

Serological surveillance of bluetongue virus in cattle, sheep and goats in Albania

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Summary

The recent spread of the bluetongue (BT) virus (BTV) in the Mediterranean Basin encouraged numerous countries to undertake entomological and serological surveillance programmes to identify affected areas and control the infection. Hitherto, no data on the presence and diffusion of BTV in Albania were available. Between October and November 2002 serum samples from 857 cattle and 870 sheep and goats were collected by the Albanian Veterinary Services in 15 districts, some bordering Yugoslavia, Macedonia and Greece, and others along the Adriatic coast. At the Albanian Veterinary Research Institute the samples were tested for the presence of BTV antibodies using a competitive enzyme-linked immunosorbent assay (c-ELISA) (bluetongue antibody test kit, IZSA&M, Teramo); in Italy, the virus neutralisation (VN) test was used to confirm the ELISA results and determine the serotype of BTV circulating. Overall seroprevalence was 18.9% in cattle and 4.4% in sheep and goats; seropositive animals occurred in all districts surveyed. The highest prevalence of BT was observed in the Tirana District, with 61% of the cattle and 20% of the sheep and goat populations BT-positive. The VN test confirmed the c-ELISA results revealing the presence of antibodies against BTV serotype 9.

Keywords

Albania – Bluetongue – Cattle – Competitive enzyme-linked immunosorbent assay – Goats – Sheep – Surveillance – Virus – Virus neutralisation.

Introduction

The incursion of bluetongue (BT) virus (BTV) into the Mediterranean Basin had a serious impact on numerous countries especially in those that had never previously experienced the infection. To identify affected areas and better control the spread of infection, some countries undertook entomological and serological surveillance programmes. Whilst four BTV serotypes (BTV-1, BTV-4, BTV-9 and BTV-16) have been reported to occur in the Balkans (Fig. 1) (4), no data on the presence and spread of BT in Albania were hitherto available. Accordingly, and under the aegis of the National Survey on Foot and Mouth Disease and Bluetongue (a joint project between the Albanian Veterinary Services, the Veterinary Research Institute of Tirana and the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' [IZSA&M]), a population of mixed domestic ruminants was surveyed for the presence of BT antibodies.

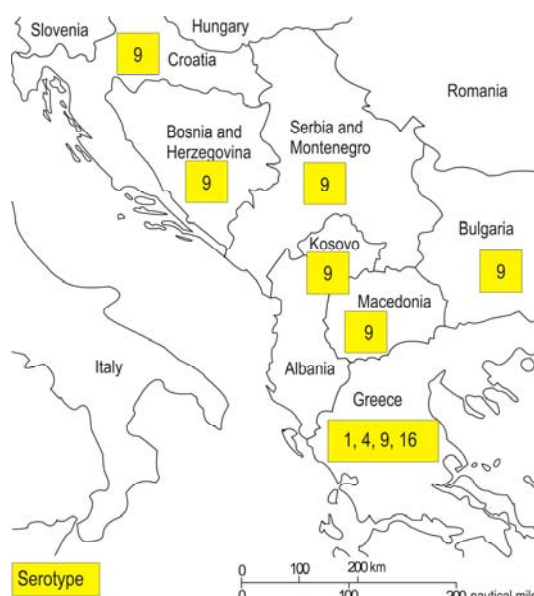


Figure 1
Bluetongue virus serotypes reported in the Balkans, 2002

Materials and methods

Sampling

Between October and November 2002, serum samples from 857 cattle and 870 sheep and goats, were collected by the Albanian Veterinary Services. Animals were randomly selected in 15 Albanian districts, some bordering Yugoslavia, Macedonia and Greece, others facing the Adriatic Sea.

Serological tests

At the Albanian Veterinary Research Institute, all serum samples were tested for the presence of BT antibodies using the competitive enzyme-linked immunosorbent assay (c-ELISA) produced by the IZSA&M in Teramo (3). The virus neutralisation (VN) test (2) was used to confirm the positive ELISA results. Positive and negative controls for the VN were kindly provided by the Onderstepoort Veterinary Institute in South Africa. The microtitre neutralisation method was used in this study. Fifty μ l of several sera dilutions, from 1:10 to 1:1 280, were added to each test well of flat-bottomed microtitre plates and mixed with an equal volume of Office International des Épizooties standard reference BTV serotypes 2, 4, 9 and 16 (100 TCID₅₀). They were incubated at 37°C in 5% CO₂. After 1 h incubation, approximately 10⁴ Vero cells were added per well in a volume of 100 μ l of minimum essential medium (MEM) containing antibiotics and, after incubation for 4 to 6 days, the test was read using an inverted

microscope. Wells were scored for the degree of cytopathic effect (CPE) observed. A sample was considered positive when it showed a CPE of more than 50% neutralisation at the lowest dilution (1:10). The serum titre represented the highest serum dilution capable of neutralising more than 50% CPE in the tissue culture.

Statistical analysis

For each district, the prevalence level and 95% confidence intervals were determined for both cattle and sheep. The prevalence level in both species was calculated through Beta (s+1, n-s+1) distribution where **s** is the total number of positives and **n** is the total number of animals tested. The probability distribution of the percentage of positive animals shows not only the most probable value of prevalence, but also the level of uncertainty due to sample size.

Results

Of the 1 727 serum samples screened, 200 were found positive for BT antibody by c-ELISA. Of the 200 ELISA-positive samples, only 71 were found positive by the VN test. All neutralising antibodies were against serotype 9 and the titres ranged from 1/10 to 1/320. Prevalence levels and confidence intervals are given in Tables I, II and III and the most probable values with the relative uncertainties of BT prevalence in Albanian cattle, sheep and goats

Table I
Prevalence levels and 95% confidence intervals of bluetongue found in Albanian cattle according to district of origin (total: 857)

District	Animals tested	Positive to VN test	Positive to c-ELISA	Prevalence level	Lower level	Upper level
Bulgise	28	2	9	32.14%	17.94%	50.83%
Devoll	50	1	2	4.00%	1.23%	13.46%
Dibre	31	0	1	3.23%	0.77%	16.22%
Durres	50	1	6	12.00%	5.70%	23.87%
Fier	50	2	5	10.00%	4.44%	21.41%
Gjirokaster	49	7	16	32.65%	21.21%	46.70%
Has	29	4	7	24.14%	12.28%	42.28%
Kolonje	50	0	7	14.00%	7.02%	26.26%
Korce	201	4	13	6.47%	3.84%	10.75%
Librazhd	50	7	26	52.00%	38.46%	65.25%
Permet	50	3	4	8.00%	3.26%	18.88%
Pogradet	70	5	10	14.29%	8.00%	24.38%
Shkoder	45	1	9	20.00%	10.95%	33.91%
Tirane	54	12	33	61.11%	47.73%	72.98%
Tropoje	50	7	14	28.00%	17.49%	41.74%
Total/average	857	56	162	18.90%	16.42%	21.66%

VN virus neutralisation

c-ELISA competitive enzyme-linked immunosorbent assay

Table II
Prevalence levels and 95% confidence intervals of bluetongue found in Albanian sheep and goats according to district of origin (total: 870)

District	Animals tested	Positive to VN test	Positive to c-ELISA	Prevalence level	Lower level	Upper level
Bulgise	30	1	1	3.33%	0.79%	16.70%
Devoll	50	2	3	6.00%	2.18%	16.24%
Dibre	44	0	3	6.82%	2.48%	18.27%
Durres	52	0	0	0.00%	0.05%	6.72%
Fier	59	0	0	0.00%	0.04%	5.96%
Gjirokaster	83	0	0	0.00%	0.03%	4.30%
Has	24	2	3	12.50%	4.54%	31.22%
Kolonje	50	0	0	0.00%	0.05%	6.98%
Korce	179	1	3	1.68%	0.61%	4.79%
Librazhd	49	0	9	18.37%	10.03%	31.44%
Permet	46	1	1	2.17%	0.52%	11.29%
Pogradet	70	0	2	2.86%	0.88%	9.81%
Shkoder	35	0	0	0.00%	0.07%	9.74%
Tirane	50	6	10	20.00%	11.29%	33.12%
Tropoje	49	2	3	6.12%	2.22%	16.55%
Total/average	870	15	38	4.37%	3.20%	5.94%

VN virus neutralisation

c-ELISA competitive enzyme-linked immunosorbent assay

Table III
Prevalence levels and 95% confidence intervals of bluetongue found in Albanian sheep, goats and cattle according to district of origin (total: 1 727)

District	Animals tested	Positive to c-ELISA	Prevalence level	Lower level	Upper level
Bulgise	58	10	17.24%	9.69%	28.97%
Devoll	100	5	5.00%	2.21%	11.18%
Dibre	75	4	5.33%	2.17%	12.93%
Durres	102	6	5.88%	2.78%	12.25%
Fier	109	5	4.59%	2.03%	10.29%
Gjirokaster	132	16	12.12%	7.63%	18.80%
Has	53	10	18.87%	10.63%	31.43%
Kolonje	100	7	7.00%	3.48%	13.76%
Korce	380	16	4.21%	2.62%	6.73%
Librazhd	99	35	35.35%	26.64%	45.18%
Permet	96	5	5.21%	2.30%	11.62%
Pogradet	140	12	8.57%	5.00%	14.39%
Shkoder	80	9	11.25%	6.08%	20.05%
Tirane	104	43	41.35%	32.34%	50.98%
Tropoje	99	17	17.17%	11.03%	25.82%
Total	1 727	200	11.58%	10.16%	13.18%

c-ELISA competitive enzyme-linked immunosorbent assay

are shown in Figure 2. The overall prevalence was 18.9% in cattle (animals tested: 857) and 4.4% in sheep (animals tested: 870). The Tirana District was the

region in which the highest prevalence was observed, with 61% of the cattle and 20% of the sheep and goat populations positive for BT antibodies (Fig. 3).

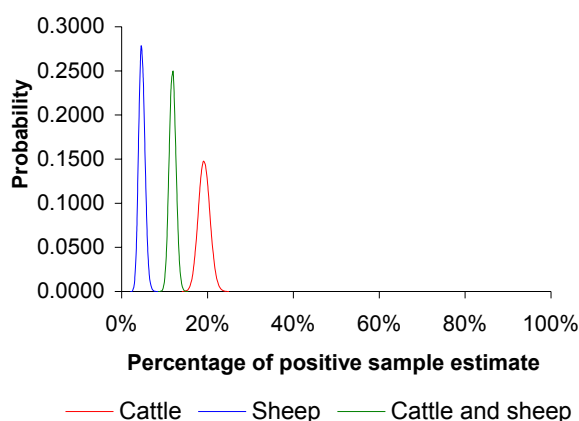


Figure 2
Probability distributions of the percentage of animals with bluetongue antibodies found in Albania (n = 1 727)

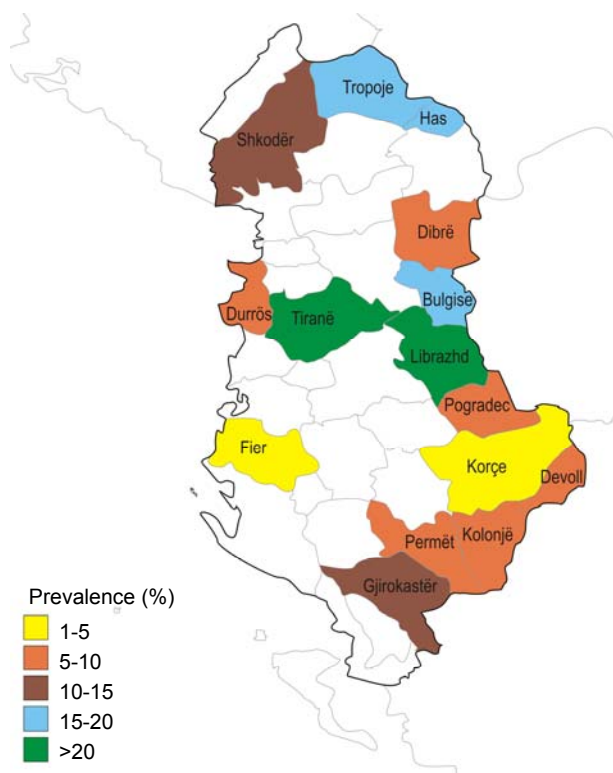


Figure 3
Prevalence of bluetongue in sheep and cattle in Albania, according to geographic origin

Discussion

The most remarkable developments of BT infection have been observed recently in south-eastern Europe. Between 1998 and 1999, BT, predominantly caused by serotype 9, occurred in north-eastern Greece, southern Bulgaria and in western Turkey. In 2001, BT was reported from north-western Greece, western Bulgaria, Kosovo, Macedonia and Montenegro and Serbia. According to the results obtained in this study, BTV was also circulating in Albania. As not all districts were included in the survey, the possible origin of BTV in Albania was

not determined. The finding that serotype 9 was the only type present would suggest that BTV infection in Albania originated from a neighbouring country. As with the prevalence levels obtained in this study, the infection was not uniformly distributed in the areas and species examined. Librazhd and Tirana were the districts with the highest infection rates and BT was found to be more prevalent in cattle than in sheep. Bluetongue is a non-contagious disease of ruminants transmitted between its vertebrate hosts by *Culicoides* biting midges. There is evidence that at least some species of *Culicoides* prefer to feed on cattle rather than on sheep (5). This preference could account for the species prevalence differences observed in this study. As mentioned before, Tirana and Librazhd were the districts with the highest BT prevalence; interestingly, Tirana was also the district in which animals showed the highest neutralising antibody titres. Moreover, Tirana was the region in which the highest number of ELISA-positive samples were confirmed by the VN test (41.9%) and double the ratio found in Librazhd. The c-ELISA is able to detect BT antibodies earlier and over a longer period than the VN test. The results of this study would suggest that, at least with regard to these two districts where a large number of animals were tested, BT occurred first in Tirana and then in Librazhd. Based on the low number of positive sera confirmed by the VN test (0.2%) and the low neutralising antibody titres found, the infection seemed to be recent. The BT outbreak reported at the end of 2001 in Librazhd provided further evidence of the presence of recent infection (1).

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