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Original article

SEROPREVALENCE OF BLUETONGUE DISEASE VIRUS (BTV) AMONG DOMESTIC RUMINANTS IN KOSOVO AND FIRST RECORD OF BTV SEROTYPE 4 IN SHEEP

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Summary

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The objective of the study was to estimate the seroprevalence and serotype of bluetongue virus (BTV) in domestic ruminants in different regions in Kosovo, in years 2014 and 2015. A total of 905 blood sera were analysed: 633 from sheep, 204 from cattle and 68 from goats, collected in 170 farms, 88 villages in 18 municipalities. All samples were analysed with c-ELISA for detection of BTV seroprevalence. From sheep with clinical signs samples were collected and were analysed with specific RT-PCR. Out of all 905 samples analysed with c-ELISA, 105 samples (11.6%) were seropositive (53 ovine, 39 bovine and 13 caprine). The 43 samples from sheep with clinical sings for bluetongue disease were confirmed by RT-PCR, and BTV-4 serotype was identified. The results indicated high seroprevalence of BTV in domestic ruminants, evidence of BTV-4 serotype in sheep, suggesting a need to strengthen national and regional scientific efforts and control strategy to meet the global challenge of this infectious disease.

Key words: bluetongue, BTV-4, domestic ruminants, Kosovo, seroprevalence

INTRODUCTION

Bluetongue is an insect-transmitted viral disease of domestic and wild ruminants (Mertens *et al.,* 2004). Bluetongue virus (BTV) is a RNA virus, belonging to the non-contagious vectorborne *Orbivirus*, family *Reoviridae*. Twenty-six BTV serotypes of the virus are reported (Maan *et al*., 2012). Multiple BTV serotypes (serotypes 1, 2, 4, 6, 8, 9, 11 and 16) have invaded Europe since 1998 (Mellor *et al*., 2009; Rodriguez-Sanchez *et al*., 2008). Bluetongue disease with clinical signs was reported in Kosovo in 2001, caused by BTV-9 serotype (Osmani *et al*., 2006).

The BTV is transmitted between its ruminant hosts by certain species of biting midges of the genus *Culicoides* (Darpel *et al*., 2007). There are approximately 30

Culicoides species that play a role in the transmission of BTV across the world (Meiswinkel *et al*., 2004; 2007). Presence of *Culicoides* containing *C. obsoletus* and *C. pulicaris* complexes but not *C. imiciola* is reported in Kosovo (Berisha *et al*., 2010).

The global distribution of individual BTV serotypes is attributed to climate change and human influence in environment (Randolph, 2008; Maclachlan, 2010), involving different mechanisms in introduction process including the movement of infected livestock, the passive movement of infected *Culicoides* by the wind (Wilson & Mellor, 2009).

The virus can infect most species of domestic and wild ruminants, but in domestic sheep clinical signs are usually most severe. Cattle are the main reservoir for the BTV, although the cattle and goats disease usually assumes a subclinical course without severe symptoms (Darpel *et al*., 2007). Serotype-specific vaccines for BTV, inactivated or live-attenuated are currently available (Savini *et al*., 2008; Zientara *et al*., 2010).

The seroprevalence of BTV using c-ELISA was reported in many studies, and it is probably the most widely used and validated method with high sensitivity and specificity (Hamblin, 2004). Sensitive and specific real-time RT-PCR assays have been developed to detect BTV types, targeting different genome-segment as reviewed by Hoffmann *et al.* (2009). Real-time RT-PCR assay for BTV-4 targeting genome segment 2, is reported to be a sensitive and reliable method for the identification and differentiation of the twenty-six BTV serotypes (Maan *et al*., 2012).

The purpose of this research is to assess the distribution, seroprevalence and BTV serotype in domestic ruminants (sheep, cattle and goats) in Republic of Kosovo.

MATERIALS AND METHODS

Samples were collected in 88 villages in 18 municipalities (Klina, Gjilan, Gjakova, Shtërpcë, Kaçanik, Drenas, Dragash, Viti, Deçan, Mitrovica, Skenderaj, Shtime, Hani i Elezit, Lipjan, Vushtrri, Peja, Novobërda and Prizren) from a total of 170 farms. The total number of serum samples collected was 905, including 633 from sheep, 68 from goats, and 204 from cattle. Samples were taken during May-September in 2014 and 2015.

Serum samples were separated from sheep blood and were kept frozen at –20 °C until used for detection of BTV-specific IgG. The testing of sera was performed by the competitive-enzyme-linked immunosorbent assay (c-ELISA) for detection of BT antibodies in ruminant sera using a commercial test (IDEXX®, Westbrook, USA). Tests were conducted according to manufacturer instructions, with the known positive and negative controls tested on each plate.

The 59 blood samples collected from sheep with clinical signs for bluetongue were also tested using type-specific real-time RT-PCR assay for BTV-4 targeting genome segment 2, for the presence of this serotype in the samples tested. Molecular analyses were done at the Pirbright Institute, UK.

Composition of reaction mixes for one-step real-time RT-PCR in total volume of 15 μL contained: EXPRESS SuperScript® qPCR SuperMix Universal (10 μL), Forward primer (0.4 μL), Reverse primer (0.4 μL), Probe (0.8 μL), RNase free water (1 μL), ROX (1/10 dilution) (0.4 μL), and EXPRESS SuperScript® mix (2 μL).

The primers for RT-PCR and thermal profile are presented in Tables 1 and 2.

Table 1. Primers and probes used for one-step RT-PCR of bluetongue virus (Hofmann *et al*., 2008)

Primer/ probe	Oligo name	Sequence (5'-3')	Working concentration (μM)
Forward	Hofm_BTV_IVI_F2	TGGAYAAAGCRATGTCAAA	20
Reverse	Hofm_BTV_IVI_R2	ACRTCATCACGAAACGCTTC	20
Probe	Hofm_BTV_IVI_P	FAM-ARGCTGCATTCGCATCGTACGC-Tamra	5

Table 2. Real time RT-PCR thermal profile

Stage of real-time RT-PCR	Temperature	Duration	Number of cycles
Reverse transcription	50 °C	15 min	1 cycle
RT inactivation/ Taq activation	95 °C	20 s	1 cycle
	95 °C	3 s	
PCR	56 °C	30 s	45 cycles
	72 °C*	30 s*	

*Read fluorescence at the end of this stage.

RESULTS

Clinical observations

In summer 2014, clinical symptoms indicative of bluetongue were observed in sheep herds in most of regions in Kosovo. The clinical findings were typical for the disease: abundant salivation, oedema of the tongue, buccal and intermaxillary regions, erosive stomatitis, elevated body temperature, coronitis with resulting lameness. Clinical symptoms of the bluetongue disease were observed in infected cattle as well.

Serological analysis

All samples were analysed by c-ELISA test. The BTV antibodies were detected in 105 from 905 samples (11.6%). Seropositive samples for BTV antibodies are detected in all domestic ruminants: sheep (8.3%), goats (19.1%) and cattle (19.1%) (Table 3).

RT-PCR analysis

The 43 seropositive samples were tested positive by RT-PCR. The RNA samples were also tested using type-specific real-time RT-PCR targeting genome Seg-2 for BTV-4. Results confirmed the BTV-4 serotype.

Table 3. Results from samples analysed with c-ELISA for BTV taken in sheep, cattle and goats in different municipalities in Kosovo in 2014 and 2015

Municipality	Animal species	Number of samples	Positive cases	%
Skenderaj	Sheep	28	0	0.0%
Gjilan	Sheep	24	3	12.5%
	Goat	9	5	55.6%
	Cattle	14	8	57.1%
Gjakova	Sheep	16	1	6.3%
Shtërpc	Cattle	20	4	20.0%
Kaçanik	Sheep	43	4	9.3%
	Goat	2	0	0.0%
Drenas	Sheep	24	1	4.2%
Dragash	Sheep	28	0	0.0%
Viti	Sheep	118	21	17.8%
	Goat	12	3	25.0%
	Cattle	6	3	50.0%
Deçan	Sheep	32	0	0.0%
	Goat	3	0	0.0%
Mitrovicë	Goat	9	0	0.0%
Shtime	Cattle	12	0	0.0%
Hani i Elezit	Sheep	16	2	12.5%
Lipjan	Sheep	9	0	0.0%
	Goat	23	1	4.3%
Vushtrri	Cattle	86	8	9.3%
Prizren	Sheep	28	4	14.3%
	Cattle	20	7	35.0%
Ferizaj	Sheep	141	1	0.7%
	Cattle	46	9	19.6%
Novobërda	Sheep	55	13	23.6%
	Goat	10	4	40.0%
Peja	Sheep	71	3	4.2%
Total		905	105	11.6%

The distribution of BTV seropositive (red dots) and BTV seronegative samples (blue dots) in farms and villages of Kosovo is shown on Fig. 1.

DISCUSSION

This report describes the first evidence of BTV-4 in sheep in Kosovo as well as BTV seroprevalence in domestic ruminants in years 2014 and 2015. After the first BT disease outbreak reported in 2001 caused by BTV serotype 9 (Osmani *et al*., 2006), in disease outbreak in 2014 in Kosovo the results of this study confirmed high seroprevalence in domestic ruminants of 11.6%: 8.3% in sheep, 19.1% in goats and 19.1% in cattle. The RT-PCR analysis in samples from sheep with clinical signs confirmed presence of BTV se-

Fig. 1. The BTV seropositive (red dots) and seronegative (blue dots) samples in domestic ruminants in different municipalities in Kosovo in 2014 and 2015.

rotype 4 involved in disease outbreak in year 2014. Both BTV-4 and BTV-9 serotypes are reported to be present in European countries (Mellor *et al*., 2009; Rodriguez-Sanchez *et al*., 2008).

The high seroprevalence of specific antibodies in domestic ruminants, the presence of disease vectors, presence of two serotypes BTV-9 and BTV-4 in domestic ruminants in Kosovo, suggest that this disease should already considered as endemic disease in Kosovo. Detection and distribution of BTV positive samples showed that the virus was widespread in different areas within the country.

The bluetongue disease is considered a major problem for veterinary medicine due to the rapid spread, mortality and incurred economic losses (Baylis & Mellor, 2001; Saegerman *et al*., 2008).

There is still no contingency plan for responding to an outbreak of bluetongue disease in Kosovo. The high rate of seroprevalence and identification of current two BTV serotypes (BTV-4 and BTV-9) suggests the need for the application of control measures for BTV in Kosovo and strengthening regional scientific efforts and control strategy for infectious diseases in animals. Further research studies are necessary to analyse different mechanisms that have been involved in the introduction process, spread of the virus, specific vector of BTV in our country, factors as-

sociated with animals and environment, factors influencing in disease outbreak, and its impact on animal production.

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