

Epizootiological control of bluetongue disease in Bulgaria in 2002

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Summary

In accordance with the National Surveillance Programme for the control of bluetongue (BT) disease in 2002, serum surveillance was performed in 22 sentinel, seronegative animal herds located in western Bulgaria. These herds were at least 40 km outside the settlements affected by the 2001 epidemic. Another 42 sentinel villages (herds) were established in southern Bulgaria in a 10 km border strip zone in the Bourgas, Yambol, Haskovo, Kardjali, Smolyan and Blagoevgrad Districts. The implementation of the programme in 2002 commenced on 15 April and continued until 15 November. More than 7 200 serum samples were tested prior to 26 August and no evidence of active BT virus (BTV) infection detected. This was confirmed by further viral, serological, epidemiological and clinico-pathological observations. In addition, there was no evidence of transborder penetration of BTV into Bulgaria by infected livestock or by infected *Culicoides*. However, on 26 August 2002, BTV seropositive sentinel animals were detected close to the southern Bulgarian border. Subsequently, animals were detected in more than 20 villages, but clinical disease was not observed.

Bulgaria was divided into 58 quadrants (50 km × 50 km) and a *Culicoides* surveillance programme established in 23 of these. A total of 92 *Culicoides* light-trap collections were made. During three years of *Culicoides* surveillance, not a single specimen of the principal BT vector *C. imicola* was captured. The dominant *Culicoides* species was *C. obsoletus*, followed by *C. pulicaris* and *C. punctatus*; in August 2001, *C. puncticollis* was recorded for the first time. Studies on the seasonal phenology of *Culicoides* were conducted in two villages (Vacevo and Bersin in the District of Kiustendil) affected in the 2001 outbreak of BT. Here trapping of *Culicoides* commenced on 1 March 2002 and continued until 15 November 2002; midges became active during the third week of April to almost cease in the second half of November. There appeared to be three peaks of activity: one during the second half of May, another in August and a third at the beginning of October.

Keywords

Bluetongue – Bulgaria – *Culicoides* – Sentinel animals – Surveillance.

Introduction

Bluetongue (BT) virus (BTV) – the aetiological agent of BT disease in ruminants, belongs to the genus *Orbivirus* of the family *Reoviridae* (5). BTV infects and replicates in the *Culicoides* vector and in domestic and wild ruminants, including cattle, buffalo, deer, antelope, sheep, goat and elk (6). The clinical and pathological symptoms vary among species and range from subclinical or mild to acute and often fatal disease with a high percentage of deaths in some sheep breeds. In 1943, BT was first recognised in Cyprus (4) and in 1956-1957, BTV infected Spain and Portugal. Approximately 180 000 animals were killed to eradicate the disease from Spain and

Portugal (1). Between 1977 and 1979, BTV infected Turkey and some of the islands of Greece (2). On the Greek islands of the Dodecanese and Rhodes, BTV infection was recognised again in late 1998. Sheep located on the island of Lesbos developed antibody against BTV but clinical disease was not observed. In the summer of 1999, BTV affected the European part of Turkey, continental Greece and for the first time Bulgaria (3). BT affected Bulgaria again in September 2001. Antibody was detected without any clinical appearance of the disease. Samples collected in previous outbreaks in the region were identified as serotypes 4, 9 and 16. This observation of various types from the past outbreaks is indicative of the high risk of entry of multiple virus types into

the surrounding countries and possible persistence of the virus in these regions.

Objectives

The objectives of the national programme in Bulgaria was to perform serum surveillance in sentinel zones, to implement *Culicoides* surveillance by trapping midges, and to perform phenology investigations by trapping *Culicoides* from the beginning to the end of the season in two villages in the Kiustendil District, which had been affected in 2001 outbreak.

Materials and methods

To detect antibodies against the group antigen of BTV, the competitive enzyme-linked immunosorbent assay (ELISA) (VMRD, USA) was used. Samples were considered positive if they had an average optical density (Δ OD) lower than or equal to the Δ OD of the positive control of the kit; otherwise, results were calculated according to a formula provided by the manufacturer. In accordance with the National Surveillance Programme for the control of BT, serum surveillance was performed in 22 sentinel serum-negative animal herds in 2002 (Fig. 1) located in western Bulgaria at least 40 km inland from the areas affected during the epidemic in 2001. The sentinel animals were tested every 30 days for the presence of BTV antibodies. Each sentinel herd consisted of 10 cattle and 10 goats; each animal was identified with an ear-tag. The settlements with sentinel herds and villages (by district) were as follows:

- Vidin Archar and Drenovet
- Montana Brusartzi, Dolno Tzerovene and Lehchevo
- Vratza Furen, Krivodol, Vratza and Zlidol
- Sofia District Milanovo, Tzerovo, Novatchene and Skravena
- Sofia town Trebich, Buchovo and Lozen
- Pernik G. Butchino, Bosnek and Dren
- Kiustendil Kraynitzi, Ovchartzi and Stob.

Another 42 sentinel villages (herds) located in southern Bulgaria in a 10 km border-strip zone in the Bourgas, Yambol, Haskovo, Kardjali, Smolyan and Blagoevgrad Districts were established (Fig. 1). Each sentinel herd consisted of 10 calves over six months of age and 10 lambs over four months of age. These sentinels were tested every 30 days for the

presence of BTV antibodies. The settlements with sentinel herds and villages (by district) were as follows:

- Bourgas Rezovo, Slivarovo, Brashlyan, Granichar, Belevren and Gorno Yabalkovo
- Yambol Strandja, Kraynovo, Goliam Dervent and Lesovo
- Haskovo Radovetz, Prisadetz, Matochina, Kap, Andreevo, Mezek, Lambuch, Slaveevo, Mandritza and Dolno Lukovo
- Kardjali Tchernichevo, Avren, Egrek, Tichomir, Lozengradtzi, Gorno Kapinovo and Drangovo
- Smolyan Zlatograd, Kushla, Mochura, Arda, Buinovo and Barutin
- Blagoevgrad Teplen, Katuntsi, Marikostinovo, Ilinden, Dzijevo, Topolnitsa, Kolarovo, Elenovo, Krupnik and Mikrevo.



- Sentinels located 40 km inside the 2001 outbreaks
- Villages with sentinel animals
- Villages with sentinel animals born in 2002

Figure 1
Sentinel animals in western and southern Bulgaria, 2002

The implementation of the 2002 programme commenced on 15 April and continued until 15 November 2002.

The blood samples taken once every 30 days from the sentinel (indicator) large and small ruminants were tested for presence of BTV antibodies at the National Reference Laboratory. All blood samples tested in 2002 (until 26 August) were negative for the presence of antibodies to BTV. Onderstepoort light traps were used to perform *Culicoides* surveillance. Midges were trapped from the beginning to the end of the season in two villages in the Kiustendil District that had been affected by the 2001 outbreak.

Results

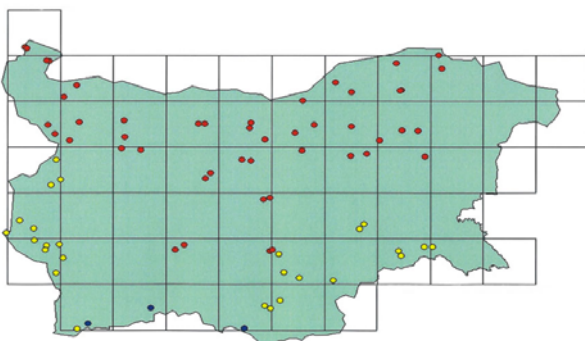
The 7 404 blood samples collected from sentinels prior to 26 August were all BT-negative. On 26 August 2002, BTV seropositive sentinel animals were detected close to the southern border of Bulgaria (Fig. 2). Serum samples positive in the c-ELISA were found in more than 20 villages but clinical disease was not observed in local animals.



- Villages with BTV-positive animals after 26 August 2002

Figure 2
Villages in southern Bulgaria with seropositive animals in 2002

The *Culicoides* trapping programme was undertaken in 23 of the 58 quadrants of 50 × 50 km at the beginning of July 2002 in conjunction with an international BTV project in collaboration with the Institute for Animal Health (IAH) in Pirbright (Fig. 3). A total of 92 *Culicoides* catches were performed (Table I).



- 2002 principal surveillance
- 2002 samples for virus isolation
- 2001 principal surveillance

Figure 3
Culicoides trapping sites and principal surveillance points in Bulgaria, 2001-2002

The phenology of *Culicoides* was studied in two villages (Vacsevo and Bersin in the District of Kiustendil) that had been affected by BT during the 2001 epizootic. *Culicoides* were trapped from 1 March 2002 to 15 November 2002. Results from trapping and *Culicoides* spp. activity during the summer of 2002 are shown in Tables II and III.

Table I
Trapped quadrants and sites:
the *Culicoides* surveillance programme, 2002

No.	Region	Village/farm	Sample code		
1	Vidin	Bregovo	BG 1	A	S1, S2
2	Vidin	Bregovo	BG 1	B	S1, S2
3	Vidin	Vidin	BG 2	A	S1, S2
4	Vidin	Vidin	BG 2	B	S1, S2
5	Montana	Lom	BG 3	A	S1, S2
6	Montana	Brusartzi	BG 3	B	S1, S2
7	Montana	Chiprovtsi	BG 13	A	S1, S2
8	Montana	Govejda	BG 13	B	S1, S2
9	Montana	Berkovitza	BG 14	A	S1, S2
10	Montana	Montana	BG 14	B	S1, S2
11	Vratza	Tichevitza	BG 15	A	S1, S2
12	Vratza	Borovan	BG 15	B	S1, S2
13	Vratza	Mezdra	BG 26	A	S1, S2
14	Vratza	Roman	BG 26	B	S1, S2
15	Pleven	Pleven	BG 16	A	S1, S2
16	Pleven	Pleven	BG 16	B	S1, S2
17	Pleven	Kozar	BG 17	A	S1, S2
18	Pleven	Levski	BG 17	B	S1, S2
19	Lovetch	Beli Osam	BG 27	A	S1, S2
20	Lovetch	Oreshak	BG 27	B	S1, S2
21	Gabrovo	Bogatovo	BG 28	A	S1, S2
22	Gabrovo	Kormiansko	BG 28	B	S1, S2
23	Pazardjik	Malo Konare	BG 46	A	S1, S2
24	Pazardjik	Glavitza	BG 46	B	S1, S2
25	Rouse	Viatovo	BG 8	A	S1, S2
26	Rouse	Chervena voda	BG 8	B	S1, S2
27	Razgrad	Isperich	BG 9	A	S1, S2
28	Razgrad	Isperich	BG 9	B	S1, S2
29	Silistra	Kalipetrovo	BG 10	A	S1, S2
30	Silistra	Alftar	BG 10	B	S1, S2
31	V. Tarnovo	Pavlikeni	BG 17	C	S1, S2
32	V. Tarnovo	P. Karavelovo	BG 18	C	S1, S2
33	Rouse	G. Ablanovo	BG 18	A	S1, S2
34	Rouse	Drianovetz	BG 18	B	S1, S2
35	Targoviste	Popovo	BG 19	A	S1, S2
36	Targoviste	Targoviste	BG 19	B	S1, S2
37	Shoumen	Tzarev brod	BG 20	A	S1, S2
38	Shoumen	Gradiste	BG 20	B	S1, S2
39	V. Tarnovo	G. Oriachovitza	BG 29	A	S1, S2
40	Targoviste	Antonovo	BG 30	A	S1, S2
41	Targoviste	Krasnoseltzi	BG 30	B	S1, S2
42	Shumen	Yangovo	BG 31	A	S1, S2
43	Shumen	Biala reka	BG 31	B	S1, S2
44	St Zagora	Zetevo	BG 38	A	S1, S2
45	St Zagora	Tzenovo	BG 38	B	S1, S2
46	St Zagora	Kran	BG 39	A	S1, S2
47	St Zagora	Dunavtzi	BG 39	B	S1, S2

It is clear from Tables II and III that the seasonal activity of *Culicoides* in 2002 started in the third week of April and continued until 15 November. It is possible to estimate two or three peaks of activity (second half of May, August and probably the beginning of October).

Table II
Phenology investigations and seasonal dynamics
of *Culicoides* spp. trapped in 2002
(Vaksevo Village, Kiustendil District)

No.	Sample code	Trap	Date	Result
1	BG 43 A	S-1	01-02.03.2002	0
2	BG 43 D	S-2	02-03.03.2002	0
3	BG 43 A	S-1	05-06.03.2002	0
4	BG 43 A	S-2	06-07.03.2002	0
5	BG 43 A	S-1	03-04.04.2002	0
6	BG 43 A	S-1	12-13.04.2002	0
7	BG 43 A	S-2	13-14.04.2002	0
8	BG 43 A	S-1	23-24.04.2002	0
9	BG 43 A	S-2	24-25.04.2002	35/82*
10	BG 43 A	S-1	25-26.04.2002	0
11	BG 43 A	S-2	26-27.04.2002	220/643
12	BG 43 A	S-1	01-02.05.2002	130/187
13	BG 43 A	S-2	03-04.05.2002	307/229
14	BG 43 A	S-1	17-18.05.2002	651/304
15	BG 43 A	S-2	18-19.05.2002	2251/2471
16	BG 43 A	S-1	24-25.05.2002	55/93
17	BG 43 A	S-2	25-26.05.2002	34/203
18	BG 43 A	S-1	13-14.06.2002	2724/32373
29	BG 43 A	S-1	12-13.07.2002	93/273
20	BG 43 A	S-1	13-14.08.2002	8/117
21	BG 43 A	S-1	14-15.08.2002	14/209
22	BG 43 A	S-1	14-15.09.2002	48/73
23	BG 43 A	S-1	14-15.10.2002	232/11
24	BG 43 A	S-2	15-16.10.2002	8/22

* *Culicoides* spp./other insects

Table III
Phenology investigations and seasonal dynamics
of *Culicoides* spp. trapped in 2002
(Bersin Village, Kiustendil District)

No.	Sample code	Trap	Date	Result
1	BG 43 B	S-1	01-02.03.2002	0
2	BG 43 B	S-2	02-03.03.2002	0
3	BG 43 B	S-1	08-09.03.2002	0
4	BG 43 B	S-2	09-10.03.2002	0
5	BG 43 B	S-1	03-04.04.2002	0
6	BG 43 B	S-2	04-05.04.2002	0
7	BG 43 B	S-1	12-13.04.2002	0
8	BG 43 B	S-2	13-14.04.2002	0
9	BG 43 B	S-1	23-24.04.2002	0
10	BG 43 B	S-2	24-25.04.2002	0
11	BG 43 B	S-1	25-26.04.2002	0
12	BG 43 B	S-2	26-27.04.2002	115/95*
13	BG 43 B	S-1	13-14.05.2002	0
14	BG 43 B	S-2	14-15.05.2002	21/25
15	BG 43 B	S-1	22-23.05.2002	17/172
16	BG 43 B	S-2	23-24.05.2002	51/59
17	BG 43 B	S-1	13-14.06.2002	68/257
18	BG 43 B	S-2	14-15.06.2002	133/467
19	BG 43 B	S-1	13-14.07.2002	137/125
20	BG 43 B	S-1	13-14.08.2002	97/93
21	BG 43 B	S-2	14-15.08.2002	47/43
22	BG 43 B	S-1	15-16.09.2002	647/36
23	BG 43 B	S-2	16-17.09.2002	651/40
24	BG 43 B	S-1	14-15.10.2002	7/7
25	BG 43 B	S-2	15-16.10.2002	21/39
26	BG 43 B	S-1	07-09.11.2002	0/2
27	BG 43 B	S-2	08-09.11.2002	3/5
28	BG 43 B	S-1	13-14.11.2002	0/3
29	BG 43 B	S-2	14-15.11.2002	4/3

* *Culicoides* spp./other insects

Discussions, hypotheses and conclusions

The absence of clinical symptoms in affected sheep, and the existence of seropositive sentinel animals only after the long summer rainfall period, suggests that insufficient ultra-violet exposure may have played a role in the absence of clinical signs of the disease. Presence of BTV seropositive sentinel animals without evidence of cross-border penetration of the infection supports the hypothesis for the special γ - δ -T-lymphocyte role of virus surviving between epidemic periods. In the past two to three years, only these regions of southern Bulgaria remained free from BTV. No BTV vaccine was used and no evidence of any virus circulation was found during this period. The entire population of sentinel ruminants remained BTV-negative but are susceptible to infection.

Firstly, the hypothesis of BTV remaining in a γ - δ -T-lymphocyte in a population of sensitive animals outside Bulgaria is also possible. Secondly, it is possible BTV penetrated the region from areas located close to the 10-km border strip. This hypothesis should be confirmed by detecting BTV by c-ELISA or by virus isolation from trapped samples of *Culicoides* spp. from these regions or after estimating a dramatic change in the vectors with *C. imicola* in the trapped *Culicoides* samples. The active *Culicoides* seasonal dynamics in 2002 started in the third week of April and two or three peaks of activity are estimated to have occurred: one during the second half of May, another in August and probably a third at the beginning of October. These peaks are equal to five or six generations throughout the season.

The following conclusions can be drawn:

- 1) Based on complex viral, serological, epidemiological and clinico-pathological investigations, evidence of the presence of active BTV infection in Bulgaria was not been found.
- 2) Prior to 26 August 2002, there was no transborder incursion of BTV into Bulgaria by persistently infected animals or by infected *Culicoides* spp.
- 3) In three years of *Culicoides* surveillance, *C. imicola* has not been detected in Bulgaria.
- 4) The dominant *Culicoides* species recorded were *C. obsoletus*, *C. pulicaris* and *C. punctatus*.
- 5) Amongst *Culicoides* midges trapped in August 2001 in south-east Bulgaria, *C. puncticollis* was recorded for the first time.

- 6) *Culicoides* phenology studies indicated two or three main peaks of *Culicoides* activity representing the occurrence of five or six generations between the beginning and end of the 2002 season.

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References

1. Mellor P.S., Jennings D.M., Wilkinson P.J. & Boorman J.P. (1985). – *Culicoides imicola*: a bluetongue vector in Spain and Portugal. *Vet. Rec.*, **116**, 589-590.
2. Mostroyannic M., Axiotis I. & Strofovos E. (1981). – Study of the first bluetongue disease in Greece. *Bull. Hell. Vet. Med. Cos.*, **32**, 138-144.
3. Office International des Épizooties (OIE) (1999). – Bluetongue in Bulgaria. *Dis. Info.*, **12**, 97-99.
4. Sellers R.F. (1975). – Bluetongue in Cyprus. *Aust. Vet. J.*, **51** (4), 198-203.
5. Urbano P. & Urbano F.G. (1994). – The *Reoviridae* family. *Comp. Immunol. Microbiol. Infect. Dis.*, **17** (3-4), 151-161.
6. Verwoerd D.W. & Erasmus B.J. (1994). – Bluetongue. *In* Infectious diseases of livestock with special reference to southern Africa (J.A.W. Coetzer, G.R. Thomson & R.C. Tustin, eds). Oxford University Press, Cape Town, 443-459.