Survey for contagious caprine pleuropneumonia in goat herds in the Thrace region of Turkey

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Ü. Özdemir [(1)], M.A. Türkyılmaz [(1)], O. Sayı [(1)], Ş.H. Erpek [(1)] & R.A.J. Nicholas [(2)*]

(1) Pendik Veterinary Control Institute, Pendik, 81480 Istanbul, Turkey

(2) The Oaks, Nutshell Lane, Farnham, Surrey GU9 0HG, United Kingdom

*Corresponding author: robin.a.j.nicholas@gmail.com

Summary

Contagious caprine pleuropneumonia (CCPP) was detected for the first time on the European continent in the Thrace region of Turkey in 2002 following outbreaks of an unusually severe respiratory disease in goats. *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp), the causative agent, was identified in many infected herds throughout the region by serological, bacteriological and molecular biological techniques. However, as no quantitative data on the prevalence and distribution of the disease have been gathered in the intervening years, the extent of infection is unknown. Consequently, in 2014, a random sample of 2,400 goats was drawn from a population of over 167,000 goats in the region by multistage sampling. Serum samples were collected and tested by a monoclonal antibody based competitive enzyme-linked immunosorbent assay (cELISA). The overall prevalence of CCPP in Thrace was found to be just over 8%. The prevalence in the individual provinces was approximately 18%, 7%, 8% and 6% for Çanakkale, Edirne, Kırklareli and Tekirdağ, respectively, while Istanbul province appeared to be CCPP-free. The

results showed that CCPP has spread throughout the region and has become endemic, and may pose a potential risk to the neighbouring countries of Greece and Bulgaria. Mccp was regularly detected from lung samples of suspect goats until 2015 but since then there has been no further detection from clinical samples.

Keywords

Competitive enzyme-linked immunosorbent assay – Contagious caprine pleuropneumonia – Goat – Polymerase chain reaction – Seroprevalence – Thrace – Turkey.

Introduction

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subspecies (subsp.) *capripneumoniae* (Mccp), is one of the most important goat diseases, causing high morbidity and mortality. It has been listed by the World Organisation for Animal Health (OIE) because of the severe socio-economic impact it has on goat farmers (1). Morbidity and mortality rates can reach 100% and 80%, respectively, when the disease is introduced to a new area containing herds not previously exposed (2). Contagious caprine pleuropneumonia occurs in the Middle East, parts of Asia and many northern and eastern African countries (3). It is, however, believed to be underreported because of the fastidiousness of the agent and the need for well-equipped laboratories and experienced personnel to make the diagnosis (3).

Although widespread in many parts of Anatolia (4), CCPP was first reported on European soil during severe outbreaks of caprine respiratory disease in 2002 in the Thrace region of Turkey (Fig. 1). This area is a buffer zone for livestock diseases, including foot and mouth disease, where animal movements are restricted because of its proximity to the European Union (EU) (5). Molecular typing of isolates from outbreaks in Thrace indicated that the source of infection was probably the Turkish mainland or closely situated Turkish islands (3).

Insert Figure 1 here

Since its first detection the Pendik Veterinary Control Institute, Istanbul has been detecting the mycoplasma annually across the region, but the exact distribution of the disease in the region is unknown because clinical signs of CCPP can sometimes resemble those of other respiratory diseases. It is therefore difficult to estimate the extent of the threat that it poses to the goat population in Thrace and neighbouring countries as well as the economic losses it causes. The objective of this work was to determine the prevalence of CCPP in Thrace and to record the results of diagnostic testing by the Mycoplasma Department in Pendik.

Materials and methods

Study design

The total goat population of the five provinces of Thrace (Fig. 1) was estimated to be 167,039 at the time of the study in 2014 by the Statistics Agency of the Republic of Turkey. Over half of the goats were found in the provinces of Erdine and Kirklari which are closest to the EU borders. *Capra hircus* are managed in herds of 100–500 in a semi-extensive way and largely comprise the Anatolian black breed known locally as Kıl Keçisi or Kilgoat; the adults are kept for milk and fibre while kids up to six months of age provide meat.

The sample size was calculated for an anticipated prevalence of 50%, confidence level of 95% and absolute precision of 2% using OpenEpi software (version 2.3). A multistage sampling method was used: in the first stage 119 villages were randomly selected, and in the second stage 20 goats were randomly selected from each village. Clinical signs were not reported.

Collection of sera and competitive enzyme-linked immunosorbent assay

About 5–10 ml of blood was collected from the jugular vein of each selected goat; sera were transferred to microcentrifuge tubes and kept at –20°C until tested.

A commercial competitive enzyme-linked immunosorbent assay (cELISA) kit for the detection of Mccp antibodies (IDEXX, Montpellier, France) was used and interpreted according to the manufacturer's instructions. An inhibition value of 55% was used as the cut-off between positive and negative samples.

Data analysis

The apparent prevalence was calculated from the cELISA results. Prevalence estimates were weighted by taking into consideration the multistage sampling plan (6).

Mycoplasma detection

Lung and pleural fluid samples from goats in Thrace presenting respiratory signs were sent to the Mycoplasma Department of the Pendik Veterinary Control Institute. For Mccp detection, the samples were cultured in H25P mycoplasma medium as previously described (7). After two to three days, culture fluids were tested by polymerase chain reaction (PCR) using the method described by Woubit *et al.* (8). For other mycoplasmas, including *M. mycoides* subsp. *capri*, *M. ovipneumoniae* and *M. arginini*, samples were cultured in Hayflick's medium and identified by growth inhibition testing using hyperimmune specific antisera (9).

Results

Seroprevalence

The estimated sample size was determined as 2,367, however 2,400 animals were actually sampled and represented 1.44% of the goat population of Thrace. Of these, 212 were found to be positive. True prevalence for the whole Thrace region was estimated to be just over 8%. Prevalence in the individual provinces was approximately 18%, 7%, 8% and 6% for Çanakkale, Edirne, Kırklareli and Tekirdağ, respectively, while the small number of goats in Istanbul province appeared to be CCPP-free (Table I).

Insert Table I here

Mycoplasma detection

The results of mycoplasma detection using the culture–PCR method on samples received from Thrace were as follows:

- in 2013, 45 lung samples were received for PCR testing, of which 19 (42.2%) were positive by Mccp PCR
- in 2014, 67 suspected lung samples were received, of which 24 samples (35.8%) were positive by PCR
- in 2015, 74 samples were received but none was positive by PCR, although other mycoplasmas were detected, including 1 *M. m. capri*, 1 *M. ovipneumoniae*, 2 *M. agalactiae* and 11 *M. arginini*
- no positive results were recorded in 2016 for Mccp from 38 lung samples and 41 serum samples, or in 2017 from 35 lung samples and 2 serum samples.

Discussion

The outbreaks of CCPP in the Thrace region on the continent of Europe in 2002 were unexpected because the importation of livestock into this region is strictly controlled owing to the proximity to EU countries, namely Bulgaria and Greece. The only previous report of CCPP in Europe occurred in Greece in the 1920s following the seizure of goats from Turkey (10). However, it is not possible to substantiate this as CCPP because accurate laboratory diagnosis was not available at that time.

There is no evidence available to date to indicate that the recent outbreaks of CCPP have spread from Thrace to neighbouring countries because no severe outbreaks of respiratory disease have been reported in goats in Greece and Bulgaria over the last few years. However, CCPP may not be easy to detect because the ubiquitous but less pathogenic *M. m. capri* can sometimes cause similar clinical signs and lesions. Moreover, *M. m. capri* grows rapidly in most culture media and is therefore relatively easy to identify, unlike the fastidious

Mccp (11). Vaccination against diseases caused by *M. m. capri* is carried out annually in some parts of Thrace but although both Mccp and *M. m. capri* are members of the *M. mycoides* cluster cross-reactions are rarely seen (2). Furthermore, blood sampling for the present survey took place at least six months after *M. m. capri* vaccination.

The present study shows serological evidence of CCPP in most provinces of Thrace, with almost 18% seropositivity in Çanakkale, a province closest to the Turkish islands and Turkish mainland. It should be noted that, although the Istanbul province appears free of disease, this area contains very few goat herds.

Interestingly, until 2014, Mccp was regularly detected in lung samples from suspect goats; however, no detections have been made over the last few years, although only small numbers of clinical samples have been submitted to the diagnostic laboratory for PCR. Recent field evidence from local veterinarians also suggests that the classical clinical signs of CCPP are now less obvious in the field (E. Özdemir, personal communication). These factors indicate that CCPP has become endemic within the goat population in Thrace, with low levels of overt clinical disease. The problem arises when these carrier animals come into contact with naïve goats with no immunological experience of CCPP. This was seen, with catastrophic consequences, when refugees returning from Sudan following the civil war in Eritrea introduced their animals to the CCPP-free native goat population in the 1990s, leading to high morbidity and mortality (12). Consequently, extra vigilance is required in countries neighbouring Thrace for any possible incursions of CCPP.

The endemic nature of CCPP means that eradication of the disease from Thrace will now be extremely difficult and will require a larger, more comprehensive serological survey to enable the identification of all infected herds. If such a study confirms the extensive nature of CCPP then vaccination, which has not yet been used in the region, should be considered as a less expensive and more long-lasting means

of control than using antibiotics, which also brings the risk of antimicrobial resistance.

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Table I
Seroprevalence of contagious caprine pleuropneumonia in the provinces of Thrace, Turkey

Province	No. positive/No. tested	Prevalence (%)*
Çanakkale	76/520	17.96
Edirne	73/1,020	7.36
İstanbul	0/40	0.00
Kırklareli	56/700	8.23
Tekirdağ	7/120	5.91
Total	212/2,400	8.26

* Prevalence estimates were weighted by taking into consideration the multistage sampling plan

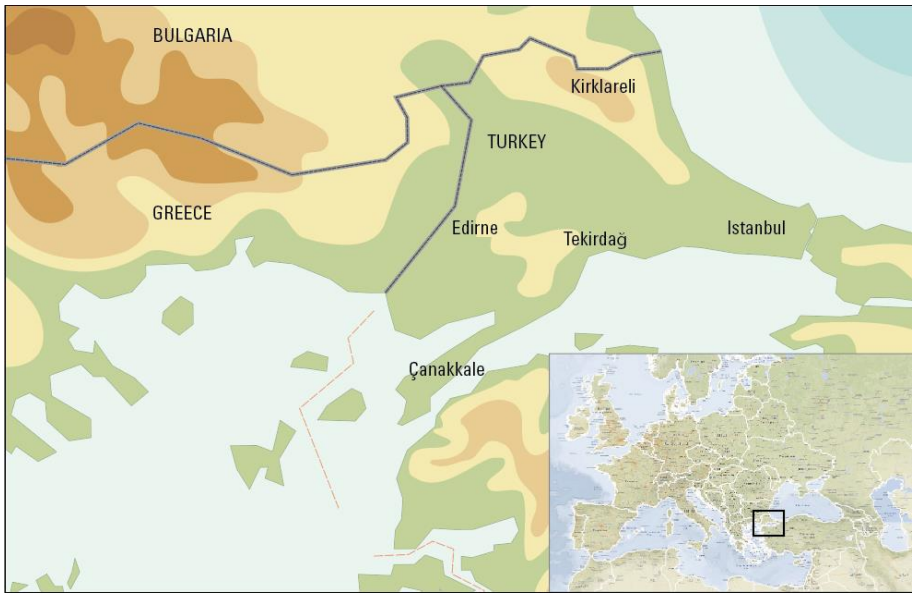

Fig. 1

Map showing the five provinces of Thrace, Turkey and its position in Europe