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Bluetongue in Bosnia: comparisons of competitive enzyme-linked immunosorbent assay and standard agar gel immunodiffusion tests

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Summary

At the end of August 2002, clinical symptoms of bluetongue (BT) (fever between 39°C and 41°C, muco-purulent or bloody nasal discharge, oedema of the lips and the intramandibular space, foot lesions including laminitis and coronitis in some cases, diarrhoea and dysentery) were recorded in Pramenka sheep flocks in north-east Bosnia in August 2002. A total of 9 599 serum samples (ovine: 8 967; bovine: 632) from 40 communities of Bosnia and Herzegovina were tested for the presence of anti-bluetongue virus (BTV) antibodies using competitive enzyme-linked immunosorbent assay (c-ELISA) and the standard agar gel immunodiffusion (AGID) test. The c-ELISA revealed BTV-seropositive reactions in 187 (1.94%) samples and the AGID test detected 141 (1.53%) cases. Complete agreement was recorded between the c-ELISA and AGID test results for bovine sera. These results indicate that the ability of c-ELISA to detect anti-bluetongue virus antibodies in ovine sera was superior to that of the AGID. All positive sera were collected from animals in the river areas of Bosnia and Herzegovina.

Keywords

Agar gel immunodiffusion test – Bluetongue – Bosnia – *Culicoides* – Enzyme-linked immunosorbent assay – Herzegovina.

Introduction

Bluetongue (BT) virus (BTV) is an arthropod-borne pathogen, transmitted by species of the genus *Culicoides*. BTV can infect several species of domestic and wild ruminants, but sheep are most susceptible (3). BTV occurs in the Americas, Africa, Asia and Australia. At least 24 serotypes of BTV have been identified worldwide. The virus can cause an acute, sub-acute, mild or inapparent disease (4). The agar gel immunodiffusion test (AGID) and competitive enzyme-linked immunosorbent assay (c-ELISA) are the most common tests for the detection of group-specific anti-BTV antibodies (1, 2, 5).

Between 1998 and 2001 incursions of BTV were recorded in countries around the Mediterranean Basin that are usually free from infection, but no occurrence was reported in Bosnia. Like many of the neighbouring countries, Bosnia had no previous record of the disease. At the end of August 2002, clinical symptoms of BT were reported for the first time in local Pramenka sheep flocks in north-east Bosnia, close to the border with Serbia. The virus spread across Bosnia between September and October, travelling through river areas.

Materials and methods

A total 9 599 field sera from 8 967 sheep and 632 cattle in 40 communities of Bosnia and Herzegovina were collected between August and December 2002. All the sera were transported on ice and were stored at –20°C until tested. Diagnosis was based on clinical signs and laboratory confirmation. For the detection of anti-BTV antibodies, all sera were tested using the c-ELISA and standard AGID tests.

Results

In August 2002, BTV was confirmed for the first time ever in Bosnia and Herzegovina, initially in Kalesija (north-east Bosnia, close to the Serbian border). During September and October, the virus

spread across the country, travelling through river areas. BT antibody was tested in sheep flocks in 40 communities (Fig. 1).

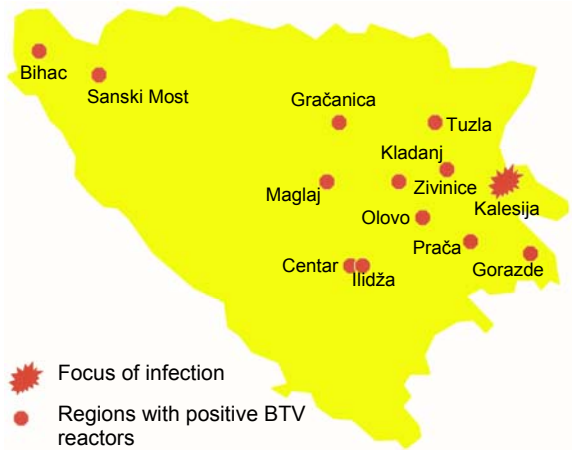

Figure 1
Communities of Bosnia and Herzegovina with seropositive reactors of bluetongue in 2002

Clinical signs were observed in 5-10% of the animals in flocks. The most common signs were fever of between 39°C and 41°C, muco-purulent or bloody nasal discharge, oedema of the lips and the intramandibular space, foot lesions, including laminitis and coronitis in some cases, diarrhoea, dysentery and death.

The comparative results of the c-ELISA and standard AGID test on 8 967 sheep and 632 cattle sera are presented in Table I.

Table I
Comparative performance of the competitive enzyme-linked immunosorbent assay and standard agar gel immunodiffusion test in the detection of antibody to bluetongue virus in sheep and cattle sera from Bosnia and Herzegovina

Test	Number of positive reactors			
	Cattle		Sheep	
	No.	Percentage	No.	Percentage
c-ELISA	6	100	187	100
AGID	6	100	141	77.9

Forty-six of the sheep sera were negative using the AGID test but positive with the c-ELISA. Complete agreement was recorded between the c-ELISA and AGID test results for cattle sera.

The Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' in Teramo confirmed the presence of BTV serotype 9.

Discussion

Currently, the most common serodiagnostic tests for BTV are the AGID test and c-ELISA that detect the VP7 group-specific antibodies (5). Although the AGID test is simple to perform and rapid, it is not highly sensitive or quantitative and has limitations regarding specificity. Sera containing antibodies to other groups of Orbiviruses (e.g. epizootic haemorrhagic disease) may give a non-specific reaction in the AGID test. Among several ELISAs that have recently been developed, the c-ELISA, in which a group-specific MAb to BTV is used, has proved to be the most sensitive and specific assay for detection of BTV antibodies. Following extensive national and international validation, the c-ELISA is gradually replacing the AGID test (1, 2). The results of our study indicate that the ability of the c-ELISA to detect anti-BTV antibodies in sheep sera was superior to that of the AGID test.

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