

Overview of bluetongue in Greece

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Summary

The history and epizootiology of bluetongue (BT) in Greece are described in detail. Three major epidemics of BT occurred in Greece, the first in 1979, due to bluetongue virus (BTV) serotype 4, in 1998-1999 due mainly to BTV-4 and BTV-9 and less to BTV-16 and in 2001 due mainly to BTV-1. The evolution of the disease, surveillance and control measures are presented, as well as the distribution of the BTV serotypes isolated.

Keywords

Bluetongue virus – *Culicoides* – Disease control – Epidemiology – Greece – Outbreaks – Sentinel – Serotypes.

Introduction

The first incursion of bluetongue (BT) into Greece occurred on the island of Lesbos in October 1979. The island is situated in the eastern part of the Aegean Sea, 10-20 km from the Asian coast. The sheep population of Lesbos (area: 1 600 km²) numbers 180 000 head, mainly of a local breed that is characterised by high milk production. The outbreak started in the north-eastern part and extended to the east of the island. From October to the end of December 1979, 17 communities, with a sheep population of 29 300, were affected. Sixty-eight flocks with about 5 000 sheep were infected. The morbidity rate in individual flocks varied from 10-90% with a mortality rate of 29% in infected sheep (11, 24). The 5 500 cattle and 18 000 goats showed no clinical signs. No clinical cases were observed in subsequent years on the island. Antibodies to BTV have been found in animals on Rhodes (6), but no clinical disease has been reported on the island or on the mainland Greece. Regionalisation of the area was applied and exports of live ruminants from the island were prohibited. In addition, the slaughter of all infected, recovered and seropositive ruminants was undertaken. One year later, an eradication programme was initiated. All 5 000 cattle on the island were serologically tested each year at the end of the vector season. Between 1980 and 1986, approximately 560 seropositive bovines were slaughtered. Exports of small

ruminants were allowed only during winter (season of low vector activity) and only after negative serological results had been received. A sentinel bovine and ovine system was applied. Restrictions continued until 1991. Surveillance and control measures were also applied on the islands of the east Aegean Sea from 1980 to 1991. The successful application of surveillance and control measures resulted in the official lifting of EU restrictions in 1991. From 1991 through to the summer of 1998, the surveillance measures continued on the islands of the east Aegean and clinical disease was not reported. A total of 5 486 sera, mainly from sheep and goats from the Dodecanese, were examined during the summer of 1998 and gave negative results.

In October 1998, clinical outbreaks of BT were reported on three east Aegean islands (Rhodes, Kos and Leros) with serological evidence of BT on another island (Samos), adjacent to the coast of Anatolian Turkey (Table I). From October until late December, 84 outbreaks were recorded. No further outbreaks of BT were reported over the following months until August 1999 when BT outbreaks occurred on mainland Greece, commencing in the north-eastern prefecture of Evros, adjacent to the Turkish and Bulgarian borders. The virus spread across northern and central Greece, affecting 16 prefectures (approximately one third of the country). Also during September 1999, a 'separate'

Table I
Incidence of outbreaks of bluetongue in Greece, 1979-2001

Year of outbreak	No. of prefectures	Location of infected prefectures	No. of prefectures with serological evidence	Location of prefectures with serological evidence	No. of outbreaks	No. of animals destroyed	Presence of <i>Culicoides</i> vectors	Serotypes isolated
1979	1	North-eastern Greece (island of Lesbos)	1	South-eastern Greece (island of Rhodes)	68	5 000	<i>C. imicola</i>	BTV-4
1998	1	South-eastern Greece (islands of Rhodes, Kos and Leros)	1	Eastern Greece (island of Samos)	84	3 000	<i>C. imicola</i>	BTV-9
1999	11	Eastern, north, and mainland Greece	5	North-eastern Greece	1 536	24 528	<i>C. imicola</i> <i>C. obsoletus</i>	BTV-4 BTV-9, BTV-16
2000	1	Western Greece			10	0	<i>C. obsoletus</i>	BTV-4
2001	11	North-western, western, north-eastern, and mainland Greece	3	Western Greece	174	1 224	<i>C. imicola</i> <i>C. obsoletus</i>	BTV-1 BTV-4 BTV-9

incursion of BT occurred on the islands of the north-east, east and south-east Aegean Sea. A total of 1 536 clinical outbreaks were reported and 24 528 ruminants destroyed. Mild symptoms were reported in some cases in bovines and goats (Table I). The most severely affected area was the island of Lesbos, where 260 clinical cases occurred from September until the end of December 1999.

In the autumn of 2000, only one prefecture was infected in central-western Greece. Ten clinical outbreaks involved 50 sick but no dead animals. Seroprevalence in this prefecture was 7.9%.

In September 2001, BT recurred but this time in north-western Greece on the border with Albania and then subsequently in western and central Greece. Outbreaks were also reported on the island of Lesbos which was heavily infected from October until the end of December 2001. During this incursion, 14 prefectures reported 174 clinical outbreaks, with 1 303 ruminants sick and 1 224 destroyed (Table I).

Measures to safeguard the trade in live animals, i.e. control of *Culicoides* spp. and other control and epidemio-surveillance measures, have been applied from 1998 to date. Serological monitoring of sentinel bovines in 17 prefectures was established in 2002. Serological surveillance of sentinel bovines for virus activity will be conducted in 14 prefectures of east, north, west and mainland Greece from May 2003 until the end of December 2003.

Materials and methods

Safeguard control and surveillance measures

Safeguard measures for live animal trade

When confronted with the epidemic, three zones were established around outbreaks, on the basis of geographical and administrative criteria, as follows:

- a zone 20 km in radius (protection zone), where a complete standstill of animal movements within the zone was implemented
- areas of affected prefectures outside the radius of 20 km (surveillance zone), where no movements were allowed unless serological tests were performed giving 100% negative results
- non-affected prefectures adjacent to affected areas (buffer zones), where clinical and serological surveillance including sentinels was applied
- prefectures far from affected areas (free zones), where no restrictions were applied to the domestic trade in live susceptible animals
- a blanket export ban on live susceptible animals was applied.

Control measures

The following control measures are applied at present:

- when faced with an epidemic(s):
 - elimination of clinically affected animals
 - large-scale and regular vector control measures.

- 2) in the absence of evidence of virus circulation:
 - a) serological monitoring of sentinel bovines
 - b) in case of seroconversion, elimination of viraemic bovines and targeted vector control measures.

Control of *Culicoides* spp.

Control of BT vectors is applied as follows:

- 1) during winter and early spring to destroy vectors at the larval stage and reduce the probability of infective vectors over-wintering
- 2) during spring and summer to destroy vectors at the adult stage and/or prevent vectors from biting naive susceptible animals.

Spraying is applied at three levels and insect repellent is provided free of charge by the local State Veterinary Services.

The three levels of spraying are as follows:

- Level 1: spraying, with insect repellent, of individual animals (bovines, sheep and goats) every 4-7 days; applied by farmers.
- Level 2: spraying, with insecticide, of damp sites inside and around animal holdings; applied by farmers.
- Level 3: spraying, with insecticide, of breeding sites; applied by local authorities and the State.

Epidemiological surveillance

Included in epidemiological surveillance are the following:

- a) Serological monitoring of sentinel bovines
- b) Isolation and serotyping of BT viruses
- c) Seroprevalence
- d) BTV vector monitoring

Sentinel animal surveillance

To study the incidence of infection in the country, a sentinel bovine system was established. Sentinel herds were selected from areas in each prefecture where vector populations are sufficient to ensure virus transmission. Insects were trapped for species identification and population trends by using light traps in the vicinity of the sentinel herd. Sentinel bovines were established in prefectures affected in previous years and in prefectures at risk. Five groups of 10 seronegative bovines, above six months of age, were placed in each prefecture. Heparinised and clotted blood samples were collected from each animal at 15-30 day intervals. When a sentinel animal seroconverted, the viraemic animal was eliminated from the group (slaughtered) and replaced with a seronegative animal. Insect collections were made for virus isolation.

Field samples

All state field veterinarians collaborate with the national laboratory in active surveillance by submitting samples from BTV cases, establishing and seromonitoring the sentinel bovine herds and submitting *Culicoides* to the Entomology Laboratory. They are also responsible for applying the measures to safeguard domestic trade of live animals and for control measures. In a case of suspected BTV infection, samples are tested for foot and mouth disease, peste des petits ruminants, orf and sheep and goat pox viruses, in accordance with the methods recommended by the Office International des Épizooties (OIE).

The samples are heparinised blood, serum and, in the case of the death of an animal, spleen and lymph nodes. Tissues are triturated and a suspension of 1:5 in buffered lactose peptone containing antibiotics is prepared. The red blood cells are washed (10) and are ruptured by lysing in sterile, distilled water. For isolation from insects in the Entomology Laboratory, the *Culicoides* are sorted into species and then into parous pools with blood-fed females pooled with non-parous females; the males are discarded. Pools of 50 to 100 parous biting midges of a single species or mixed species are placed into vials containing MEM with antibiotics and are sent to the virology laboratory for BTV isolation and identification. The lysed blood cells, tissues and insects are held at +4°C or -70°C until inoculated.

Serological testing

From 1979 to 1985, the serum samples were tested using the immunodiffusion test (16), while from 1986 to date, competitive enzyme-linked immunosorbent assay (c-ELISA) is performed (1), using the serogroup reactive monoclonal antibody 3-17-A3. The BTV serotype-specific antibodies are determined using the serum neutralisation test in BHK-21 cells (8).

Differential diagnosis

Differential diagnosis is performed in clinical cases for the following purposes:

- 1 to detect antibodies against epizootic haemorrhagic disease of deer virus (EHDV), using the c-ELISA (23)
- 2) to detect antibodies against orf and against sheep and goat pox viruses using the immunodiffusion or serum neutralisation test (15).

Virus isolation and identification

The samples thus prepared are inoculated intravenously into 11- to 12-day-old embryonated chicken eggs (7). Embryos found dead between

2 and 7 days post inoculation (pi) are collected for inoculation into BHK-21 tissue cultures and/or Vero cells (14). For identification of the isolated virus, indirect immunofluorescence was used (9, 10), but this was replaced with the antigen-capture ELISA for BTV (14) and for EHDV (22), as well as the polymerase chain reaction (PCR) test (3). The isolated BTV is serotyped with the microtitre virus neutralisation test (14). Each time a new BTV serotype is identified, results are confirmed by the OIE Reference Laboratories (Onderstepoort Veterinary Institute, 1979 for BTV-4 and the Institute of Animal Health, Pirbright Laboratory, 1998 for BTV-9, 1999 for BTV-16 and 2001 for BTV-1).

Vector monitoring for *Culicoides* spp.

The methodology of the vector monitoring system is as follows:

- 1) operation of at least one light trap in each 'affected' area, with preference for affected premises, breeding sites, etc.
- 2) collection and classification of insects every 15 to 30 days
- 3) creation of a database.

In regard to the trapping protocol, mainland Greece was composed of 59 quadrants of 50 km × 50 km² (labelled 1-59), which were sampled for *Culicoides* over two years (Fig. 1). Two farms (at least 10 km apart) were sampled in each quadrant. During the summer (July to October), each farm was sampled for two nights. During the winter (December to March), farms where *C. imicola* was found in the summer were sampled for a further five to seven nights.

Results

The results of serological monitoring of the sentinel bovines are presented in Table II and Figure 2. In the first incursion of BTV in 1979, BTV was confirmed as serotype 4 (24) and the vector responsible was *Culicoides imicola* (4, 5, 12). BTV serotype 9 was first isolated in 1998, apparently with *C. imicola* again involved in transmission (M. Patakakis, personal communication). During the 1999 epidemic, three serotypes were isolated, namely: BTV-4, BTV-9 and, for the first time, BTV-16. The three insect vectors involved were *C. imicola*, *C. obsoletus*, *C. pulicaris* on the eastern

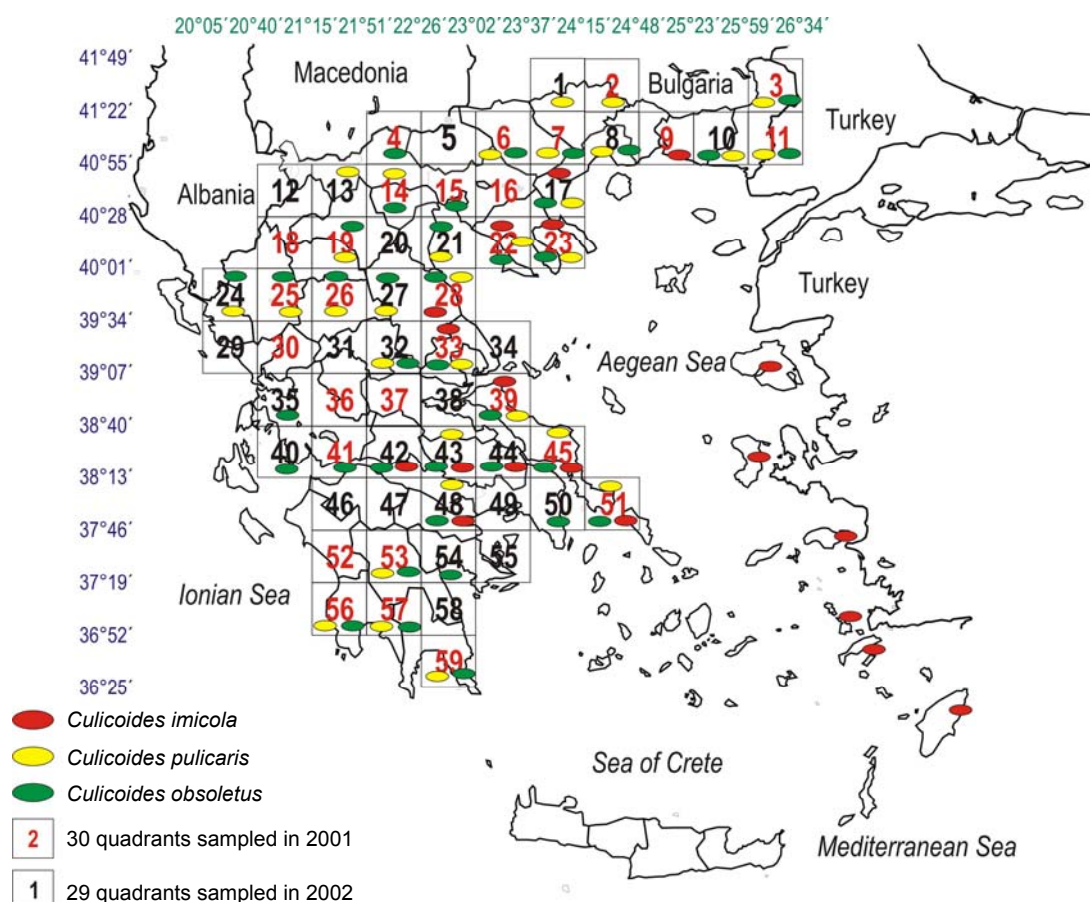


Figure 1
Distribution of bluetongue virus vectors in Greece, 2000-2002

islands and on mainland Greece. *C. imicola* was not found in northern Greece (M. Patakakis, personal communication). Only BTV-4 was isolated in the 2000 outbreak and *C. imicola* was not detected (M. Patakakis, personal communication). In 2001, BTV-1 was isolated for the first time in the western, north-western and eastern regions of Greece, while

BTV serotypes 4 and 9 were isolated in north-west and central Greece. In the eastern and central prefectures, *C. imicola* was involved, while *C. obsoletus* was involved in the western, north-western and central prefectures (M. Patakakis, personal communication). Results of the BTV serotypes identified are presented in Table III and Figure 3.

Table II
Results of serological monitoring of sentinel bovines for bluetongue antibodies in Greece, 1980-2003

Year	No. of prefectures with sentinels	Location of prefectures with sentinels	Period of sampling starting date	End date	Results (positive/total samples tested)	Seroconversion months
1980-1990	4	Eastern Greece	June	December	560/approx. 24 000	
1999	4	South-eastern Greece	May 1999	End of September	12/639	August, September
2000	17	South-eastern, eastern, north-eastern, north-central, central Greece	March 2000	End of December 2000	23/12 638	March until September
2001	18	South-eastern, eastern, north-eastern, north western, north-central, central Greece	June 2001	June 2002	47/4 340	September until January
2002	17	North, north-eastern, north-western, north-central, central Greece	June 2002	End of November 2002	0/4 418	
2003	14	South-eastern, eastern, north-eastern, north-western, western, north-central, central Greece	June 2003	End of September	0/1 999	

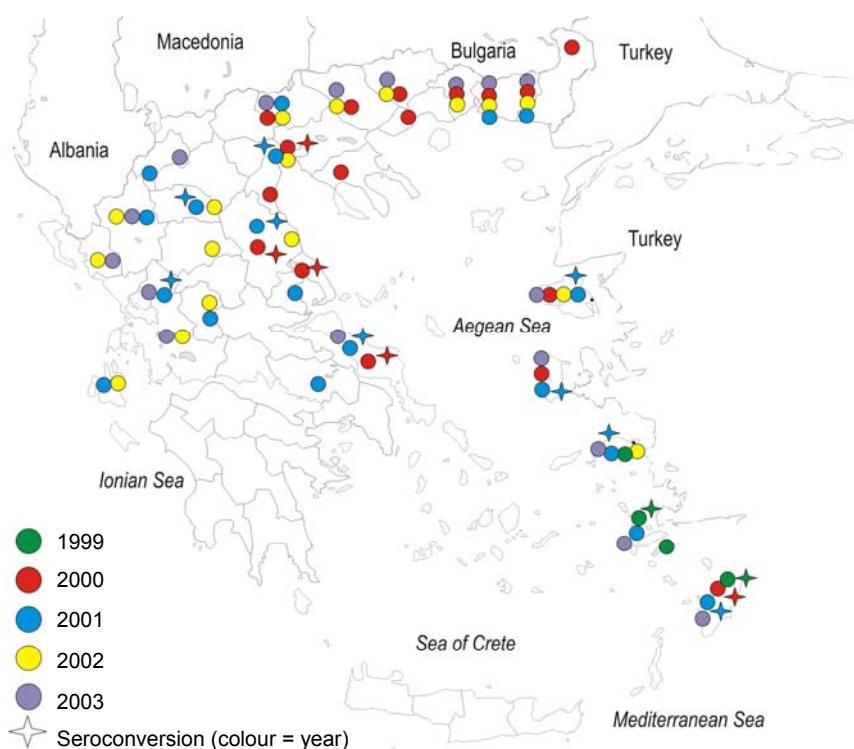


Figure 2
Serological monitoring of sentinels for bluetongue in Greece, 1999-2003

Table III
Distribution of bluetongue virus serotypes in Greece, 1979-2001

Prefectures	1979		1998		1999		2000		2001	
	Virus isolated	Serology	Virus isolated	Serology	Virus isolated	Serology	Virus isolated	Serology	Virus isolated	Serology
Dodekanissa (south-east)			BTV-9		BTV-4, BTV-16		BTV-9, BTV-16		BTV 1	
Samos (east)							BTV-4, BTV-16			
Chios (east)					BTV-4					
Lesbos (north-east)	BTV-4				BTV-4	BTV-4, BTV-9	BTV-4, BTV-16		BTV-1	
Evros (north-east)					BTV-4		BTV-4			
Rodopi (north)					BTV-4	BTV-9	BTV-9			
Drama (north)							BTV-9			
Kavala (north)							BTV-4			
Serres (north)							BTV-9			
Chalkidiki (north)					BTV-9, BTV-16	BTV-9				
Picira (north)					BTV-4					
Grevena (north-west)									BTV-1	BTV-4, BTV-9
Kastoria (north-west)										BTV-4, BTV-9
Ioannina (north-west)									BTV-1	BTV-4, BTV-9
Thesprotia (west)									BTV-1	
Arta (west)							BTV-4			
Larisa (mainland)					BTV-9	BTV-9				
Magnesia (mainland)					BTV-4		BTV-4			
Evia (mainland)					BTV-4	BTV-9				

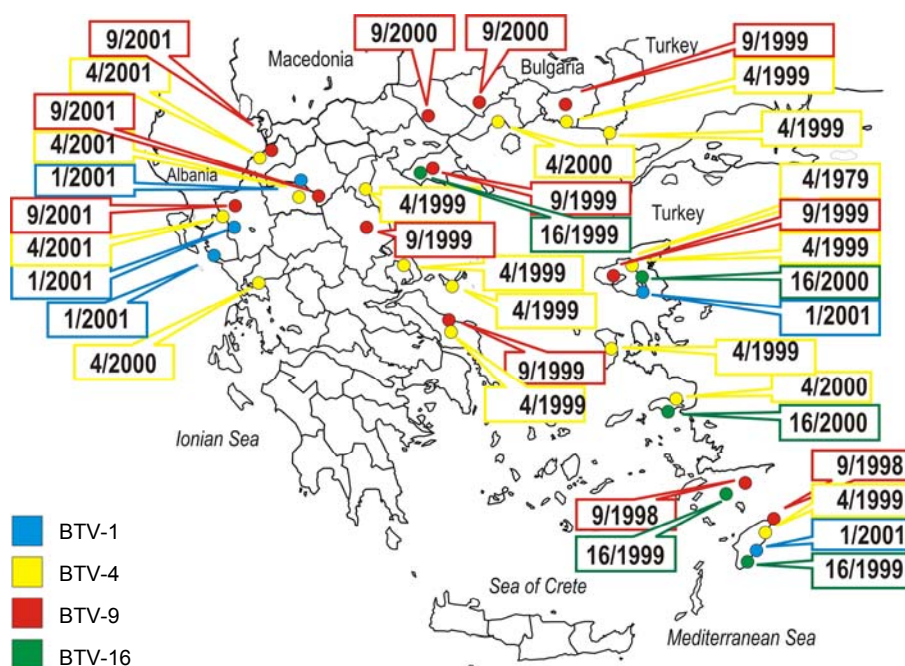


Figure 3
Distribution of bluetongue serotypes in Greece, 1979-2001

Results of the distribution of *Culicoides* are presented in Figure 1 and, finally, results of seroprevalence for 2002 are presented in Figure 4.

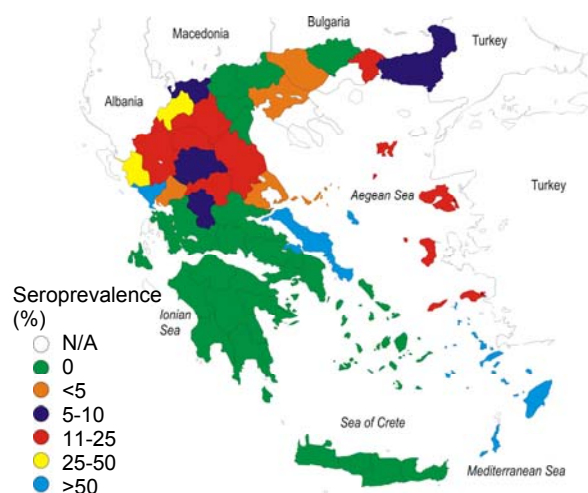


Figure 4
Accumulative seroprevalence (%) of bluetongue in Greece, 2002

Discussion

The major epidemics of 1998-1999 and 2001 were due to new independent incursions of BTV. In October 1998, after an absence of almost 20 years, BT was confirmed on four islands in the south-east near the western coast of Turkey. Serotype 9 was identified and was the first incursion of this serotype into Greece, although in 1979-1980 it had already been found to occur serologically in western and south Anatolian Turkey (21).

In August 1999, BTV serotype 4 and serotype 9 were introduced into Greece from the north-east. Initially, BTV-4 outbreaks were reported from near the border with European Turkey but later BTV-4 was distributed more widely by prevailing winds. BTV-9 was isolated initially from outbreaks in northern Greece and later BTV-9 was also isolated more widely. In June 1999, BTV-9 was confirmed in southern and eastern Bulgaria (2), while in July 1999, Turkey reported the incursion of BT into European Turkey in provinces bordering Bulgaria and mainland Greece (13). BTV-4, BTV-9 and BTV-16 were also isolated from south-eastern prefectures in Greece; BTV-4 had previously been reported in Anatolian Turkey (21). BTV-16 occurs regularly in Israel (19, 21). The source of the introduction of BTV-4, BTV-9 and BTV-16 into Greece in 1999 is difficult to determine. BTV serotypes 2, 4, 6, 9, 10, 13 and 16 have been reported in previous years in Anatolian Turkey (21), Syria, Jordan (20) and Israel. The movement of some of these viruses or of others (e.g. Akabane) from east to west is well documented

(21, 24). At this time, BTV-2, BTV-6, BTV-10 and BTV-13 have not been isolated in Greece. Work on the serotyping of BTV isolates from previous outbreaks is in progress.

In September 2001, BTV serotype 1 invaded north-west Greece along the Albanian border and then subsequently spread to western Greece, although it was limited in its spread due to natural barriers (mountains). In late October 2001, a BTV-1 incursion was reported on the island of Lesbos in the same areas that were severely infected during the 1999 BT epidemic. The isolation of BTV-1 was made from sick animals that had recovered from clinical disease in 1999. BTV-1 has never been reported in neighbouring countries or in eastern Turkey, Syria, Jordan or Israel; BTV-1 has been isolated in India (17, 18). Efficient laboratories must be established in neighbouring countries as well as in all Middle Eastern and North African countries so that BTV can be isolated and typed.

Overall, in 1979 and between 1998 and 2001, BTV-4 appears to have been the most widely distributed serotype, and was isolated consistently every year. BTV-16 was identified for the first time in 1999 and appears to be limited to the islands of the east Aegean Sea, with the notable exception of isolation from insects on mainland Greece in 1999.

It seems that several independent incursions of BTV-4, BTV-9, BTV-16 and BTV-1 occurred in Greece between 1998 and 2001. The islands of the east Aegean Sea were exposed to repeated incursions throughout the period 1979 to 2001. Results of the nationwide seroprevalence survey undertaken in 2002 are shown in Figure 4 indicating that despite repeated waves of disease, a large proportion of uninfected susceptible animals remain. Results of vector monitoring indicate there is an abundant and persistent presence of competent and/or potential BTV vectors in most areas of Greece. *Culicoides imicola*, in particular, is abundant in south and eastern Greece and was not found in the north, north-west and west.

Considering all the problems associated with the use of the only commercially available BTV vaccines manufactured in South Africa (13), the Greek Ministry of Agriculture refused to apply vaccination against BTV.

The nationwide seroprevalence survey indicates that large parts of Greece remain free of BTV infection, which suggests that the control and safeguard measures applied were appropriate and efficient.

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