Bluetongue virus surveillance in a newly infected area

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Summary

The occurrence of bluetongue virus (BTV) in areas in which intensive animal production is practised and where there is extensive movement of animals may have a substantial impact on both animal trade and husbandry. This situation occurred in Italy after the detection of bluetongue (BT) in August 2000. In such situations, surveillance can be used to delineate with precision those areas in which the virus is circulating and, consequently, to enforce the appropriate animal movement restrictions. Furthermore, surveillance can provide the data required to assess the risk associated with animal movement and trade. A structured surveillance system for the detection of BTV has been in place in Italy since August 2001. The system is based on the periodical testing of unvaccinated sentinel cattle that are uniformly scattered throughout Italy in a grid of 400 km^2 cells. The initial number of sentinel sites and sentinel animals, together with the width of the restricted area generated by the finding of a single seroconversion in a sentinel animal, were based on conservative criteria. Animal movement was restricted in a 20 km radius buffer zone around any positive serological result. This buffer area extends about 1 257 km^2, equivalent to the area of three grid cells. After the commencement of the BT vaccination campaign in Italy, the sentinel surveillance system was the only way in which the effectiveness of vaccination and the incidence of infection in the non-immunised strata of ruminant animals could be estimated. Data collected over two years was used to assess the risks posed by the adoption of less conservative criteria for the delineation of infected areas and by the progressive relaxation of movement restrictions of vaccinated animals. In regard to the delineation of restricted areas, a new approach was tested and validated in the field, based on a Bayesian analysis of the positive and negative results obtained by the testing of sentinel animals from defined regions. For the risks related to animal movement, the surveillance data was used in risk assessment analyses to address the movement of slaughter and breeding animals from vaccinated/infected and surrounding areas to free areas. These risk assessments led to an amendment of the relevant European Union legislation. Finally, a Montecarlo simulation model was developed to simulate different sentinel system scenarios and to decrease the total number of sentinel animals and sites required by the surveillance system. The sentinel surveillance system was complemented by an entomological surveillance system based on the use of a number of permanent blacklight traps run weekly year-round and a number of mobile blacklight traps moved through the grid cells during the summer and autumn of each year. The aim of entomological surveillance was to define the maximum distribution of vectors and their seasonal population dynamics. Furthermore, the permanent trap system provides an early warning of the start of new epidemics. The data from the entomological surveillance system were also analysed to generate probability maps of the presence of the principal BTV vector (*Culicoides imicola*) and to define the geographical risk of BT on a nationwide basis, and to predict the geographical distribution and the short-term spread of *C. imicola* in Sardinia, using spatio-temporal data. The detection, since 2001, of BT outbreaks in the absence of *C. imicola* and the recent identification of BTV in midges of the Obsoletus Complex also stimulated investigations on other vector *Culicoides*, including *C. obsoletus* and *C. pulicaris*.

Keywords

Bluetongue – *Culicoides* spp. – Europe – Italy – Sentinel animals – Surveillance.

Since 1998, Southern Europe has been affected by the largest epidemic of bluetongue (BT) ever recorded. The disease initially affected Greece in 1998 with 84 outbreaks and 2 990 cases and then spread to Bulgaria in 1999 (1 651 outbreaks in the two countries with 11 960 cases), France (Corsica), Italy and Spain (Balearic islands) in 2000 (7 423 outbreaks in the three countries and 268 205 cases, 98% of which were recorded in Italy), the Balkans and southern Italy in 2001 (7 358 outbreaks and 261 839 cases, 94% of which were in Italy), and in 2002 the disease continued to spread to the Balkans and through much of southern Italy (Figs 1 and 2).

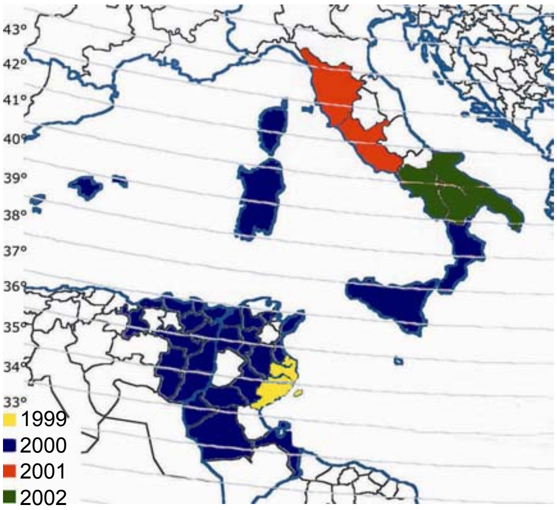

Figure 1
Spread of bluetongue, caused by BTV-2, in Europe, 1999-2002

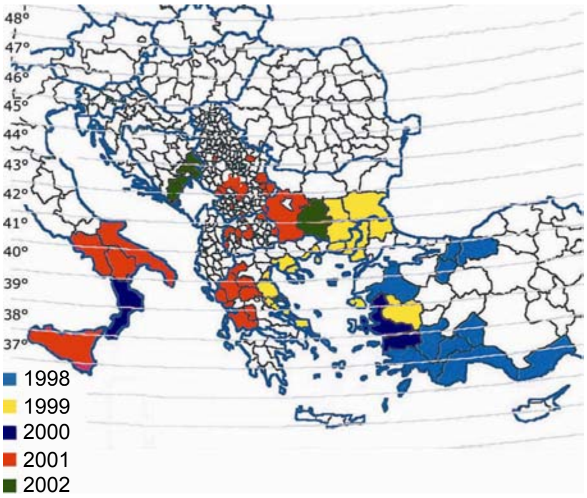

Figure 2
Spread of bluetongue, caused by BTV-9, in Europe, 1998-2002

In terms of both control actions and surveillance, neither the European Union (EU), nor the affected European countries were adequately prepared to cope with the problems posed by a vector-borne disease such as BT when it first appeared. This inadequate level of preparedness of Europe was reflected in existing regulations, because BT was given in Directive 92/119/EEC (22), together with other OIE List A diseases, such as rinderpest, sheep pox, swine vesicular disease etc., although Directive 1992/35/EEC (21) had already been issued to define measures against African horse sickness. Directive 92/119/EEC foresaw exclusive and direct control measures and the demarcation of a 3-km radius protection zone and a 10-km radius surveillance zone around each infected farm. Direct control measures included the slaughter of all susceptible animals on the farm and the possible extension of such measures to neighbouring farms suspected of housing infected animals.

In November 2000, after the incursion of BT into Sardinia (Italy) on 18 August (9), the Balearic islands on 29 September (4) and Corsica on 18 October (3), the EU considered provisions already stipulated in Directive 92/35/EEC (21) and issued Directive 2000/75/EC (23) which fixes specific rules for the control and eradication of BT, and in particular the following:

- the demarcation of a protection zone with a radius of 100 km around the outbreaks or any farm on which virus circulation was confirmed
- the establishment of a surveillance zone of 50 km around the protection zone
- the slaughter of animals deemed necessary to prevent the spread of the epidemic and the destruction, elimination, incineration or burial of the carcasses of those animals
- the implementation of serological and entomological surveillance programmes in the protection and surveillance zones
- the prohibition of animal movement from protection and surveillance zones.

To complement these prescribed measures, the Directive foresaw the possibility of a vaccination programme in the protection zone. Since no specific criteria were provided in the Directive for serological and entomological surveillance, each country can propose its own programme to the European Commission, taking into account specific needs and geographical features or husbandry practices.

The application of Directive 2000/75/EC (23), through the adoption of Decision 2001/138/EC (13), has disrupted animal trade in at least a third of Italy. Long-term application of these regulations would probably have caused the irreversible decline of the entire cattle and small ruminant production system. Decision 2001/138/EC (13) instituted protection and surveillance zones in accordance with the criteria established by Directive 2000/75/EC

(23) and more than a third of Italy was subjected to movement restrictions: 26% in the protection zone and 9% in the surveillance zone (Fig. 3). Animal movement was prohibited from the regions of Sicily, Sardinia and southern Italy (protection and surveillance zones) to all disease-free regions. Consequently, it was impossible to fatten and cull cattle, activities which traditionally take place in the Po Valley (free zone). Moreover, animal transhumance from the winter pastures in Puglia (surveillance zone) to the summer pastures in Abruzzi and Molise (free zones) was also stopped.

Figure 3
Protection and surveillance zones identified in Italy, in accordance with Decision 2001/138/EC (13)

All existing EU legislation regarding the compensation of farmers was developed in relation to contagious diseases of OIE List A, mainly foot and mouth disease and hog cholera (classical swine fever). The control strategy used in Europe for outbreaks of these diseases resorts to stamping-out of infected and in-contact animals and, since 1990, vaccination is only an ancillary measure. In this context, the principal economic losses are direct, due to the slaughter of infected and in-contact animals. According to European legislation, any compensation for indirect losses would perturb the market. In the case of vector-borne disease, especially when vaccination is the principal control measure, direct losses are virtually negligible, whereas indirect losses due to movement restrictions become considerable. Moreover, losses suffered by farmers whose livestock is subjected to movement restrictions also affect the earnings of farmers living in free areas. This has been recognised only very recently (July 2003) by the European Commission that enacted the Decision C(2003)2519fin (20), authorising the region of Sardinia to compensate cattle farmers for indirect losses due to movement restrictions imposed from 6 September 2000 to 31 December 2001.

Prior to the 1998 outbreak in the Mediterranean (Figs 1 and 2), little information was available on the distribution of BTV vectors, i.e. of areas at risk of infection. In the early 1980s *C. imicola* was first identified in Spain, Portugal and on the Greek islands of Lesbos and Rhodes (5, 7, 29, 30). The presence of *C. imicola* was neither reported from the Balearic islands, Corsica, Sardinia, Sicily and Malta, nor was it found in the mainland territories of Greece and Italy (7). Further studies conducted in southern Italy, Sicily and the island of Pantelleria (Italy) in 1996 also did not detect the presence of *C. imicola* (33). It was only in June 2000 that *C. imicola* was first identified in western Sicily (26). It is noteworthy that, at least as far as Italy is concerned, the records show that in the past, *C. imicola* probably escaped notice in Italy as the majority of historical collections were made outside its current range (10). Even less was known regarding the distribution of other potential vectors of BTV, namely species of the Obsoletus and Pulicaris Complexes. Therefore, the Italian government decided to design a surveillance system that could delineate with precision the areas in which virus circulates and thus would enforce the appropriate movement restrictions on animal populations. The attempt to define, as precisely as possible, those areas that should be subjected to movement restrictions (i.e. less than the 100-km radius stipulated by the Directive), implies the existence of an effective early warning system, because the buffer territory of movement restrictions around areas of virus circulation is reduced. Furthermore, surveillance data were also used to provide information needed to assess the risks associated with animal movement and trade.

The surveillance system comprised the following:

- clinical surveillance
- serological surveillance
- entomological surveillance.

The surveillance programme

Clinical surveillance

Bluetongue spreads quickly after it enters a susceptible population. In 2000, the virus initially spread at a rate of around 30 km per week in Sardinia (Fig. 4) (9). When BTV serotype 4 (BTV-4) invaded Sardinia in August 2003, it spread at a rate similar to that recorded three years previously with BTV-2 (Fig. 5).

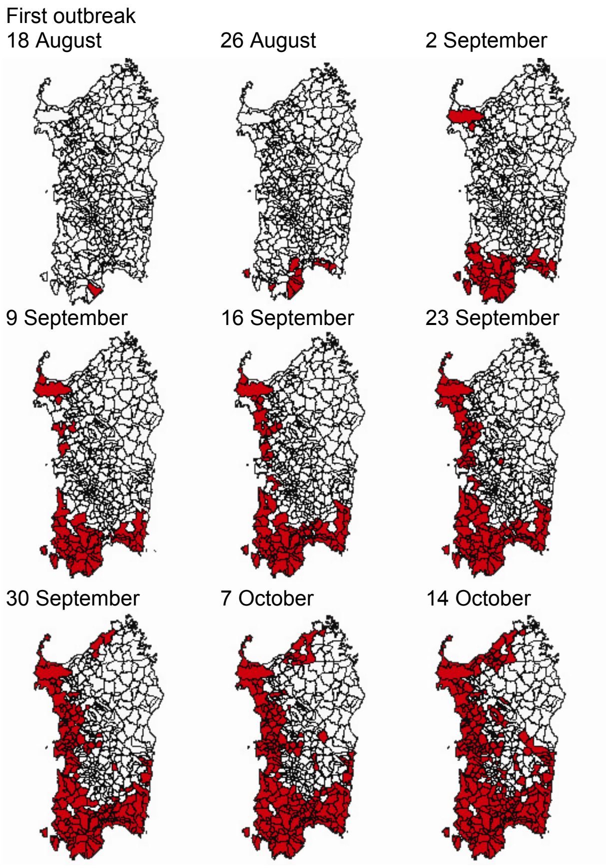

Figure 4
Weekly spread of bluetongue outbreaks in Sardinia, 18 August-14 October 2000

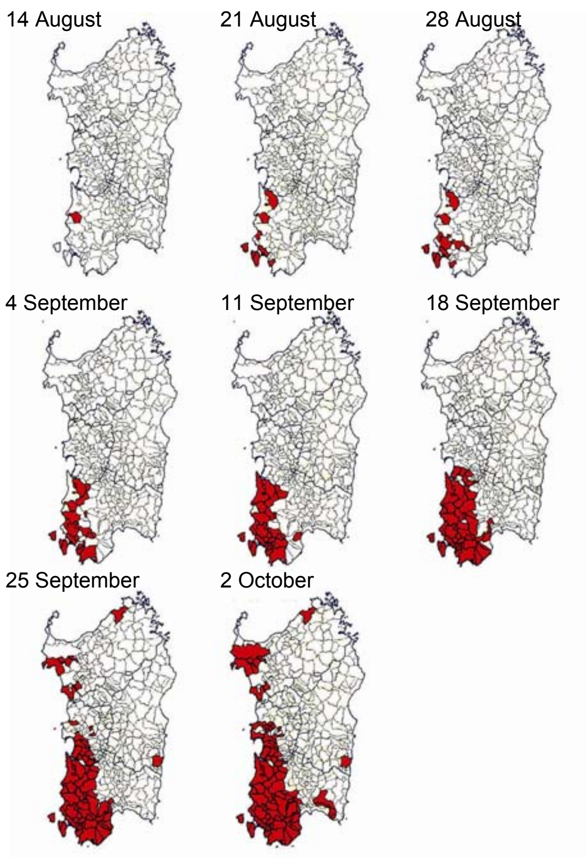

Figure 5
Weekly spread of bluetongue outbreaks in Sardinia, 14 August-2 October 2003

The incubation period of clinical BT disease is between 5 and 20 days. Thus, diseased animals are detected more rapidly by clinical examination than by serology. Therefore, in the summer and early autumn of 2000 (the epidemic peak), BT surveillance in Italy was based on clinical examination of sheep. Serological and virological diagnoses were only used as confirmatory tools on randomly sampled animals in newly infected areas (9). As the disease becomes established in a given territory, the importance of clinical surveillance decreases and the importance of serological surveillance increases.

The presence of clinical BT was verified in suspected outbreaks; all susceptible animals were subjected to clinical examination and appropriate samples were collected for laboratory confirmation. Weekly clinical visits were made to farms to monitor the disease pattern and to update the census. Clinical visits were discontinued when diagnosis was not confirmed or when there was no evidence of virus circulation. When the presence of BT was confirmed in an area, clinical visits were extended to all sheep flocks within a radius of 20 km or 4 km in cases of clinical illness or sub-clinical infection, respectively. Clinical visits, sampling of animals and insect trappings were also performed on farms in BT-free regions that contained animals that had been imported from recently infected areas. During the first epidemic (August 2000-May 2001), a total of 22 691 clinical visits (84% in Sardinia) were made. During the second epidemic (May 2001-April 2002), a total of 10 070 clinical visits (2.4% in Sardinia) were made.

Serological surveillance

Based on the Italian experience, structured serological surveillance in a newly infected area can only start in the decreasing phase of the epidemic peak (Fig. 6). As already stated, clinical evaluation is more reliable than serology in detecting incursion of BTV in the initial phases of an epidemic. A sentinel network was already in place for more than 18 months prior to the 2003 epidemic of BTV-4 infection in Sardinia when 740 outbreaks involving animals in most of the southern and western coast of Sardinia were detected in 49 days (Fig. 5), whereas only 2 of more than 2 000 tested sentinel animals seroconverted to BTV-4.

Serological surveillance was based on a set of ad hoc surveys and a network of sentinel animals. Ad hoc surveys were the first serological activities;

subsequently, based on the results of clinical surveillance and of ad hoc surveys, the network of sentinel animals was developed and implemented.

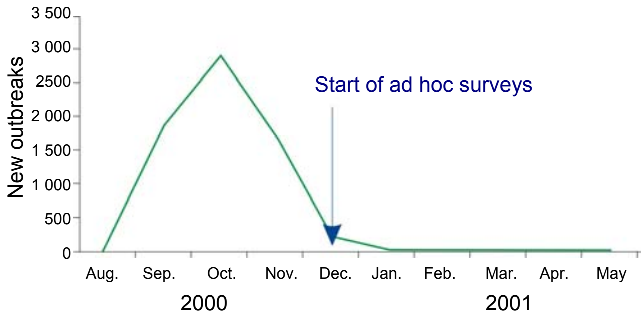

Figure 6
Monthy outbreaks of bluetongue, showing the peak of the first epidemic in Italy and ad hoc surveys, August 2000-May 2001

Ad hoc surveys

The objectives of the ad hoc surveys conducted in the winter of 2000-2001 were to determine the actual geographic distribution and the prevalence of BTV infection in sheep and cattle populations in the areas involved. Target populations and sampling design varied in the various regions that were monitored, depending on behaviour of the epidemic and existing knowledge (9). The objective of the monitoring, target population, sampling criteria and features of the infection are summarised in Table I. Sardinia was the first region affected and recorded the greatest number of outbreaks (91% of the outbreaks occurred in Sardinia). In the winter of 2000-2001, the geographic distribution of BTV infection appeared to be well defined, involving virtually the entire region, except the highest mountains. Therefore, the objectives of the monitoring programme, were to define the following:

- the prevalence of sub-clinical infection in infected flocks
- the involvement of cattle.

These data were considered important for two main reasons, namely:

- to predict the likely outcome of the next epidemic, based on the level of immunity in the animal populations
- to evaluate the possible epidemiological role of cattle in the Sardinian environment.

Table I
Ad hoc monitoring performed during the winter of 2000-2001
Epidemiological behaviour of infection, objectives of monitoring, target population and sampling criteria

Region	No. of clinical outbreaks	Date of first outbreak	Objective of monitoring	Target population	Sampling criteria
Sardinia	6 264	18 August 2000	Prevalence of infection in the cattle population	Cattle	Cluster sampling, stratified by cattle population density and by date of first detection of infection in the municipality
			Prevalence of subclinical infection in the sheep population	Sheep	Serological testing of animals in flocks clinically affected during the epidemic
Sicily	16	10 October 2000	Geographic distribution of infection in the cattle population	Cattle	Random sampling of the entire region, with 3 animals/km², so as to detect one infected animal per 100 km²
			Geographic distribution of infection in the sheep population	Sheep	Random sampling of the entire region, with 3 animals/km², so as to detect one infected animal per 100 km²
Calabria	589	10 October 2000	Geographic distribution of infection in the cattle population	Cattle	Random sampling of the entire region, with 3 animals/km², so as to detect one infected animal per 100 km²
			Prevalence of subclinical infection in the sheep population	Sheep	Serological testing of all animals in flocks within a radius of 20 km around any clinically affected flock during the epidemic
Basilicata	0	–	Possible presence and geographic distribution of infection (region bordering infected areas)	Cattle	Random sampling of the entire region, with 3 animals/km², so as to detect one infected animal per 100 km²
Salerno Province	0	–	Possible presence and geographic distribution of infection (province bordering infected areas)	Cattle	Random sampling of the entire province, with 3 animals/km², so as to detect one infected animal per 100 km²

In Sicily, the infection commenced on 10 October and only a few scattered outbreaks were observed. The objectives of the monitoring programme, were to determine the following:

- the actual geographic distribution of the infection
- the involvement of the cattle population.

In Calabria the infection started on 10 October as in Sicily, but the number of outbreaks observed was higher (589) than in Sicily (16) and the infection was clustered along the Ionian coast. The objectives of the monitoring programme, therefore, were to determine the following:

- whether or not the Tyrrhenian coast was affected
- the prevalence of sub-clinical infection in ruminants
- the involvement of cattle.

In Basilicata and in the Salerno Province, the disease was not detected during the summer and autumn of 2000, but as these regions border Calabria, Basilicata and Salerno were monitored to determine if subclincal infections had occurred.

Other ad hoc surveys were subsequently performed (2001-2003) to verify the following:

- antibody coverage of vaccinated populations
- the risk linked to seasonal grazing practices
- the extent of virus circulation in certain zones in which individual animals seroconverted or where unexpected positive results were found.

The results obtained from monitoring activities performed in the winter of 2000-2001 were also used to plan the sentinel network adopted for Italy.

Sentinel network

Networks of sentinel animals have been implemented in several countries to monitor the presence and spread of vector-borne diseases. None of them, however, were designed to delineate the areas where virus circulation occurs with a precision significantly lower than 100 km.

A sentinel system has been used in Australia since late 1975, but the number of sentinel sites was very limited. From 1988 to 1990, there were between 27 and 28 sites uniformly scattered within the known range of the vector (*C. brevitarsis*), as well as along the margins and outside its range (28). The total number of sites in 2001 was 94 (Fig. 7) (1), with the maximum density in New South Wales (one sentinel site every 22 900 km²).

In Canada, a sentinel programme has been in place in the Okanagan Valley since 1988 (22). In 1988, the system was composed of five sentinel sites with up to 10 animals at each. The aim was to monitor activity of BTV and related orbiviruses (epizootic haemorrhagic disease viruses) in locations where BTV infection had previously been documented (since 1975).

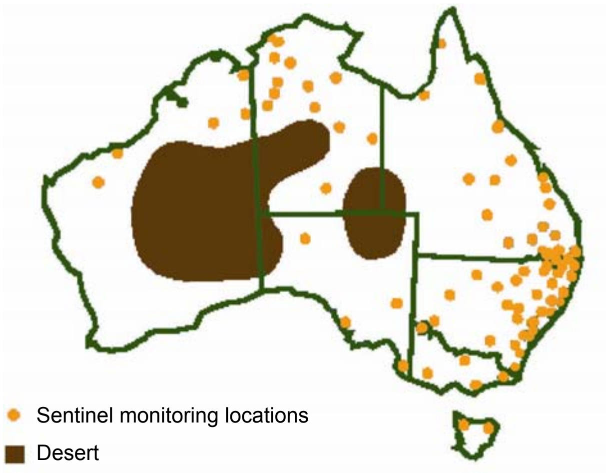

Figure 7
Location of sentinel sites in Australia, 2001

In the United States of America (USA), periodical surveys are performed, usually serological surveys of slaughter cattle for antibody against BTV. These surveys are conducted on sera from animals from low prevalence states or geographic areas (anticipated less than 2% antibody prevalence) (2, 32).

The serological surveillance networks in place in other countries were not consistent with the requirements for serological surveillance of BTV infection in Italy. The Italian system was based on the following:

- the OIE *Terrestrial animal health code*, which states in Chapter 2.1.9. (31) that 'random and targeted serological surveillance should provide at least a 95% level of confidence of detecting an annual seroconversion incidence of 2% in cattle (or other ruminant species if sufficient cattle are not available)'
- a serological survey undertaken in the state of Queensland (Australia) in 1989 showed that the prevalence of serological positivity in cattle from locations with low prevalence of infection was on average 6.45% (34).

The value of about 5% prevalence in areas of low prevalence of BTV infection was confirmed by the ad hoc monitoring conducted in Sardinia to evaluate the involvement of the cattle population in the BT epidemic. Therefore, it was decided to divide Italy into two main zones, based on the risk of infection (Fig. 8). The lower risk zone was subdivided into a grid of square cells of 400 km per side (1 600 km² per cell) and 148 sentinel animals were monitored in each cell (in compliance with the OIE requirements for free countries or zones to provide a confidence level of at least 95% to detect annual seroconversion incidence of 2% in cattle). In the higher risk area, a finer grid was designed, with squares of 20 km per side (400 km²), to have a more precise definition of the distribution of infection. A total of 58 sentinel animals were included in each cell to confirm that the prevalence of infection was less than that observed in low prevalence areas (i.e. to provide at least a 95% level of confidence of detecting an annual seroconversion incidence of 5% in cattle). Since the minimal movement restricted zone is a circle of 20 km radius around the infected holding, the geographic density of sentinels is able, for any area of 1 256.6 km² (equivalent to a circle with a radius of 20 km), to provide at least a 95% level of confidence of detecting a seroconversion incidence of 1.6% in cattle.

Figure 8
Subdivision of Italy into high- and low-risk zones, September 2001
Area A: high risk zone (squares of 400 km²)
Area B: low risk zone (squares of 1 600 km²)

Entomological surveillance

Given the lack of knowledge on the distribution of vectors in Italy, an entomological surveillance programme was implemented in infected and adjoining areas at the beginning of the BT epidemic in October 2001 to map the distribution of vectors (with particular reference to *C. imicola*). Blacklight traps were moved around the study areas to define the distribution of *C. imicola,* and permanent traps were operated at different sites in Italy from June to October 2001 to evaluate the effect of soil type on the presence of *C. imicola*. Entomological surveillance has been extended nationwide since October 2001. Blacklight traps were positioned in fixed locations in each province (Fig. 9) and operated weekly to monitor the population dynamics of *Culicoides* spp. Blacklight traps were also operated on a temporary basis in suspected or confirmed cases of virus circulation and whenever a more specific understanding of vector distribution was required.

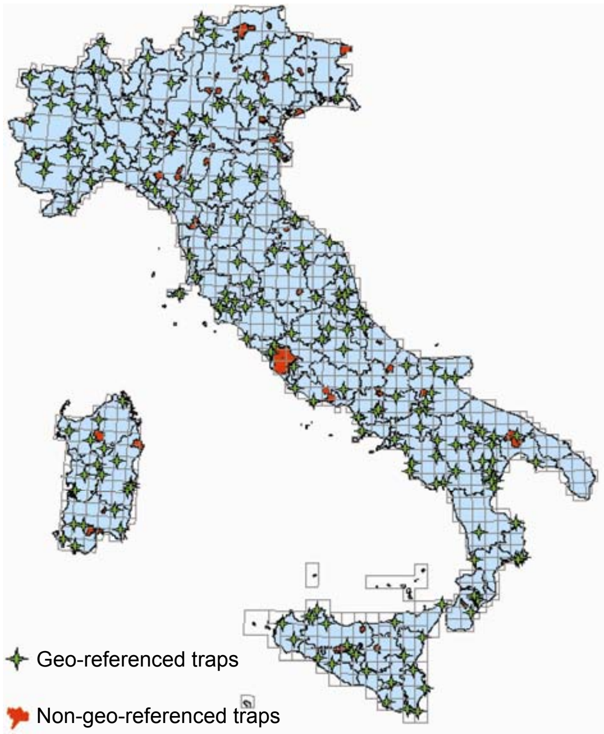

Figure 9
Location of permanent blacklight traps in Italy, 2001

Discussion

Decision 2001/138/EC (13) instituted protection and surveillance zones according to the criteria established by Directive 2000/75/EC (23) and a third of Italy was subjected to movement restrictions: 26% in the protection zone and 9% in the surveillance zone. Animal movement to disease-free regions was prohibited from the regions of Sicily, Sardinia and southern Italy (protection and surveillance zones). Therefore, it was clearly essential from the beginning of the outbreak that the epidemiology of BTV infection in Italy needed to be defined, and that a comprehensive surveillance system be developed to accurately define the status of infection in the various regions. The implementation of a viable surveillance system that addressed the prescribed requirements was logistically challenging, and required the gathering of vast quantities of data and very intensive field activities. It involved the regular clinical evaluation (in most cases fortnightly) of more than 30 000 sentinel animals and the placing of about 250 permanent insect traps throughout Italy. Information and data produced by the surveillance system constitutes the information base of the early warning system for BT in Italy. The system also has accurately established the epidemiology of BTV infection and the distribution and dynamics of BT vectors (11, 27). In addition, monitoring of the spread of BTV infection was facilitated (9, 24), and risk factors linked to the spread of the vectors and to animal movement were evaluated.

The surveillance systems implemented in the countries of southern Europe, and particularly in Italy where BTV had spread and persisted more than in any other EU country, produced information that was critical to the development of the flexibility that now characterises the European Union legislation on BT.

- Decision 2001/783/EC of 11 September 2001 (14), has included two procedures foreseen by the OIE *Terrestrial animal health code* (31) in the European provisions, namely:
  - Article 2.1.9.8. for animal movements from infected zones
  - Article 2.1.9.3. to define seasonally free areas.

Furthermore, the decision has reduced the radius of the zone from which slaughter animals cannot be sent to free zones from 100 km to 20 km, provided that a surveillance system is in place.

- Subsequently, from 16 January 2002, a series of Decisions (2002/35/EC, 2002/189/EC, 2002/543/EC (15, 16, 17) have excluded some Italian provinces from those surveillance zones in which the surveillance system had documented the absence of virus circulation.
- From 10 January 2003 (Decision 2003/14/EC) (18), the despatch of slaughter animals from infected to free areas was permitted, provided that the vaccination coverage is at least 80% in the province of origin and a risk assessment has been made.
- Decision 2003/218/EC (19) of 27 March 2003 introduced the concept of 'risk' in the European provisions and has subdivided the territories in areas of higher and lower epidemiological risk.

The decision, therefore, allows the following:

- the despatch of live animals from the 'lower risk areas', where viral circulation has not been detected, to the remainder of the European Union
- the despatch of slaughter animals from 'lower risk areas' even with active infection and from 'higher risk areas' where viral circulation has not been detected to free areas in the national territory. The latter is allowed only if the animals have been vaccinated at least 30 days previously, if they belong to a herd where all the animals have been vaccinated and if their transport occurs during daylight hours only. According to Decision 2003/218/EC, the Member State can demarcate 'epidemiologically relevant areas of origin'; in other words, on the basis of surveillance results, it can reduce or increase the protection zones to a lower or higher radius than 20 km and it can evaluate the possibility of demarcating lower risk areas in higher risk territories.

The data and knowledge obtained in these studies has also facilitated risk assessments to:

- define the optimal national BT control strategy
- define the risk arising from movement of slaughter and live animals from restricted zones as related to the presence or absence of viral circulation and to population immunity from vaccination (24)
- define the minimum level of serological surveillance able to detect ongoing BTV infection with comparable sensitivity to the existing surveillance programme (8).

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