Regional overview of bluetongue viruses, vectors, surveillance and unique features in Eastern Europe between 1998 and 2003

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Summary

Between 1998 and 2002, successive epidemic waves of bluetongue (BT) virus infection were recorded in the Balkans giving rise to clinical outbreaks of BT that caused severe direct losses of livestock in several countries, namely: Greece, Bulgaria, Yugoslavia, Kosovo, the Former Yugoslav Republic of Macedonia, Bosnia-Herzegovina and Albania and probably Turkey and Croatia. Affected countries resorted to different control, safeguard, prevention and epidemiological/ surveillance measures against BT but comprehensive and reliable data are by and large lacking. This review attempts an analysis and extrapolation of the local epidemiological profiles and patterns documented in some countries in south-eastern Europe and – assuming that the evolution of BT in these countries reflects the situation of BT in the wider region – considers some relevant and timely questions of epidemiological significance.

Keywords

Bluetongue – *Culicoides* – Eastern Europe – Epidemiology – Surveillance – Virus.

After a prolonged period of historical freedom, presumed freedom or, at worst, minor, sporadic and geographically confined incidents of seroconversion to bluetongue (BT) virus (BTV) in livestock, massive and multiple epidemics of the disease were recorded in south-eastern Europe starting in 1998 and continuing over subsequent years. Between the autumn of 1998 and winter of 2002, successive waves of BT epidemics were recorded in the Balkans, giving rise to a number of clinical outbreaks, causing severe direct losses in several countries, namely: Greece, Bulgaria, Yugoslavia, Kosovo, the Former Yugoslav Republic of Macedonia, Bosnia-Herzegovina and Albania and probably Turkey and Croatia.

Affected countries used different control, safeguard, prevention and epidemiological surveillance measures against BT. With the exception of Greece and Bulgaria, comprehensive and reliable epidemiological data are by and large lacking. However, through an analysis and extrapolation of the epidemiological profiles and patterns observed in Greece and Bulgaria, and assuming that the evolution of BT in these two countries reflects closely the BT situation throughout the Balkans, certain general comments can be made and some questions of epidemiological significance emerge which shed new light on the conventional perceptions and clearly call for a new risk assessment and prevention and control strategy against BT.

Outstanding questions arising from the study of BT in the Balkans include the following:

a) the geographical occurrence and abundance of efficient vector(s)
b) the potential involvement of other, more common and widely spread vectors
c) the occurrence and distribution of BTV serotypes, in particular those perceived as 'exotic'
d) the most appropriate prevention, control and safeguard measures.

Temporal and spatial occurrence of bluetongue in Eastern Europe

Epidemiological conditions and perceptions preceding primary incursion

In the wake of the 1979 epidemic of BT which affected the Greek Island of Lesbos in the eastern

Aegean Sea (12, 22), the Greek veterinary authorities were acutely aware of the risk of re-incursion and, consequently, routinely applied active serological monitoring on all Greek islands opposite and along the western Turkish coastline. During 1997 and up until late September 1998 (1), approximately 5 500 serum samples collected in the Dodecanese islands alone were tested, with negative results. Consequently, the estimated date of primary incursion of BTV into Eastern Europe can be determined with some accuracy. These encouraging findings, however, were shadowed by sporadic reports of BT outbreaks in Turkey throughout the 1990s as well as by the inherent risk factor posed by the documented presence of efficient BTV vectors (*Culicoides imicola*) on most Greek islands of the eastern Aegean Sea (9, 10). It is worth mentioning that prior to 1998, no other country in eastern Europe, or the rest of Europe, considered BT a relevant and potentially emerging disease.

History of recent outbreaks reported in Eastern Europe

Against this background, BT was confirmed on four Greek islands in October 1998, namely Rhodes, Kos, Samos and Leros (Fig. 1) adjacent to the western coast of Turkey. The causative virus was identified as BTV serotype 9, which had only been reported previously in 1979-1980 in western and southern Turkey. The vector involved was definitely *C. imicola*. The means of introduction was presumed to be airborne infective vectors carried by the prevailing easterly winds. The source of infection was not identified (1). Between mid-October and late December 1998, 84 outbreaks (flocks) were recorded in the four Greek islands and a total of approximately 3 000 animals (exclusively sheep) died or were culled due to BT (1). This was the first incursion of BT into Europe since 1979 and caused no real surprise since it involved a known risk area located well within the vector zone between 35°N and 40°N.

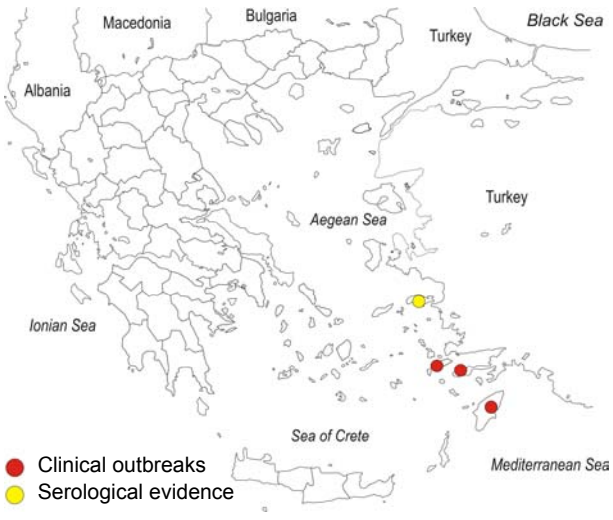

Figure 1
Regions of Eastern Europe clinically affected by bluetongue in 1998

In late June 1999, BT was reported for the first time in the region of Burgas in south-eastern Bulgaria. By the end of December, the disease had spread in a south-south westerly direction affecting four Bulgarian regions, namely: Burgas, Yambol, Haskovo and Kardjali (Fig. 2).

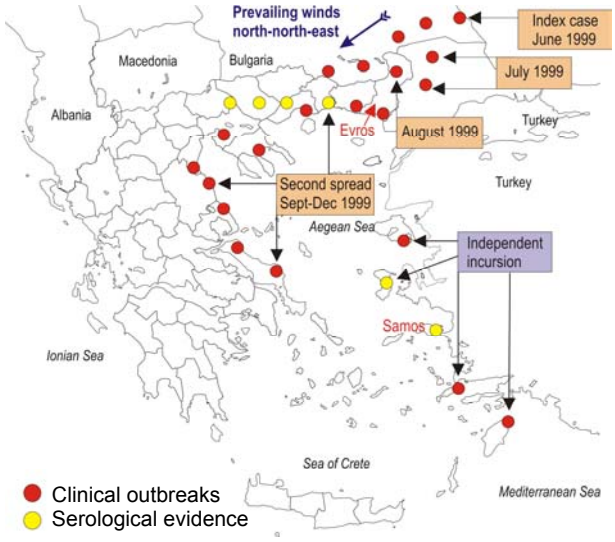

Figure 2
Regions of Eastern Europe clinically affected by bluetongue in 1999

BTV serotype 9 was identified. Vector surveillance failed to confirm the presence of vectors, but revealed an abundance of *C. pulicaris* and *C. obsoletus* which have long been suspected as potential vectors of BTV (2, 13). Both the means of introduction and the source of infection remain unclear. In total, 85 outbreaks (or villages) were reported in Bulgaria in 1999 and 667 animals (sheep) died or were culled due to BT (2). This second incursion of BT into Europe does not appear to be linked to the 1998 epidemic of BT in the Greek islands some 600 km to the south, as areas affected were as far north as 42°30´N and supported the hypothesis that other vectors besides *C. imicola* may have been involved in some cases (13). In July 1999, Turkey reported the presence of BT, supposedly having originated in Bulgaria, in two provinces bordering Bulgaria and Greece, namely Kirklareli and Edirne (Fig. 2) and responded by vaccinating some 60 000 sheep with a locally produced live virus vaccine against BTV serotype 4. However, despite the implied success of the vaccination campaign, the field virus isolated in

1999 in the European part of Turkey was later identified as being serotype 9 (13). No systematic entomological studies had been undertaken until this time in the European part of Turkey to determine the presence, geographical distribution and seasonal variation of BTV vectors. Subsequent evolution of BT in Turkey during 1999 remains unclear and the only comment that can be made is the contradiction between official reports, or absence of such reports, and unofficial personal communications.

In August 1999, BT was predictably reported in the prefecture of Evros, north-eastern Greece, adjacent to the borders to Bulgaria and Turkey (3). By December, the disease had spread in a south-south-westerly direction along the prevailing wind patterns and involved nine prefectures in mainland Greece. In addition, serological evidence of BTV infection was found in four additional prefectures (4). Incursion and spread was traced along the valley of the Ardas River that flows from Burgas (Bulgaria) to Evros. As in Bulgaria, BTV serotype 9 was involved and *C. imicola* was not identified (at the time) in northern Greece, thus lending support to the hypothesis that other species of *Culicoides* may be involved in transmission (13). In the same epidemic, however, outbreaks of BT were reported on the south-eastern tip of Evros, which is far from the predicted direction of spread (Fig. 2). Intensive epidemiological inquires ruled out any link with the known sources of infection and laboratory tests identified BTV serotype 4 in this sub-cluster of outbreaks and subsequently in other outbreaks in mainland Greece (3). This was a novel and unexpected occurrence and, since no official information was available suggesting the recent presence of serotype 4 in the region, the original source of BTV-4 was designated as 'unknown'. It was evident, however, that an incursion of multiple BTV serotypes was in progress and from that point onwards the isolation and typing of as many field strains as possible became a necessity. The conclusion that multiple serotypes of BTV had entered the region was confirmed in September 1999 when, shortly after an official but flimsy report of BT in the vicinity of Smyrna, Turkey, a massive epidemic of BT swept the islands of Lesbos and the Dodecanese in the eastern Aegean Sea, adjacent to the Turkish coast. Again, intensive epidemiological inquiries ruled out any link with the known sources of infection on mainland Greece and laboratory tests identified BTV serotypes 4 and, astonishingly, 16. By December 1999, two additional prefectures on the islands were clinically affected, specifically Chios and Lesbos, while serological evidence of BTV infection was found in another prefecture, namely Samos (Fig. 2). In total, 1 536 outbreaks were recorded in Greece in 1999 causing the death or culling of 24 528 sheep (3).

The following year, 2000, was relatively quiet, with no clinical evidence of BT anywhere in Eastern Europe, except in the prefecture of Arta in central-western Greece, where a cluster of ten BT outbreaks caused by BTV serotype 4 involving 50 animals was linked to a known internal source of infection through illegal movement of viraemic bovines (5) (Fig. 3). Prompt identification and efficient application of targeted vector control measures prevented any spread and the epidemic burned itself out.

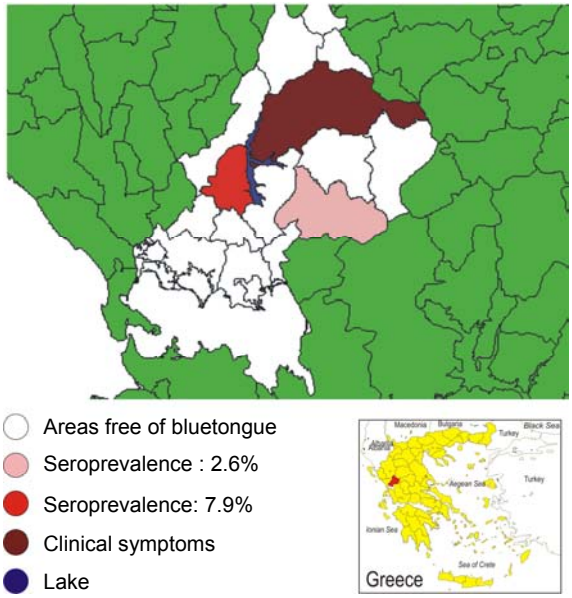

Figure 3
Regions of Greece that were clinically affected by bluetongue in 2000

In late September 2001, BT was first reported in north-western Greece, adjacent to the borders with the Former Yugoslav Republic of Macedonia and Albania. The epidemic gradually expanded to the south, eventually involving 11 prefectures, most of which were not affected during the 1999 epidemic (6) (Fig. 4). *C. imicola* was found in the eastern and coastal prefectures and *C. obsoletus* in the northern and central mountainous areas. Remarkably, however, BTV serotype 1 was identified in both the primary and northern-most outbreaks. This particular BTV serotype has never been reported anywhere near the Mediterranean Basin or Middle East and the epidemiological picture is further obscured by the failure to identify this serotype anywhere else in Eastern Europe in the course of the 2001 epidemic. In total, 174 outbreaks were recorded in Greece in 2001 accounting for the death or culling of 1 224 sheep.

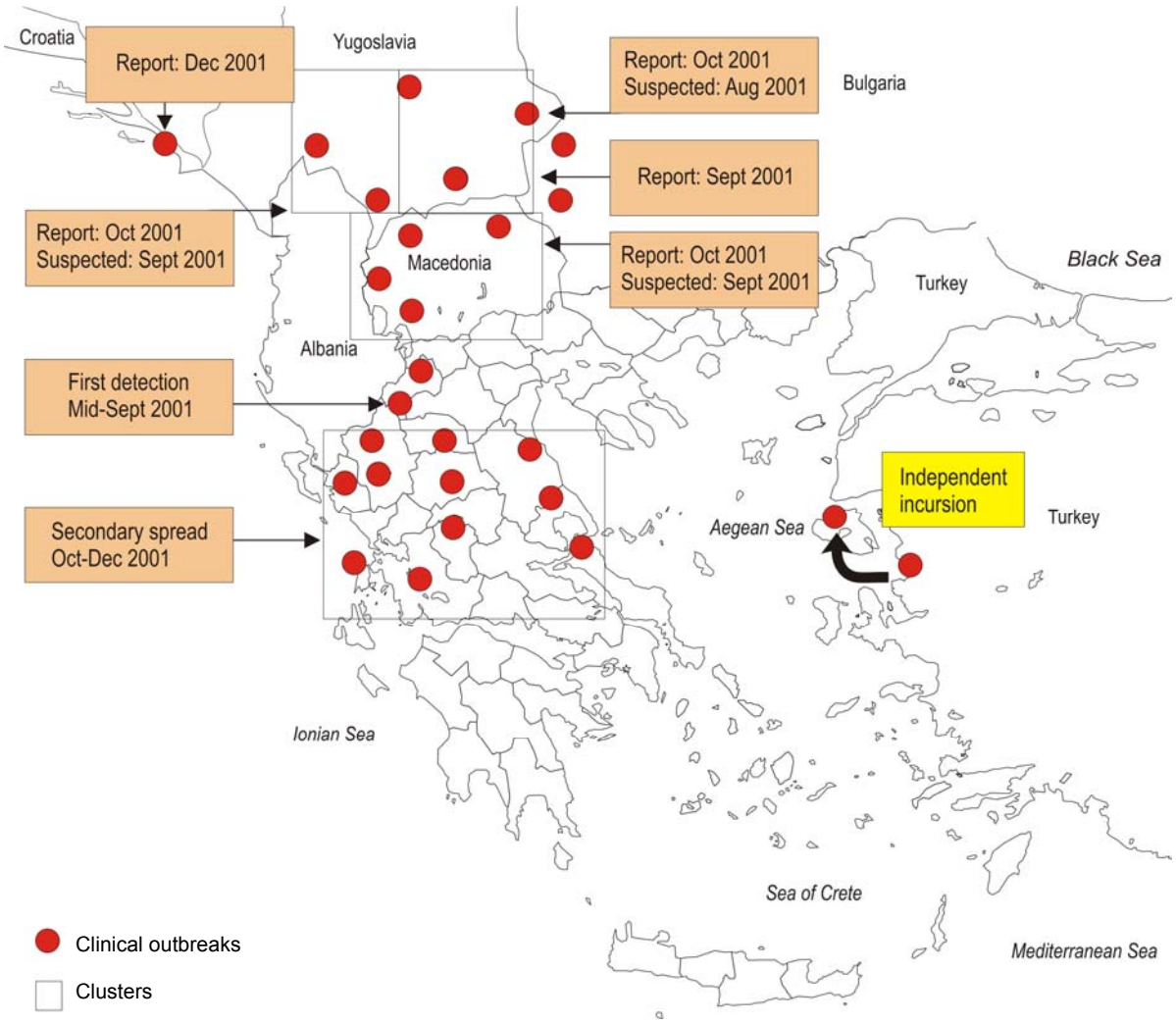

Figure 4
Regions of Eastern Europe clinically affected by bluetongue in 2001

Following the alert from Greece, reports came in from various countries in the region within a matter of days, retrospectively announcing the presence of BT in their territories in 2001, as follows:

a) In early October 2001, Bulgaria announced the widespread presence of BTV along the entire length of its western border with the Former Yugoslav Republic of Macedonia and Serbia, and in particular in the Kiustendil Province, extending as far north as 43°36´N on the Bulgarian-Romanian border (Fig. 4). The BTV serotype involved has not been identified and, again, no *C. imicola* was detected in the region. In total, 75 outbreaks (or villages) were affected by BT in Bulgaria in 2001 and 23 severely affected sheep died or were culled (4).

b) In October and November 2001, the Former Yugoslav Republic of Macedonia reported a total of 36 outbreaks, starting from the district of Kriva Palanka on the borders with Bulgaria and Serbia and eventually spreading along the entire length of the northern, western and eastern borders with Albania, Kosovo, Yugoslavia and Bulgaria (16) (Fig. 4). A total of 178 sheep died or were culled and the BTV serotype involved was not identified.

c) In October 2001, Kosovo reported six outbreaks of BT in as many villages in the provinces of Strpce, Podujevo, Glogovac and Vitina (14) (Fig. 4). The BTV serotype was not identified.

d) In late October 2001, Yugoslavia confirmed the presence of BT (suspected since late August 2001) in 37 outbreaks extending along the southern borders with Bulgaria and Kosovo (15) (Fig. 4). BTV serotype 9 was identified.

e) In mid-December 2001, Croatia announced the suspicion of BT in three outbreaks in the region of Dubrovnik (17) (Fig. 4). The suspicion was later confirmed but the BTV serotype was not identified.

In the following year, 2002, evidence of BTV circulation was reported from several countries, as follows:

a) In early September 2002, Bulgaria reported seroconversion in three sentinel animals in the Smolian region, near the border with Greece (18) (Fig. 5). The BTV serotype involved was not identified.

b) At the same time (early September 2002), Bosnia-Herzegovina reported 19 outbreaks for the first time, involving 169 animals in 11 villages (18) (Fig. 5). BTV serotype 9 was identified.

c) In September and October 2002, Yugoslavia reported 9 outbreaks involving 25 animals in the regions of Sebac (44°40´N) and Kraljevo (43°43´N) (19) (Fig. 5). The BTV serotype involved was not identified.

d) In December 2002, Albania reported one outbreak in the region of Librazhd, near the border with the Former Yugoslav Republic of Macedonia (8) (Fig. 5), in which apparently no animals died or were culled. The BTV serotype involved was not identified.

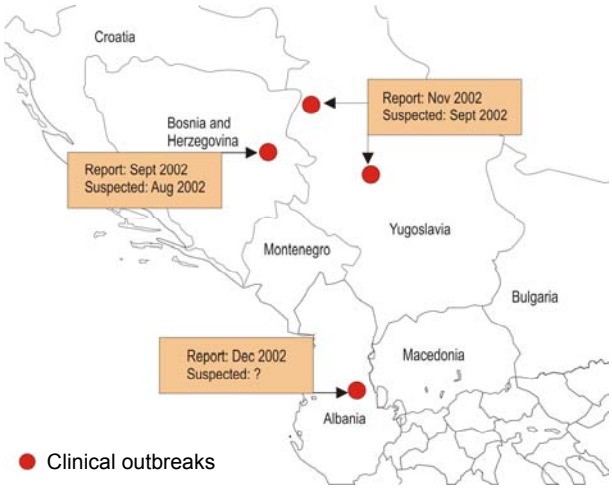

Figure 5
Regions of Eastern Europe clinically affected by bluetongue in 2002

Since then, there have been no reports of BTV circulation anywhere in Eastern Europe (until the time of writing at the end of September 2003).

Serotypes isolated in Eastern Europe

Considering the history of BT in Eastern Europe from 1998 to 2002, one of the most striking and unexpected findings is the multitude of different BTV serotypes that were identified in the region. Indeed, at the beginning of the primary incursion, the only historical data was that BTV serotype 4 was identified on the island of Lesbos in 1979 (9, 22) and that BTV serotypes 2, 4, 6, 9, 10, 13 and 16 had been reported over a number of years in Anatolian Turkey, Syria, Jordan and Israel (13). However, although the westward movement of some of these serotypes is documented, there was no hint that they may already be at the threshold of Europe. BTV serotype 1, in particular, has never previously been reported anywhere near Europe or the Middle East.

The unravelling of the mystery was triggered in Greece in 1999 as a result of the failure to explain some outbreaks on epidemiological grounds. Once it was understood that more than one BTV serotype may occur in the same country or region, the Greek veterinary authorities undertook to isolate and type as many field strains of the virus as possible. Unfortunately, due to inadequate resources and laboratory capabilities, this policy was not adopted by other countries in the region and, consequently, only a very limited number of BTV field strains have been isolated and typed from Eastern Europe.

In summary, the BTV serotypes identified in Eastern Europe, except Greece, between 1999 and 2002, are as follows (Fig. 6):

- Bulgaria: serotype 9 (1999: 1 typing)
- Turkey: serotype 9 (1999: 1 typing) serotype 16 (2000: 1 typing)
- Serbia: serotype 9 (2001: 1 typing)
- Bosnia-Herzegovina: serotype 9 (2002: 4 typings).

The BTV serotypes identified in Greece between 1998 and 2001 are summarised as follows (Fig. 6):

- 1998 : serotype 9 (5 typings)
- 1999 : serotype 9 (10 typings)
  : serotype 4 (12 typings)
  : serotype 16 (4 typings)
- 2000 : serotype 4 (1 typing)
- 2001 : serotype 1 (4 typings)
  : serotype 4 (3 typings)
  : serotype 9 (3 typings).

Furthermore, isolation and typing of >100 frozen field samples collected between 1999 and 2001 is in progress.

Vectors identified in Eastern Europe

The distribution and vectorial capacity of efficient and potential BTV vectors, as well as the impact of climatic changes on these factors has been comprehensively reviewed (13). Prior to 1998, however, knowledge on the abundance, geographical distribution and seasonal variation of BTV vectors in Eastern Europe was fragmented and limited to certain Greek islands of the eastern Aegean Sea (9,

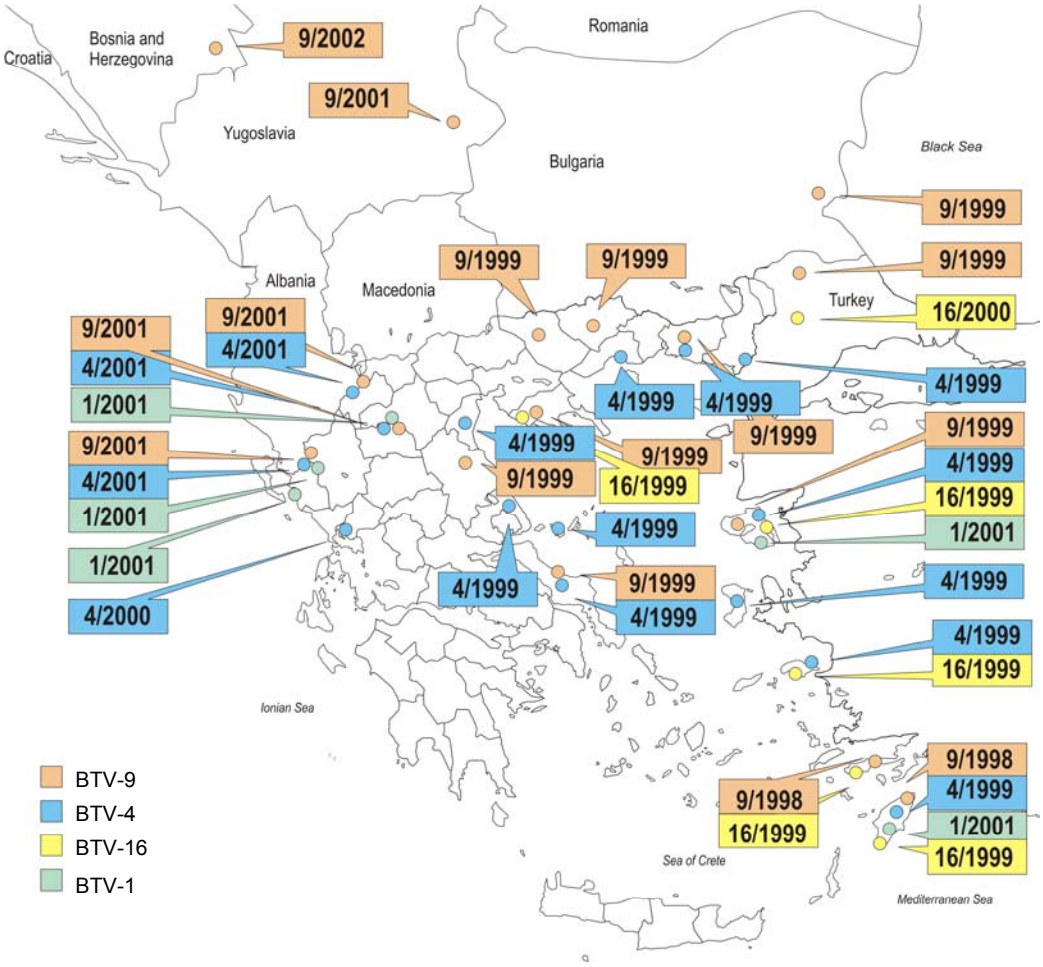

Figure 6
Bluetongue virus (BTV) serotypes identified in Eastern Europe, 1998-2001

10). Furthermore, previous random catches had failed to identify *C. imicola* in mainland Greece. In the context of the BT epidemics that occurred in Eastern Europe in the period from 1998 to 2001, systematic entomological surveys were undertaken in Greece and Bulgaria and they are still ongoing.

All available findings in Bulgaria, as well as preliminary findings in Greece in 1999 and 2000, failed to identify *C. imicola* anywhere north of 40°N while they revealed an abundance of *C. obsoletus* and *C. pulicaris*. This led to the working hypothesis that the latter two species may be potential, though much less efficient, vectors of BTV but that they compensate for their low efficacy by their large populations. If this hypothesis proves correct, then BT becomes very relevant for large parts of western and northern Europe previously considered to be free of the disease due to the absence of efficient vectors.

Accumulated results of vector monitoring in Greece from 1999 to 2002 are summarised in Figure 7 (7). The results indicated that:

- *C. imicola* occurs regularly in mainland Greece, particularly in the eastern coastal areas
- *C. imicola* has been identified in northern Greece, north of 40°N, near the border with Bulgaria.

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. 7). 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.

Serological monitoring of sentinel animals

Serological monitoring (surveillance) of sentinel herds was introduced in Bulgaria and Greece and routinely applied after each annual BT epidemic so as to detect residual BTV circulation in the affected

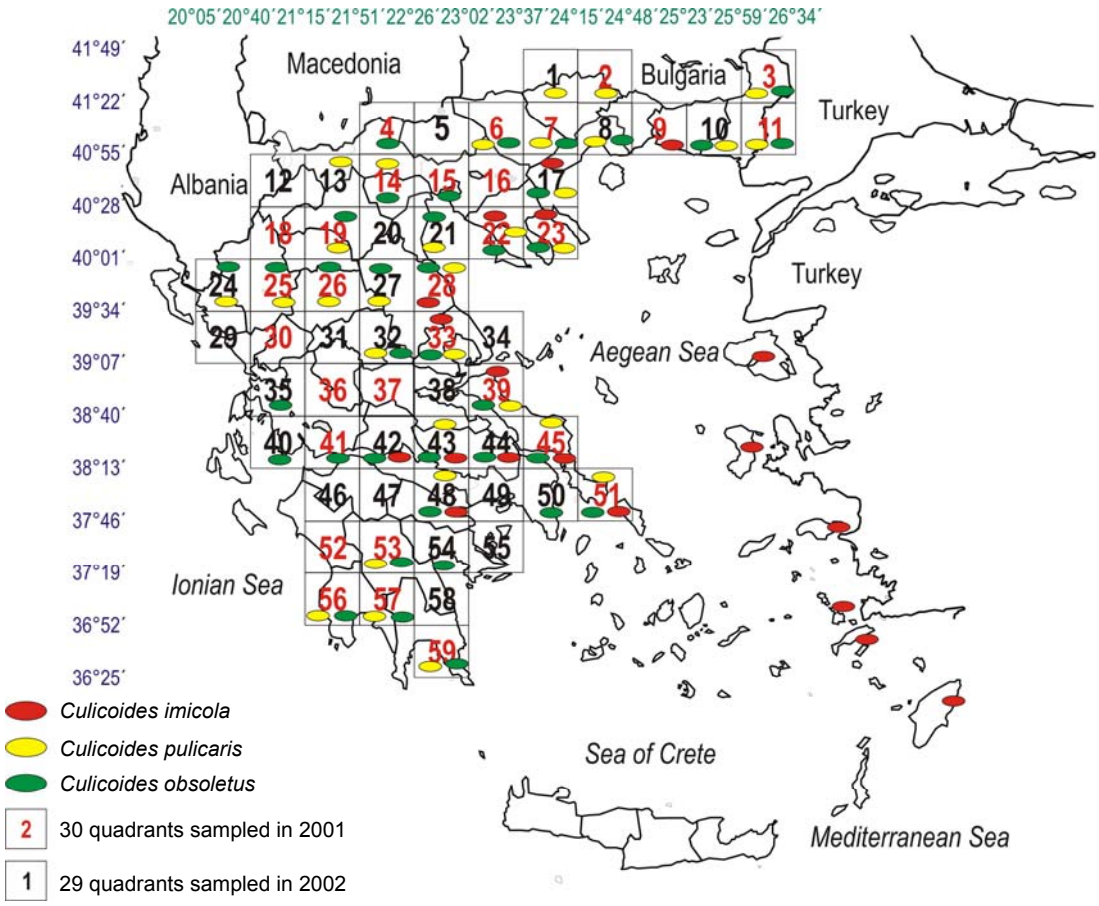

Figure 7
Vectors of bluetongue identified in Greece, 2000-2002

areas and/or re-incursion of BTV in areas at risk. The principle was the same in both countries but the objectives and the methods were different, as follows:

a) In Bulgaria, sentinel herds were widely distributed and comprised 10 cattle and 10 goats each. From 2000 to 2001 some 40 sentinel herds were deployed solely along the Greek-Bulgarian and Turkish-Bulgarian borders in an effort to detect external re-incursion rather than internal residual infection. Following the 2001 epidemic in western Bulgaria, a further 22 sentinel herds were also deployed along the western borders following the same rationale (Fig. 8).

b) In Greece, sentinel herds were established in both affected areas and areas at risk and involved naive cattle exclusively. As a rule, five groups of 10 cattle each were placed in each targeted prefecture near vector breeding or outbreak sites and the list of serologically monitored prefectures was added to address the issue of the annual evolution of BTV (Fig. 8).

c) In both countries, serological monitoring was performed seasonally (from April to December) and sentinels were sampled every 30 days and tested for antibodies to BTV. In case of seroconversion of sentinel animals in Greece, virus isolation was attempted for typing and the viraemic animal was eliminated.

The results of serological monitoring of sentinel herds from 1999 to 2003 are summarised as follows (the location of seroconverting sentinels indicated in Figure 8):

a) In Bulgaria, in 2000 and 2001, approximately 10 000 samples were tested with consistently negative results. In 2002, approximately 9 000 samples were tested and three seroconversions were detected in late August in the district of Smolyan. The BTV serotype involved was not identified. In the absence of any other evidence of virus circulation, seroconversion was attributed to recurrence from previously infected animals through a 'carrier' state mechanism involving the γδ T-cells (21). In 2003, serological monitoring of sentinel animals continued, with presumably negative results.

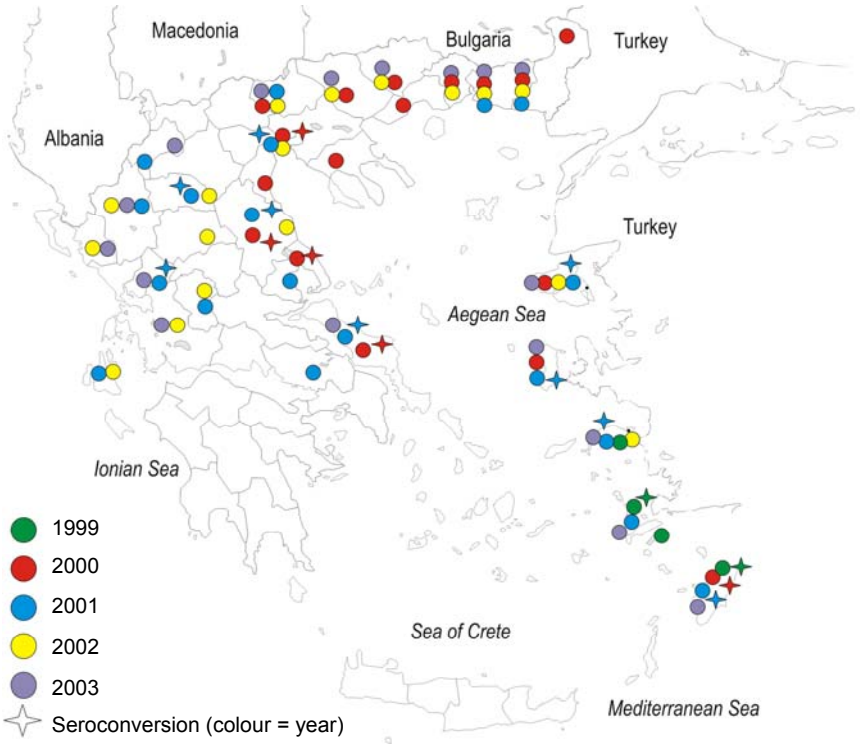

Figure 8
Serological monitoring of sentinels for bluetongue in Greece, 1999-2003

b) In Greece, in 1999, 12 seroconversions were observed from 639 samples collected in two prefectures. In 2000, 23 seroconversions were observed in 12 638 samples from 17 prefectures. In 2001, 46 seroconversions were detected in 3 716 samples from 18 prefectures. In 2002, no seroconversion was observed from 4 418 samples taken in 17 prefectures. In 2003 (until the end of September), no seroconversion was observed in 1 959 samples from 14 prefectures.

Seroprevalence in susceptible livestock

Insofar as is known, large-scale serological surveys for antibodies to BTV in the general livestock population have only been undertaken in Greece as part of internal safeguard measures requiring pre-movement testing (with negative results) of animals. Although more than 500 000 samples have been serologically tested since 1998, Figure 9 represents only the results of approximately 60 000 serological tests carried out between April and May 2002. This selective presentation is justified because it reflects the accumulated seroprevalence over successive waves of BT epidemics (7).

On the basis of results presented in Figure 9, the following comments can be made specifically for Greece:

a) Despite multiple incursions and successive BT epidemics, large areas of the country have not been affected by the disease. Arguably, this provides a measure of the success of disease control and safeguard measures.

b) Seroprevalence in most affected areas ranges from between 1% and 25%, leaving enough naive animals to sustain a new epidemic should a re-incursion or recurrence of BTV infection occur.

Figure 9
Seroprevalence in susceptible livestock in Greece, 2002

c) Seroprevalence in areas at greatest risk is 50% or higher (as high as 90% in the islands) and, therefore, the animals have already developed a natural, lasting and effective immune response

against the BTV serotype(s) that prevail in the area.

Control-safeguard and preventive measures against bluetongue in Eastern Europe

Control measures

With the notable exception of Kosovo, where clinically affected animals were spared due to financial constraints, all countries of Eastern Europe applied similar control measures when confronted with the epidemic, namely:

a) modified 'stamping-out' policy by slaughter and destruction of clinically affected animals
b) vector control measures using insecticides and/or insect repellents
c) intensive clinical, sometimes augmented by serological, surveillance.

Safeguard measures

All affected countries in Eastern Europe established protection and surveillance zones, extending over a radius varying from 20 km to 100 km, and introduced movement restrictions of animals and germplasm products from these zones.

In some countries, a curfew was imposed on animal movements from dusk to dawn and gatherings of animals (e.g. trade fairs, exhibitions etc.) were suspended.

Preventive measures and vaccination

As mentioned above, in the autumn of 1999, Turkey vaccinated some 60 000 sheep along the borders with Greece and Bulgaria. The vaccine used was a nationally produced live virus vaccine containing serotype 4 but information is lacking concerning the application and results of vaccination. It should be remembered, however, that the BTV serotype circulating in the area at the time was later identified as serotype 9. Bulgaria resorted to vaccination as a means to prevent recurrence of BT in the areas affected in 1999. The vaccination campaign was conducted in early 2000, after the lambing season, and involved some 100 000 lambs. A commercially available pentavalent live-attenuated vaccine containing serotypes 3, 8, 9, 10 and 11 was used. With the exception of seroconversions observed in sentinel animals in 2002, subsequent evolution of BT in the vaccination area was no different from that in the adjacent prefectures of northern Greece where no vaccination was practised and, therefore, the effects of vaccination are uncertain. It is noted, however, that Bulgaria refrained from vaccination during the 2001 BT epidemic. Kosovo announced its intention to apply mass vaccination in 2002, but no follow-up information is available. The rest of the affected counties in Eastern Europe did not resort to vaccination.

Discussion of the main epidemiological features of bluetongue in Eastern Europe

Although clinical manifestation of BT is a far from safe and accurate criterion of BTV circulation, understandably in Eastern Europe it was the one most commonly relied upon to signify presence and delineate spread of BTV infection. This partly explains the lack of uniform, comprehensive and consistent epidemiological data, further aggravated by a likely under-detection and/or under-reporting of the disease. A further complicating factor was the different definition of an 'outbreak' used by different countries in the region to describe the spatial distribution of BT, with some countries attributing the term to individual flocks and other countries encompassing entire villages. Apparent differences in the virulence of different BTV serotypes and strains may also have complicated reporting of BT in Eastern Europe. Clinical observations made in Greece suggest the following:

a) BTV serotype 9 is consistently highly virulent, causing severe clinical symptoms and high morbidity and mortality (on average 25% and 10%, respectively). BTV-9 is the only serotype identified so far in Eastern Europe, with the exception of Greece
b) BTV serotypes 4 and 16 are generally less virulent, causing mild and transitory clinical symptoms, low morbidity (<10%) and almost no mortality. As an example, it is noted that during the 2000 epidemic in Greece (due to serotype 4), BT was obscured by concurrent Orf infection and the combined morbidity/mortality rates were 10% and 0%, respectively

- BTV serotype 1 displays varying virulence depending on the species and abundance of local vectors, breed of affected animals (sheep) and local climatic conditions and terrain. As an example, it is noted that during the 2001 epidemic in the north-western part of Greece, morbidity and mortality attributed to BTV-1 were 4.5% and 0.7%, respectively, while in the epidemic that occurred at the same time due to the same serotype on the island of Lesbos, morbidity and mortality were approximately 30% and 15%, respectively.

Available serotyping data from strains of BTV in Greece indicated that it is not uncommon for two or more BTV serotypes to be identified in the same country, region or flock. It is difficult to accept that the multitude of BTV serotypes identified so far in Greece (i.e. serotypes 1, 4, 9 and 16) simply appeared out of nowhere and that these are self-restricted inside national boundaries. On the contrary, epidemiological reasoning strongly suggests that the situation of BT in Greece reflects the evolution and spread of the disease in the wider region and appears to be more complicated than elsewhere because of more stringent epidemiological surveillance. If this working hypothesis is valid, complete and accurate mapping of BTV serotypes circulating in Eastern Europe is an essential prerequisite to the design and implementation of efficient disease control/prevention policies and, therefore, should become one of the priorities of surveillance.

Another provisional finding of epidemiological significance emerging from the epidemics of BT in Eastern Europe between 1998 and 2002 is the potential implication of less efficient, but more abundant and widely occurring BTV vectors, notably *C. obsoletus* and *C. pulicaris*. Recent publications support this possibility (11, 20). Arguably, the recent identification of *C. imicola* in northern Greece, near the border with Bulgaria casts doubt on the exact vector species implicated in the Balkans and clearly warrants further vector monitoring in the region. In connection with vector monitoring, it is understood that BTV vectors are certainly a risk factor and merit close monitoring but do not signify the presence of BTV per se, as long as they are not infective. In this respect, the consistent presence of efficient BTV vectors in a disease-free/seroconversion-free region where sufficient numbers of naive susceptible animals are present, might be construed to imply absence of BTV circulation in this region.

Serological monitoring of sentinel animals is an indispensable component of epidemiological surveillance in areas at risk for either re-introduction or recurrence of BT, although the scheme must be correctly deployed and properly managed. Seroconversion in sentinels not only provides early warning of BTV circulation, but also may be used as an indicator for initiating early and targeted control/prevention measures, e.g. vector control in the vicinity. The success of such early measures depends largely on local conditions, such as species and abundance of vectors, climate and terrain but, under certain circumstances, this approach may provide the basis for an alternative prevention policy. This was probably the case in northern mainland Greece in 2001 and in southern Bulgaria in 2002, where seroconversions in sentinel animals were not followed by an epidemic, and serological evidence indicated that BTV did not persist either in the sentinel animals or in contiguous herds.

Random serological surveys in the livestock population are considered impractical and of little relevance when faced with an active epidemic but they may provide valuable information in the aftermath or between epidemics of BT. The value of such surveys includes the following:

a) helping to accurately delineate affected areas
b) evaluating the success of safeguard and control measures
c) providing insight to the probable evolution of BTV in areas at risk and to applicable surveillance and prevention measures. To qualify this last statement, where seroprevalence is low, clinical surveillance is a meaningful component of overall epidemiological surveillance, while where seroprevalence is high, the animals have already developed a natural, lasting and effective immune response to the particular BTV serotype(s) circulating in the region and, consequently, vaccination is superfluous.

Control measures must reflect the complex and elusive epidemiology of a vector-borne disease, such as BT, thus conventional control measures, such as culling of clinically affected animals, are of psychological and financial rather than of epidemiological significance. However, field experience gained in Greece suggests that vector control measures may have a beneficial effect in reducing BTV circulation and proliferation if applied in a timely, targeted and multi-level manner, i.e. at breeding sites, inside and around animal holdings and on individual animals. Safeguard measures, on the other hand, aim to prevent or reduce the spread of infection through movements of viraemic animals, their semen and embryos, and may be effective as long as they are strictly observed. Finally, with vaccines which are commercially available today, vaccination remains a controversial tool to control or eradicate the disease and its apparent value is limited to reducing direct losses and culling due to severe clinical symptoms.

Conclusions and recommendations

With the exception of the Greek islands of the eastern Aegean Sea, which are constantly at risk due to either the re-introduction or year-round persistence of *C. imicola*, and without prejudice to the situation in the northern Balkans and Turkey, it would appear that BTV has not become endemic and may even be diminishing in south-eastern Europe. The recession of the epidemic, however, is

no reason for complacency but should be a stimulus to enrich collective and shared knowledge and understanding of the complicated factors that influence the disease. Furthermore, a co-ordinated regional approach that will strengthen multilateral co-operation and ensure prompt dissemination of reliable information is an essential component for a meaningful disease control/prevention strategy.

To this end, the European Union is sponsoring three timely and relevant research programmes aiming, respectively, to achieve the following:

a) develop predictive models allowing identification of regions at risk and mapping of BTV vectors therein.

b) assess the safety and efficacy of existing live virus vaccines and develop inactivated whole-virus or sub-unit vaccines.

c) establish a database of BTV genome segments to allow tracing back of BTV incursions and to enable detection of live vaccine strain reversion to virulence.

Additional efforts must be made, however, to attract a broader participation in and a firmer commitment to these programmes.

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References

1. Anon. (1998). – Report on the incursion and evolution of bluetongue in Greece in 1998. Ministry of Agriculture, Directorate General of Veterinary Services, Department of Infectious Diseases, Athens.
2. Anon. (2002). – 2nd Annual Report: Bluetongue and other *Culicoides*-borne diseases threatening the EU. Ministry of Agriculture, State Veterinary Service, Sofia.
3. Anon. (1999). – Final report on the incursion and evolution of bluetongue in Greece in 1999. Ministry of Agriculture, Directorate General of Veterinary Services, Department of Infectious Diseases, Athens.
4. Anon. (1999). – Preliminary report on the incursion and evolution of bluetongue in Greece in 1999. Ministry of Agriculture, Directorate General of Veterinary Services, Department of Infectious Diseases, Athens.
5. Anon. (2000). – Report on the recurrence of bluetongue in Greece in 2000. Ministry of Agriculture, Directorate General of Veterinary Services, Department of Infectious Diseases, Athens.
6. Anon. (2001). – Report on the incursion and evolution of bluetongue in Greece in 2001. Ministry of Agriculture, Directorate General of Veterinary Services, Department of Infectious Diseases, Athens.
7. Anon. (2002). – 2nd Annual Report: Bluetongue and other *Culicoides*-borne diseases threatening the EU. Ministry of Agriculture, Directorate General of Veterinary Services, Athens.
8. Anon. (2003). – OIE Handistatus II database. OIE, Paris.
9. Boorman J.P.T. (1986). – Presence of bluetongue virus vectors on Rhodes. *Vet. Rec.*, **118**, 21.
10. Boorman J.P.T. & Wilkinson P.J. (1983). – Potential vectors of bluetongue in Lesbos, Greece. *Vet. Rec.*, **113**, 395-396.
11. Caracappa S., Torina A., Guercio A., Vitale F., Calabro A., Purpati G., Ferantelli V., Vitale M. & Mellor P.S. (2003). – Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *Vet. Rec.*, **153**, 71-74.
12. Mastroyanni M., Axiotis I. & Stoforos E. (1981). – Study of the first outbreak of bluetongue disease in sheep in Greece. *Bull. Hell. Vet. Med. Assoc.*, **32** (2), 138.
13. Mellor P.S. & Wittmann E.J. (2002). – Bluetongue virus in the Mediterranean Basin 1998-2001. *Vet. Rec.*, **164** (1), 20-37.
14. ProMED-mail (2001). – Bluetongue – Kosovo: OIE Report. Archive No. 20011012.2500, 12 October (promedmail.org/pls/askus/f?p=2400:1001:1049503 4095116438030::::f2400_p1001_back_page,f2400_p1 001_archive_number,f2400_p1001_use_archive:1202, 20011012.2500,y accessed on 23 June 2004).
15. ProMED-mail (2001). – Bluetongue – Yugoslavia. Archive No. 20011106.2750, 6 November (promedmail.org/pls/askus/f?p=2400:1202:1049503 4095116438030::no::f2400_p1202_check_display,f24 00_p1202_pub_mail_id:x,16508 accessed on 23 June 2004).
16. ProMED-mail (2001). – Bluetongue – Bulgaria, Greece, Macedonia: OIE Report. Archive No. 20011114.2800, 14 November (promedmail.org/pls/ askus/f?p=2400:1202:10495034095116438030::no::f2 400_p1202_check_display,f2400_p1202_pub_mail_id :x,16556 accessed on 23 June 2004).
17. ProMED-mail (2001). – Bluetongue, sheep – Croatia (Dubrovnik): suspected. Archive No. 20011223.3104, 23 December (promedmail.org/pls/askus/f?p=2400: 1202:10495034095116438030::no::f2400_p1202_chec k_display,f2400_p1202_pub_mail_id:x,17104 accessed on 23 June 2004).
18. ProMED-mail (2002). – Bluetongue, ruminants – Bosnia & Herzegovina, Bulgaria. Archive No.

20020906.5244, 6 September (promedmail.org/pls/askus/f?p=2400:1202:10495034095116z438030::no::f2400_p1202_check_display,f2400_p1202_pub_mail_id:x,19245 accessed on 23 June 2004).

19. ProMED-mail (2003). – Bluetongue – Yugoslavia: OIE. Archive No. 20030203.0297, 3 February (promedmail.org/pls/askus/f?p=2400:1202:10495034095116438030::no::f2400_p1202_check_display,f2400_p1202_pub_mail_id:x,20590 accessed on 23 June 2004).
20. Savini G., Goffredo M., Monaco F., de Santis P. & Meiswinkel R. (2003). – Transmission of bluetongue virus in Italy. *Vet. Rec.*, **152**, 119.
21. Takamatsu H., Mellor P.S., Mertens P.P.C., Kirkham P.A., Burroughs J.N. & Parkhouse R.M.E. (2003). – A possible overwintering mechanism for bluetongue virus in the absence of insect vectors. *J. Gen. Virol.*, **84**, 227-235.
22. Vassalos M. (1980). – Cas de fièvre catarrhale du mouton dans l'Ile de Lesbos (Grèce). XLVIIIth General Session, Report No. 2.11. *Bull. Off. Int. Épiz.*, (7-8), 547-555.