till use. After cELISA was performed, 5 representative strongest samples from five districts were sent to the reference bluetongue laboratory[3].

Serological test

Competitive ELISA test was performed at infectious disease laboratory at Veterinary Faculty, Tirana. Each sample were identifying by unique number and were recorded available data such as farm address, animal ID etc. After samples and kit reagents reached the environment temperature the samples were analyzed according the manufacturer instruction.

The ELISA test was carried out in accordance with manufacturer instruction. In short, 50 µl from each sample negative and positive control were transferee to ELISA microplate covered by antigen. After 1h incubation in wet camera at 37°C, 50 µl conjugate was add in each well and the plate was incubating for 30 minutes in dark environment. Three times washing step was done between incubation times.

[2] Bluetongue antibody test kit, IZSA&M, Teramo, Italy

[3] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Via Campo Boario,Teramo, Italy

In each well was add 100 µl substrate and after 30 minutes' incubation in each well 50 µl stop solution was added, 10 minutes later the results were read. The results of the ELISA tests were expressed as the value of the sample (S) divided by value of the positive control serum (P) supplied in the ELISA kit, as determined by measurement of the optical density (OD_{450}) with a "TECAN" ELISA plate reader[4]. The same procedure was conducted for 42 samples of follow on survey. Criteria for classification of animal's health status are presented in Table 1.

Table 1. Animal health status of tested animals based on competitive ELISA results (Celisa)

Criteria	Animal status	Number of animals in national survey	Number of animals in follow on survey	Total
S/P value ≤Difference Positive control- Negative control	Positive	90	14	104
S/P value >Difference Positive control- negative control	Negative	1258	28	1286
Total		**1348**	**42**	**1390**

Competitive ELISA test and Serum neutralization test carry out at reference laboratory,

The five selected BT positive samples were analyzed in serial by using a commercial competitive ELISA test and serum neutralization test at Bluetongue reference laboratory test, Teramo, Italy according to standard operation procedure. The positive ELISA samples were tested for nine BT serotypes known circulate in Europe, respectively BTV1, BTV 2, BTV 4, BT6, BTV 8, BTV 9, BTV 14 –BTV16.

RESULTS

The competitive ELISA results of national survey are presented in Table 2, the results of cELISA follow on survey are presented in Table 3, and in Table 4 there are results of serial testing of

[4] Manufacturer, Tecan Austria GmbH ("Tecan") 5082 Grödig, Austria

cELISA and serum neutralization assays performed at BT reference laboratory.

Table 2. Serological results of national survey based on cELISA method

District	Number of tested animals	Number of positive samples	Percentage (%)of positive samples
Berat	37	0	0
Bulqizë	57	0	0
Dibër	123	8	6.5
Durrës	125	14	11.2
Elbasan	90	4	4.4
Fier	135	12	8. 9
Korcë	40	0	0
Krujë	43	3	7
Lushnjë	108	7	6.4
Mat	107	14	13.1
Përmet	34	2	5.9
Pogradec	54	4	7.4
Sarandë	52	1	1.9
Tepelenë	77	0	0
Tiranë	188	16	8.5
Vlorë	78	5	6.4
Total	**1348**	**90**	**6.67**
Mean of district level	***84.3***	***5.6***	***5.5***

Table 3. Serological results of follow up survey based on cELISA method

District	Number of animal sampled (farms)	Competitive ELISA Test results			
		Negative	Positive (%)	Strong positive	Weak positive
Mat	9 (5)	6	3 (33.3)	2	1
Dibër	7 (3)	5	2 (28.6)	1	1
Tiranë	8 (5)	6	2 (25.0)	1	1
Lushnjë	9 (4)	6	3 (33.3)	2	1
Fier	6 (2)	4	2 (33.3)	0	2
Elbasan	3 (1)	1	2 (66.7)	0	2
Total	**42 (20)**	**28**	**14 (33.3)**	**6 (42.9%)**	**8 (56.1%)**

Table 3. Serological results based on cELISA and serum neutralization tests

District	Tested samples	cELISA Positive results	Seroneutralization Test Results		
			Positive	Serotype	Titer
Mat	1	1	1	BTV-9	1:10
Dibër	1	1	1	BTV-9	1:10
Tiranë	1	1	1	BTV-9	1:10
Lushnjë	1	1	1	BTV-9	1:10
Elbasan	1	0	NA	-	
Total	**5**	**1**	**1**	**All BVT-9**	**All 1:10**

DISCUSSION

Since 1998, there is not any report on bluetongue disease outbreak in Albania (Ventura *et al.,* 2004). Out of 1348 sera samples 90 reveal positive results, a prevalence at national level 6.7% (Table 2), whereas the average prevalence between districts is 5.47 % ($\bar{x}$ ±SD; 5.47±4.12%). The highest prevalence was recorded at Mat, Durrës, Fier, Tiranë, Pogradec, Krujë, Dibër. There was no evidence of anti-bluetongue virus antibodies in Bulqizë, Tepelenë, Berat and Korçë. Detailed analyzing the optic density (OD) value shown that in general the positive value was close to cut off, which indicate a low titer of antibodies. We have not any explanation for this, however it could be related with end of epizootic wave of certain serotype. In other hand in some districts we noticed presence of cluster positive samples (more than one animal in the same farm or very close farms). We didn't know which serotype is circulating in Albania, as long as cELISA do not discriminate them. In other hand, in Europe during 2005 -2008 a BTV 8 serotype outbreak occurred in Europe and it was remarkable that cattle population were highly affected and both mortality and morbidity were significantly higher compare to other serotypes. There are several reports that indicate circulation of different BTV serotypes in Balkan region, for examples in Greece BTV-1, BTV-4, BTV-9 and BTV-16, while in other neighboring countries there were data only for BTV-9 serotype. Comparing to a survey carried out in 2004 (M. Di Ventura et al, 2004) the prevalence in cattle was approximately 18%, obviously higher than results in our study (6.67%). In addition, the prevalence between districts was much higher (close to 21.5%) compare to our study (almost 5.5%). Climatic changes (Purse BV, 2005, Wilson A, 2008), insect circulation and animal movement are the main risk factors for ruminant population exposure to new serotypes of BTV.

The follow-on survey results indicate that the percentage of positive samples was 33.3% (14), whereas the strongest positive samples in cELISA test represent only 42% of them. Both positive samples in Elbasan were positive, but the OD and S/P value was close to threshold level. Four out of five representative positive samples were confirmed in reference laboratory as positive in competitive ELISA test, only one sample (weak positive) resulted negative. All four other positive samples were positive in serum neutralization test and BTV serotype 9 was confirmed that circulate in Albania. All four samples have a low titer, 1:10, exactly the titer at the cutoff limit.

CONCLUSION

There are strong evidences that BTV serotype 9 circulated in 2012 - 2013 in cattle population. The prevalence level was low and the titer was as low as threshold level.

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