Bluetongue in Italy: Part I

P. Calistri[(1)], A. Giovannini[(1)], A. Conte[(1)], D. Nannini[(1)], U. Santucci[(2)], C. Patta[(3)], S. Rolesu[(3)] & V. Caporale[(1)]

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Via Campo Boario, 64100 Teramo, Italy
(2) Ministero della Salute, Direzione Generale della Sanità Pubblica Veterinaria, Alimenti e Nutrizione, Piazzale Marconi, 00144 Rome, Italy
(3) Istituto Zooprofilattico Sperimentale della Sardegna 'G. Pegreffi', Via Duca degli Abruzzi N° 8, 07100 Sassari, Italy

Summary

The eastern focus of the current outbreak of bluetongue (BT) in the Mediterranean Basin commenced in late 1998, infecting Turkey and some of the eastern islands of Greece. In the summer of 1999 it moved to continental Greece and for the first time to Bulgaria. By the late summer of 2000, BT spread progressively through Greece and to the Balkan states. The BT virus (BTV) serotypes involved were BTV-4, BTV-9 and BTV-16. The west-central focus of the outbreak, involving BTV-2, appeared in Tunisia in December 1999 and the following summer also in Algeria. In August 2000, BTV-2 was reported for the first time in Italy (in Sardinia) and soon thereafter in France (Corsica) and in Spain (the Balearic Isles). In the autumn of 2000, a second serotype (BTV-9) emerged in southern peninsular Italy. Eventually this incursion of virus into the central Mediterranean region resulted in the largest epidemic of BT ever to affect Europe. Some features of this epidemic differ significantly from those observed previously, namely:

a) its deep penetration northwards (reaching 44°N both in Italy and in the Balkans)

b) its persistence across four seasons in various zones of Italy and the Balkans, implying that BT could become endemic over a wide geographic area

c) its successful invasion of areas separated from previously infected ones by fairly large distances (Sardinia, Sicily, Calabria, and the Balearic islands).

The pattern of the spread of BT across Italy, before the introduction of vaccination, is described. The possible role of climate, soil and insect vectors on the incidence of the disease, and the overwintering of the virus, are discussed. Some hypotheses on the possible origins and modes of introduction of BTV into Italy are postulated.

Keywords

Bluetongue – *Culicoides* – Epidemiology – Italy – Risk factors – Virus.

Introduction

In the Mediterranean region, bluetongue (BT) was first reported in Cyprus and Israel in 1943 but is thought to have occurred in those countries as early as 1924 (6). Six serotypes of BT virus (BTV), namely BTV-2, BTV-4, BTV-6, BTV-10 and BTV-16, recur at various intervals in Turkey, Syria, Israel and Egypt, where BT is endemic (10, 19). A BTV-10 epidemic occurred between 1956 and 1960 in Spain and Portugal causing the death of approximately 179 000 sheep (9). BT epidemics have been reported, although irregularly, from 1979 to 1999 in Greece, in the eastern islands (Lesbos, Leros and Kos) and the Dodecanese archipelago (3). In 1999, BT was reported in mainland Greece and south-eastern Bulgaria, close to the Turkish and Greek borders (3). From August to September 2000, BT spread progressively through the Balkan region to Albania, Bosnia Herzegovina, Croatia, Kosovo, Macedonia Republic, Republic of Serbia and Montenegro. BTV-9 is the sole serotype detected in the Balkan countries, while BTV-1, BTV-4, BTV-9 and BTV-16 have been isolated in Greece (13, 14, 15, 16).

In December 1999, BTV-2 was recorded in the north and east of Tunisia, spreading to Algeria

during the summer of 2000 (3). Also in the summer of 2000, BTV-2 appeared in Italy (Sardinia), France (Corsica) and Spain (Balearic islands) (12), while in November 2000, BTV-9 was isolated in Calabria. The infection of these central Mediterranean countries involved the largest BT epidemic ever recorded in Europe, characterised by the following:

a) a deep northward penetration (up to 44°N in Italy and the Balkans)
b) continuous virus circulation in large geographical areas over four years
c) occurrence in territories separated from each other by more than 100 km of sea (Sardinia, Sicily, Corsica and the Balearic islands).

The authors describe the spread of BTV infection in Italy before the introduction of vaccination campaigns. The influence of climate, type of soil and insect vectors on the incidence of the disease and the persistence of infection through the winter are discussed. Possible sources and routes of BTV introduction into Italy are also evaluated.

Materials and methods

The history of BT in Italy can be described as having occurred in four separate seasonal epidemic waves, as follows:

a) first epidemic (18 August 2000 to 14 May 2001)
b) second epidemic (15 May 2001 to 14 April 2002)
c) third epidemic (15 April 2002 to 14 April 2003)
d) fourth epidemic (15 April 2003 to 14 April 2004).

Only the first and second epidemics (before the implementation of vaccination in Italy) are described here.

Data sources

Data on outbreaks, serological and entomological surveillance and ad hoc surveys, were derived from the archives of the National Information System (7). Meteorological data were provided by the Italian Air Force Meteorological Service. Satellite images on dust storms over the Mediterranean Sea were derived from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) Project (NASA/Goddard Space Flight Center Orbimage) (seawifs.gsfc.nasa.gov/seawifs.html).

Clinical outbreaks

The diagnosis of BT in infected areas during the epidemic was mainly clinical. During the first epidemic (2000-2001), before the implementation of the sentinel network, all flocks in the protection zones were clinically inspected every fortnight. Suspect cases of BT in free areas were confirmed by virus isolation and identification.

Ad hoc surveys

On 9 February 2001, in Decision 2001/138/EC, the European Commission defined Sardinia, Sicily, Calabria, the Basilicata Regions and Salerno Province to be in the BT protection zone (4); the Apulia Region and the Campania Provinces of Avellino, Benevento, Caserta and Naples formed the BT surveillance zones (11).

A survey was conducted in the winter of 2000-2001 to determine the actual geographic distribution and the prevalence of infection in sheep and cattle populations in the zone where the disease had been reported and in those zones bordering them. Target populations and sampling design differed in the various zones, based on the behaviour of the epidemic and on other data and information already available. Blood samples were screened by competitive enzyme-linked immunosorbent assay (c-ELISA) and confirmed by virus neutralisation (VN).

Sardinia

Prevalence of infection in the cattle population was estimated by cluster sampling, stratified by cattle population density and by date of first detection of infection in a given municipality. The prevalence of subclinical infection in the sheep population was estimated by testing a sample of sheep and goats in flocks clinically affected during the epidemic season. The number of animals to be sampled in each flock was calculated in accordance with the morbidity rate in the outbreak.

Sicily/Calabria

A serological survey was conducted by selecting three bovine animals and three sheep/km^2 at random. This would reveal infection (if present) in at least one animal per 100 km^2 and would enable the geographical distribution of BTV circulation in the region to be determined. In Calabria, all sheep within a 20-km radius from the outbreaks were tested.

Basilicata and Salerno Provinces

The same procedure was applied as described above for Sicily/Calabria except that within the surveillance zone (Apulia Region and Avellino, Benevento, Caserta and Naples Provinces), all susceptible animals were tested serologically before moving.

Sentinel animals

Italy was divided into a grid of square units of either 400 km² or 1 600 km², according to the occurrence of BT or according to the risk of introducing BTV infection, respectively. To be able to detect at least a 5% infection rate with a 95% confidence level in each 400-km² unit, a sample of 58 bovine animals was selected from 5 to 8 farms. To be able to detect an infection rate of at least 2% with a 95% confidence level in each unit of 1 600 km², a sample of 148 bovine animals was selected from 8 to 12 farms. If cattle were not present in the area, sheep were selected as sentinel animals. Blood samples from sentinels were collected regularly with variable frequency, dependent on the season and occurrence of infection in an area (7).

Entomological surveillance

During the first epidemic (2000-2001), ad hoc surveys were performed in Sardinia, Sicily and Calabria to define the geographical distribution of *C. imicola*. Since 2001, entomological surveillance has been extended country-wide, using approximately 250 permanently sited traps to define the seasonal dynamics of the *Culicoides* spp. populations (8).

Results

During the first two epidemics, the disease occurred in seven regions (Fig. 1), and were those with the highest populations of sheep and goats (Fig. 2). During the first epidemic (2000-2001), BT occurred in Sardinia, Sicily and Calabria (Fig. 1a), causing 6 869 clinical outbreaks; morbidity and mortality rates were 18.2% and 3.3%, respectively. More than 275 000 sheep and goats died or were slaughtered (Table I). During the second epidemic (2001-2002), BT occurred again in Sardinia, Sicily and Calabria but now spread into Basilicata, Campania, Latium and Tuscany (Fig. 1b), causing 6 807 clinical outbreaks. Morbidity and mortality rates were 17.8% and 5.2%, respectively, causing the loss of approximately 250 000 sheep and goats (Table I). Details of the disease pattern in the BT-infected regions are given below.

Figure 1
Distribution of bluetongue outbreaks in Italy during the first and second epidemics, 15 August 2000-14 April 2002

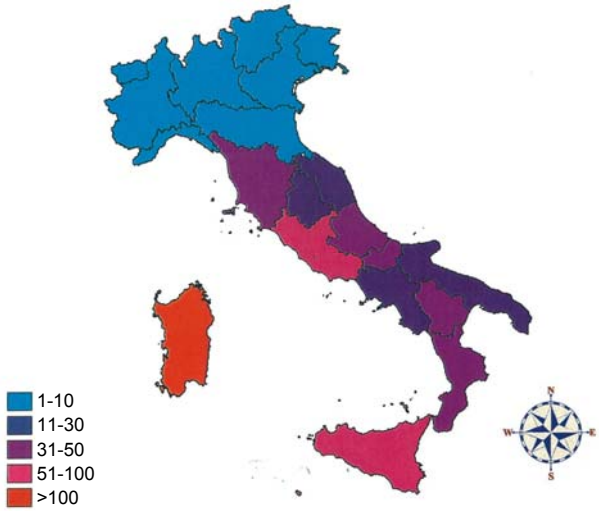

Figure 2
Density of sheep and goats in Italy per km²

Sardinia

Bluetongue was first reported on 18 August 2000 in a flock located in the south-western part of the island in the Cagliari Province. Serological confirmation of BT was obtained on 24 August and BTV-2 was isolated by the National Reference Centre for Exotic Diseases (CESME: *Centro Studi Malattie Esotiche*) on 1 September and identified subsequently by the Onderstepoort Veterinary Institute. In the 15 days following notification of the first case, 262 infected flocks were detected along the southern and western coast of the island (Fig. 3). The disease spread rapidly, about 30 km per week, along the coast and the river basins (Flumendosa, Tirso and Coghinas). In April 2001, when the last case of BT during the first epidemic was reported, 80% of the island had been infected. The only area not infected was the central mountainous region. During the first epidemic, the disease was observed in 6 264 flocks (25% of the total flocks in Sardinia) causing 293 901 cases. Losses incurred, either through death or the slaughter of sick animals, amounted to 260 856 (Table I). At the peak of the epidemic (October 2000) 2 755 new outbreaks were reported (Fig. 4). During the period between epidemics from January to July 2001, new cases of

Table I
Clinical outbreaks of bluetongue in Italy, 15 August 2000-14 April 2002

Regions	No. of outbreaks		Number of sheep and goats							
			In diseased flocks		Diseased		Dead		Slaughtered	
	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002
Basilicata	–	63	–	4 766	–	152	–	16	–	–
Calabria	589	427	89 166	52 722	15 676	10 176	1 011	236	16 485	9 882
Campania	–	1	–	64	–	3	–	2	–	–
Latium	–	62	–	23 040	–	431	–	69	–	371
Sardinia	6 264	6 090	1 360 614	1 294 365	246 908	239 178	46 993	72 952	213 863	159 819
Sicily	16	6	1 760	853	175	29	106	29	94	–
Tuscany	–	158	–	33 988	–	693	–	119	–	560
Total	6 869	6 807	1 451 540	1 409 798	262 759	250 662	48 110	73 423	230 442	170 632

First epidemic: 24 August 2000-14 May 2001 Second epidemic: 15 May 2001-14 April 2002

BT were reported in Cagliari and in the other provinces until March 2001. After a five-month seasonal interruption, BT reappeared in August 2001 both suddenly and intensively (300 outbreaks and 6 859 diseased animals). This second epidemic of BTV-2 recrudesced on the north-eastern coast of the island and spread eventually across the entire region. A total of 6 090 outbreaks, with 312 130 cases, were reported and by the end of the season, the dead and slaughtered animals numbered 232 771 (Table I). In October 2001, at the peak of the epidemic, 3 177 new outbreaks were reported (Fig. 4). In October 2001, serological surveillance of sentinel animals commenced and the information integrated with that derived from clinical and serological surveillance. The data showed BTV circulation to be more extensive than revealed through clinical surveillance. Furthermore, the sentinel system demonstrated that virus circulation during winter and spring was not limited to the Cagliari Province but occurred also in the other Provinces of Sardinia (Fig. 5).

Sicily

BT disease was first reported in a sheep flock in the Palermo Province on 10 October 2000. Serological confirmation was made on 13 October and was confirmed by virus isolation by CESME on 23 October (BTV-2). In Sicily, there were a few scattered outbreaks involving 281 cases in 16 flocks (12 in Palermo, 2 in Trapani and 2 in Agrigento) (Fig. 1 and Table I) with 200 animals dead or slaughtered (Table I); the last case was reported in November 2000. No further cases occurred until the next epidemic when the disease re-appeared in eastern Sicily (2 outbreaks in October 2001 in the Messina Province and four outbreaks in November in the Siracusa Province), affecting a total of 58 animals; these were the only outbreaks reported during the 2001-2002 epidemic. In Sicily, as in

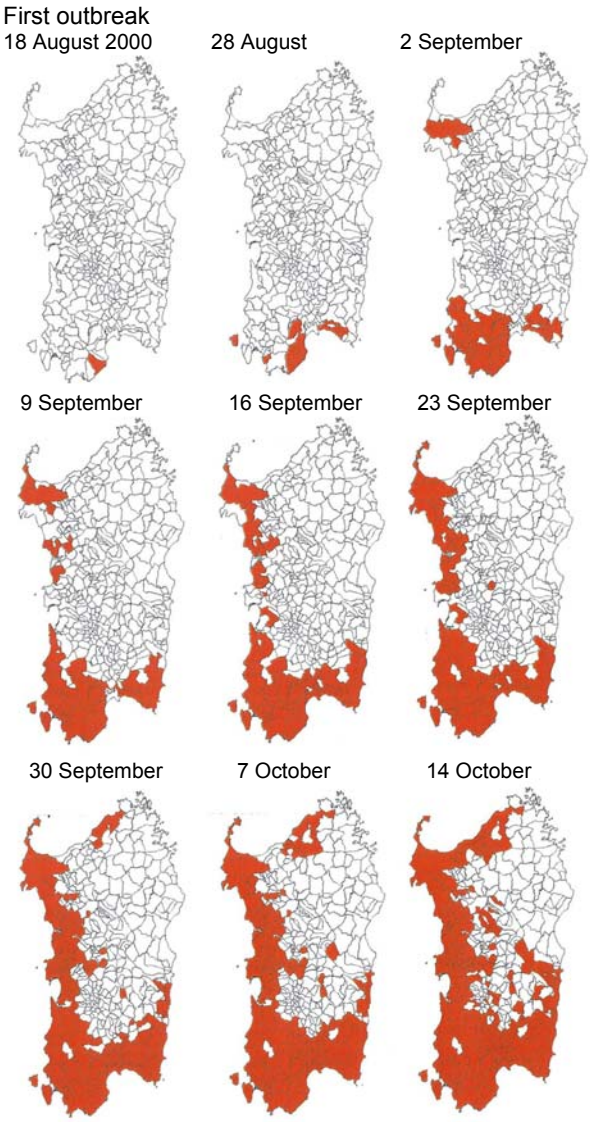

Figure 3
Weekly spread of bluetongue outbreaks across Sardinia, 18 August-14 October 2000

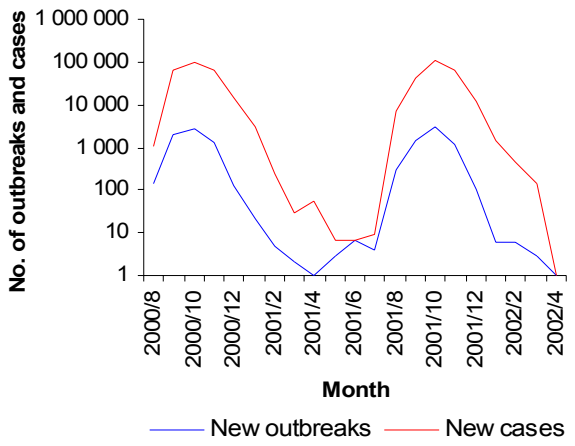

Figure 4
Number of new cases and outbreaks of bluetongue per month (logarithmic scale) in Sardinia during the first and second epidemics

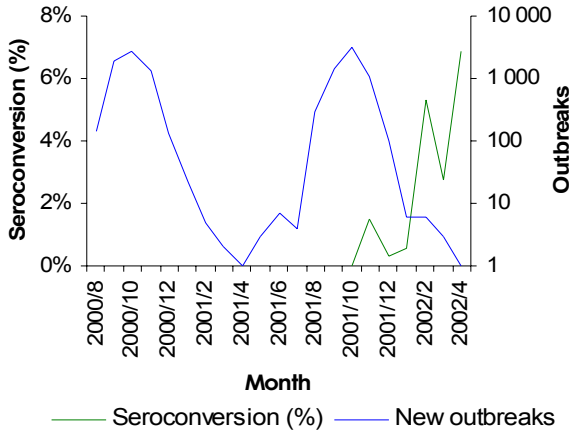

Figure 5
Number of outbreaks (logarithmic scale) and percentage of seroconversions per month in Sardinia during the first and second epidemics

Sardinia, surveillance detected virus circulation to be wider than that demonstrated by clinical surveillance.

Calabria and other southern regions of Italy

The first case of BT was reported on 11 October 2000 in a flock in the Province of Reggio Calabria. Serology confirmed BTV presence on 13 October and on 23 October virus isolation by CESME confirmed that BTV-2 was again involved. From October to November 2000 the disease spread 8-9 km per week along the eastern (Ionian) coast. Commencing on 2 November, new outbreaks were reported in the Provinces of Catanzaro, Cosenza and Crotone, 70 km from the first disease focus in Reggio Calabria (Fig. 6). One of these new outbreaks, in the Municipality of Cariati (Cosenza Province) involved BTV-9, confirmed by virus isolation. During this first season, BTV-9 was detected in only five holdings.

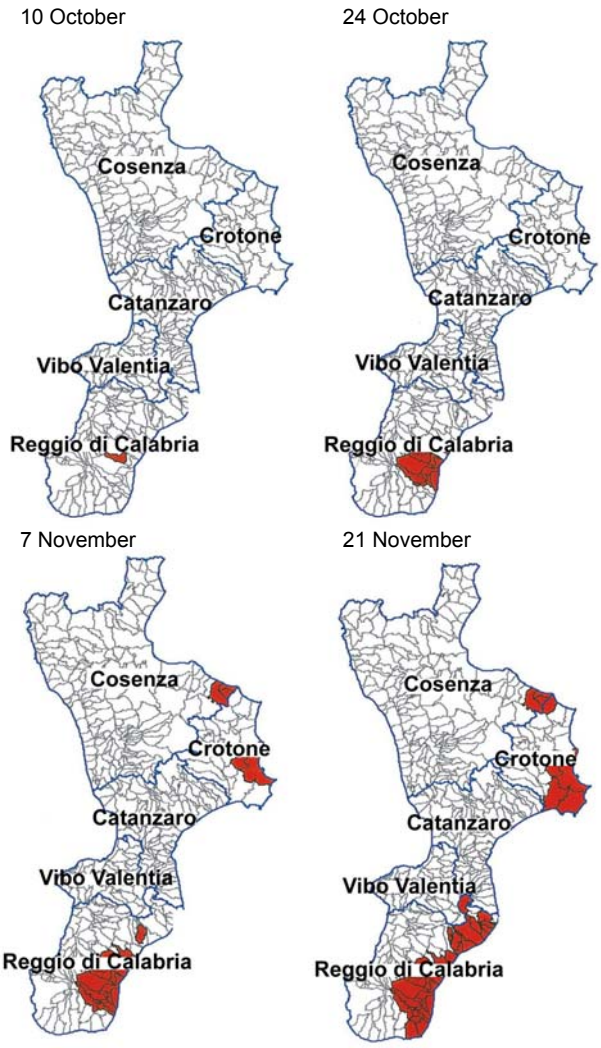

Figure 6
Weekly spread of bluetongue outbreaks in Calabria, southern Italy, 10 October-21 November 2000

The epidemic peak occurred in November 2000, with 7 862 diseased animals in 349 flocks. Subsequently, disease incidence decreased progressively and the last clinical cases were reported on 1 February 2001 (Fig. 7). A total of 16 687 diseased animals in 589 clinical outbreaks was reported, of which 17 496 died or were slaughtered (Table I). The disease reappeared in June 2001 in the Catanzaro, Cosenza and Crotone Provinces (1 303 cases in 92 flocks), spreading along the Tyrrhenian coast for the first time. From August to November 2001, the disease moved into the regions of Basilicata (Potenza and Matera Provinces) and Campania (Salerno Province) (Fig. 1) with a total of 491 clinical outbreaks (427 in Calabria) reported (Table I); the number of cases was 10 412, of which 10 136 died or were slaughtered. In Calabria and southern Italy, the epidemic peak occurred in July 2001 (Fig. 7).

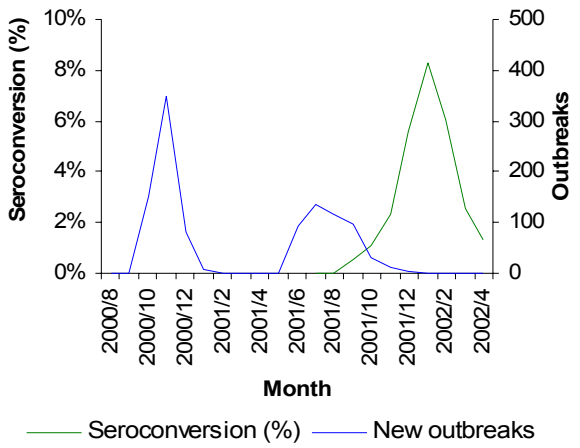

Figure 7
Number of new bluetongue cases and outbreaks per month during the first and second epidemics in southern Italy, August 2000-April 2002

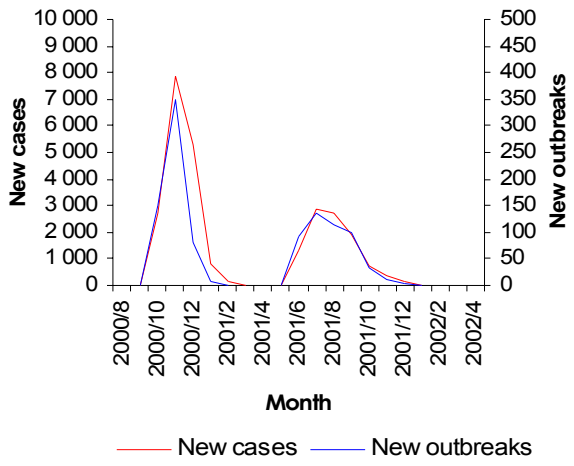

Figure 8
Number of outbreaks and percentage of seroconversions per month during the first and second epidemics in southern Italy, August 2000-April 2002

As in the previous epidemic, the last clinical case of the second epidemic was reported in February in the Province of Reggio Calabria. During the second epidemic, BTV-9 was the prevalent serotype in southern Italy (of 183 outbreaks in Calabria and Basilicata, 179 were due to BTV-9). The infection was detected in sentinel animals in several areas where clinical disease was not observed. Seroconversions occurred in the Province of Naples in September 2001, and in the southernmost Provinces of Apulia (Brindisi, Lecce and Taranto), the virus circulated continuously between September 2001 and January 2002 (Fig. 8). Furthermore, serological surveillance demonstrated the constant persistence of infection in the inter-epidemic period along the Ionian coast of Calabria, in Basilicata, in the Province of Salerno and in Apulia.

Northern Tyrrhenian coast

BT infection was detected in Tuscany for the first time in August 2001 when sentinel animals in a herd in the Grosseto Province seroconverted. The first clinical outbreaks were reported in the same province the following month (Fig. 1). In September, the disease was reported also in the Latium Region (Latina and Viterbo Provinces) (Fig. 1). The epidemic peak occurred in October (115 outbreaks with 502 diseased animals) (Fig. 9) and the last outbreak reported in November. The total number of outbreaks and diseased animals was 220 and 1 312, respectively (Table I). During the inter-epidemic period of 2002, serological surveillance detected the infection also in other areas along the northern Tyrrhenian coast (Rome and Pisa Provinces) (Fig. 10).

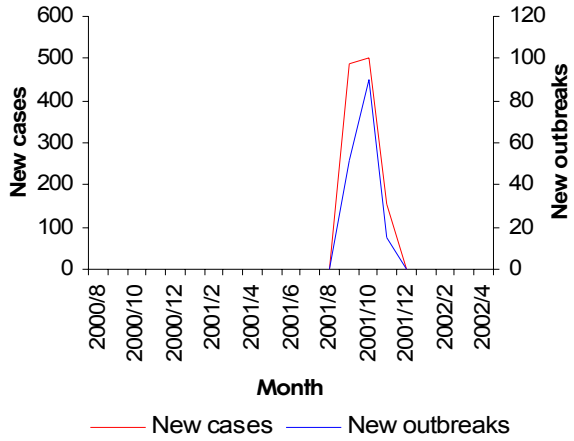

Figure 9
Number of new cases of bluetongue and outbreaks per month during the first and second epidemics in Latium and Tuscany, August 2000-April 2002

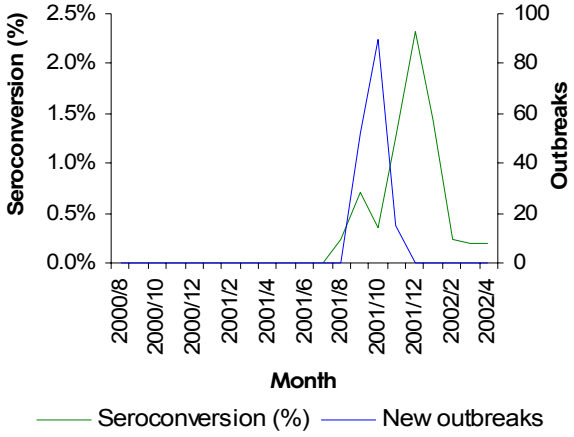

Figure 10
Number of outbreaks and percentage of seroconversions per month during the first and second epidemics in Latium and Tuscany, August 2000-April 2002

Origin of infection

The introduction of BT into Sardinia by the movement of animals or animal products has been ruled out. The most likely route is the passive wind-borne transportation of infected vectors from BTV-2-infected regions of North Africa. The reasons for the exclusion of BT introduction by live animals are as follows:

- there is no record of live animals, semen or embryos being introduced from an infected or suspect country or zone
- during the first 15 days after the notification of the first case, 262 infected flocks were detected along more than 200 km of the west coast of the island
- the BT incubation period is compatible with the hypothesis of a common source for the outbreaks occurred during the first 15 days
- in the same period, the disease was also reported in the Balearic islands
- meteorological evidence would appear to support the hypothesis of wind-borne introduction. The summer of 2000 in Italy was characterised by unusual climatic conditions. Several dust storms occurred, originating in North Africa and moving across to southern Italy and the Italian islands. One of these was recorded on 24 July 2000 (Fig. 11), 25 days before the first case of BT was reported in Sardinia. If this dust storm carried infected *Culicoides* midges into Sardinia, and based on the length of the incubation period, the disease could subsequently appear in sheep in the first half of August and correlates well with the first clinical signs reported in flocks from around 15 August.
- given its geographic position in relation to North Africa, the infection was more likely to first occur in the southernmost part of Sardinia, exactly where the disease first appeared.

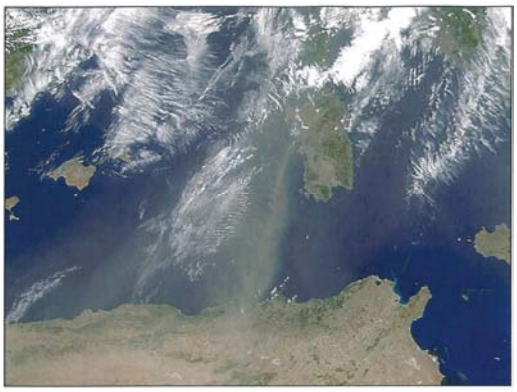

Figure 11
Dust storm over Sardinia on 24 July 2000 originating in Algeria and Tunisia
Source: 'SeaWiFS Project' (NASA/Goddard Space Flight Center) and ORBIMAGE, OrbView-2 satellite

For Sicily, the origin of infection is unknown. The Veterinary Services found no evidence of the introduction of animals, semen or embryos from infected or suspected areas; the scattered occurrence of infection does not exclude the illegal introduction of infected animals as the possible source of the epidemic. The dust storms from North Africa during the summer of 2000 also included Sicily (Fig. 12) so the scattered pattern of infection could be consistent with a wind-borne origin. In Calabria, the origin of BTV-2 infection was linked to the introduction of animals from Sardinia.

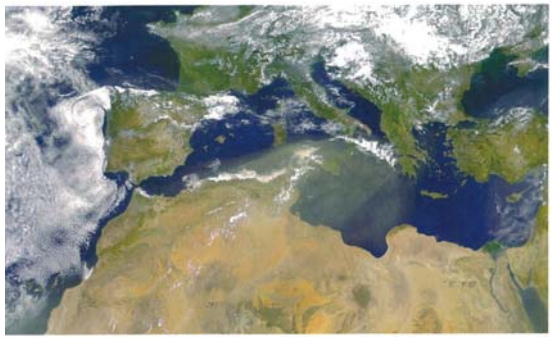

Figure 12
Dust storm over the Mediterranean Sea on 18 July 2000 originating in North Africa
Source: 'SeaWiFS Project' (NASA/Goddard Space Flight Center) and ORBIMAGE, OrbView-2 satellite

On 11 August 2000, 15 bovines were moved from Sardinia to a herd close to the first outbreaks of BT in the Province of Reggio Calabria. During the epidemiological investigations, all 15 cattle were tested; of which 5 were serologically positive for BTV-2. In addition, on 28 August, 105 sheep from the Municipality of Guasila (Cagliari Province, Sardinia), were transferred to a flock in the Municipality of Isola Capo Rizzuto (Crotone Province), only 3 km from the first outbreak of BT in Crotone, where clinical signs were observed in sheep in September.

The most probable hypothesis on the origin of BTV-9 infection in Calabria is the illegal introduction of infected animals from eastern Mediterranean countries, where BTV-9 had been circulating previously. However, official investigations failed to reveal any introduction of animals, semen or embryos from infected or suspected zones. The introduction of BTV-9 into Calabria by infected midges carried on winds blowing from the Middle East or the eastern Mediterranean is unlikely, whereas the clandestine movement of refugees and immigrants from the Middle East by sea is well known and documented.

The origin of infection in the Northern Tyrrhenian coast, in Latium and Tuscany, is probably linked to the infection in Sardinia and Corsica, as follows:

- the two islands are close to the Tyrrhenian coast (about 170 km from Sardinia and 60 km from Corsica)
- the same serotype (BTV-2) was involved
- during the summer of 2001, winds blew for several days along a line connecting Sardinia (Nuoro Province) to the Tyrrhenian coast of continental Italy (Grosseto and Viterbo Provinces).

Distribution of vectors

Culicoides imicola were caught in all infected regions (8) and in 95% of the zones infected with the disease. Entomological surveillance data and climatic data provided by the Italian Air Force Meteorological Service were used to develop a BT risk map for Italy (2) in order to predict the probability of finding *C. imicola* in different zones of Italy. The availability of climatic data meant that the accuracy of other forecasting models under Italian conditions could be tested (1). In some areas of Italy where BTV was widespread, *C. imicola* was scarce. In particular, in large areas of Apulia and Campania, *C. imicola* has never been caught. However, in these two regions, BTV was isolated from specimens of the *Culicoides obsoletus* complex (17). Nevertheless, the role of species of *Culicoides* other than *C. imicola* in virus transmission is not completely clear. It has not been established whether *Culicoides obsoletus* midges are able to sustain BT infection for a long time or just for a limited chain of infections. In addition, the minimal *C. imicola* population density required to sustain the infection is also unknown (whether small populations of midges, as reflected in blacklight trap collections, can sustain the infection).

Discussion

When BT entered the European Union, member countries were ill-prepared to cope adequately with the problems posed by a vector-borne disease. The European legislation that was in force in the summer of 2000 to control BT required the slaughter of all susceptible animals in an outbreak with the possible extension of such measures to neighbouring farms suspected of exposure (5). The application of these draconian measures probably would not have led to an effective reduction of BTV circulation, but if the Directive had been applied, at least one-third of the domestic ruminant population in Sardinia would have had to be slaughtered. Unfortunately, the knowledge of the distribution of vectors was virtually nil when BT occurred for the first time in Italy. In particular, the data available at that time regarding *C. imicola* distribution in the Mediterranean did not cover the Italy (1). The experience on BT, acquired in Italy over these two years, opened some very intriguing perspectives on infectious disease control in Europe. As an example, the hypothesis of the potential role played by winds and dust storms in the transport of vectors over the southern Mediterranean, if confirmed, appears of relevant impact not only for the future control of BT and related diseases such as African horse sickness, but also for other vector-borne infections. The evidence of BTV transmission by midges of the *C. obsoletus* complex (17) makes BT an infection with the potential of spreading well beyond the Alps. Bluetongue has a heavy economic impact on both the sheep and cattle industry for the following reasons:

- direct losses in sheep and goats
- application of animal movement restrictions leading to an almost complete ban on the movement of ruminant species from infected to free zones.

An example of the consequence of the movement restrictions was that all calves and most culled cows from Sardinia, Sicily and southern Italy were normally moved to northern Italy for fattening and slaughter. However, in 2000, these animals were forced to remain in the territories of origin, which had no facilities for fattening and for the slaughter of these animals. This has caused not only heavy economic losses, but also severe social and political tension in the regions affected. The Italian experience, therefore, demonstrated the high economic, social and political impact that a disease like BT can have on animal husbandry, forcing farmers to change their methods and cycles of production and commercial routes. It also seems to prove that the scientific basis of the present international standards and European Union legislation calling for complete animal movement ban from countries and/or zones where BTV is circulating, irrespective of the immunity status of the animals concerned, might prove not to be completely justifiable (18).

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