Spatial distribution of bluetongue in cattle in southern Croatia in the

last quarter of 2002

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Summary

The domestic ruminant population of southern Croatia was affected by bluetongue (BT) in late 2001. A sentinel cattle scheme was developed to detect the presence of bluetongue virus (BTV) activity in the domestic cattle population in the protection zone (based on the distribution of BT in 2001: Dubrovacko-Neretvanska County and the southern area of the Splitsko-Dalmatinska County) as well as in the surveillance zone (the northern area of the Splitsko-Dalmatinska County). Twenty-five villages were selected to serve as sentinel locations during the observation period which lasted from 15 September to 15 December 2002. Seroconversion was not detected in cattle in sentinel locations in the surveillance zone. However, in the protection zone, serum antibodies to BTV serotype 9 were detected in eight cattle in five of the ten sentinel locations. Although no clinical case of BT disease was detected in sheep on mainland Croatia in late 2002, BTV activity was present in sentinel cattle in the protection zone. When compared with 2001, spatial distribution of the locations in which cattle seroconverted to BTV-9 in the last quarter of the 2002 suggests a northward trend to the spread of BTV in the cattle of southern Croatia.

Keywords

Bluetongue virus - Cattle - Croatia - Sentinel herd - Spatial data.

Introduction

Over the last several years a significant change has been recorded in the epizootiological situation of bluetongue (BT) in Europe. In Croatia, there is no documented evidence of BT virus (BTV) infection in the ruminant population prior to 2001. While BTV can infect most ruminants, BT disease primarily affects sheep (8), with the incidence of clinical disease highly variable (5). While natural and experimental BTVinfection of cattle is asymptomatic in the vast majority of cases, rare and authentic instances of disease are likely to occur (7). BTV infection of cattle often results in prolonged viraemia, hence cattle serve as a reservoir from which the virus may be recovered by the haematophagous insect vectors and then transmited to other ruminants (6). This could explain why bovines are often used as 'early warning' sentinel animals to determine the prevalence of BTV activity in an area in which clinical BT disease has not been endemic.

Sentinel surveillance can provide clearly defined data (usually incidence data) on a regular basis. Although not all outbreaks are likely to be detected at an early stage if surveillance is limited to sentinel sites, a sentinel herd scheme may enable the detection of BTV circulation between domestic ruminants and vectors and can assist in the identification of risk factors associated with the occurrence of BT disease. Furthermore, when sentinel sites are randomly selected, generalisation of the results can be applied to a wider population.

Sentinel herd schemes are particularly effective surveillance and monitoring tools in regions where clinical BT disease is uncommon (10). In Queensland, Australia, 47 sentinel herds (with 10-20 cattle in each herd), most of which were bled monthly over the period from 1990 to 1992 (10), gave valuable information on BTV serotypes present in Queensland. In 1988, the official BT policy adopted by Canada included a surveillance mechanism for determining BTV seroprevalence by

using a sentinel herd programme (2). The sentinel herd programme consisted of six herds of cattle (with 7 animals in each herd), which were distributed along or within 48 km of the Canada-United States border (2). The identification of two infected animals illustrated the effectiveness of the sentinel herd programme. Greece initiated a sentinel cattle 1999 programme in (4). The programme commenced on 30 June 1999 and animals were sampled approximately every 15 days. The sentinels were posted in four locations: Rhodes, Kos, Leros and Samos, with 50, 51, 18 and 18 animals per location, respectively, in two to five villages per location. This programme also proved to be effective in detecting animals that seroconverted.

The main objective of the 2002 sentinel cattle scheme implemented in the two southernmost counties of Croatia (Dubrovacko-Neretvanska and Splitsko-Dalmatinska) was to investigate whether there was BTV activity present in cattle populations. In addition, one of the aims of the scheme was to obtain baseline data required for the planning of the 2003 sentinel cattle programme to more accurately inform decision-makers on ruminant movement control.

Materials and methods

A sentinel cattle pilot scheme was conducted from 15 September to 15 December 2002. Surveillance of sentinel cattle in the protection zone (defined as having a 100-km radius around the infected holdings and so included Dubrovacko-Neretvanska County and the southern part of Splitsko-Dalmatinska County) was designed to detect seroconversion in cattle at a prevalence of 5%. For this purpose, 60 cattle in 10 sentinel locations (6 cattle per location) were sampled approximately every 15 days.

Sentinel cattle in the surveillance zone (defined as having a 100-km radius around the protection zone and so included the northern area of Splitsko-Dalmatinska County) were monitored to detect seroconversion in cattle at a prevalence of 2%. For this purpose, 150 cattle in 15 sentinel locations (10 cattle per location) were sampled approximately every 30 days.

The three criteria for selecting locations where sentinel animals should be placed were ruminant population density (e.g. the higher the number of cattle in the village, the higher the probability that the village would be selected), locations which the local Veterinary Service knows, or suspects, to be suitable for vectors, and to have owners willing to participate in the sentinel scheme. The criterion for inclusion of cattle in a sentinel study was that the animals had to be serologically negative and over six months of age. Those cattle which became seropositive to BT were replaced with seronegative cattle.

To determine the number of cattle (sample size) that would serve as sentinel animals in the surveillance and protection zones, the formula of Cannon and Roe was used to detect the presence of the disease (based on the assumption of a perfect test) (1). This formula may be used either for a herd or for a welldefined geographic zone (9). The number of animals required for the purpose of estimating the prevalence of BTV antibodies at levels of 2% and 5% were 147 and 59, respectively. This enables detection of at least one seropositive animal if BT infection is present in the cattle population at and above the specified prevalence level.

Serum samples were screened with competitiveenzyme-linked immunosorbent assay (c-ELISA,) (VMRD Inc, USA). To declare a bovine animal positive, two positive reactions to the c-ELISA were necessary. Visualisation of spatial data was performed using ArcView 8.2 (3).

Results

The spatial distribution of sheep flocks showing clinical signs of BT, as well as cattle herds found to have BTV antibodies in late 2001, are presented in Figure 1.



Figure 1

Spatial distribution of flocks in which sheep showed clinical signs of bluetongue as well as herds in which cattle had antibodies to BTV-9 Last guarter of 2001

The spatial distribution of sentinel cattle in the protection and the surveillance zones from September to December 2002 is shown in Figure 2.



Figure 2 Spatial distribution of sentinel villages in the protection and surveillance zones 15 September-15 December 2002

During the observation period in the protection zone, serum antibodies to BTV were detected in eight cattle in 5 of the 10 sentinel locations. More specifically, from 14 to 17 October 2002, BTV antibodies were detected in one sentinel cow in Krstatice, Runovici and Krvavac and in two sentinel cows in Podgorje. Further BTV activity in sentinel cattle in the protection zone was recorded between 2 and 4 November 2002 in the sentinel locations of Podgorje and Krvavac (BTV antibodies being detected in one sentinel cow per location). On 16 November, BTV antibodies were detected in a cow in Mihanici. No clinical sign of BT disease was reported in any ruminant animal in the locations where BT-seropositive cattle were detected.

The spatial distribution of locations in the protection zone in which BT seroconversion was detected in sentinel cattle during the 2002 sentinel programme is shown in Figure 3. Antibodies to BTV were not detected in sentinel cattle in the surveillance zone.

Discussion

The finding that BTV antibodies were present in sentinel cattle in southern Croatia in late 2002 suggests that viral activity continues in the absence of the disease in the areas of mainland Croatia that were affected by BT in 2001. However, in December 2002, only one isolated outbreak of BT disease in one sheep flock was detected on the Island of Hvar. This could be explained by the fact that BTV is probably maintained by a cycle of infection in the insect vector and cattle, and only when the vector population is very high does the virus 'spill over' into other species such as sheep (6).

The results of the 2002 sentinel cattle scheme in southern Croatia also suggest that although the cattle densities in the Counties of Dubrovacko-Neretvanska Splitsko-Dalmatinska are very low, they still seem high enough to sustain the virus. However, since a detailed analysis of the factors contributing to the presence of BTV activity in 2002 was not performed, we can only speculate as to whether BTV had been present because it overwintered in vectors and/or in hosts, or because of new BTV incursions that occurred in 2002.



Spatial distribution of sentinel cattle that seroconverted to BTV-9 14 October-16 November 2002 Better utilisation of data collected during the 2002 sentinel programme as well as the sentinel programme that commenced on 15 July 2003, might provide more comprehensive information on the epidemiology of BT in southern Croatia.

Although the nature of the disease and the clustering level might require a different approach as well as adjustments of the sample size (a 'perfect' test in detection of BTV antibodies was not used), the results of the 2002 sentinel cattle scheme provided valuable information which served to aid in the decision to not only continue but to extend further the BT surveillance programme to the north-western areas of Croatia. This information also proved to be valuable for animal movement control measures.

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