Small Ruminant Research 142 (2016) 61–68

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Experiences from the 2014 outbreak of bluetongue in Greece

N.G.C. Vasileiou[a], G.C. Fthenakis[a,*], G.S. Amiridis[a,1], L.V. Athanasiou[a,1], P. Birtsas[b,1], D.C. Chatzopoulos[a,1], T.M. Chouzouris[a,1], A. Giannakopoulos[a,1], K.S. Ioannidi[a,1], S.N. Kalonaki[a,1], A.I. Katsafadou[a,1], C.S. Kyriakis[c,1], V.S. Mavrogianni[a,1], E. Papadopoulos[d,1], V. Spyrou[b,1], G. Valiakos[a,1], A.P. Venianaki[a,1], C. Billinis[a]

[a] Veterinary Faculty, University of Thessaly, Karditsa, Greece
[b] Technological Education Institute of Thessaly, Larissa, Greece
[c] College of Veterinary Medicine, University of Georgia, Athens, GA, USA
[d] School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 10 February 2016
Accepted 13 February 2016
Available online 22 February 2016

Keywords:
Anaemia
Balkan peninsula
Bluetongue
Citrobacter
Culicoides
Goat
Greece
Sheep
Vaccination

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.

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

* Corresponding author.
E-mail address: gcf@vet.uth.gr (G.C. Fthenakis).
[1] These authors have contributed equally to the manuscript and their names are listed alphabetically.

http://dx.doi.org/10.1016/j.smallrumres.2016.02.010
0921-4488/

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 reasssortment 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© system eye colour chart (Vatta et al., 2001; Papadopoulos 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) (Athanasiou et al., 2013). The following parametres 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 parametres: erythrocyte count, haemoglobin concentration, mean corpuscular volume and mean corpuscular haemoglobin concentration. Detailed results of haematological examinations (Vasileiou et al., 2015), with