



RETROSPECTIVE ANALYSIS OF THE BLUETONGUE OUTBREAK IN SERBIA

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Received 27 January 2016; Received in revised form 20 August 2016; Accepted 10 October 2016

ABSTRACT

Bluetongue, a vector-borne disease caused by the Bluetongue virus (BTV) and transmitted by *Culicoides* biting midges, is considered to be one of the most important diseases of domestic ruminants. The first outbreak of bluetongue in Serbia was reported in 2001, when BTV serotype 9 was identified in sampled materials. In 2014, outbreak of BTV-4 in Serbia caused considerable economic losses affecting sheep, cattle and goats. During this outbreak, BTV-4 was recorded in 644 outbreaks within 49 municipalities, part of 17 administrative regions. From the total number of sheep kept in areas affected by bluetongue (n=1 748 110), 2 083 cases (0.2%) were proven to be BTV-4 infected. Total of 206 infected cattle and 24 infected goats were reported during this investigation period, which represents 0.06% and 0.03% of the total number of cattle and goats kept in affected areas, respectively. The highest incidence of infected sheep, cattle and goats was recorded on the territory covered by veterinary institute of Nis. Recorded lethality in cattle, sheep and goats was 18.45% (n=38), 48.10% (n=1002) and 54.17% (n=13), respectively. The peak of the outbreak was in September and October when 94.43% of the confirmed positive cases, regardless of the species, was recorded. Monitoring of bluetongue disease in Serbia relies on active surveillance programmes aimed at: (i) identification and tracing of susceptible and potentially infected animals and (ii) detection, distribution and prevalence of insect vectors. Vaccination of sheep is planned to be implemented as a control measure against bluetongue in Serbia.

Key words: bluetongue, BTV-4, outbreak, Serbia

INTRODUCTION

Bluetongue (*Febris catarrhalis ovium*) is a non-contiguous vector-borne viral disease of domestic and wild ruminants with a wide distribution and considerable impact on the health status and production (1, 2). The disease is caused by the Bluetongue Virus (BTV), a double stranded RNA

virus of the *Orbivirus* genus in the *Reoviridae* family (3, 4). There are currently 26 different BTV serotypes (5). The most important route of BTV transmission is primarily through the bite of haematophagous midges of the genus *Culicoides* (Diptera: Ceratopogonidae), the major biological vector of the virus (6, 7). In endemic regions the disease displays a seasonal pattern, with highest incidence in late summer when the vector population reaches its peak (8). The severity of clinical symptoms depends mainly on the infected host, the virulence of the BTV strain and the immunological status of the affected animals (2, 3). The clinical form of bluetongue is most commonly observed in domestic sheep, and it is characterised by fever, excessive salivation, hyperaemia of the nasal and labial mucous membranes, oedema and swelling of the face, severe dyspnoea, asphyxia, oedemas and a blue swollen tongue partly hanging out of the

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Competing Interests: The authors have declared that no competing interests exist.

Available Online First: 15 November 2016

Published on: 15 March 2017

<http://dx.doi.org/10.1515/macvetrev-2016-0094>

mouth (9, 10, 11). In cattle, goats and wild ruminants, bluetongue is most often present in a subclinical form or with mild or transient clinical signs (2, 12). Occurrence of bluetongue is closely related with the activity of competent *Culicoides* vectors, thus the emergence of this disease was traditionally reported in a geographic band between the latitudes 40°N and 35°S, which is its natural habitat (14).

Until 1998, only short outbreaks of bluetongue were occasionally recorded in South European countries (10). However, after nearly 20 years of absence BTV was recorded again on four Greek islands, where BTV-9 was isolated and that was the first report of this serotype on the European continent (15, 16). Since 1998, multiple emergences of bluetongue have been evidenced in Southern Europe, caused by various serotypes of the virus: 1, 2, 4, 8, 9, 10 and 16 (14, 17, 18). From 2006-2010 an epidemic of bluetongue caused by BTV serotype 8 was recorded in many countries of Central and Northern Europe (14, 17, 19). There have been indications that serotypes BTV-6 (detected in Germany and Holland) and BTV-11 (detected in Belgium) could have been introduced by illegal import of live attenuated vaccines (20, 21), as no virus has been isolated and no clinical signs of bluetongue disease have been observed (22).

The first report of bluetongue in Serbia was in 2001 (23), when BTV-9 was detected. In the same year, BTV-9 positive cases were also reported in Bulgaria, Macedonia, Montenegro and Croatia (10, 24, 25), and in the following year, the same serotype was confirmed in Bosnia and Herzegovina (10). In September 2014, following the outbreak of clinical symptoms of bluetongue in sheep and cattle in Serbia, the Institute of Veterinary Medicine Belgrade (Serbia) and the Pirbright Reference Laboratory (England), confirmed the presence of BTV-4 for the first time in this country. Date of the first confirmed case was September 3, 2014 (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=15987). The outbreak of BTV in Europe has caused considerable economic losses, including not only direct damage due to mortality and reduced production, but also indirect damage because of trade bans of ruminants between BTV infected and non-infected areas (17, 26). Due to the increasing importance of bluetongue, the fast spreading of the causative agent and the abovementioned direct and indirect losses, this disease has been included in the List A of notifiable diseases compiled by the OIE (27).

This paper presents a retrospective analysis of the incidence and geographical distribution of BTV in sheep, cattle and goats in the 2014 BTV outbreak in Serbia.

MATERIAL AND METHODS

Investigation of BTV positive animals

The epizootiological situation on Serbian territory is under the surveillance of 12 veterinary institutes, where each institute covers specific area (Fig. 1). The presence of bluetongue was investigated in sheep, cattle and goats. Detection of clinically infected animals throughout Serbian territory was carried out in a joint action by field veterinarians, specialists from veterinary institutes and representatives of the Veterinary Directorate (Ministry of Agriculture and Environmental Protection of Republic of Serbia). In an extensive action that lasted from 03.09 - 31.12.2014, all cases suspected for bluetongue disease were reported by field veterinarians to the veterinary institute responsible for each district. Blood sample from each suspect animal was subjected to serological and molecular testing according to the recommendation from OIE (28). Subsequently, blood from the rest of animals from herds with confirmed BTV positive cases was also tested in the same manner. Testing for the presence of antibodies against BTV was performed using BTV antibody competitive ELISA (cELISA) (IDVET, Montpellier, France). In each seropositive case detection of BTV RNA was performed using real-time RT-PCR (29). All laboratory analyses were performed by the National Reference Laboratory for bluetongue disease, The Institute of Veterinary Medicine - Belgrade (Serbia).

Statistical analysis

Results are shown in a form of descriptive statistical parameters. Differences were tested using t-test in cases of two independent samples. Data analysis was performed using software package PASW Statistics 18.

RESULTS

In 2014, 644 outbreaks were reported in 49 municipalities in 17 (out of 29) administrative regions in Serbia (source: Veterinary Department, Ministry of Agriculture and Environmental Protection, Republic of Serbia, http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=15987). Bluetongue cases were confirmed on territories under the surveillance of the following 9 veterinary institutes: Nis, Pozarevac, Zajecar, Pancevo, Zrenjanin, Kraljevo, Sabac, Jagodina and Belgrade (Fig. 1). From the total number of animals with clinical signs of bluetongue, 91.45% were positive on cELISA testing. Results of molecular identification confirmed the presence

of BTV virus serotype 4 in 56.78% of seropositive samples. With regards to the total number of susceptible animals (1 484 725) kept in the areas of BTV outbreak recorded in 2014, the overall incidence proportion of BTV in Serbia reaches 0.16% (2 313 infected animals).



Figure 1. Distribution map of bluetongue cases in territories covered by veterinary institutes in Serbia in 2014

Bluetongue in sheep

Bluetongue cases in sheep were reported on territories covered by 9 veterinary institutes. From the total number of sheep in Serbia ($n=1\,748\,110$), 58.96% ($n=1\,030\,655$) were located in areas affected by bluetongue (Table 1). Total of 2 083 cases of infected sheep were reported during the investigation period, which represents 0.2% of the total number of sheep in affected areas. The highest incidence proportion with 1 224 cases per 139 872 sheep or 0.88%, was recorded in the territory under surveillance of Veterinary Institute of Nis (Table 1).

Bluetongue in cattle

In cattle, BTV-4 was confirmed in 6 out of 12 areas covered by veterinary institutes. From the total number of cattle in Serbia ($n=920\,068$), 39.28% ($n=361\,042$) were located in areas affected by bluetongue. A total of 206 infected cases per 361 042 of cattle were reported, which gives incidence proportion of 0.06%. The highest incidence proportion with 79 cases per 100 923 of cattle or 0.08%, was recorded in the territory under surveillance of the Veterinary Institute of Nis.

Table 1. Distribution of bluetongue infection in 2014 on territories under the surveillance of veterinary institutes in Serbia

Veterinary institute	cattle		sheep		goats		Total (cattle+sheep+goats)	
	total	infected	total	infected	total	infected	total	infected
“Nis”	100 923	79 (0.08%)	139 872	1 224 (0.86%)	53 311	16 (0.03%)	294 106	1 319 (0.45%)
“Zajecar”	35 750	46 (0.13%)	78 777	290 (0.37%)	16 598	2 (0.012%)	131 125	338 (0.26%)
“Pozarevac”	32 528	3 (0.01%)	79 452	125 (0.16%)	12 299	1 (0.008%)	124 279	129 (0.10%)
“Pancevo”	27 021	67 (0.25%)	48 030	408 (0.85%)	/	/	75 051	475 (0.63%)
“Zrenjanin”	77 262	5 (0.01%)	83 123	14 (0.02%)	10 450	5 (0.05%)	170 835	24 (0.01%)
“Kraljevo”	87 918	6 (0.01%)	165 510	4 (0.002%)	/	/	253 428	10 (0.004%)
“Sabac”	/	/	161 878	3 (0.002%)	/	/	161 878	3 (0.002%)
“Jagodina”	/	/	195 049	9 (0.005%)	/	/	195 049	9 (0.005%)
“Beograd”	/	/	78 974	6 (0.008%)	/	/	78 974	6 (0.007%)
Total	361 042	206 (0.06%)	1 030 655	2 083 (0.2%)	92 658	24 (0.03%)	1 484 725	2313 (0.16%)

total - the total number of animals by species

infected - number and % of confirmed BTV cases

Bluetongue in goats

Bluetongue in goats was confirmed on territories under the surveillance of 4 veterinary institutes. Total of 24 infected cases per 92 658 goats were reported, which gives incidence proportion of 0.03%. The highest incidence proportion with 16 cases per 53 311 goats or 0.02%, was recorded in the territory under surveillance of the Veterinary Institute of Nis.

Monthly distribution of bluetongue cases

The highest incidence of BTV in all susceptible species was documented during the period September-December 2014, with different incidence rates for each species. Bluetongue cases in cattle and sheep were not recorded only in December and August, respectively. BTV positive goats were reported only in September and October. Results of monthly incidence proportion of bluetongue cases, regardless of the species, showed 94.43% positive cases in September and October, which is significantly higher compared to other months ($p < 0.01$). With regards to the species, the highest incidence of BTV and mortality were detected in sheep. Recorded lethality (%; died/infected x 100) in cattle, sheep and goats was 18.45% (38/206 x 100), 48.10% (1002/2083 x 100) and 54.17% (13/24 x 100), respectively (Fig. 2A, B).

presence and geographic distribution of *Culicoides* biting midges have proven to be critical aspects in BTV transmission among susceptible animals, and also the limiting factor in occurrence of the disease (3). Prior to 1998, the appearance of BTV infections were limited to a geographic band between the latitudes 40°N and 35°S, habitat of its main vector, *C. imicola* (14). The breaking point for emergence of bluetongue on the Balkans was the 1998 BTV outbreak on 4 Greek islands (30, 31). At the same time, with the exception of Turkey, this was the first report of BTV-9 in this part of the Europe (32). During the following years, in addition to BTV-9, the presence of BTV-1, BTV-2, BTV-4 and BTV-16 was also confirmed (10, 17).

The first outbreak of bluetongue in Serbia was reported at the end of 2001, where BTV-9 was detected (23). There is a presumption that BTV-9 reached Serbia through infected host animals from Macedonia, Albania and Bulgaria (10), from where it spread further to Croatia (33, 34) and Bosnia and Herzegovina (10). During the outbreak in 2002, bluetongue cases in Serbia were recorded in 37 focal points within 16 municipalities (23). The most recent outbreak of BTV in Serbia was reported at the end of August 2014 (35), and the virus was identified as BTV-4. This was the first report of BTV-4 in Serbia, after which its presence was reported in Montenegro, Bosnia and

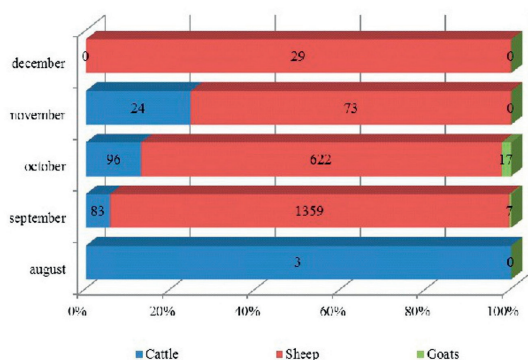


Figure 2A. Morbidity - lethality ratio of bluetongue infected ruminants in Serbia in 2014

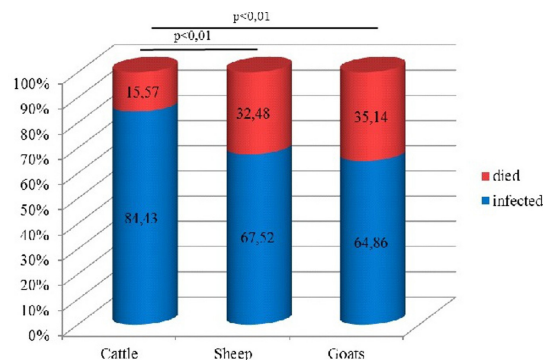


Figure 2B. Morbidity - lethality ratio of bluetongue infected ruminants in Serbia in 2014

DISCUSSION

During the past 20 years, the distribution and characteristics of BTV infection have changed substantially in Europe. It has been suggested, but not proven, that global climate change is responsible for spreading the areal of BTV vectors and enabling the vector competence to indigenous *Culicoides* species, which influenced the occurrence of different BTV serotypes in geographic areas above 50°N (11). The

Herzegovina and Croatia (36). Outbreaks of BTV-9 and BTV-4 in Serbia are most likely connected to the vector competence of other species of *Culicoides* other than *C. imicola* (7). Isolation of BTV-9 and BTV-4 from Palaearctic *Culicoides* species, especially *C. obsoletus*, *C. pulicaris* and *C. scoticus* indigenous to the Balkans (17) supports the hypothesis that other species of biting midges act as primary BTV vectors.

Bluetongue cases in Serbian ruminants were recorded during 644 outbreaks in 49 municipalities,

i.e. 17 administrative regions covered by 9 veterinary institutes. With regards to the total number of susceptible animals kept in the areas of BTV outbreak recorded in 2014, the overall incidence of BTV in Serbia reaches 0.16%. Individual incidence for sheep, cattle and goats was 0.2%, 0.06% and 0.03%, respectively. This is consistent with the incidence proportions recorded in Greece, Bulgaria and Macedonia (37). BTV-4 outbreak was reported in Croatia and Bosnia and Herzegovina in 2014, but official statistics concerning number of infected and deceased animals has not been available to the authors of this paper.

The highest number of bluetongue cases was documented in September and October. These results support the observation that the highest incidence of BTV corresponds to the period of greatest vector abundance (38). Investigation of the bluetongue outbreak indicated higher number of infected animals in municipalities positioned in the basins of major rivers that flow through Serbia (Danube, Sava, Velika Morava, Timok). Highest number of fatality cases due to BTV infections in Serbia, in 2014, was recorded in sheep, followed by goats and cattle. This is not surprising given that sheep develop the most severe clinical conditions, while cattle are the most susceptible host (10). Surprisingly high lethality recorded in goats is in disagreement with the traditional views that bluetongue in goats and cattle often remains subclinical or exhibits mild and transient signs (12, 13).

In comparison with the BTV-9 outbreak in Serbia in 2002, BTV-4 outbreak in 2014 resulted in substantial increase in the number of affected areas. Potential reasons for this could be : (i) spread of *Culicoides* biting midges with regards to their abundance and vector competence due to climate changes (39); (ii) increase in ungoverned and illegal trade of ruminants from BTV affected surrounding areas (Bulgaria, Macedonia and Albania). For the purpose of increasing the understanding of occurrence and distribution of BTV in Serbia, along with investigation of infected animals, a survey aimed at defining the *Culicoides* species present in Serbia, their vector competence and infection status is urgently needed.

CONCLUSION

From the above stated, monitoring of bluetongue disease in Serbia relies on active surveillance programmes aimed at: (i) identification and tracing of susceptible and potentially infected animals and (ii) detection, distribution and prevalence of insect

vectors. In addition, restriction and trade bans of ruminants between BTV infected and non-infected areas are conducted. For 2016, vaccination of sheep against BTV is planned to be implemented in areas affected by bluetongue in 2014 and 2015.

CONFLICT OF INTEREST STATEMENT

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

ACKNOWLEDGMENT

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. III 46002).

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