VIROLOGICAL SUPERVISION OF BLUETONGUE DISEASE IN THE SOUTH-EAST REGION OF ROMANIA

Ana SAMSON (TUDOSE)[1], Cristina RÎMBU[1], Cătălin CARP-CĂRARE[1], Andrei TUDOSE [2], Cristina HORHOGEA[1], Mihai CARP-CĂRARE[1]

[1]Microbiology-Immunology Laboratory, Department of Public Health, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad,700489,Aleea Mihail Sadoveanu, No.8., Iași, Romania

[2] Sanitary-Veterinary Circumscription Smardan, Galați

Abstract

Bluetongue is an infectious, non-contagious, vector-transmitted viral disease affecting domestic ruminants (sheep, goats, cattle) and wild (buffaloes, deer, several species of African antelopes and other species of the Artiodactyla order). The economic importance of the disease lies in the important economic losses following the decrease in the productive capacity of the animals, mortality and fetal malformations, immunization costs of the receptive animals, trade restrictions, reduction of the economic recovery price of the receptive animals and products thereof. Our study aimed at identifying by virological examinations the presence and circulation of BLA virus in the SE region of Romania. For viral isolation and identification were used blood samples collected from domestic ruminants in the counties: Galati, Braila, Tulcea and Vrancea. According to the working chart of the BT Diagnostic Manual (LNR Arboviroze Bucharest), the samples collected from suspect animals were processed and tested by RT-PCR. In the period 2015-2016, 517 blood samples with anticoagulant from 282 cattle and 235 sheep suspected of Bluetongue were tested for the identification of the viral genome by RT-PCR technique. There were no suspicions of Bluetongue disease in goats in the counties included in the study. In bovines in the SE of Romania, the viral genome was identified in 171 (60.64%) blood samples with anticoagulant. In sheep in the SE of Romania, the viral genome was identified in 209 (88.93%) blood samples with anticoagulant. Most positive samples confirmed by the detection of the BT viral genome came from Vrancea, both in cattle (161 positive samples) and in sheep (209 positive samples). Because of the pathogenicity, bluetongue virus infection can not be diagnosed for a certain period of time, the period in which the disease may exist and evolve, the infected animals being sources of infection for vectoric culicoid insects.

Key words: *Bluetongue, virological supervision, domestic ruminants*

Introduction

Bluetongue (BT) is an infectious but non contagious viral disease caused by *Bluetongue virus* (BTV), family *Reoviridae*, genus *Orbivirus* and there are 24 serotypes (Carp Carare M.,2001; Saegerman C., et al., 2008, ICTV,2016). The viral genome consists of 10 double-stranded RNA segments that encode for 4 nonstructural proteins (NS1, NS2, NS3, and NS3A) and 7 structural (VP1-VP7) proteins (Roy P., 1992; Verwoerd D.W. et al., 1970, Saegerman C., et al., 2008). The most important BTV serotypes are 1,2,3,4,6 and 10 but their pathogenicity may vary significantly across each serotype (Saegerman C., et al., 2008, Mann N.S. et.al, 2015).

Bluetongue (BT) is a major disease with major socio-economic implications that cause serious financial losses for both the country and the livestock (Saegerman C., et al., 2008 , Gonciarov Magda et al, 2015; Niedblaki W., 2015).

Because of pathogenicity, Bluetongue virus infection may not be diagnosed for a certain period of time, but the disease still exists and evolves, the infected animals being sources of infection for culicoid insects (Perianu T., 2012; Saegerman C., et al., 2008). In most animals, bluetongue virus infection (BTV) evolves inappropriately but sometimes causes fatal cases in sheep, deer and wild ruminants. In cattle with bluetongue virus infection it usually does not show clinical signs, except for serotype 8 (BTV8) infection in Europe (Dal Pazzo F. et al., 2009; Perianu T., 2012;Saegerman C., et al., 2008; OIE, 2018).

In Romania, ANSVSA's Strategic Program for Bluetongue Surveillance (Bluetongue) in 2017 aims to highlight the lack of viral circulation. In order to accomplish this goal, procedures were structured on two epidemiological directions: passive surveillance and active surveillance, each with clear application methodologies (Ord.32/2006, Ord.124/2018).

The purpose of this paper was to highlight the reports in the period 2016-2017 regarding the presence and circulation of the Bluetongue virus in the SE region of Romania, the border area of the Buzau county, where the first outbreak of Bluetongue in Romania was reported.

Materials and methods

Serological and virological tests are performed at designated LSVSA and / or IDSA (Ord.32/2006). Samples for confirmation of viral serotype are sent by IDSA with the approval of ANSVSA to the European Union Reference Laboratory for Bluetongue (DC_2000/75/CE).

Isolation and viral identification by classical virological examination was performed according to the common virological diagnosis model for all other viruses. Since in vitro virus isolation is more convenient than in vivo, the EU recommended virological monitoring of animals by detecting the specific viral genome in anticoagulated blood samples (EDTA) using the RT-PCR test (Schudel A, 2004).

Diagram of virological diagnosis for Bluetongue disease was adapted to the requirements of the Laboratory Application Chart for Virological Surveillance according to the Terrestrial Animal Health Code. This chart was transposed into the Bluetongue Diagnostic Manual initiated by LSR Arboviroze IDSA Bucharest. According to this charts obtained from suspect animals are processed and tested by PCR, and a positive result is followed by an attempt to isolate the virus by inoculation on embryonated eggs, cellular monolayer cultivation, cytopathic effect and identification of viral serotype and genotype.

***Reverse transcription-polymerase chain reaction* (*Recommended Testing for Virological Confirmation*)**

The use of the RT-PCR technique (polymerase chain reaction) allowed rapid amplification of viral genomic RNA with clinical samples starting, and the current available PCR procedure allows obtaining information on the serotype, serotype, and topotip of the virus (OIE , Terrestrial Manual, 2009). The RT-PCR test, which has a specificity of 92% and a 95% sensitivity (Cetre Sossah et al., 2008).

The reaction involves direct amplification of viral RNA by primers. The results indicate that RT-PCR can be used not only for the detection of viral nucleic acid but also for the serogrouping of *Orbiviruses*, providing information on the genotype of isolated BTV strains only a few days after the onset of signs of disease, through blood samples collected.

Traditional viral isolation procedures, followed by identification, may take 3-4 weeks to provide indications about serotype and serogroup.

In the first step, BTV RNA is extracted from the blood, then transformed into DNA by reverse transcriptase, and then amplified by PCR. The last step is to analyze the PCR product by electrophoresis. Test sensitivity may vary between laboratories, but in general the viral genome can be detected in blood samples 3 days after infection (real-time PCR), antibodies can be detected by ELISA, but 5-7 days after infection .

Results and discussions

Virological surveillance is done by viral genome detection and virus isolation from samples taken from bluetongue suspect animals (Ord.35/2016). Virological diagnosis procedures definitely establish, if samples are harvested from animals infected with Bluetongue virus. Virological investigations were performed on blood samples taken from suspected animals, where

blood serum samples were positive for serological testing, performed by the ELISA technique (Tudose A. et al., 2018).

In the period 2014-2015, the number of positive samples for the serological surveillance of ruminants in Galaţi, Braila, Tulcea and Vrancea counties, varied by area and species (Table 1, fig.1). All results obtained from serological and virological testing were included in Annex 5, developed by each county DSVSA

Table 1

Annual distribution of samples taken from domestic ruminants in the SE region of Romania si testate prin RT-PCR

		2014			2015			
County	Species	No. samples	Results of RT-PCR		No. samples	Results of RT-PCR		TOTAL TEST SAMPLES
			POS.	NEG.		POS.	NEG.	
Galaţi	Cattle	5	3	2	0	0	0	
	Sheep	0	0	0	0	0	0	
	Goats	0	0	0	0	0	0	
Vrancea	Cattle	179	161	18	1	0	1	
	Sheep	235	209	26	1	0	1	
	Goats	0	0	0	3	0	3	
Brăila	Cattle	6	5	1	91	2	89	
	Sheep	0	0	0	0	0	0	
	Goats	0	0	0	0	0	0	
Tulcea	Cattle	0	0	0	0	0	0	
	Sheep	0	0	0	0	0	0	
	Goats	0	0	0	0	0	0	
Total		425	378	47	96	2	94	

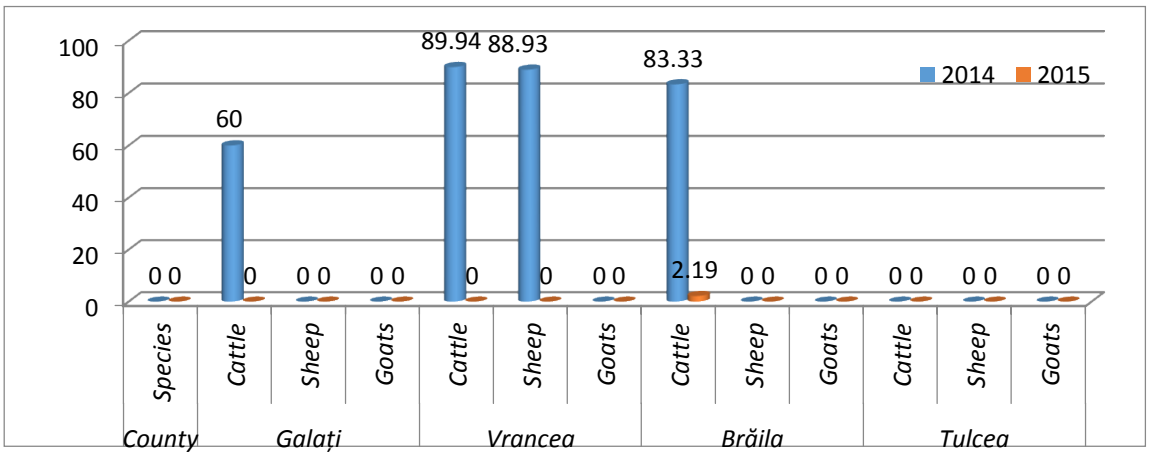

Fig.1. Incidence of positive virological samples tested by RT-PCR

In Galati County, in 2014, 5 blood samples were tested using the RT-PCR technique to identify the viral genome. Samples were harvested only from animals suspected of Bluetongue disease. The virological examination revealed the presence of the viral genome in 3 (60%) of the 5 samples tested. In 2015, no samples were taken to perform the virological examination.

In Vrancea County, in 2014, suspicion of disease was reported in 179 cattle and 235 sheep, animals from which blood samples were taken for serological and virological examinations.

The virological examination revealed the presence of the viral genome in 161 (89.94%) cattle and 209 (88.93%) sheep. In 2015, the number of blood samples collected for the virological examination was significantly lower, with three samples of goats blood taken from a cattle and sheep sample. No positive samples were identified.

In Braila County, only 6 samples of bovine blood were tested in 2014 and no samples from other domestic ruminant species were tested. Of these, 5 (83.33%) were positive for the virological examination. In 2015, tests were performed only on blood samples from bovine animals suspected of being ill. Of the 91 samples tested, 2 (2.19%) of the samples showed the viral genome.

In Tulcea County, Virological examinations for the confirmation of Bluetongue virus were not performed during 2014-2015. Even if in 2014 in Tulcea did not confirm the cases of illness, the epidemiological situation near the county imposed preventive measures, similar to the other counties.

Virological examinations carried out during 2014-2015 revealed the viral genome in blood samples from bovine and ovine animals in the SE of Romania. During this time, blood samples from the goat species were not tested. Most positive samples came from Vrancea County and the most receptive animals to viral infection were bovines, followed by sheep. In bluetongue epidemiology, bovine animals play a particularly important role due to prolonged viraemia in the absence of clinical signs of the disease. However, there is no explanation for the very high morbidity rate in bovine animals in Romania.

Because of pathogenicity, Bluetongue virus infection may not be diagnosed for a certain period of time, but the disease still exists and evolves, and the infected animals are sources of infection for *Culicoides* insects. Ruminants from non-professional farms are the most exposed animals to bluetongue virus infection, as these holdings do not have biosecurity systems or these conditions are very poor.

In Romania, the circulating virus BTV belongs to the 4th serotype, like other countries in this region of Europe, such as Bulgaria and Greece, and have been circulating in recent years in the countries of the Western Mediterranean and North Africa (http://www.bluetonguevirus.org/). According to the European Commission, the data presented in the control and restriction document on Bluetongue, the circulating serotype in Romania has been identified in other parts of Europe

Conclusions

1. The Bluetongue viral genome has been highlighted in suspected disease ruminants in the SE region of Romania
2. There were no suspicions of Bluetongue disease in goats in the SE region of Romania.
3. In Vrancea County, the viral genome was identified in 89.94% of the cattle and 88.93% of the sheep tested in 2014.
4. In Galati county, there were suspicions of Bluetongue disease in cattle in 2014, with 60% of the tested animals being positive.
5. In Braila County there were suspicions of Bluetongue disease in cattle, 83.33% (in 2014) and 2.19% (in 2015) of the tested animals being positive.
6. In Tulcea County, no suspected Bluetongue disease was reported during 2014-2015

References

1. Carp Cărare M., (2001) - *Microbiologie generală Virusologie* – Casa de editură "Venus" Iaşi
2. Cetre-Sossah, C., B. Mathieu, M. L. Setier-Rio, C. Grillet, T. Baldet, J. C. Delecolle and E. Albina (2008) - *Development and evaluation of a real-time quantitative PCR assay for Culicoides imicola, one of the main vectors of bluetongue (BT) and African horse sickness (AHS) in Africa and Europe*.Veterinary Research 35 (2004) 325-337

3. Dal Pazzo, F.,și col.. (2009)- *Bovine infection with bluetongue virus with special emphasis on European serotype* 8. The Veterinary Journal, 182(2), 142–151. http://dx.doi.org/10.1016/j.tvjl.2009.05.004
4. Gonciarov, M., Coman, C. (2015). *Risk factors, incidence and prevalence of bluetongue in Romania and worldwide in the last decade*, Scientific Works. Series C. Veterinary Medicine., Vol. LXI (2), USAMV Bucureşti, pp.42-47
5. Niedbalski W., (2015) - *Bluetongue in Europe and the role of wildlife in the epidemiology of disease.* Polish Journal of Veterinary Sciences Vol. 18, No. 2 (2015), 455–461
6. Perianu T., (2012) – *Tratat de boli infecțioase ale animalelor,* Viroze vol I, Ed. Universitas XXI Iasi, pag.920
7. Roy P.,(1992)- *Bluetongue virus proteins*, Journal of General Virology;73:3051–64.
8. Saegerman C., Bervens D., Melor S. P., (2008) – *Bluetongue Epidemiology in the European Union*, Emerging Infectious diseases, Vol. 14,no.4, 539-544
9. Schudel A., Wilson D. & Pearson J.E. (2004). – *Office International des Epizooties international standards for bluetongue*. In Proc. Third International Symposium on bluetongue (N. James MacLachlan & James E. Pearson, eds). 26-29 October 2003, Taormina. Vet. Ital., 40 (4), 676-68
10. Tudose (Samson) A., Rimbu C., Carp-Carare C., Tudose A., Cozma A., Carp-Carare M., (2018) – *Serological supervisions of Bluetongue disease in the South-East Region of Romania*, Animal Breeding and Pathology to day, USAMV Timisoara,
11. Verwoerd DW, Louw H, Oellermann RA.,(1970)- *Characterization of bluetongue virus ribonucleic acid*. Journal of Virology.;5:1–7
12. Xxx Directive 2000/75/EC as regards the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue.
13. xxx International Committee on Taxonomy of Viruses (ICTV). (2016)- Virus taxonomy: 2014 Release. Retrieved from http://www.ictvonline.org/virusTaxonomy.asp?taxnode_id=20143496
14. xxx Ord.124/2018 Order No. 124/2018 on completing the Order of the President of the National Sanitary Veterinary and Food Safety Authority no. 35/2016 for the approval of the Methodological Norms for the application of the Program of actions for surveillance, prevention, control and eradication of animal diseases, of disease Transmitted from Animals to Human, protection of animals and environment protection, identification and registration of bovine, swine, sheep , goats and equidae, as well as the Methodological Norms for the implementation of the Food Safety Supervision and Control Program
15. xxx Ordinul nr. 32/2006 for the approval of the Sanitary Veterinary Norm, which establishes specific provisions for the control and eradication of bluetongue, transposed in Directive 2000/75/
16. xxx World Organisation for Animal Health_OIE, 2018 – Bluetongue, scientific.dept@oie.int