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## Experiences from the 2014 outbreak of bluetongue in Greece



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### ABSTRACT

Objective of this paper was to review relevant work and to present a general account of the bluetongue outbreak, which occurred in Greece in 2014. In total, 2895 outbreaks of the disease have been reported by the veterinary authorities of Greece; sheep, goats and cattle were affected with officially reported morbidity rates of 11.0%, 2.0% and 3.5%, respectively. No vaccinations were allowed and conservative measures were implemented to attempt to limit the disease, which at the end had expanded throughout the country. In field investigations, a significantly higher bluetongue morbidity rate (27.5%) in sheep has been reported. During that work, clinical anaemia was encountered, which was characterised as macrocytic, hypochromic, regenerative and non-haemolytic. Other investigations, which are reviewed in this paper, have described an outbreak of *Citrobacter freundii*-associated enteritis in newborn kids, offspring of goats subclinically infected with *Bluetongue virus*, increased rate of early embryonic deaths, reduced conception rates, increased incidence risk of mastitis and reduced milk yield in herds of subclinically-infected cattle and detection of the virus from hunter-harvested tissue samples of roe-deer. In 2015, vaccines against the disease have been licenced; vaccinations started in May 2015. Then, in 2015, only one outbreak of the disease was confirmed, which could have been the result of a combination of reasons acting concurrently to prevent further cases.

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## 1. Introduction

Bluetongue is a vector-borne viral disease of ruminants, which nevertheless affects clinically mainly sheep. Causal agent is the *Bluetongue virus*, which is classified in the genus *Orbivirus* (family Reoviridae) and includes 26 serotypes. The virus is principally transmitted by biting midges of the genus *Culicoides*.

In Europe, a significant outbreak of the disease was caused by serotype 8 of the virus, between 2006 and 2009. It occurred in north and west Europe, up to Scandinavia and Great Britain, with very severe financial losses (Kyriakis et al., 2015). Another extensive outbreak of the disease started in Greece and has occurred in

south-east European countries during 2014 and 2015 caused by serotype 4 of the virus (Kyriakis et al., 2015).

Objective of this paper is to review previously published evidence describing various facets of the disease in Greece and that way to present a general account of the outbreak. Appropriate updates have been included, in order to present information up to the end of January 2016. This is the first comprehensive description of the outbreak in Greece.

## 2. Bluetongue in Greece in 2014

At the end of May 2014, a case of bluetongue was clinically diagnosed in sheep in southern Peloponnese, over 400 km away from the nearest previously recorded case of the disease. Clinical diagnosis was confirmed at the official diagnostic laboratory of the State Veterinary Service, by using competitive ELISA and RT-PCR. Initial laboratory tests revealed that the outbreak was caused by a serotype 4 strain of the virus. The situation has been

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immediately reported to the international veterinary authorities (World Organisation for Animal Health, 2014).

By the end of June 2014, the disease had spread across the Peloponnese. The authorities opted to control the outbreak by (i) enforcing restriction of movements of animals across the country, (ii) instituting insecticide sprayings in the environment, especially directed at breeding sites of insects and (iii) recommending use of insect repellents in farms with susceptible livestock (Vasileiou and Fthenakis, 2014; World Organisation for Animal Health, 2014). Nevertheless, by end of August 2014, cases of the disease had been reported in most areas of mainland Greece and later, by end of November 2014, cases of the disease had also been reported in the islands of the country (World Organisation for Animal Health, 2014).

Cumulatively, 2895 outbreaks of the disease were reported (Tasioudi et al., 2015a,b). These involved sheep, goats and cattle, with reported morbidity rates of 11.0%, 2.0% and 3.5%, respectively. Reported case fatalities were 39.0%, 26.0% and 14.5% (World Organisation for Animal Health, 2014) (Table 1). Entomological studies reported that the principal vector of the virus was identified as *Culicoides obsoletus* (Tasioudi et al., 2015a).

### 3. Bluetongue in other countries of south-east Europe in 2014

In July 2014, cases of bluetongue were reported in Bulgaria (World Organisation for Animal Health, 2014). Thereafter, cases of the disease were reported in Albania, Croatia, Former Yugoslavian Republic of Macedonia, Hungary, Montenegro, Romania, Serbia and Turkey (Kyriakis et al., 2015; Niedbalski, 2015). In total, 7068 outbreaks were reported in all countries, which involved sheep, goats, mouflon, cattle, bison and roe deer (World Organisation for Animal Health, 2014, 2015; Kyriakis et al., 2015) (Table 2).

In Turkey, subsequently to an initial outbreak in August 2014, vaccinations of susceptible animals were applied and the outbreak was considered resolved; then, in October 2014, new outbreaks developed with vaccinations of susceptible animals applied again. In total, over 700,000 animals were vaccinated. In contrast, all other countries of the region did not opt to apply vaccinations metaphylactically and attempted to control the outbreak by other measures.

Full length sequencing of genome segment 2 and phylogenetic comparisons of virus indicated that isolates from samples collected in Greece or in Bulgaria shared a 99.9% nucleotide similarity between them (Mertens et al., 2014). Further comparisons showed that closest match within serotype 4 was with a strain isolated in Sudan in 1983 (94.2%–95.7% sequence similarity) (Mertens et al., 2014). In segment 4 of the virus, there was a more close relation with two serotype 2 strains isolated in Tunisia or Italy, respectively (98.8% sequence similarity). Based on the evidence, it was suggested that the causal strain of the outbreak was a reassortant strain with genome segments from lineages of serotype 1, 2 and 4 (Mertens et al., 2014). This would not be unusual in the *Bluetongue virus* family, where gene reassortment occurs frequently (Roberts et al., 2014).

### 4. Case studies during the outbreak in Greece

#### 4.1. Clinical investigations in sheep flocks in central Greece

Vasileiou and Fthenakis (2014) were the first to report, during the outbreak, investigations performed in four closely monitored sheep flocks located in central Greece. Methodology and initial results of the investigation have been reported by Vasileiou and Fthenakis (2014) and are reviewed herein, with an update of the findings also presented in this paper. The first cases were diagnosed

clinically in mid-August 2014. In all flocks, appropriate laboratory tests (competitive ELISA and/or RT-PCR) confirmed bluetongue caused by a serotype 4 strain of the virus. In these flocks with a total population of 560 sheep, 155 clinical cases were recorded, hence a morbidity rate of 27.5% (Table 3), which was significantly higher ( $P < 0.001$ ) than the overall morbidity rate reported throughout Greece (Table 1).

Consistent clinical signs in affected animals included anorexia and depression, nasal discharge, tachypnoea, salivation and frothing. Other signs frequently observed (in 50%–75% of affected animals) were haemorrhagic lesions on the lips and the buccal mucosa, abnormal auscultatory findings, fever (up to 42.5 °C) and clinical anaemia. Less frequent findings were abortion, locomotion disorders and regurgitation (Vasileiou and Fthenakis, 2014).

As a means of improving the general condition of the affected animals and providing some relief from the adverse effects of the disease, long-acting oxytetracycline and non-steroid anti-inflammatory agents were prescribed. Although these were of no curative action against the causal agent, often they appeared to contribute to minimise the adverse effects of potential secondary infections. Other measures that were implemented to limit the disease in flocks with affected sheep were the extended housing of affected animals, the provision of high-energy, soft type feeds and the regular external application of insect repellents. In fact, occasionally, some animals recovered fully from the disease (Vasileiou and Fthenakis, 2014).

#### 4.2. Investigations into the presence of anaemia in affected sheep

Vasileiou et al. (2015) have reported that during clinical examination of the animals in the flocks described above (4.1.) (Vasileiou and Fthenakis, 2014), they also observed frequently severe pallor of the mucous membranes ('clinical anaemia') in the affected sheep. They performed detailed investigation of 75 such clinical cases in the four sheep flocks; methodology has been presented by Vasileiou et al. (2015). During the examination of the mucous membranes of the eyes, they assigned one of the five scores available in the FAMACHA<sup>®</sup> system eye colour chart (Vatta et al., 2001; Papadopoulou et al., 2013), with scores ranging from '1' (=red, non-anaemic mucous membrane) to '5' (=white, severely anaemic mucous membrane); in order to avoid inter-observer error, clinical examination of the animals and score assignment was always performed by the same investigator, a principal author in the current paper (NGCV). Modal score was '4' (=pink-white, anaemic mucous membrane) and 87% of animals examined were assigned a score indicating anaemia (Fig. 1). In no case, jaundice or haematuria were evident during clinical examinations. Finally, no ticks were evident during the examination on any sheep examined.

The authors (Vasileiou et al., 2015) have reported that blood samples were collected for haematological examination from the above animals, as well as from clinically healthy sheep in the same flocks. Blood smears were prepared and evaluated for detection of morphological abnormalities and leucocyte differential count. A complete blood count was also performed by an automated haematological analyser (Abbott Cell-Dyn 3500 System; Abbott, USA) (Athanasidou et al., 2013). The following parameters were determined: haematocrit, erythrocyte count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, total leucocyte count and thrombocyte count. Total bilirubin concentration was measured by an automated biochemical analyser (Reflovet; Scil Diagnostics, Germany).

In sheep with clinical anaemia, values outside the reference ranges were recorded in the following parameters: erythrocyte count, haemoglobin concentration, mean corpuscular volume and mean corpuscular haemoglobin concentration. Detailed results of haematological examinations (Vasileiou et al., 2015), with

**Table 1**

Cumulative epidemiological data of the 2014 outbreak of bluetongue (serotype 4) in Greece; cases that occurred up to 31 December 2014 [data based on information from World Organisation for Animal Health (2014)] (Kyriakis et al., 2015).

	Animals in farms with reported outbreaks	Reported clinical cases	Reported morbidity rate	Reported fatalities	Reported fatality rate
Sheep	661,126	73,806	11.0%	28,861	39.0%
Goats	78,678	1,492	2.0%	385	26.0%
Sheep/Goats	410	25	6.0%	1	4.0%
Cattle	2,500	89	3.5%	13	14.5%

**Table 2**

Cumulative epidemiological data of the 2014 outbreak of bluetongue (serotype 4) in south-east European countries\*; cases that occurred up to 31 December 2014 [data based on information from World Organisation for Animal Health (2014, 2015)] (Kyriakis et al., 2015).

	Animals in farms with reported outbreaks	Reported clinical cases	Reported morbidity rate	Reported fatalities	Reported fatality rate
Sheep	1,191,903	103,454	8.5%	38,510	37.0%
Goats	136,494	1,713	1.5%	418	24.5%
Sheep/Goats	54,569	2,774	5.0%	531	19.0%
Mouflon	488	60	10.5%	47	96.0%
Cattle	70,792	1,764	2.5%	119	6.5%
European bison	12	2	16.5%	2	100.0%
Buffaloes	3	0	0.0%	0	
Roe deer	60	1	1.5%	1	100.0%
Red deer	20	0	0.0%	0	

**Table 3**

Cumulative epidemiological data of investigations in four flocks of sheep in central Greece during the 2014 outbreak of bluetongue (serotype 4) in Greece [data based on information from Vasileiou and Fthenakis (2014), updated with results of continuing investigations up to 31 December 2014].

	Animals in the flocks	Reported clinical cases	Reported morbidity rate	Reported fatalities	Reported fatality case
Sheep	560	155	27.5% <sup>a</sup>	53	34.0% <sup>b</sup>

<sup>a</sup>  $P < 0.001$  compared to respective national figure (details in Table 1).

<sup>b</sup>  $P = 0.218$  compared to respective national figure (details in Table 1).

**Table 4**

Haematological results in sheep affected with bluetongue that showed clinical anaemia (Vasileiou et al., 2015).

Haematological parametre	Mean ( $\pm$ s.e.) findings	Reference range
Haematocrit (%)	31.1 $\pm$ 1.5	25.0–45.0
Erythrocyte count ( $\times 10^6$ cells $\mu\text{L}^{-1}$ )	8.15 $\pm$ 0.25	10.0–15.0
Haemoglobin concentration (g $\text{dL}^{-1}$ )	8.79 $\pm$ 0.33	9.0–15.0
Mean corpuscular volume (fL)	38.16 $\pm$ 1.13	25.0–30.0
Mean corpuscular haemoglobin concentration (g $\text{dL}^{-1}$ )	29.18 $\pm$ 0.54	33.0–36.0
Total leucocyte count (cells $\mu\text{L}^{-1}$ )	9,703 $\pm$ 485	4,000–12,000
Neutrophil count (cells $\mu\text{L}^{-1}$ )	4,954 $\pm$ 291	600–6,000
Neutrophils (% of total leucocytes)	51.8 $\pm$ 3.2	11.0–47.0
Band neutrophil count (cells $\mu\text{L}^{-1}$ )	64 $\pm$ 22	
Band neutrophils (% of total leucocytes)	0.6 $\pm$ 0.2	
Lymphocyte count (cells $\mu\text{L}^{-1}$ )	3,958 $\pm$ 410	1,500–9,000
Lymphocytes (% of total leucocytes)	41.2 $\pm$ 2.9	41.0–83.0
Monocyte count (cells $\mu\text{L}^{-1}$ )	455 $\pm$ 90	
Monocytes (% of total leucocytes)	4.9 $\pm$ 0.9	0.0–13.0
Eosinophil count (cells $\mu\text{L}^{-1}$ )	122 $\pm$ 46	<1,000
Eosinophils (% of total leucocytes)	1.2 $\pm$ 0.5	0.0–15.0
Basophil count (cells $\mu\text{L}^{-1}$ )	5 $\pm$ 5	
Basophils (% of total leucocytes)	0.1 $\pm$ 0.1	0.0–3.0
Thrombocyte count (cells $\mu\text{L}^{-1}$ )	345,624 $\pm$ 38,297	180,000–750,000
Total bilirubin concentration (mg $\text{dL}^{-1}$ )	0.28	<0.40

s.e.: standard error of the mean.

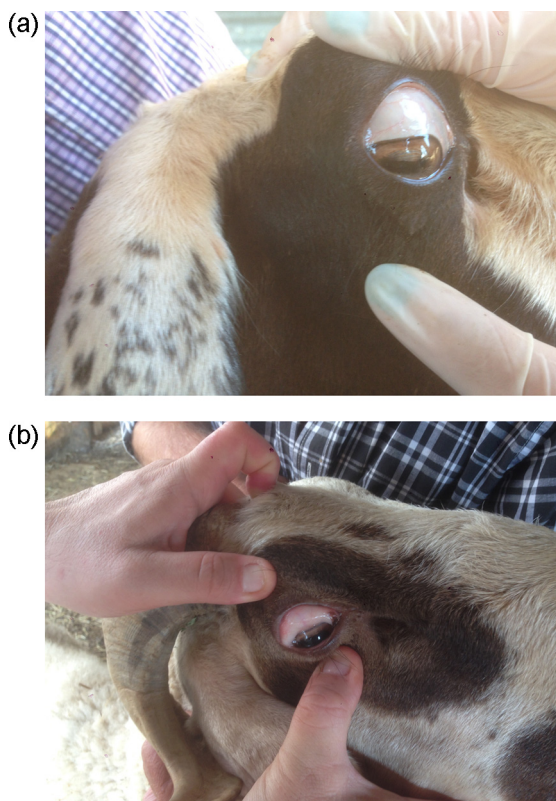
Sources for reference range of haematological results: Kramer (2000), Martin and Aitken (2000) and Roger (2008).

Mean values outside the reference range, are marked in red colour.

comparisons to suggested reference values (Kramer, 2000; Martin and Aitken, 2000; Roger, 2008) are in Table 4. In contrast, in clinically healthy sheep during the visit in the same flocks, from which samples were also tested, haematological parameters were always within the respective reference ranges. During examination of Giemsa-stained blood films, macrocytosis, main evidence

of regenerative anaemia, was observed. However, other indicators of regeneration, e.g., apolychromasia, presence of nucleated erythrocytes or Howell-Jolly bodies, were not detected. Further, no evidence of haemolysis (e.g., schistocytes) was observed, while the total bilirubin concentration was within the normal range. Based on the above findings, anaemia was classified as macrocytic,





**Fig. 1.** Severe pallor (clinical anaemia) of the mucous membranes in ewes affected with bluetongue (Vasileiou et al., 2015).

hypochromic, regenerative and non-haemolytic (Vasileiou et al., 2015).

Finally, appropriate laboratory examinations did not reveal presence of *Anaplasma* or *Babesia* infection in any of these animals, whilst in faecal samples mean counts for trichostrongylids were 285 epg (100–450), mean proportion of *Haemonchus contortus* larvae was 7.8% (3.5%–13.0%) and mean counts of *Dicrocoelium dendriticum* were 17.5 epg (10–70) and of *Fasciola hepatica* 3.5 epg (0–5) (Vasileiou et al., 2015).

#### 4.3. Investigations into cases of enteritis associated with *Citrobacter freundii* in kids born from subclinically infected dams

Chatzopoulos et al. (2015b) have reported that increased incidence risk (~65%) of diarrhoea was recorded among newborn kids (younger than 5 days) in a goat herd in central Greece. Accompanying clinical manifestations of the disorder included sudden loss of appetite, depression and evidence of abdominal pain; faeces were white-coloured, often blood-tinted or frank haemorrhagic; further, neurological signs (intense muscle tremors, nystagmus, blindness, ataxia, torticollis) were also seen. Increased fatality rate (~40%) was recorded, although kids that did not develop neurological signs and survived the acute phase of the disease, often recovered fully after 5–7 days (Chatzopoulos et al., 2015b).

The authors (Chatzopoulos et al., 2015b) have reported that faecal samples had been collected from kids with diarrhoea (n = 30) or from healthy individuals (n = 10) and were subsequently examined by appropriate bacteriological, parasitological and virological techniques. Samples from kids with diarrhoea yielded consistently *Citrobacter freundii* in heavy growth, in mixed culture with smaller populations of *Clostridium* spp. and/or *Escherichia coli*, but with no other pathogens (e.g., *Cryptosporidium*, *Giardia*, *Rotavirus*, *Adenovirus*, *Coronavirus*), whilst samples from healthy individuals



**Fig. 2.** Milk content in the stomach and intestinal tract of kids with diarrhoea likely associated with *C. freundii* infection (Chatzopoulos et al., 2015b).

yielded only *Clostridium* spp. and *E. coli*, findings which indicate a likely role of *C. freundii* in causation of the disease (Chatzopoulos et al., 2015b).

Gross pathological examination of dead animals revealed increased milk content in the stomach and intestinal tract, with evidence of only mild inflammatory lesions (Fig. 2). In blood samples collected from the affected kids (7/10) and their dams (19/20), increased antibody titres against *Bluetongue virus* were evident. In contrast, no evidence of infection by *Small Ruminant Lentivirus* (virus or antibodies) was recorded in any blood sample (doe or kid) (Chatzopoulos et al., 2015b).

#### 4.4. Investigations into effects in performance of subclinically infected cows

Chouzouris et al. (2015) have reported the results of investigations performed in four dairy herds in central or northern Greece, in close proximity with populations of small ruminants. Total number of cows in those herds was 903 animals. The investigations were performed as part of routine monitoring of the herds, which included pregnancy diagnosis [by means of laboratory tests (Paré et al., 2008) and clinical examination], evaluation of milk production records and blood testing for viral diseases (including bovine virus diarrhoea, infectious bovine rhinotracheitis and bluetongue).

The authors (Chouzouris et al., 2015) have reported that no clinical signs characteristic of bluetongue had been observed in any cattle in the farms. In general, morbidity rate in three of these herds had not increased compared to previous periods; in one herd only, incidence of clinical mastitis increased abruptly during the study period (6% versus 2.5% in the immediately preceding period). In total, in 36 of the 60 cows sampled among animals of the four herds, increased antibody titres against *Bluetongue virus* were evident (Chouzouris et al., 2015).

Compared to those in the preceding and following periods, during the bluetongue incursion period, conception rates in all herds decreased to <27% and remained at such reduced rates for 4–5 months. In one herd, increased rates of early embryonic losses were also recorded; however, no significantly increased abortion rates were evident in any herd. Further, milk yields of cows decreased in all herds up to 8.5%. Detailed results of performance of animals in the herds studied are in Table 5 (Chouzouris et al., 2015).

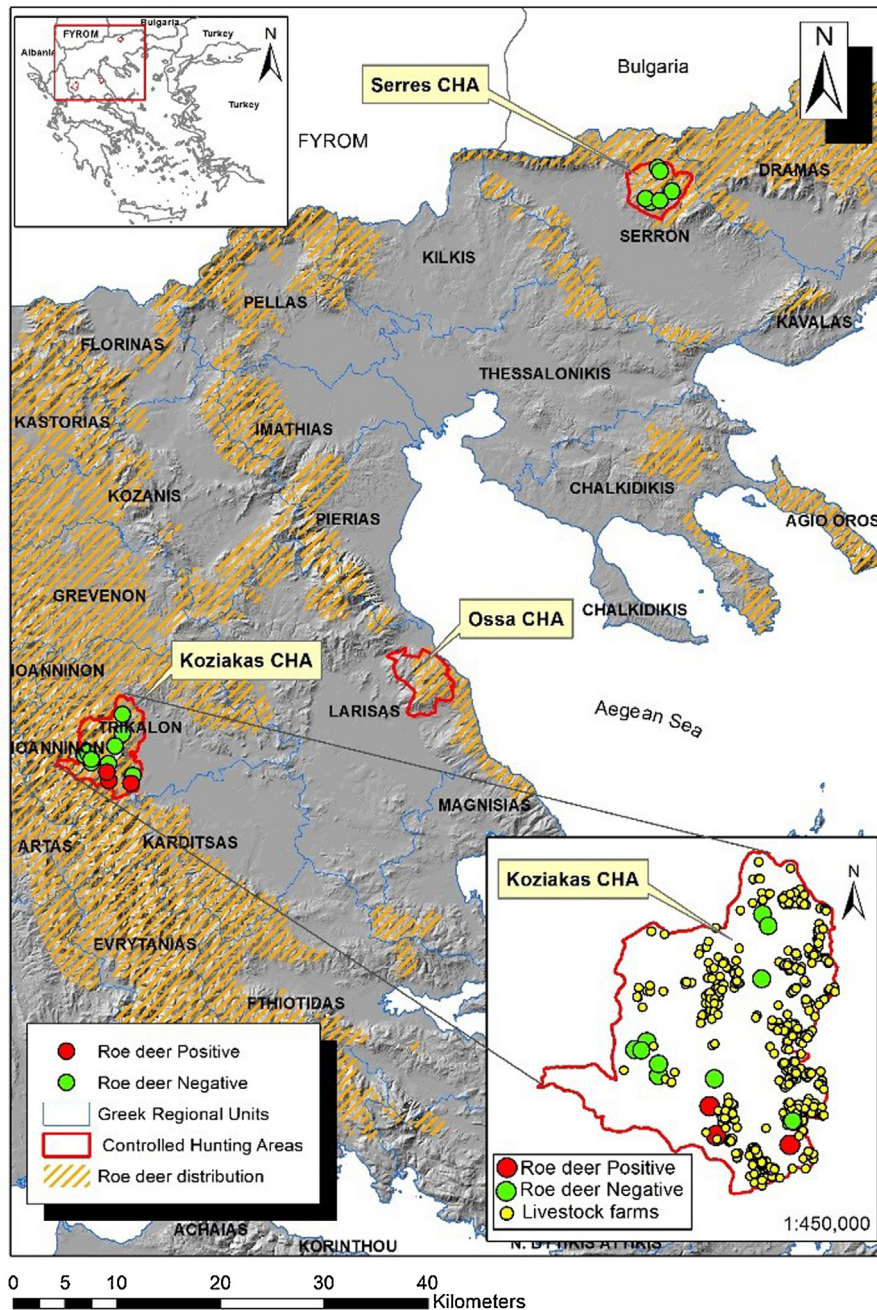
#### 4.5. Investigations into infection of wild cervids with Bluetongue virus

Chatzopoulos et al. (2015a) have described an investigation into infection by *Bluetongue Virus* in wild cervids in Greece during the period of the outbreak. Samples of spleen and whole blood were collected from hunter-harvested roe deer during the period September to November 2014. In total, 19 samples, 13 from the

**Table 5**  
Findings in production parameters in four herds of dairy cows in central or northern Greece, subclinically infected with bluetongue (Chouzouris et al., 2015).

	2015								2016
	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	
Mean conception rates (%) (4 herds)	33.3 <sup>a</sup>	36.4 <sup>a</sup>	25.1 <sup>a</sup>	17.6 <sup>b</sup>	22.4 <sup>b</sup>	17.2 <sup>b</sup>	29.6 <sup>a</sup>	36.8 <sup>a</sup>	
	33.9 <sup>a</sup>	30.9 <sup>a</sup>	26.4 <sup>a</sup>	15.1 <sup>b</sup>	18.9 <sup>b</sup>	17.1 <sup>b</sup>	26.4 <sup>a</sup>	31.5 <sup>a</sup>	
	30.4 <sup>a</sup>	24.3 <sup>a,b</sup>	21.7 <sup>b</sup>	22.6 <sup>a,b</sup>	22.1 <sup>b</sup>	31.3 <sup>a</sup>	29.4 <sup>a,b</sup>	36.5 <sup>a</sup>	
	24.2 <sup>a</sup>	25.3 <sup>a</sup>	26.5 <sup>a</sup>	16.0 <sup>b</sup>	17.4 <sup>b</sup>	25.8 <sup>a</sup>	30.7 <sup>a</sup>	28.7 <sup>a</sup>	
Rates (%) of early embryonic losses (1 herd)	7.4 <sup>a</sup>	8.8 <sup>a</sup>	24.0 <sup>b</sup>	36.8 <sup>b</sup>	30 <sup>b</sup>	30.1 <sup>b</sup>	10.3 <sup>a</sup>	8.3 <sup>a</sup>	
Mean daily milk yield (L animal <sup>-1</sup> ) (4 herds)			26.0 <sup>a</sup>	26.3 <sup>a</sup>	25.6 <sup>a</sup>	26.8 <sup>a</sup>	27.8 <sup>a,b</sup>	29.0 <sup>b</sup>	
			31.3 <sup>a</sup>	28.9 <sup>a</sup>	27.0 <sup>b</sup>	26.4 <sup>b</sup>	29.4 <sup>a</sup>	31.2 <sup>a</sup>	
			30.5 <sup>a,b</sup>	31.0 <sup>a,b</sup>	29.2 <sup>a</sup>	31.2 <sup>a,b</sup>	31.5 <sup>a,b</sup>	32.2 <sup>b</sup>	
			28.8 <sup>a,b</sup>	27.1 <sup>a,b</sup>	26.4 <sup>a</sup>	26.3 <sup>a,b</sup>	28.6 <sup>a,b</sup>	30.3 <sup>b</sup>	

<sup>a,b</sup>  $P < 0.05$  for values marked with different superscripts within a row.



**Fig. 3.** Map of Greece with details of the areas, from where samples from roe deer have been collected for *Bluetongue Virus* detection (Chatzopoulos et al., 2015a). Green/red dots indicate points of collection of negative/positive samples, respectively. The Koziakas hunting area, where positive samples have been collected, is zoomed in and yellow dots indicate livestock farms in the area (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).



Koziakas hunting area and 6 from the Serres hunting area (Fig. 3) were tested for presence of *Bluetongue Virus* RNA, by using RT-PCR. In total, three samples collected in the Koziakas hunting area were found positive. Subsequently, presence of *Bluetongue virus* nucleic acid was confirmed by sequencing. In the general area, where samples were collected, there were 44,215 sheep and goats in 478 farms, as well as 5,949 cattle (Chatzopoulos et al., 2015a). The results of that study indicated the exposure of roe deer to *Bluetongue Virus* in Greece and confirmed the implication of wildlife species in the outbreak currently ongoing in the country (Chatzopoulos et al., 2015a).

## 5. Bluetongue in the region in 2015 and 2016

### 5.1. Bluetongue in Greece

In early 2015, the regulatory authorities in Greece have licenced for use in ruminants in Greece the following three vaccines: (i) BTVPUR AISap 4 inj. sol. for sheep (Merial, France), (ii) BLUEVAC-4 for sheep and cattle (CZ Veterinaria, Spain) and (iii) ZULVAC 4 (Zoetis, USA). The vaccines have become available commercially at the end of May 2015.

In 2015, only one confirmed case of bluetongue (serotype 4) was recorded in Greece. The incident developed in the island of Rhodes and involved 7 goats in a small farm with ruminants (sheep, goats, cattle). Clinical diagnosis was confirmed at the official diagnostic laboratory of the State Veterinary Service, by using competitive ELISA and RT-PCR. Initial laboratory tests revealed that the outbreak was caused by a serotype 4 strain of the virus. The situation has been immediately reported to the international veterinary authorities (World Organisation for Animal Health, 2015).

### 5.2. Bluetongue in other countries of Europe

During 2015, cases of bluetongue have also been recorded in other countries of the region, specifically in Austria, Croatia, Hungary, Slovenia, Romania and Turkey. In total, 75 outbreaks were reported in all countries (99% fewer than in 2014), which involved sheep, goats and cattle (World Organisation for Animal Health, 2015,2016) (Table 6).

In Turkey, vaccinations of susceptible animals were again applied and the outbreak was considered resolved. In response to the outbreak, vaccinations were also implemented in Croatia and Hungary, whilst in Greece vaccinations had already started before the outbreak. In contrast, Austria, Romania and Slovenia did not opt to apply vaccinations and attempted to control the outbreak by other measures.

Finally, in January 2016, the disease has been reported in Austria (in cattle) and Georgia (in sheep).

## 6. Discussion

In Greece, bluetongue is endemic in the islands of the eastern part of the Aegean sea, adjacent to the Asian coast. In the past, incursions of the disease caused by various serotypes (1, 4, 8, 9, 16) of the virus have occurred in the country (Billinis et al., 2001; Giovannini et al., 2004; Gómez-Tejedor, 2004; Nomikou et al., 2004; Kyriakis et al., 2015), but never before with as rapid and extensive dissemination and severity as recorded during the 2014 outbreak.

The outbreak of 2014 had been an extremely serious event, similar to which the agricultural sector in Greece had not faced for many years. The disease spread quickly and caused very significant losses primarily in sheep. Field experience (e.g., Vasileiou and Fthenakis, 2014) coupled with anecdotal evidence from veterinarians around

the country indicated that many sheep farmers underwent tremendous damages in their animal populations and production.

After the first cases of the disease in the Peloponnese, control of the outbreak had been attempted by limiting insect vector activity, to be succeeded by the use of insecticides and/or insect repellents. Unfortunately however, this approach did not limit expansion of the disease to other regions of Greece, well beyond the area of the initial outbreak, as well as to neighbouring countries. Similar approaches were undertaken by other countries in the region (bar Turkey). Ultimately, the disease had spread widely and significantly, over 1500 km from the initial outbreak in southern Peloponnese to northern Romania and Hungary (World Organisation for Animal Health, 2014), favoured by the warm weather conditions, which supported flight activity of the vectors and the consequent transmission of the virus. One may possibly consider that the vaccinations performed in susceptible livestock in Turkey contributed to limiting the disease at the initial stages, soon after the first cases of the disease were diagnosed in that country.

In clinical investigations, it has been found that bluetongue morbidity rate in the sheep flocks studied was significantly higher (Vasileiou and Fthenakis, 2014) than the national average reported to the World Organisation for Animal Health (2014,2015). Therefore, one may suggest that under-reporting by farmers and field veterinarians has occurred. Possible reasons for this can be (i) fears of farmers for complications after reporting disease cases, e.g. reduced support payments, social reactions in small communities or legal requirements for disposal of dead animals, (ii) uncertainty from the part of those involved that declaration of cases to the authorities would lead to any improvement of the situation, (iii) under-staffing of the veterinary service or even (iv) fatigue of those involved consequently to the long duration of the outbreak.

During field work, clinical anaemia has been often recorded in affected animals, which was characterised as macrocytic, hypochromic, regenerative and non-haemolytic. Clinical anaemia had rarely been reported as associated with the disease. In the past, only Balaro et al. (2014) have reported this finding in cases of the disease caused by serotype 4 of the virus. In fact, Dercksen and Lewis (2007) have described that absence of anaemia can be used diagnostically for clinical differentiation of bluetongue from other diseases manifested with oedema in the head, e.g., *H. contortus* or *F. hepatica* infections. As this outbreak seems to have been caused by a novel, reassortant strain of the virus (Mertens et al., 2014), the findings confirm the many clinical facets of the disorder, which may differ accordingly to the causative serotype or even strain of the virus. Presence of macrocytic erythrocytes with no other evidence of regenerative anaemia has been described in horses or cats (Thrall, 2004). In the latter species, the findings have been associated with potential viral effect on bone marrow (Munoz, 2005); indeed, *Bluetongue virus* has been found to be able to destroy haemopoietic stem cells (Rodriguez-Calvo et al., 2014). Findings in such cases would be compatible with the results of haematological tests (Vasileiou et al., 2015). This would also explain absence of all other findings related to regenerative anaemia (e.g., polychromasia). Moreover, the virus causes endothelial injury (Maclachlan et al., 2009): changes in affected vessels include endothelial hypertrophy with perivascular oedema and/or mild haemorrhages, which would further account for the reduced number of erythrocytes. Maclachlan et al. (2009) expressed the opinion that, possibly, *Bluetongue virus* may have similarities with viruses causing haemorrhagic diseases (e.g., *Ebola virus*) in causing endothelial damage and vascular injury and damage. Possibly, the extent of these lesions can be accentuated by release of host-mediated vasoactive molecules, as has been reported in other haemorrhagic viral diseases (Hoenen et al., 2006; Aleksandrowicz et al., 2008).

Chatzopoulos et al. (2015b) were first to report enteritis in newborn kids associated with *C. freundii*, although in the past,

**Table 6**

Cumulative epidemiological data of the 2014 outbreak of bluetongue (serotype 4) in European countries\*; cases that occurred during the year 2015 [data based on information from World Organisation for Animal Health (2015, 2016)].

	Animals in farms with reported outbreaks	Reported clinical cases	Reported morbidity rate	Reported fatalities	Reported fatality rate
Sheep	1,927	11	0.5%	2	18.0%
Goats	152	7	4.5%	4	57.0%
Cattle	8,365	120	1.5%	0	0.0%

the disorder had been reported in calves (Ambrosim et al., 2002) and puppies (Galarneau et al., 2003). In general, this organism is considered to be an opportunistic pathogen, which causes clinical disease under particular circumstances (e.g., in immunocompromised individuals). The organism exerts its pathogenic action after aggregative adherence onto the gastrointestinal mucosa, where it multiplies. In general, mechanisms of infection are similar to those of enteropathogenic strains of *E. coli* (Bai et al., 2012); production of Shiga-like and/or heat stable toxins by some strains has also been reported (Guarino et al., 1987). *C. freundii* may also invade into the central nervous system, leading to development of neurological signs by affected individuals, but the relevant mechanisms are not fully understood, although it has been suggested that they were related to blood-brain barrier permeability and intracellular proliferation; the organism has a predilection for the meninges, which explains the characteristic neurological signs (Badger et al., 1999).

Immunosuppression associated with *Bluetongue virus* infection has been well documented (Maclachlan et al., 2009; Umeshappa et al., 2010) and may lead to increased incidence of secondary bacterial infections in affected animals. The extended viraemia, the replication of the virus in lymphoid cells and the endothelial damage and vascular injury caused by the virus lead to malfunctions of hematopoietic organs, including bone marrow and spleen. This might have contributed to the severity of disease observed in kids infected with *C. freundii*, as possibly infected and immunocompromised dams might have not provided to their kids qualitatively adequate colostrum, which led to reduced protection against an opportunistic pathogen.

Increased incidence of mastitis in a cow herd might also be the consequence of immunosuppression as the direct effect of *Bluetongue virus* infection. A potential hypothesis is that the effect of the virus in lymphoid cells of the hosts could have compromised the lymphoid follicles present at the border between teat duct – teat cistern. In fact, in previous studies, it has been established that these defensive structures of the udder (Mavroggianni et al., 2005) are compromised in cases of viral infections, thus predisposing animals to mastitis (Mavroggianni et al., 2006).

Infection of cattle with *Bluetongue virus* frequently remains sub-clinical (Maclachlan, 1994), although clinical cases, even fatalities, due to bluetongue have been reported (Parsonson, 1993), as was also recorded in the current outbreak (Tables 1, 2 and 6). Reproductive failure can be another adverse effect of the disease, likely occurring as a direct foetopathic effect of the virus (Vanroose et al., 2000; Daniel Givens and Marley, 2008). However, the main financial losses in cases of bovine bluetongue are caused by the significant reduction of milk production, as has been recorded during the outbreak in Greece by Chouzouris et al. (2015). This drop was evident three to four months after likely infection of the animals (Chouzouris et al., 2015).

Detection of the virus in wild ruminants (Chatzopoulos et al., 2015a) indicated that new reservoirs of the virus should also be monitored. Wild deer, especially red deer, which is most relevant to *Bluetongue virus* infections, presents several features, due to which, they might be appropriate host of the virus (Chapman et al., 1993; Grego et al., 2014). Red deer live in populations with increased density and often at the same altitude with livestock. They are animals

larger than sheep or goats, with life habits and feeding habitats similar to domesticated small ruminants, hence exposed to bites of insects (Acevedo et al., 2008; Apollonio et al., 2010). Therefore, they can be infected to a similar extent as domestic ruminants, which indicates that systematic surveillance can be significant for monitoring and prediction of possible re-emergence. These findings also point out to a requirement to change surveillance of the disease. In fact, bluetongue has been confirmed in wild cervids prior to diagnosing it in domestic ruminant species (Garcia et al., 2009; Lorca-Oro et al., 2011), a finding that may be used as an early marker of an upcoming outbreak. In endemic regions, seropositivity rates in wild cervids were similar to those in domestic ruminants (Ruiz-Fons et al., 2014).

Despite the very high bluetongue morbidity rate in 2014, significantly fewer (<99%) cases of the disease have been reported in 2015. There may be a combination of reasons for this: (i) increased antibody titres in animals infected during 2014, which might have provided protection, (ii) immunoprotection of susceptible animals, which had been vaccinated subsequently to licencing appropriate immunological products, and (iii) reduced numbers of infected vectors, as the result of widespread spraying of insecticides in the spring and early summer of the year. Nevertheless, there is a need to set up effective surveillance for the disease. It is noteworthy that in 2015, in France, new cases of bluetongue caused by serotype 8 of the virus have been diagnosed (World Organisation for Animal Health, 2015), only some years after the country had been declared free from the previous incursion of the disease.

## 7. Concluding remarks

An outbreak of bluetongue (serotype 4) started in Greece in May 2014. No vaccinations were allowed and the disease spread throughout the country and beyond within three months. Features of the disease have been described and the current paper reviews and combines the previous work, providing an overall account of the outbreak of the disease in Greece. Presence of clinical anaemia, which had rarely been associated with the disease in the past, is noteworthy. The disease had further repercussions, including disorders caused by unusual pathogens and adverse effects in production of subclinically infected animals. In the spring of 2015, vaccines have been licenced. Very few new cases of the disease have been officially reported to the international authorities in 2015.

## Conflict of interest

The authors have nothing to disclose.

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