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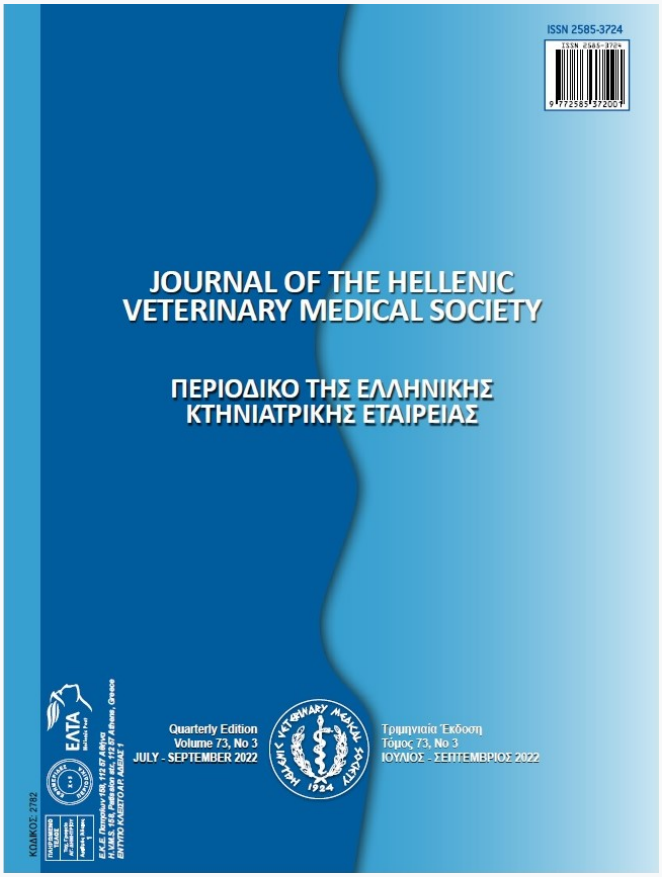

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Research article
Ερευνητικό άρθρο

Serological survey of *Chlamydia abortus* in Greek dairy sheep flocks

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ABSTRACT: Chlamydiosis due to *Chlamydia abortus* is one of the most common causes of abortion in small ruminant flocks worldwide. Although the causative agent is zoonotic, chlamydiosis is not a reportable disease. There is lack of recent data concerning sheep chlamydiosis in Greece.

In the current study, a serological investigation for *Chlamydia abortus* was conducted. Blood samples from 26 randomly selected sheep flocks not vaccinated against chlamydiosis were collected. From each flock, 15 to 20 blood samples were taken from adult female sheep. In total 464 blood samples were examined. One hundred and six samples were positive (22.8%), while 24 samples (5.24%) were doubtful. Moreover, at farm level, in 18 out of the total number of 26 farms, at least one positive animal was detected (69.2%).

Chlamydiosis is considered a highly likely cause of sheep abortion in Greece. Therefore, vaccination of the sheep flocks is strongly recommended for the prevention and control of the disease.

***Keywords*:** sheep, *Chlamydia abortus*, serology, zoonoses, enzootic abortion

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INTRODUCTION

Chlamydia abortus is the causative agent of enzootic abortion in ewes. It causes abortions at the last month of gestation or delivery of weak lambs or stillbirths (Sargison, 2008). Sometimes there is retained placenta with or without ewe mortality (Mamlouk et al., 2020). Infection in rams can cause orchitis, seminal vesiculitis and infertility (Aitken and Longbottom, 2007; Radostits et al., 2007). The disease is therefore the cause of important economic losses worldwide in sheep related meat and dairy industry. Specifically, the annual losses caused by the disease in the United Kingdom during the early 90's were estimated to be about 15-20 million £ (Radostits et al., 2007). About 47% of ovine abortions in Scotland for the early 2019 were attributed to chlamydiosis (SRUCVS 2019). *Chlamydia abortus* is also a concern for public health as it is a zoonotic agent; in humans it causes abortion or a para-influenza syndrome (Mobini et al., 2002; Sargison, 2008).

Although chlamydiosis is an important clinical condition, the existing epidemiological studies in Greece are few, relatively old and of local interest. This short communication aims to investigate the *C. abortus* seroprevalence in different geographical areas of Greece. Chlamydial abortions are controlled in ewe flocks by hygienic measures and vaccination. Licensed vaccines are not designed to differentiate vaccinated from infected animals and thus *C. abortus* circulation cannot be accurately investigated in vaccinated farms by means of serological tests (O'Neill et al., 2018). For this reason, the flocks recruited by this study were not vaccinated against chlamydiosis.

MATERIALS AND METHODS

Animals and Sampling

The study was carried out from March 2019 until August of the same year. During this period 464 blood samples were collected from 26 randomly selected sheep flocks located in different geographical areas of Greece. The only criteria that were used for the recruitment were: i) the tested flocks had never been vaccinated against chlamydiosis and ii) the sampled animals were >3 years old ewes. The selected flocks were distributed as: four (4) in Thrace (north-eastern Greece), ten (10) in Macedonia (northern Greece), five (5) in Thessaly (central Greece), three (3) in Peloponnese (southern Greece) and four (4) in Crete (southern Greece).

Blood samples were collected from 14-21 ewes per flock during morning hours by jugular venipuncture with vacuum tubes (BD Vacutainer®-CAT). Sera were separated by centrifugation at 3,000 rpm for 20 minutes. All sera samples were stored in plastic vials and kept frozen (-18°C) until they were tested.

Sample examination

The antibody response against *C. abortus* was evaluated by using the indirect enzyme-linked immunoassay (ELISA) test kit CIVTEST® RUMI CHLAMYDIA (Laboratorios HIPRA S.A., Spain) following the manufacturer's instructions. The Relative Index x 100 (IRPC) was calculated to interpret the results. Sera samples were stated as positive (greater or equal to 40), suspicious (between 30 and 40) or negative (less than or equal to 30) depending on their IRPC levels.

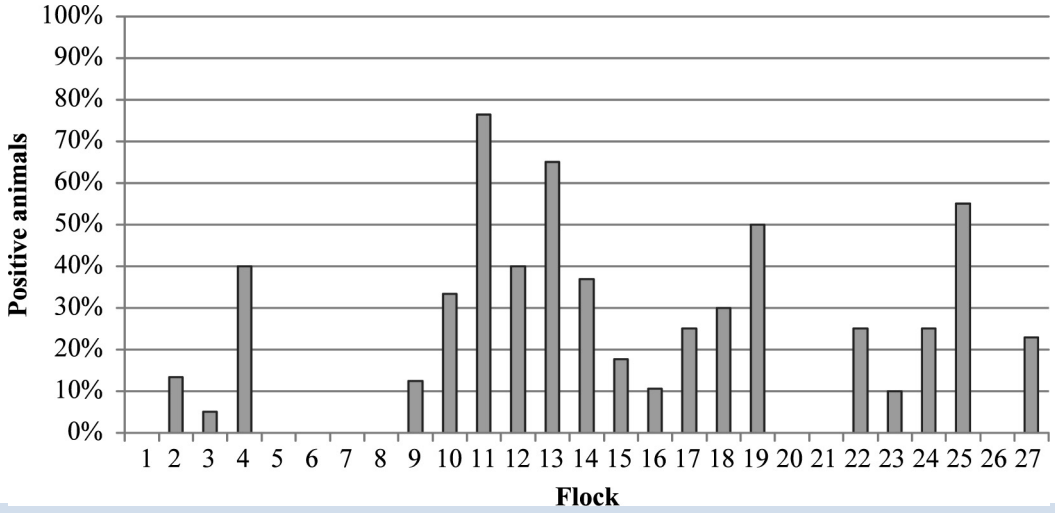

Figure 1. Percentage of *C. aborus* seropositive animals in the studied flocks.

RESULTS

The examination of the antibody response in the sera samples showed that 69.23% of the flocks (n=18) showed positive ewes (at least one), 3.84% of the flocks (n=1) showed just a suspicious ewe, and 26.92 % of the flocks (n=7) showed negative ewes (Figure 1). The percentage of positive ewes per flock was ranging between 5% and 76.47% with an estimated 95% confidence interval of 21.73-41.67%, when just positive flocks were considered.

When the entire sera sample collection was considered, 22.84% (n=106) were stated as positive, 5.24% (n=24) suspicious and 71.86% (n=334) were stated as negative (Table 1). The level of antibodies against *C. abortus* of positive ewes were ranging between 40 and 93 IRPC and it showed an average of 57.81 IRPC (95%CI: 55.25-60.37 IRPC).

Table 1. Data of the examined flocks

Area (Flocks)	Flocks' size*	Examined animals	Positive
Thrace (4)	1,000	81	10
Macedonia (10)	2,800	177	76
Thessaly (5)	2,500	99	18
Peloponnese (3)	700	54	12
Crete (4)	1,600	53	0

*Estimated by the farmers. 172

DISCUSSION

Abortions are considered as an important issue in sheep flocks worldwide, mainly due to economic losses from lamb loss, decrease of replacement stock and milk production (Giadinis, 2012). Chlamydiosis, due to *C. abortus*, is a significant cause of abortion in sheep and goats and less common (sporadic) in cows and deer (Aitken and Longbottom, 2007). Moreover, a report of epizootic abortion in llamas has been published by Nietfeld in 2001.

The main routes of sheep infection due to *C. abortus* are the oral and respiratory route. Infected placenta and vaginal excretions, as well as aerosol in infected flocks are the main sources of the bacterium spreading. The elementary bodies are very resistant in the environment, especially in low temperatures and humidity. The disease usually is introduced into the flocks with the entrance of new replacement stock from infected flocks. Moreover, the wild ruminants seem to play an important role in the epidemiology of the disease (Radostits et al., 2007; Qin et al., 2014; Mamlouk et al., 2020).

Sheep can be infected by two (2) *Chlamydia* species. *Chlamydia abortus*, that can cause abortion, retained placenta, stillbirths and orchitis and *C. pecorum*, that can cause conjunctivitis, pneumonia, encephalomyelitis, arthritis and diarrhoea or the infected animals can remain asymptomatic (Tsakos et al., 2001a; Mobini et al., 2002). The latest is not considered zoonotic (Nietfeld, 2001).

Of course, it is always useful to identify the causative agent of chlamydiosis at the species level. The golden standard to distinguish *C. abortus* from *C. pecorum* remains the use of PCR analysis (Bommana et al., 2019). Unfortunately, this is not always applicable since it requires the presence of the antigen at the blood sample or the shipping of biohazardous biological material from, in most cases, inaccessible areas to diagnostic labs equipped with biosafety level 3 facility. For the above reasons, in the present study a specific for *C. abortus*, according to the manufacturer, ELISA kit has been selected. The last decades, there is a consistent trend for improvement of ELISA in terms of specificity concerning *C. abortus* detection (Tsakos et al., 2001b; McCauley et al., 2010; Mamlouk et al., 2020). Other serological methods like complement fixation test (CFT) and indirect haemagglutination assay (IHA), are less specific and sensitive than ELISA, but some of them i. e. IHA remains cheaper (Qin et al., 2014). In any case, serological methods are useful diagnostic tools mainly for flock screening, while molecular methods should be applied for diagnosis at animal level (Nietfield et al., 2001).

Vaccination as well as the enzootic abortion disease caused by *C. abortus* generally produce an antibody response in ewes (Garcia-Seco et al 2016, O'Neill et al 2018), which is not distinguishable by currently available serological tests. For this reason, flocks vaccinated against enzootic abortion were not recruited by the present study. A previous epidemiological study about *C. abortus* prevalence in China showed that older sheep were more likely to be seropositive against the pathogen, probably as consequence of infection (Qin et al. 2014). Based on this finding and considering that it has been described that some animals may not seroconvert after infection (O'Neill et al 2018), ewes older than 3 years were sampled in the present study; these animals were expected describe more precisely the exposure of the flock to *C. abortus*.

In the present study, 69.2% of the examined flocks

had at least one seropositive animal, while 22.9% of the animals were found seropositive and in 5.24% the results were doubtful to *C. abortus*. The prevalence of chlamydiosis recorded in the present study was like that of a recent study in Turkey (Malal et al., 2020), that reported a seropositivity of 63 % at flock level and 25% at individual animal level. In the study of Tsakos et al (2001a) in Greece, 82% of the tested flocks and 20% of the tested animals were found seropositive, a finding that resembles that of Iran, where 84% of the flocks and 24% of the animals were tested positive (Esmaeli et al., 2015). In Tunisia, 58% of the flocks and 6.6% of the animals have been tested positive in a recent study (Mamlouk et al., 2020), while in China a much lower percentage (18.65%) of serum samples were positive in a past study (Qin et al., 2014). Surprisingly, the prevalence in Costa-Rica in individual sheep was low (5.4%), without a satisfactory documentation by the authors (Villagra-Blanco et al., 2015).

The variability of the proportion of positive ewes per flock widely ranged between 5% and 74%, suggesting that the prevalence of the pathogen might variate widely depending on the flock; unfortunately, it was not possible to correlate these values with the disease or with the management practice of the farms to determinate whether there was any important influencing factor. Despite this, it was estimated that the proportion of seropositive animals of flocks was 21.73-41.67%. Based on this, it can be inferred that in the worst-case scenario between 1 and 2 over 5 ewes per flock might have had at least one abortion during their previous 3 years of life.

In conclusion, the epidemiological data of this study showed that *C. abortus* is still highly circulating in Greek sheep flocks. It was estimated that *C. abortus* still represents a great risk for the health of the sheep flocks and the production of the farms in Greece. Based on this and considering that *C. abortus* can be transmitted to and cause disease in other species like goat and even humans, the implementation of vaccination schemes and hygienic measures in terms of biosafety and biosecurity is strongly recommended.

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