ORIGINAL ARTICLE

Serological prevalence of *Coxiella burnetii* in dairy goats and ewes diagnosed with adverse pregnancy outcomes in Greece

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Abstract

Introduction. *Coxiella burnetii* is an obligatory intracellular bacterial pathogen causing the zoonotic disease Q fever. The most common reservoirs of *C. burnetii* are wild mammals, birds and ticks. Pregnant domestic ruminants infected with this bacterium are also a major source of human infection.
Materials and method. The serological prevalence of *C. burnetii* in goats and sheep diagnosed with adverse pregnancy outcomes was assessed by undertaking a survey on 800 dairy goats and 800 dairy ewes reared in four different regions of Greece (Macedonia, Thrace, Thessaly, and Peloponnese). A stratified sampling was carried out, taking also as a criterion the age of the animals. Serum antibodies were analyzed by a commercial ELISA according to the manufacturer's recommendations.
Results. Generally, there was a statistically significantly higher serological prevalence of *C. burnetii* (14.4%) in goats compared to sheep (8%). Serological prevalence was higher in adults (15.5% in goats and 8.5% in sheep) compared to yearlings (7.4% in goats and 4.6% in sheep). The prevalence increased significantly with age only in goats. Finally, all animals reared in Peloponnese had a prevalence significantly higher (21% in goats and 18% in sheep) than animals reared in the other three regions.
Conclusion. To the best of the authors' knowledge, this is the first report that associates *C. burnetii* with reproductive disturbances of domestic ruminants in Greece. However, considering the importance of coxiellosis for public health, further investigations are required on its epidemiology regarding abortion, premature delivery, stillbirth and weak offspring in small ruminants, as well as in other domestic and wild animal species.

Key words

Coxiella burnetii, reproduction, dairy sheep, dairy goats

INTRODUCTION

Coxiella burnetii (*C. burnetii*) is a small, short pleomorphic and obligate intracellular Gram-negative coccobacillus causing the zoonotic disease Q fever. The most common reservoirs of *C. burnetii* are wild mammals, birds and ticks. The dominant mode of transmission to healthy animals is through direct contact with excretions of infected animals or through tick bites [1]. In domestic ruminants, *C. burnetii* infection is often asymptomatic. However, infection of pregnant animals is associated with adverse pregnancy outcomes, such as abortion, premature delivery, stillbirth and weak offspring. The environment is contaminated by infected placentas at the time of parturition, leading to the persistence of the bacterium for a long time [2]. Aerosols from infected placentas, secretions or excretions of animals are a major source of transmission to humans [3]. Infected

humans develop Q fever, a zoonosis characterized by an acute form, flu-like symptoms and a chronic form with severe complications such as pneumonia, hepatitis and endocarditis [4].

Culture and molecular detection of *C.burnetii* are only available in reference laboratories. However, serology is considered a simple, cheap and accurate method for the diagnosis of coxiellosis in humans and animals via the detection of specific IgM and IgG antibodies [5]. The presence of *C. burnetii* in asymptomatic small ruminant populations has been known in Greece since 2008 when a survey showed a seroprevalence of 10.4% in sheep (n=554) and 6.5% in goats (n=61) [6].

OBJECTIVE

The aim of the study was to determine the serological evidence of coxiellosis in dairy ewes and goats in Greece diagnosed with adverse pregnancy outcomes, considering the scarcity of relative data and the role that such data can play in developing preventive measures for public health.

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Annals of Agricultural and Environmental Medicine 2017, Vol 24, No 4

703

George Filioussis, Alexandros Theodoridis, Dimitrios Papadopoulos, Athanasios I Gelasakis, Sotiria Vouraki, George Bramis, Georgios Arsenos. Serological prevalence…

MATERIALS AND METHOD

The study was carried out between January 2014 – June 2015 in four different regions of Greece that host the largest national population of sheep and goat flocks. The regions were Macedonia, Thrace, Thessaly, and Peloponnese. Dairy sheep and goat farming is an important activity for these regions with socio-economic, environmental and cultural impact. A population of 200 dairy goats and 200 dairy ewes per region were included in the study. Following communication with local veterinarians in each region, the criteria for including animals in the study was clinical diagnosis of any of the following adverse pregnancy outcomes: abortion, premature delivery, stillbirth and weak offspring (known as APSW complex). All animals diagnosed with any of the latter were subjected to blood sampling within 10–15 days at the latest. Blood samples were collected from the jugular vein in designated tubes of 8 ml (Greiner Bio-One, Kremsmunster, Austria), using a 20 G needle (Greiner Bio-One, Kremsmunster, Austria). The collected blood samples were classified into two groups: yearling animals (<2 years old) and adult animals (>2 years old). After blood sampling, the tubes were left at ambient temperature for 30 min and then stored in a portable fridge (icebox). All blood samples were centrifuged at local veterinary laboratories within 6 h at 2,500 g for 15 min; the serum samples obtained were stored at -20 °C.

Serum samples were sent to the Laboratory of Microbiology and Infectious Diseases of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece. The serum antibody titres against $C.$ $burnetii$ were assayed using a Q fever indirect ELISA kit (ELISA Cox kit, Laboratoire Service International, Lissieu, France), according to the manufacturer's instructions. Briefly, the serum samples were incubated at room temperature for 30 min and then diluted in a 1:20 sample diluent. In order to detect total immunoglobulin G antibodies (IgG), 100 μl of the diluted samples were placed on commercial ELISA plates coated with a mixture of $C.burnetii$ phases I and II antigen. After overnight incubation at 4 °C, the plates were washed with a washing buffer. Finally, 100 μl of horseradish peroxidase (HRP) conjugated antibody was added and the absorbance read using an ELISA reader at a wavelength of 450 nm. Negative and positive controls were always included while examining serum samples. The manufacturer had previously validated the ELISA kit estimating sensitivity (Se) of 100% and specificity (Sp) of 95%, respectively. The criterion for considering a serum sample as positive was based on ELISA value calculated as follows: ELISA value = 100 x (Absorbance of test sample – Absorbance value of negative control)/ (Absorbance value of positive control – Absorbance value of negative control).

Serum samples with indices ≤40 were considered negative, whereas samples with indices >40 were considered positive. The differences of IgG between sheep and goats, age classes and regions were statistically assessed by Chi-square tests. Rogan-Gladen correction (RGC) according to the sensitivity and specificity of the ELISA test was calculated [7] using EpiTools epidemiological calculators [8]. Calculating the 95% confidence interval for each of the proportions assessed statistical uncertainty.

RESULTS AND DISCUSSION

A summary of ELISA test results on the presence of $C.burnetii$ antibodies in animals' serum that exposed clinical signs of APSW complex is presented in Table 1. In detail, ELISA test showed in total $C.$ $burnetii$ seroprevalence of 14.4%, CI: 12.1–17% (RGC: 9.9%, CI: 7.3–12.4%) in goats and 8%, CI: 6.3–10.1% (RGC: 3.2%, CI: 1.2–5.1%) in sheep. Similar studies dealing with epidemic reproductive disorders have indicated that $C.$ $burnetii$ seroprevalence ranges between 31–93% in goat flocks and 9–25% in sheep flocks [9]. Furthermore, in the presented study, statistically significant differences were found between goat and sheep seroprevalences (χ^2=16.36, p-value=0.000≤0.05). The latter result can be attributed to the intrinsic susceptibility of goats to coxiellosis that is clinically manifested with abortion and/or reproductive problems [10].

ELISA seroprevalence-age associated patterns differed between the two ruminant species tested in the present study (Tab. 1). Seroprevalence of $C.$ $burnetii$ appeared to be higher in adults goats (15.5%, CI: 13–18.3%, RGC: 11%, CI: 8.2–13.8%) than in yearlings (7.4%, CI: 3.8–13.9%, RGC: 2.5%, CI: -2.7–7.7%), as well as in adults sheep (8.5%, CI: 6.7–10.8%, RGC: 3.7%, CI: 1.5–5.9%) than in yearlings (4.6%, CI: 2–10.4%, RGC: <0.0). Differences, however, were statistically significant only for goats (χ^2=16.36, p-value=0.001≤0.05). Similar age-related $C.$ $burnetii$ serological patterns in sheep and goats that exposed clinical signs of APSW complex have been reported previously [11]. Such results indicate that females used as replacements (less than 1 year old) are probably less exposed to the pathogen until the lambing period when the agent shows higher exposure rates due to increased excretion [12]. Moreover, as a consequence of a higher probability of contact, pathogen contact rate increases with age, a feature observed mainly in goats.

The region of herd origin was also indicated as a significant risk factor for seropositivity (Tab. 1). The lowest seroprevalence was observed in goats (11%, CI: 7.4–16.1%, RGC: 6.3%, CI: 1.8–10.9%) and sheep (3.5%, CI: 1.7–7%, RGC: <0.0), reared in Macedonia. Additionally, the highest seroprevalence was observed in goats (21%, CI: 15.9–21.2%, RGC: 16.8%, CI: 10.9–22.8%) and sheep (18%, CI: 13.9–23.9%, RGC: 13.7%, CI: 8.1–19.3%) reared in Peloponnese.

Finally, significant differences were found between regional seroprevalence, both in sheep (χ^2=36.82, p-value =0.000≤0.05) and goats (χ^2=10.02, p-value=0.018≤0.05). The latter was expected since the Greek sheep farming sector is characterized by a diversity of production systems, ranging from extensive, pastoral and semi-extensive to intensive. Specifically, the extensive production systems that are situated mainly in mountainous areas, influence the transmission of $C.$ $burnetii$ in small ruminants through wildlife/livestock contact or through arthropod vectors.

In the presented study, ELISA was used as a diagnostic tool because compared to other serological techniques its reliability is not limited to the high variability of $C.$ $burnetii$ excretion by animals throughout the year. During recent decades, different serological techniques, such as indirect fluorescence assay (IFA), complement fixation tests (CFT) and ELISA, have been employed worldwide to detect $C.$ $burnetii$ antibodies. In most cases, contact of this pathogen at the population level was screened by a highly sensitive test; positive samples were confirmed by using a highly special tool. In contrast, there has been no need for any

704   **Annals of Agricultural and Environmental Medicine 2017, Vol 24, No 4**

George Filioussis, Alexandros Theodoridis, Dimitrios Papadopoulos, Athanasios I Gelasakis, Sotiria Vouraki, George Bramis, Georgios Arsenos. Serological prevalence…

Table 1. Seroprevalence of *Coxiella burnetii* in dairy ewes and dairy goats with adverse pregnancy outcomes in Greece

Animal	class	Peloponnese			Thessaly			Macedonia			Thrace			Total		
		(+)*	Prevalence (95% CI§)	RGC†- Prevalence (95% CI)	(+)*	Prevalence (95% CI§)	RGC†- Prevalence (95% CI)	(+)*	Prevalence (95% CI§)	RGC†- Prevalence (95% CI)	(+)*	Prevalence (95% CI§)	RGC†- Prevalence (95% CI)	(+)*	Prevalence (95% CI§)	RGC†- Prevalence (95% CI)
Goat	Young	5/48	10.4 (4.5-22.2)	5.7 (-3.4-14.8)	2/24	8.3 (2.3-25.8)	3.5 (-8.1-15.1)	0/22	0 (22)	0.0 (0-14.9)	1/14	7.1 (1.3-31.5)	2.3 (-11.9-16.5)	8/108	7.4 (3.8-13.9)	2.5 (-2.7-7.7)
	Adults	37/152	24.3 (18.2-31.7)	20.4 (13.2-27.5)	25/176	14.2 (9.8-20.1)	9.7 (4.3-15.1)	22/178	12.4 (8.3-18.0)	7.7 (2.7-12.8)	23/186	12.4 (8.4-17.9)	7.8 (2.8-12.7)	107/692	15.5 (13.0-18.3)	11.0 (8.2-13.8)
	Subtotal	42/200	21.0 (15.9-21.2)	16.8 (10.9-22.8)	27/200	13.5 (9.4-18.9)	8.9 (4.0-13.9)	22/200	11.0 (7.4-16.1)	6.3 (1.8-10.9)	24/200	12.0 (8.2-17.2)	7.4 (2.6-12.1)	115/800	14.4 (12.1-17.0)	9.9 (7.3-12.4)
Sheep	Young	4/36	11.1 (4.4-25.3)	6.4 (-4.4-17.2)	1/27	3.7 (0.7-18.3)	<0.0‡	0/33	0 (33)	0.0 (0-10.4)	0/12	0.0 (0.0-24.2)	<0.0	5/108	4.6 (2.0-10.4)	<0.0‡
	Adults	32/164	19.5 (14.2-26.2)	15.3 (8.9-21.7)	10/173	5.8 (3.2-10.3)	0.8 (-2.8-4.5)	7/167	4.2 (2.0-8.4)	<0.0	10/188	5.3 (2.9-9.5)	0.3 (-3.0-3.7)	59/692	8.5 (6.7-10.8)	3.7 (1.5-5.9)
	Subtotal	36/200	18.0 (13.3-23.9)	13.7 (8.1-19.3)	11/200	5.5 (3.1-9.6)	0.5 (-2.8-3.9)	7/200	3.5 (1.7-7.0)	<0.0	11/200	5.0 (2.7-9.0)	0.0 (-3.2-3.2)	65/800	8.0 (6.3-10.1)	3.2 (1.2-5.1)

*(+) - Seropositives; §CI - confidence interval; †RGC - Rogan-Gladen corrected;
‡true prevalence estimates less than zero are not consistent with assumed sensitivity and specificity values, indicated by <0.0

Annals of Agricultural and Environmental Medicine 2017, Vol 24, No 4

705

George Filioussis, Alexandros Theodoridis, Dimitrios Papadopoulos, Athanasios I Gelasakis, Sotiria Vouraki, George Bramis, Georgios Arsenos. Serological prevalence…

further confirmation of the results in this study since the validated commercial ELISA kit used had high sensitivity and specificity.

CONCLUSION

To the best of the authors' knowledge, this is the first report that associates *C. burnetii* with reproductive disturbances of domestic ruminants in Greece. Considering the importance of coxiellosis for public health, further investigations are required on its epidemiology regarding APSW syndrome in small ruminants, as well as in other domestic and wild animal species. Such studies should focus on the isolation of the pathogen from animals with adverse pregnancy outcomes, as well as genomic evaluation of the isolates. As Q fever can be a fatal disease, professionals working in the health system need to regularly monitor and report the occurrence of the pathogen. In particular, the results of the presented study suggest that domestic ruminants with reproductive disturbances should be checked for the presence of *C. burnetii*.

Acknowledgements

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