

Post-epidemic Occurrence of Bluetongue Virus Vectors (*Culicoides* Species) în Iași County

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Abstract

Culicoides is a genus of biting midges in the family *Ceratopogonidae*, representing a big threat, giving that several species serve as biological vectors of pathogens of veterinary importance. BTV distribution and persistence is thought to be almost entirely dependent upon the presence or absence of *Culicoides* adults vector. In this study, biting midges were collected during 2015-2016, once a week, from April to December. In the first year, 2617 BTV vector specimens were collected and identified as *Culicoides obsoletus* (41.2%; 95% CI: 39-43), *Culicoides pulicaris* (58%; 95% CI: 56-59) and *Culicoides nubeculosus* (0.8%; 95% CI: 0.4-1.1). In the second year, 165 BTV vector specimens were collected and identified as *Culicoides obsoletus* (44.25%; 95% CI: 36-51), *Culicoides pulicaris* (50.3%; 95% CI: 42-57), *Culicoides dewulfi* (4.84%; 95% CI: 1.5-8.1) and *Culicoides nubeculosus* (0.61%; 95% CI: 0.5-1.7). *Culicoides pulicaris* and *Culicoides obsoletus* complex were especially abundant within the two collection sites in Iași County. Our data on post-epidemic occurrence and composition of BTV vector species in the studied area will facilitate a better understanding of *Culicoides* vectors distribution and the possible role in bluetongue virus overwintering.

Keywords: biting midges, *Culicoides*, Iași County, vectors

Introduction

Biting midges of the genus *Culicoides* (Diptera: *Ceratopogonidae*) are among the smallest hematophagous dipteran insects. These flies are of medical, veterinary, and economic importance because their blood-feeding behavior can transmit disease agents. Some species of *Culicoides* may act as vectors of many disease pathogens affecting livestock worldwide, including at least 66 viruses, 15 protozoa and 23 nematodes (Sick *et al.*, 2019). Adult *Culicoides* are poor fliers (Mellor *et al.*, 2000), dispersal being limited to within several kilometres from the emergence site (Kluiters *et al.*, 2015). However, individuals can be carried over far greater distances through

wind dispersal, largely inferred from the spread of *Culicoides*-borne disease (Gloster *et al.*, 2008). This semi-passive flight is one of the reasons why *Culicoides*-borne arboviruses are capable of rapid spread (Burgin *et al.*, 2013). Most of the *Culicoides*-borne viruses belong to the families *Reoviridae* (e.g., African horse sickness virus, bluetongue virus, or epizootic hemorrhagic disease virus), *Rhabdoviridae* (e.g., bovine ephemeral fever virus) and *Peribunyaviridae* (e.g., Akabane virus, Schmallenberg virus, or Oropouche virus). This study examines the distribution and abundance of *Culicoides* vectors involved in BTV transmission in Iași County, one of the counties in the eastern focus of infection of the last bluetongue epidemic.

During this study, surveillance on biting midges was carried in two years, during 35 weeks each year.

Materials and methods

The research has been carried out during 2015-2016, once a week, from April to December in order to collect biting midges. The capture was made using modified CDC traps placed on the meadow, 1.8 m above the ground, in front of the stable, in the vicinity of stables with cattle. The sampling sites (Figure 1) span altitudes of 48 m (Vlădeni) to 62 m (Zaboloteni). Vlădeni collection site is located in the northeast of the county, on the shores of the Jijia river, in an area with multiple lakes. The second collection site is located also in the northeast of the Iași county, on the shores of the Prut river.

All individuals were identified in samples that contained less than 500 specimens. For the morphological identification, the Interactive IIC key (Mathieu *et al.*, 2012) was used for livestock-associated *Culicoides* species based on wing pattern and palpi. Identification was performed under a stereomicroscope with 10× and 20× magnification.

Results and discussions

Some *Culicoides* species (among these the most abundant being *C. obsoletus*) are known to act as vectors for bluetongue virus (BTV). This Orbivirus of the family *Reoviridae* causes an infectious disease called bluetongue disease (BTD) in ruminants – mainly sheep, but cattle and goats are affected as well.

First cases of bluetongue virus infection occurred in August 2014 in cattle, in South-East of Romania, at an altitude of 1000 meters. The animals have shown signs of disease: hyperthermia, hyperemia, congestion and erosions of the skin and mucosae, especially oral mucosa, salivation, epiphora, and nasal discharge. Forward the BTV serotype identified as BTV 4 was confirmed by the Pirbright Reference Laboratory on September 2014. In 2014, there were registered 1885 outbreaks disseminated all over the country (www.oie.int). This frequency decreased to 30 outbreaks of BTV 4 in 2015 of which three outbreaks were registered in Iași County and 18 in Botoșani County. The last outbreak of bluetongue was notified on November 2015 in the Eastern region of the country.

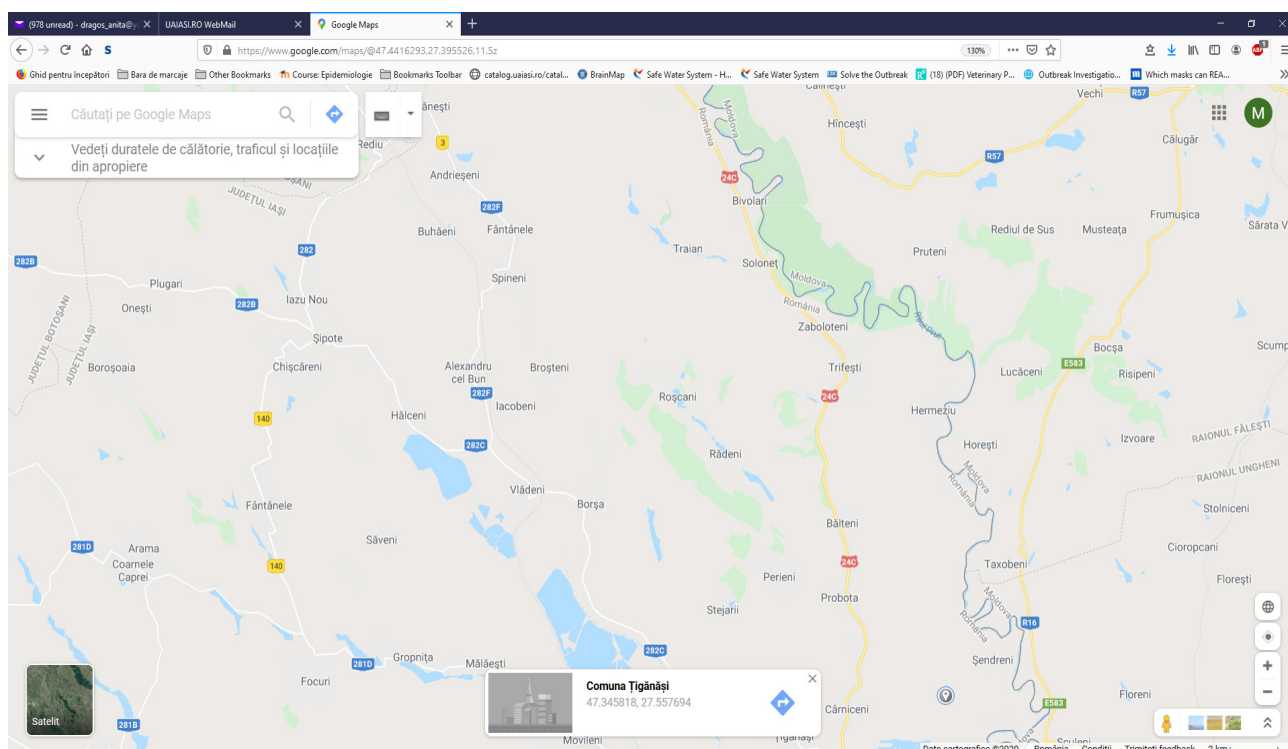


Figure 1. Geographical origin of the sampled vectors: A. in 2015; B in 2016

On the basis of this epidemiological context of BTV4 circulation, a total of 2782 *Culicoides* specimens were collected during 35 consecutive weeks in each year (2015 and 2016) of the studied period. In the first year, 2617 BTV vector specimens (table 1) were collected and identified as *Culicoides obsoletus* (41.2%), *Culicoides pulicaris* (58%) and *Culicoides nubeculosus* (0.80%).

In the second year, 165 BTV vector specimens (Table 2) were collected and identified as *Culicoides obsoletus* (44.25%), *Culicoides pulicaris* (50.3%), *Culicoides dewulfi* (4.84%) and *Culicoides nubeculosus* (0.61%).

Results of the vector monitoring carried out from 2015 to 2016, with a particular attention to the *Culicoides* species/complexes most involved in bluetongue virus transmission in Romania (*C. obsoletus*, *C. pulicaris*, *C. nubeculosus*, *C. dewulfi*), are statistically analyzed in Table 3. *Culicoides*

pulicaris and *Culicoides obsoletus* complex were especially abundant within the two collection sites in Iași County.

The *Culicoides pulicaris* complex was most abundant, representing 57.5% of all individuals trapped, followed by the *Culicoides obsoletus* complex (41.5% of individuals). The other *Culicoides* complexes (*C. nubeculosus* and *C. dewulfi*) represented only a very low proportion of all individuals and the major vector, *C. imicola*, was absent from all catches and sites. The maximum catch of *C. pulicaris* complex in a trap, at 1186, was two times that of the *C. obsoletus* complex, at 596. These two species complexes were widespread across both sites and more abundant in 2015.

The current involvement of these two species complexes in BTV transmission in Europe is well confirmed: by fine scale overlap of their geographic distributions with outbreaks (De Liberato *et al.*,

Table 1. Results of the entomological surveillance performed in Iași County in 2015

Date	Target species	T °C min/max	C. obsoletus	C. pulicaris	C. nubeculosus	C. dewulfi
			No.	No.	No.	No.
17.04.2015	bovine	8°C/ 5°C	9	0	0	0
24.04.2015	bovine	12°C/ 7°C	3	1	0	0
30.04.2015	bovine	4°C/ 7°C	0	0	0	0
06.05.2015	bovine	14°C/ 12°C	596	1186	0	0
13.05.2015	bovine	14°C/ 12°C	8	21	0	0
21.05.2015	bovine	24°C/ 10°C	394	252	3	0
3.06.2015	bovine	24°C/ 10°C	6	4	0	0
11.06.2015	bovine	24°C/ 18°C	26	5	0	0
24.06.2015	bovine	23°C/ 17°C	27	16	0	0
09.07.2015	bovine	28°C/ 25°C	8	2	2	0
17.07.2015	bovine	24°C/ 17°C	0	0	0	0
22.07.2015	bovine	25°C/ 18°C	0	0	0	0
30.07.2015	bovine	22°C/ 17°C	0	3	0	0
04.08.2015	bovine	22°C/ 17°C	0	0	0	0
12.08.2015	bovine	22°C/ 17°C	0	0	0	0
04.09.2015	bovine	22°C/ 17°C	0	0	0	0
17.09.2015	bovine	20°C/ 11°C	1	8	8	0
24.09.2015	bovine	20°C/ 11°C	0	10	4	0
09.10.2015	bovine	12°C/ 09°C	0	10	4	0
30.10.2015	bovine	10°C/ 5°C	0	0	0	0
09.11.2015	bovine	9°C/ 2°C	0	0	0	0
12.11.2015	bovine	9°C/ 2°C	0	0	0	0
19.11.2015	bovine	9°C/ 2°C	0	0	0	0
Total			1078	1518	21	0

2005), by vector competence studies (Paslaru *et al.*, 2018; Carpenter *et al.*, 2006) and by virus isolation from wild-caught adults of *C. pulicaris* and *C. obsoletus* complex (Savini *et al.*, 2005). Climate

change has been implicated in the spread of BTV vectors in Europe, with an increasing incidence of BTV outbreaks in areas that have warmed over the last decade. A cross-sectional study demonstrated

Table 2. Results of the entomological surveillance performed in Iași County in 2016

Date	Target Species	T °C min/max	<i>C. obsoletus</i>	<i>C. pulicaris</i>	<i>C. nubeculosus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
			No.	No.	No.	No.	No.
07.04.2016	bovine	23°C/10°C	10	1	0	0	0
14.04.2016	bovine	23°C/10°C	10	72	0	0	0
22.04.2016	bovine	11°C/8°C	0	0	0	0	0
22.04.2016	bovine	11°C/8°C	0	0	0	0	0
28.04.2016	bovine	14°C/6°C	0	1	0	0	0
05.05.2016	bovine	13°C/14°C	1	1	1	0	0
12.05.2016	bovine	13°C/14°C	0	0	0	0	0
25.05.2016	bovine	15°C/16°C	3	0	0	0	0
30.05.2016	bovine	23°C/16°C	0	0	0	0	0
03.06.2016	bovine	15°C/16°C	0	0	0	0	0
10.06.2016	bovine	15°C/16°C	0	0	0	0	0
17.06.2016	bovine	25°C/18°C	0	0	0	0	0
22.06.2016	bovine	28°C/23°C	8	5	0	3	0
23.06.2016	bovine	25°C/18°C	2	1	0	0	0
30.06.2016	bovine	25°C/20°C	0	0	0	0	0
07.07.2016	bovine	25°C/20°C	9	1	0	2	0
13.07.2016	bovine	26°C/19°C	27	0	0	3	0
14.07.2016	bovine	27°C/18°C	0	0	0	0	0
22.07.2016	bovine	27°C/18°C	0	0	0	0	0
28.07.2016	bovine	27°C/21°C	1	1	0	0	0
05.08.2016	bovine	27°C/18°C	1	0	0	0	0
11.08.2016	bovine	27°C/14°C	0	0	0	0	0
18.08.2016	bovine	27°C/18°C	0	0	0	0	0
23.08.2016	bovine	27°C/19°C	0	0	0	0	0
25.08.2016	bovine	26°C/15°C	0	0	0	0	0
01.09.2016	bovine	26°C/13°C	1	0	0	0	0
08.09.2016	bovine	28°C/15°C	2	1	0	0	0
14.09.2016	bovine	27°C/14°C	2	1	0	0	0
22.09.2016	bovine	16°C/5°C	0	0	0	0	0
12.10.2016	bovine	8°C/8°C	0	0	0	0	0
Total			73	83	1	8	0

Table 3. The abundance of *Culicoides* species in the two collections sites from Iași County

Sampling year	<i>C. obsoletus</i>		<i>C. pulicaris</i>		<i>C. nubeculosus</i>		<i>C. dewulfi</i>	
	No.	Prevalence (CI 95%)	No.	Prevalence (CI 95%)	No.	Prevalence (CI 95%)	No.	Prevalence (CI 95%)
2015	1078	41.2% (39-43)	1518	58% (56-59)	21	0.8% (0.4-1.1)	0	0
2016	73	44.25% (36-51)	83	50.3% (42-57)	1	0.6% (-0.5-1.7)	8	4.84% (1.5-8.1)

that *Culicoides* vectors are active during peak delivering periods inside sheds. A longitudinal study the following delivery season demonstrated that *Culicoides* were more abundant indoors than outdoors, and demonstrated activity of gravid and parous *Culicoides* over the winter (Magliano *et al.*, 2018). This demonstrates a possible mechanism for overwintering of BTV4 in the east of Romania during 2014-2015.

Conclusions

The *Culicoides* abundance analysed in this study is exclusively based on the abundance observed on cattle farms. BTV distribution and persistence is thought to be almost entirely dependent upon the presence or absence of *Culicoides* adults vector. Thus, our data on post-epidemic occurrence and composition of BTV vector species in the studied area is the first step for a better understanding of *Culicoides* vectors distribution and the possible role in virus overwintering. Further indepth studies are to be undertaken in order to conclude if there is still a risk of BTV or other *Culicoides* transmitted diseases in Iași County.

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