Bluetongue surveillance in Switzerland in 2003: a serological and entomological survey

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Summary

At present, Switzerland is considered officially free from bluetongue (BT) disease. Recently reported outbreaks have recorded BT moving north as far as latitude 44°30´N in Europe and 49°N in Kazakhstan. The absence of clinical disease does not prove freedom from BT virus (BTV) infection. In addition, the occurrence and distribution of the only known biological vector, certain species of *Culicoides* biting midges (Diptera: Ceratopogonidae), is poorly understood for Switzerland. Consequently the Swiss Veterinary Office initiated a project on BT surveillance in April 2003 on cattle farms. The study comprised serological and entomological activities; initial results are presented.

Keywords

Blacklight trap – Bluetongue – Competitive enzyme-linked immunoassay – *Culicoides* – Serological surveys – Surveillance – Switzerland – Vector.

Introduction

Bluetongue (BT) is an infectious, non-contagious vector-borne viral disease of ruminants (5) that has been designated a 'List A' disease by the Office International des Épizooties (OIE). To date, certain species of *Culicoides* biting midges (Diptera: Ceratopogonidae) are the only known biological vectors of bluetongue virus (BTV). Areas in which the climatic and environmental conditions favour the survival of *Culicoides* spp. are considered to be at risk; these areas occur approximately between 40°N and 35°S although in parts of western North America and in China may extend to almost 50°N (3). In Europe, the major vector species is *C. imicola*, but the *C. obsoletus* and *C. pulicaris* species groups of midges may also be involved in some areas (4).

Recently, outbreaks of BT have been reported from as far north as latitude 44°30´N in Europe (4) and 49°N in Kazakhstan (2); the absence of clinical disease does not prove freedom from BTV infection (7). At present, Switzerland is considered to be officially free from BT disease. However, the occurrence and distribution of vector *Culicoides* in Switzerland is poorly understood. With climate as a major determinant of insect distribution and vector competence, BTV competent populations of *Culicoides* may already be present in Switzerland or could become established as global warming proceeds. Furthermore, livestock populations in Switzerland have not been tested for antibodies against BTV. Consequently, the Swiss Veterinary Office initiated a BT surveillance project in Switzerland in April 2003. As *C. imicola*, the major Old World BTV vector apparently prefers feeding on cattle rather than on sheep (6), the system was based around selected cattle farms. The study was divided into serological and entomological activities. The objectives of this project were to investigate the immune status of cattle regarding BTV and to obtain baseline data on vector populations, predominantly *C. imicola*, *C. pulicaris* and *C. obsoletus*, and their distribution within areas of Switzerland considered to be most at risk.

Materials and methods

Serological study, test validation and testing protocol

Blood samples were taken from cattle on farms selected randomly. The sample size for the surveillance study was calculated using OIE recommendations, so that a minimal prevalence of 2% could be detected with a probability of 95% (8) assuming that Switzerland is free from BT. Switzerland was divided into 46 equal quadrants (40 km × 40 km). Taking into account that quadrants have a different acreage because of lakes and mountains, a proportion of km² pasture/quadrant was calculated for each quadrant. The number of farms to be sampled within each quadrant was calculated according to pasture size. A two-stage cluster sampling scheme was used. Of 2 384 cattle farms sampled for routine disease surveillance purposes in 2003, 660 farms with cattle over 24 months of age were selected randomly and five blood samples taken on each. Almost half of a total of 3 300 blood samples were analysed by competitive ELISA (c-ELISA); prevalence of BT antibodies was calculated using a survey toolbox (1).

As the prevalence of BTV-specific antibodies in the Swiss cattle population was expected to be very low, a sensitive serological test for antibody detection was required. Two commercial ELISAs, c-ELISA VMRD (Pullmann, Washington, USA) and c-ELISA BDSL (Irvine, UK) were compared for sensitivity. Comparison of the sensitivity of each test was conducted using international standards (weak positive control: Institute of Animal Health [IAH] Pirbright [OIE Bluetongue Reference Laboratory], United Kingdom, and weak positive control: Onderstepoort Veterinary Institute [OVI], Onderstepoort, Republic of South Africa). Initial serological screening was performed with c-ELISA BDSL as it was more sensitive than c-ELISA VMRD. Positive results thus obtained were to be re-tested with the c-ELISA VMRD using a lower cut-off than given in the instructions of the manufacturer so as to also detect weak positive samples or international standards, respectively. As a consequence of a c-ELISA VMRD positive result, all animals of the herd in which a positive sample originated would be sampled and tested. Because prevalence of BTV-antibodies is expected to be very low, and because imperfect serological tests were to be used, it would be necessary to confirm positive herd screening results by virus neutralisation (VN) tests. Therefore, the two strongest positive samples would be sent to the IAH in Pirbright to confirm seropositivity and to determine the BTV serotype involved.

Test specificity and its 95% confidence interval were determined, based on serum samples originating from the 1998 serum bank consisting of samples obtained from animals over 24 months of age during routine serological surveillance. It was assumed that the Swiss cattle population was free of BT in 1998.

Entomological study

Vector trapping was performed on cattle farms within areas considered to be at most risk as calculated by Rawdon (9) in a mathematical model. For the entomological study, blacklight traps manufactured by the OVI were used to capture *Culicoides* for one night in high-risk areas. The locations of the trapping sites are shown in Figure 1.

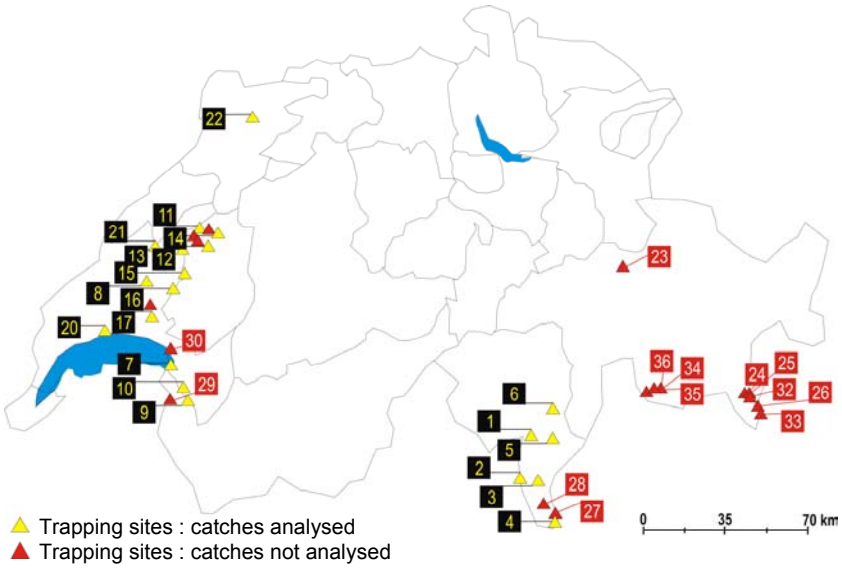

Figure 1
Locations of 36 *Culicoides* trapping sites in Switzerland, 2003

High-risk areas were defined as areas with the following:

- annual average temperature ≥12.5°C
- the most intensive rainfall
- adjacent to Italy without natural barriers (e.g. high mountains) to prevent the introduction of insects.

Trapping site criteria were as follows:

- cattle farm (>3 cattle on farm during collection)
- altitude lower than 1 100 m above sea level.

Farm managers were asked to participate by telephone interview. As July (*C. obsoletus*) and September (*C. imicola*) is the peak season for midge activity, traps were set during this period. On each selected farm, a trap was set for one night (single point/single night collection modus). At each trapping site, the same trapping protocol (modified trapping protocol of the OVI) was followed. In addition to collection site parameters, an interview based on a questionnaire was conducted with the farm manager to obtain information on livestock management. The midges collected were analysed by the IAH to identify the abundance of *Culicoides* species found, specifically *C. imicola*, *C. obsoletus* and *C. pulicaris*.

Meteorological data were obtained from the national Swiss weather service; daily temperatures are recorded at 25 weather stations distributed across Switzerland.

Results

Preliminary results of the serological study

Analysis of sera collected in 2003

Of 1 492 sera analysed by BDSL ELISA, 1 426 were negative, 303 gave a non-specific result, while 33 were positive. To date, 40 samples giving either questionable results or a positive response to the BDSL ELISA have been re-analysed with the VMRD ELISA, resulting in 11 positive samples. For a definite positive result, additional analyses using the VN test are required; the results of these tests are pending.

Preliminary results of the entomological study

A total of 15 664 *Culicoides* were caught at 21 trapping sites on 21 trapping nights (range in number of *Culicoides* per trapping site: 3-4 905). The total *Culicoides* catch consisted of 13 090 *C. obsoletus* (range per trapping site: 2-1 560), 957 *C. pulicaris* (range per trapping site: 0-240) and 1 617 other *Culicoides* of unidentified species.

Discussion

Serological study

Based on the information available at present, c-ELISA VMRD positive results are likely to be false positives, because both tests are not 100% specific. Additionally, positive samples originated from different farms distributed across Switzerland. However, all samples need to be re-analysed and confirmed by the IAH in Pirbright before conclusions can be made.

Entomological study

A further 15 *Culicoides* collections remain to be analysed before final conclusions can be drawn.

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