Vet. Ital., **40** (3), 274-277

Association between the 2001-2003 bluetongue epidemic in Lazio and Tuscany (central Italy) and distribution and abundance of *Culicoides imicola* and *C. obsoletus* vectors

G. Scavia, G.L. Autorino, C. De Liberato, F. Farina, G. Ferrari, M. Guidoni, A. Magliano, M. Miceli, F. Scholl, M.T. Scicluna & P. Scaramozzino

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Via Appia Nuova 1411, 00178 Rome, Italy

Summary

During the epidemic of bluetongue (BT) in Lazio and Tuscany between 2001 and 2003, the distribution pattern of *Culicoides imicola* did not always correspond either geographically or seasonally, with virus circulation. *Culicoides obsoletus* was observed to be abundant, ubiquitous and active throughout the year. The geographical and seasonal distribution of BT virus (BTV), *C. imicola* and *C. obsoletus* was compared. The territory of the two regions was divided into 30 cells each measuring 1 600 km². The presence of *C. obsoletus* was recorded in every cell, while *C. imicola* was detected in 18 of the 30 cells, but was absent in 6 of the 21 cells that indicated the presence of BTV. The occurrence of seroconversions appeared to be positively correlated with maximum *C. obsoletus* catches. Seroconversions were recorded throughout the year, even when *C. imicola* was not active, whereas *C. obsoletus* was detected during the entire period. The occurrence of BTV circulation in areas and periods where *C. imicola* was absent, and the abundant and constant presence of adult *C. obsoletus* in all the cells, suggest the active role of the latter species in BTV circulation in central Italy.

Keywords

Bluetongue – *Culicoides imicola* – *Culicoides obsoletus* – Italy – Lazio – Tuscany.

Introduction

Since 2000, the circulation of bluetongue (BT) virus (BTV) has been recorded in central and southern Italy, causing one of the largest epidemics of BT in recent decades in the Mediterranean Basin. At present, the serotypes involved are mainly 2, 4 and 9. In the summer-autumn of 2001, the Lazio and Tuscany regions of central Italy experienced 220 clinical outbreaks of BT caused by BTV serotype 2, which was also involved in the 15 outbreaks observed in southern Lazio in 2002. Surveillance, based on the regular assessment of the serological status of sentinel animals (58 in each of the 20 km × 20 km quadrants) and on extensive *Culicoides* collections, commenced in the two regions in the summer of 2001, in the framework of a BT National Surveillance Programme. During the same year, because of the occurrence of BT outbreaks in the study area, serological surveillance of sentinels was suspended between September and December and surveillance concentrated on the detection of clinical cases.

In 2002, the Health Ministry authorised the vaccination, using a live attenuated vaccine, of all susceptible domestic species in areas considered at risk for BT. In both 2002 and 2003, over 80% of the susceptible animal population in large areas of Lazio and Tuscany was vaccinated in two consecutive campaigns.

Culicoides imicola Kieffer, 1913 is the only known and confirmed BTV vector in the Mediterranean region, and its presence in Sardinia, Sicily and mainland Italy was confirmed during the entomological surveillance programme (4). However, *C. imicola* was absent in many sites in Lazio and Tuscany where BTV circulated and, if present, its populations were small

(3). On the other hand, *C. obsoletus* (Meigen), 1818 was almost ubiquitous and active throughout the year (C. De Liberato, unpublished findings). *Culicoides obsoletus* is a species known to be capable of sustaining BTV replication (6) and also strongly suspected of being the BT vector in Eastern Europe (5, 9). In fact, *C. obsoletus* is known to be a group of species and, when *C. obsoletus* is referred to in this paper, the Obsoletus Complex is intended.

The aim of this study was to investigate the role of *C. imicola* and *C. obsoletus* as BTV vectors by evaluating the association between the geographical and seasonal distribution of both species, together with BTV circulation in the two regions.

Materials and methods

The study was conducted from July 2001 to August 2003. According to the criteria of the entomological surveillance programme in the study area, which includes the territories of Lazio and Tuscany, the region was divided into thirty 1 600 km² quadrants. Some of the outer quadrants had different shapes and areas (Fig. 1).

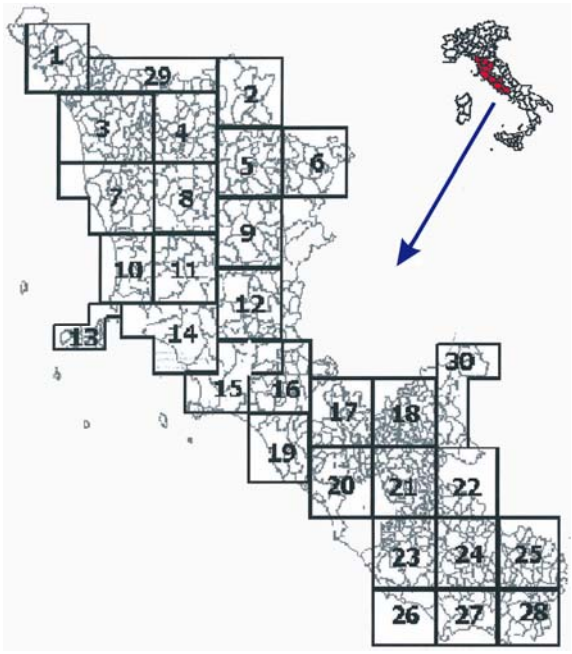

Figure 1
Regions of Lazio and Tuscany divided into thirty geographical units

The assessment of BTV circulation was made through BT clinical outbreaks and seroconversions, the latter defined as a positive serological diagnosis in a recent previously seronegative tested sentinel, according to the BT National Surveillance Programme. For serological diagnosis a 'positive and confirmed' scheme was used with ELISA (7) as the first screening step, followed by serum neutralisation; the latter was performed by the National Reference Centre for Exotic Diseases (CESME: *Centro Studi Malattie Esotiche*), to confirm seropositivity and serotype. Each seroconversion detected was included in the present data set, regardless of BTV serotype and whether or not it was a field or vaccine virus.

Entomological surveillance was based on *Culicoides* collections, with trapping and sorting activities as described by standardised methodologies (1). A total of 2 808 catches were performed in 415 different trapping sites over the entire study area. *Culicoides imicola* was counted in the whole set, whereas *C. obsoletus* in only a subset of 1 515 catches made at 160 trapping sites. For statistical analysis, all data were grouped according to the 30 geographical units (Fig. 1). The relationship between presence and abundance of *C. imicola* and/or *C. obsoletus* and virus circulation was evaluated. The amount of BTV circulation in each cell was estimated by a seroconversion rate (SR) per month, calculated as the mean number of seroconversions per month, over a period (follow-up) ranging from the first seroconversion observed in every cell to 100 days after the last. Counts of *C. obsoletus* and *C. imicola* were log transformed because of the high abundance variability. Maximum catches of *C. obsoletus* (CobMax) and *C. imicola* (CimMax) were considered more appropriate than mean catches to express population sizes (2). All catches for *C. obsoletus* were classed into three equally sized categories. The proportion of *C. obsoletus* catches in the maximum class (P-obs) was calculated for each cell. Due to the generally scarce catches of *C. imicola*, only presence-absence was considered for each cell. Nine cells that had no evidence of BTV circulation were not included in the statistical analysis.

Pearson's correlation coefficient was calculated between SR and P-obs, CobMax and CimMax. The mean SR value was compared between *C. imicola*-present and *C. imicola*-absent cells. Multiple linear regression analysis was performed to assess the association between SR and *C. imicola* presence and P-obs, as well as with CobMax and CimMax. Analysis of variance (ANOVA) was performed to compare P-obs, CobMax and CimMax mean values between cells with and without clinical outbreaks. In this analysis, only outbreaks that occurred in 2001 (before the commencement of the vaccination campaign), were considered. The seasonality of seroconversions and *C. obsoletus* and *C. imicola* activity in the entire study area are presented in Figure 2.

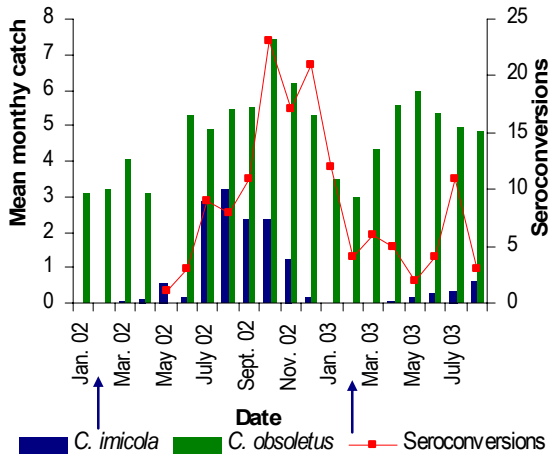

Figure 2
Mean monthly catches of *Culicoides imicola* and *C. obsoletus* and number of bluetongue virus seroconversions per month, January 2002-August 2003
The commencement of the 2002 and 2003 vaccination campaigns are indicated by arrows

Results

Table I summarises the results of serological, clinical and entomological surveillance in the 30 cells. *Culicoides obsoletus* was present in all cells, whereas *C. imicola* was found in only 18 of the 30 cells. In 6 of the 21 cells (28.6%) in which BTV circulation was detected, *C. imicola* was not found. On the other hand, this species was detected in 6 of the 9 cells with clinical outbreaks.

The SR was significantly positively correlated ($r=0.46$, $p<0.05$) only with CobMax. Results from regression analysis confirm the statistically significant ($\beta=0.46$, $p<0.05$) association between SR and CobMax, even when taking into account CimMax. Linear regression showed a positive, although not statistically significant, association between SR and P-obs, even when taking into account the presence

Table I
Virus circulation (seroconversions and outbreaks) and entomological data (number of catches, *C. imicola* and *C. obsoletus* maximum catch) for each cell

Cell	Seroconversions (2002-2003)	Follow-up (months)	SR[a]	Outbreaks[b]	Catches[c]	No. of *Culicoides*	CimMax[d]	CobMax[e]	P-obs[f]
1	8	9.6	0.84		69 (49)	35 249	7	3 733	65.3%
2[g]					31 (15)	12 171	0	2 897	46.7%
3	1	3.3	0.30		120 (50)	14 738	1	791	12.0%
4[g]					112 (46)	14 915	0	1 638	21.7%
5[g]					11 (4)	2 772	0	342	100.0%
6[g]					13 (8)	4 645	0	520	75.0%
7	3	6.0	0.50		51 (15)	2 908	5	789	6.7%
8[g]					92 (46)	84 588	3	6 600	47.8%
9[g]					197 (88)	54 744	0	2 991	23.9%
10	8	14.4	0.56	3	117 (75)	30 539	54	4 458	37.3%
11	5	13.1	0.38		26 (18)	14 571	1	8 414	44.4%
12	5	13.5	0.37		181 (72)	25 846	3	1 699	34.7%
13[g]					93 (65)	30 160	260	2 219	50.8%
14	2	11.3	0.18	100	285 (156)	80 181	37	1 532	32.7%
15	11	12.4	0.89	55	291 (165)	76 481	31	7 352	32.1%
16	1	3.3	0.30	56	120 (49)	24 082	6	9 602	30.6%
17	2	3.3	0.60	1	26 (14)	39 583	0	24 018	78.6%
18	7	6.8	1.03		82 (43)	25 965	0	1 745	37.2%
19			0	4	70 (48)	7 273	28	125	10.4%
20	12	12.8	0.94		165 (102)	32 012	1 477	3 950	34.3%
21	8	8.2	0.98		88 (28)	12 142	1	152	7.1%
22[g]					57 (23)	3 794	0	147	30.4%
23	15	12.2	1.23	1 (1)	58 (49)	15 204	12	4 041	30.6%
24	10	13.1	0.77		18 (9)	4 360	0	805	77.8%
25	9	6.1	1.47	(7)	22 (18)	89 755	0	23 069	88.9%
26	8	12.9	0.62		103 (75)	13 065	6	3 137	33.3%
27	7	9.7	0.72		209 (142)	33 321	1 498	733	9.2%
28	15	10.1	1.49	(7)	92 (36)	163 911	10	34 157	63.9%
29	2	11.5	0.17		6 (4)	8 259	0	1 134	100.0%
30[g]					3 (3)	1 206	0	272	100.0%

a) Seroconversion rate per month
b) In brackets: 2002 outbreaks not included in statistical analysis
c) In brackets: number of catches of *C. obsoletus*
d) *C. imicola* maximum catch
e) *C. obsoletus* maximum catch
f) Proportion of *C. obsoletus* catches in the highest abundance class
g) Cells not included in statistical analysis

of *C. imicola*. A slightly lower mean SR was found in cells with *C. imicola* presence, if compared with cells without this species, although this was not significant.

Mean monthly catches of *C. imicola* and *C. obsoletus* and seroconversion numbers for each month from January 2002 to August 2003 are shown in Figure 2. Seroconversions in sentinel bovines were recorded throughout the year, even during the winter months when *C. imicola* was not active, whereas activity of *C. obsoletus* was detected during the entire period.

Discussion

Occurrence of BTV circulation was recorded in areas from which *C. imicola* was absent and during periods of the year when they were not found to be active elsewhere. On the other hand, the abundant and constant presence of adult, active *C. obsoletus*, indicates it played a role in the epidemic considered here. This evidence appears to be confirmed by the positive association between *C. obsoletus* abundance and BTV circulation, as indicated by the correlationship between SR and CobMax and, even if not statistically significant, P-obs. *Culicoides imicola*, the only proven BT vector in the Palaearctic Region, did not affect the SR in these two regions. A possible limit of the present study could be the geographical units being too large, hence too limited in number. Despite the large quantities of data provided by the present study (especially by entomological surveillance), which meant very 'robust' indicators were obtained for every cell, the low number of units reduces the possibility of performing a more powerful statistical analysis. Further analysis, based on the same surveillance system, could be performed using single sites.

In October 2002, BTV serotype 2 was isolated from a pool of *C. obsoletus*, caught on a farm in southern Lazio clinically affected by BT (C. De Liberato, unpublished findings). Isolation of BTV from *C. obsoletus*, together with data from serological, clinical and entomological surveillance, could prove to be an important breakthrough in BT epidemiology. Areas until now considered risk-free, could now be considered at risk. The presence of *C. obsoletus* extends from southern Italy to Great Britain and can be recorded throughout the year in the southern areas of distribution (5, 8). Furthermore, the presence of this species throughout the year in the study area (Fig. 2) suggests the possibility of BTV overwintering in infected midges.

Finally, the contemporaneous presence of large numbers of viraemic animals (i.e. during vaccination campaigns), in an atypical season for BTV circulation, together with the concomitant presence of active *C. obsoletus*, could explain the occurrence of many seroconversions at the beginning of 2003 (Fig. 2). This finding raises the question about possible vaccine virus transmission among susceptible animals through active vectors. Finally, the bovine population was also included in the vaccination programme, thus introducing a new element in the interaction between vaccine virus hosts and vectors on a large scale.

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