Veterinaria Italiana, **46** (4), 431-437

Entomological research on the vectors of bluetongue disease and the monitoring of activity of *Culicoides* in the Prishtinë region of Kosova

Betim Berisha[1], Izedin Goga[2], Beqë Hulaj[2], Driton Çaushi[3], Kurtesh Sherifi[4], Anthony J. Wilsmore[5] & William P. Taylor[6]

Summary

Clinical bluetongue (BT) caused by BT virus serotype 9 (BTV-9) was observed in Kosova in 2001 and, although subsequently no further clinical cases was diagnosed, its continuing presence has been demonstrated by serological tests in cattle, sheep and goats. In this study, light traps were placed in stables near Prishtinë to identify possible vectors of BTV in Kosova. Samples were collected from October 2004 until the end of 2006. *Culicoides* were identified and speciated and results were plotted against temperature data. Samples contained Obsoletus and Pulicaris Complexes but not *C. imicola*. The first specimens of *Culicoides* were collected in April and they continued to be detected until November. Generally, Obsoletus Complex was present in the largest numbers, with the exception of the middle of the year when the Pulicaris Complex predominated. The number of *Culicoides* trapped was directly linked to temperature ($p<0.05$) and records indicated that *Culicoides* activity ceased when minimum temperatures fell below 0°C; activity recommenced when minimum temperatures rose to approximately 6°C. These results indicate that there was a lack of a vector for BTV during winter for a period lasting approximately five months.

Keywords

Bluetongue, BTV, *Culicoides*, Kosova, Obsoletus Complex, Pulicaris Complex, Virus.

Febbre catarrale degli ovini (BT): ricerca entomologica dei vettori e monitoraggio dell'attività di *Culicoides* nella regione di Pristina, Kosovo

Riassunto

E' stata studiata nel 2001, in Kosovo, la febbre catarrale degli ovini (BT) causata dal virus BT sierotipo 9 (BTV-9). Anche se non sono stati diagnosticati nuovi casi clinici, la costante presenza del virus è stata dimostrata attraverso l'accertamento sierologico in capi di allevamenti ovini e caprini. Durante questa fase di studio, sono state posizionate trappole luminose nelle stalle presenti nelle vicinanze di Pristina, Kosovo, per individuare i possibili vettori del BTV. I campioni sono stati raccolti nel periodo ottobre 2004-dicembre 2006. I Culicoides *catturati sono stati identificati e*

(1) Str. 28 Nentori, L. Dardania, Villa 2, 10000 Prishtinë, Kosova
b.betim@gmail.com
(2) Kosova Veterinary Laboratory (Laboratori i Veterinarisë dhe i Ushqimit), Kosova Veterinary and Food Agency (Agjencia e Ushqimit dhe Veterinarisë), Zona Industriale, 10 000 Prishtinë, Kosova
(3) Rr. Nene Tereza nr 218, 50000 Gjakove, Kosova
(4) L. Lakrishte 1 Blvd. Bill Clinton Nr. 14, 10000 Prishtinë, Kosova
(5) Veterinary Epidemiology and Economics Research Unit, School of Agriculture, Policy and Development, Earley Gate, P.O. Box 237, Reading RG6 6AR, United Kingdom
tonywilsmore@yahoo.co.uk
(6) 16 Mill Road, Angmering, Littlehampton, West Sussex BN16 4HT, England, United Kingdom

classificati per specie. I risultati sono stati registrati tenendo conto della temperatura. Sono state rinvenute le seguenti specie: Culicoides obsoletus *Complex e* Culicoides pulicaris *Complex, ma non la specie* Culicoides imicola. *Gli esemplari sono stati raccolti nel mese di aprile fino a novembre. Il numero di* Culicoides *catturati è risultato significativamente correlato alla temperatura (p<0,05). Si è rilevato, infatti, che la loro attività cessa quando le temperature minime scendono al di sotto dello 0°C, per riprendere quando le temperature minime si attestano intorno ai 6°C. I risultati hanno evidenziato la mancanza di un vettore per il virus durante il periodo invernale.*

Parole chiave
Bluetongue, BTV, *Culicoides*, *Culicoides obsoletus* Complex, *Culicoides pulicaris* Complex, Kosovo.

Introduction

In 2001, clinical cases of bluetongue (BT) were observed in sheep in a few regions of Kosova. BT virus serotype 9 BTV-9 was identified by the Institute of Animal Health Pirbright Laboratory in the United Kingdom. Serological screening of serum samples of cattle, sheep and goats collected in late 2003, and of serum samples of cattle and sheep collected in the periods of April-May and September to November 2004, revealed that high BT prevalence was noted in some of the animals (7).

Culicoides imicola is the vector responsible for the transmission of BT in the Mediterranean Basin, but there is evidence that the species groups of *Culicoides*, namely: the Obsoletus Complex (6 species) and Pulicaris Complexes (6 species) are becoming vectors of BTV as they have been observed in regions of BT outbreaks where *C. imicola* is rare or absent (2, 3, 4, 5, 8). Reporting from Cyprus, Mellor and Pitzolis (6) suggested that *C. obsoletus* was a vector for BTV and Carpenter *et al.* (1) demonstrated BTV infection and excretion in cases of *C. obsoletus* and *C. pulicaris* that was comparable with that of *C. imicola*.

The epidemiological aims of this study were to identify potential vectors for BT in Kosova, using an entomological screening system based on the permanent use of light traps, as described by Venter and Meiswinkel (cited by Goffredo and Meiswinkel [4]) and to monitor their seasonal activity. An additional objective was to determine whether or not the persistence of BTV from one year to the next might be explicable by continuing vector activity during the winter in the Balkans.

In research systems for the vectors of bluetongue, a common technique is to catch the adult form of *Culicoides* in the vicinity of animal housing, using powerful light traps to ensure trapping sensitivity and to acquire data on the scale and variety of the *Culicoides* population.

Materials and methods

In this study, a blacklight trap was placed inside the cowshed of the Agricultural and Veterinary Faculty in the Municipality of Prishtinë in October 2004 where the first collections were made. Samples were collected every week from October 2004 until the end of 2005. In total, 55 collections were made during this period.

During 2006, operation of the light traps continued and *Culicoides* were collected every two weeks, with a total of 23 collections being made during the year but the location selected for the light trap in 2006 was inside a cowshed in a farm that raised more than 10 cows in a farming area near Prishtinë.

The sites were chosen based on their location, i.e. places with suitable rainfall patterns and areas of dampness in which *Culicoides* would be likely to breed. The sites also had electricity which was necessary to operate the light traps.

Light traps were installed at a height of 1.5 m-2 m and as close as possible to the animals. A 500 ml container filled with 200 ml of water and a small amount of detergent, was placed inside the net of the lamp before it was activated. The light trap was switched on one hour before sunset and was switched off the next day after sunrise. The container was removed the same day, with care to ensure that insects that had settled on the sides of the container would fall into the suspension (4).

Each sample collected from the light trap was despatched to the Kosova Veterinary Laboratory and registered with an identification number and date of collection. Samples were examined using a stereo microscope, observing the laboratory protocol developed at the National Reference Centre for Exotic Diseases (*Centro di Referenza Nazionale per lo studio e l'accertamento delle malattie esotiche degli animali*: CESME) in Italy, in cooperation with the Onderstepoort Veterinary Institute in South Africa (4). *Culicoides* were identified based on the morphological characteristics of their wings, thoracic veins and spots. Data from the results of laboratory examinations were recorded.

Results of the analysis included the total number of insects (*Culicoides* and other insects), total number of *Culicoides* (*C. imicola* + Obsoletus Complex + Pulicaris Complex + *Culicoides* spp.) and individual numbers of *C. imicola,* Obsoletus Complex, Pulicaris Complex and *Culicoides* spp. *Culicoides* identified as *Culicoides* spp. were those which could not be identified as *C. imicola,* or Obsoletus and Pulicaris Complexes.

Three collections in July and one in August 2005 were examined using a sub-sampling method (9) because of the large number of insects collected.

Results of the analyses were evaluated against temperature data obtained from the Meteorology Institute of Kosova in Prishtinë.

Results

From October 2004 to the end of 2004, 10 samples were collected from the Faculty cowshed in Prishtinë. Some of the samples contained species of the Obsoletus and Pulicaris Complexes, but not the recognised vector of bluetongue, *C. imicola.* The numbers and proportions of the different samples of *Culicoides* collected in 2004, together with minimum and maximum temperatures recorded by the Meteorology Institute on the days of collection, are shown in Table I. During 2004, *Culicoides* were collected until the night of 17/18 November when the maximum temperature had fallen to 11.3°C and the minimum temperature had fallen to –3.6°C. More Obsoletus Complex samples were collected over this period than their Pulicaris counterparts.

In 2005, weekly sampling continued resulting in the collection of 45 samples. An examination of the samples revealed the presence of Obsoletus Complex and Pulicaris Complexes but there was no evidence of the presence of *C. imicola*. The results of these observations are shown in Table II. In this monitoring period, the first substantial *Culicoides* collection was

Table I
Samples collected in 2004 (October-December)

Date of collection		Temperature Max (°C)	Temperature Min (°C)	Total no. of insects	Total no. of *Culicoides*	Results Total no. of Obsoletus Complex (%)	Results Total no. of Pulicaris Complex (%)	Results Total no. of *Culicoides* spp. (%)
Oct	19-20	22.4	9.0	552	128	71 (55.5)	56 (43.8)	1 (0.8)
	20-21	22.4	9.3	588	91	58 (63.7)	35 (38.5)	0
	21-22	23.1	9.3	543	64	42 (65.6)	22 (34.4)	0
	28-29	21.4	7.8	1 485	193	155 (80.3)	35 (18.1)	3 (1.6)
Nov	04-05	18.1	10.4	155	17	16 (94.1)	1 (5.9)	0
	17-18	11.3	-3.6	10	0	0	0	0
	25-26	5.1	-9.1	0	0	0	0	0
Dec	08-09	6.4	-3.2	0	0	0	0	0
	20-21	2.2	-3.1	4	0	0	0	0
	27-28	11.4	-0.4	0	0	0	0	0
Total	10 collections			3 337	495	342 (69.1)	149 (30.1)	4 (0.8)

Table II
Samples collected in 2005 (January-December)

Date of collection		Temperature		Total no. of insects	Total no. of *Culicoides*	Results		
		Max (°C)	Min (°C)			Total no. of Obsoletus Complex (%)	Total no. of Pulicaris Complex (%)	Total no. of *Culicoides* spp. (%)
Jan	04-05	12.4	–2.3	24	0	0	0	0
	10-11	11.4	–7.0	0	0	0	0	0
	17-18	3.8	–9.8	0	0	0	0	0
	26-27	3.7	–3.8	77	0	0	0	0
Feb	07-08	–4.5	–19.9	0	0	0	0	0
	14-15	7.4	–1.2	0	0	0	0	0
	21-22	11.2	–5.2	0	0	0	0	0
Mar	01-02	–3.3	–11.6	0	0	0	0	0
	14-15	15.1	–0.6	0	0	0	0	0
	22-23	18.2	–2.6	17	0	0	0	0
	30-31	16.1	3.5	249	0	0	0	0
Apr	04-05	16.1	–1.4	187	1	1(100)	0	0
	12-13	16.9	6.6	32	0	0	0	0
	19-20	16.6	6.1	74	13	10 (76.9)	3 (23.1)	0
	25-26	19.9	7.7	149	22	14 (63.6)	6 (27.3)	2 (9.1)
May	02-03	26.6	4.9	526	79	49 (62.0)	28 (35.4)	2 (2.5)
	09-10	20.1	5.2	272	21	15 (71.4)	5 (23.8)	1 (1.3)
	17-18	25.7	11.3	607	164	62 (37.8)	95 (57.9)	7 (4.2)
	24-25	24.6	12.5	448	35	21 (60)	13 (37.1)	1 (2.9)
Jun	07-08	16.3	6.6	187	29	18 (62.1)	11 (37.9)	0
	14-15	25.5	9.1	563	43	25 (58.1)	16 (37.2)	2 (4.6)
	21-22	25.3	8.4	759	35	15 (42.9)	14(40)	6 (17.1)
	29-30	33.1	13.4	702	78	37 (47.4)	38 (48.7)	3 (3.8)
Jul	06-07	25.3	11.2	347	11	2 (18.2)	8 (72.7)	1 (9.1)
	18-19	32.4	13.5	4998	114	68 (59.6)	34 (29.82)	12 (10.5)
	26-27	32.4	13.7	2698	214	57 (26.63)	133(62.14)	24 (11.21)
Aug	2-3	35.3	18.6	1413	213	108 (50.7)	81 (38.0)	24 (11.3)
	10-11	27.4	8.6	316	51	14 (27.5)	31 (60.8)	6 (11.8)
	17-18	21.2	10.7	249	20	9 (45)	8 (40)	3 (15)
	30-31	27.2	15.5	272	4	2 (33.3)	1 (16.7)	1 (16.7)
Sept	2-3	24.9	10.7	517	22	6 (27.3)	14 (63.6)	2 (9.09)
	10-11	28.4	13.5	112	16	10 (62.5)	5 (31.3)	1 (6.3)
	17-18	27.1	10.8	309	17	12 (70.6)	3 (17.6)	2 (11.8)
Oct	03-04	22.6	6.6	432	38	23 (60.5)	13 (34.2)	2 (5.3)
	10-11	17.1	5.3	361	20	11 (55)	7 (35)	2 (10)
	18-19	12.9	–3.4	421	25	19 (76)	4 (16)	2 (8)
	25-26	22.2	3.3	319	26	17 (65.4)	7 (28)	2 (7.7)
Nov	03-04	16.3	–2.3	106	4	4 (100)	0	0
	10-11	12.7	–4.3	42	2	2 (100)	0	0
	18-19	4.5	–0.9	17	0	0	0	0
	28-29	11.9	3.1	11	0	0	0	0
Dec	7-8	9.0	2.1	29	0	0	0	0
	13-14	3.8	–1.4	0	0	0	0	0
	21-22	0.8)	–10.6	0	0	0	0	0
	28-29	12.1	4.3	17	0	0	0	0
Total	45 collections			17 859	1 317	631 (47.9)	578 (43.9)	108 (8.2)

made on the night of 19/20 April when the maximum temperature had risen to 16.6°C and the minimum temperature had risen to 6.1°C. *Culicoides* remained active until the last identification was made on the night of 10/11 November when the maximum temperature was 12.7°C whereas the minimum temperature had been falling to below 0°C since the beginning of the month. A week later (the night of 18/19 November), no *Culicoides* were collected and the maximum temperature was 4.5°C while the minimum temperature was just below 0°C.

The Obsoletus Complex was usually present in larger numbers than the Pulicaris Complex, except for one collection in July when there was more than double the number of Pulicaris Complex samples captured in comparison to the Obsoletus Complex. Furthermore, the season during which the Obsoletus Complex could be trapped was longer, extending to mid-November, compared to the end of October for the Pulicaris Complex. While other unspeciated *Culicoides* were trapped, they were fewer in number and recorded over a shorter season than either the Obsoletus Complex or the Pulicaris Complex.

From January to December 2006, samples were collected from another farm every two weeks and a total of 23 samples were collected. The results of the study are shown in Table III. During this period, the first *Culicoides* samples were collected on the night of 11/12 April, a week earlier than in 2005 (barring the singleton

Table III
Samples collected in 2006 (January-December)

Date of collection		Temperature		Total no. of insects	Total no. of *Culicoides*	Results		
		Max (°C)	Min (°C)			Total no. of Obsoletus Complex (%)	Total no. of Pulicaris Complex (%)	Total no. of *Culicoides* spp. (%)
Jan	13-14	–1.0	–9.4	0	0	0	0	0
	25-26	–7.4	–20.3	0	0	0	0	0
Feb	22-23	7.1	–1.2	5	0	0	0	0
Mar	11-12	7.8	1.0	9	0	0	0	0
	27-28	22.1	5.6	17	0	0	0	0
Apr	11-12	18.9	6.4	327	24	8 (33.3)	12 (50)	4 (16.7)
	27-28	19.3	10.6	562	31	23 (74.2)	7 (22.6)	1 (3.2)
May	9-10	20.3	7.3	288	11	4 (36.4)	7 (63.6)	0
	29-30	26.9	12.4	465	86	17 (19.8)	61 (70.9)	8 (9.3)
Jun	13-14	21.9	9.0	255	19	11 (57.9)	5 (26.3)	3 (15.8)
	28-29	33.6	16.2	737	59	7 (11.9)	49 (83.1)	3 (5.1)
Jul	1-2	26.6	17.0	532	46	22 (47.8)	20 (43.47)	4 (8.7)
	14-15	27.6	12.2	1 248	81	34 (42.0)	42 (51.9)	5 (6.2)
Aug	16-17	31.2	11.6	389	26	15 (57.7)	9 (34.6)	2 (7.7)
	29-30	23.9	10.9	309	33	18 (54.5)	13 (39.4)	2 (6.1)
Sept	11-12	24.6	11.9	216	16	11 (68.8)	5 (31.3)	0
	25-26	24.8	5.3	524	23	12 (52.2)	8 (34.8)	3 (13.0)
Oct	13-14	19.3	5.3	484	38	26 (68.4)	11 (28.9)	1 (2.6)
	23-24	24.5	7.8	319	29	20 (69.0)	7 (24.1)	2 (6.9)
Nov	06-07	15.6	3.8	33	3	2 (66.7)	0	1 (33.3)
	29-30	6.1	–1.3	10	0	0	0	0
Dec	14-15	3.1	–5.1	33	3	2 (66.7)	0	1 (33.3)
	28-29	7.8	–10.7	10	0	0	0	0
Total	23 collections			6 659	525	230 (43.8)	256 (48.8)	39 (7.4)

seen on the night of 4/5 April) but maximum and minimum temperatures were similar (18.9°C and 6.4°C, respectively).

The Pulicaris Complex was identified for the last time in 2006 on the night of 23-24 October when the maximum temperature was 24.5°C and the minimum temperature was 7.8°C, but the Obsoletus Complex was collected until 14/15 December when maximum and minimum temperatures had dropped to 3.1°C and –5.1°C, respectively.

In 2006, the Pulicaris Complex was trapped in greater numbers in May, June and July, but otherwise the Obsoletus Complex was trapped more frequently and for longer periods. Small numbers of unspeciated *Culicoides* were trapped over the entire duration of trapping of the Obsoletus and Pulicaris Complexes.

Using linear regression, above a minimum temperature of 3°C and a maximum temperature of 12°C, the number of *Culicoides* trapped was closely linked to temperature ($p<0.05$). However, r^2 values (a measure of proportion of variation in numbers trapped that is explained by temperature) were all low (0.19-0.32) which indicated that there were other factors affecting the numbers of *Culicoides* trapped.

Discussion

The temperature records displayed in Tables I, II and III indicate that *Culicoides* activity ceases when minimum temperatures fall below 0°C and activity begins again when minimum temperatures rise to approximately 6°C.

The Obsoletus and Pulicaris Complexes are potential vectors of BT and, in the apparent absence of *C. imicola*, we can suspect that these species have been transmitters of BTV in Kosova.

In 2004, *Culicoides* were last trapped on the night of 4 November and were not trapped again until 4 April 2005. In 2005 they were last trapped on the night of 10 November and were not trapped again until 11 April 2006. The data assembled from the results of light trapping are insufficient to draw precise conclusions on the periods of inactivity of the vector. Nevertheless, they do suggest that there was a lack of a vector for BTV for about a five-month period in both winters.

Conclusion

The results of a previous study that indicated that BTV-9 was present in Kosova (7), raises the important question of how did BTV persistently overwinter in Kosova in the absence of a vector detectable by light traps (nocturnal activity) for approximately five months? Clearly an alternative mechanism existed.

Acknowledgments

The authors would like to thank Maria Goffredo, *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molize 'G. Caporale'* in Teramo, for her training and N.M. Taylor from the Veterinary Epidemiology and Economics Research Unit (VEERU) for statistical analysis. The help and support of the Director and staff of the Kosova Veterinary and Food Agency and the Veterinary and Agricultural Faculty of the University of Prishtinë for the use of their livestock facility are gratefully acknowledged.

References

1. Carpenter S., Lunt H. L, Arav D., Venter G.J. & Mellor P.S. 2006. Oral susceptibility to bluetongue virus of *Culicoides* (Diptera: Ceratopogonidae) from the United Kingdom. *J Med Entomol*, **43**, 73-78.
2. Goffredo M., Conte A. & Meiswinkel R. 2004. Distribution and abundance of *Culicoides imicola*, Obsoletus Complex and Pulicaris Complex (Diptera: Ceratopogonidae) in Italy. *Vet Ital*, **40**, 270-272 (www.izs.it/vet_italiana/2004/40_3/270.htm accessed on 6 November 2010).
3. Goffredo M., Delécolle J.-C., Semproni G. & Lika A. 2004. *Culicoides* (Diptera: Ceratopogonidae) in Albania: results of the 2002 entomological survey for bluetongue. *Vet Ital*, **40**, 209-211 (www.izs.it/vet_italiana/2004/40_3/209.htm accessed on 6 November 2010).

4. Goffredo M. & Meiswinkel R. 2004. Entomological surveillance of bluetongue in Italy: methods of capture, catch analysis and identification of *Culicoides* biting midges. *Vet Ital*, **40**, 260-265 (www.izs.it/vet_italiana/2004/40_3/260.htm accessed on 6 November 2010).
5. Goffredo M., Romeo G., Monaco F., Gennaro A. & Savini G. 2004. Laboratory survival and blood feeding response of wild-caught *Culicoides obsoletus* Complex (Diptera: Ceratopogonidae) through natural and artificial membranes. *Vet Ital*, **40**, 282-285 (www.izs.it/vet_italiana/2004/40_3/282.htm accessed on 6 November 2010).
6. Mellor P.S. & Pitzolis G. 1979. Observations on breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bull Entomol Res*, **69**, 229-234.
7. Osmani A., Murati B., Kabashi Q., Goga I., Berisha B., Wilsmore A.J. & Hamblin C. 2006. Evidence for the presence of bluetongue virus in Kosova between 2001 and 2004. *Vet Rec*, **158**, 393-396.
8. Scavia G., Autorino G.L., Liberato C., de Farina F., Ferrari G., Guidoni M., Magliano A., Miceli M., Scholl F., Scicluna M.T. & Scaramozzino P. 2004. Association between the 2001-2003 bluetongue epidemic in Lazio and Tuscany (central Italy) and distribution and abundance of *Culicoides imicola* and *C. obsoletus* vectors. *Vet Ital*, **40**, 274-277 (www.izs.it/vet_italiana/2004/40_3/274.htm accessed on 6 November 2010).
9. Van Ark H. & Meiswinkel R. 1992. Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort J Vet Res*, **59**, 183-189.