

PREVALENCE OF *COXIELLA BURNETII* ANTIBODIES IN WILD RUMINANTS IN KAVEČANY ZOO, KOŠICE, EASTERN SLOVAKIA

Erik Dorko¹, Kvetoslava Rimárová¹, Emil Pilipčinec², Milan Trávniček³

¹Department of Public Health, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovak Republic

²Department of Microbiology and Immunology, University of Veterinary Medicine, Košice, Slovak Republic

³Department of Epizootiology and Infectious Diseases, University of Veterinary Medicine, Košice, Slovak Republic

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Abstract: *Coxiella burnetii* is an obligate intracellular agent that causes Q fever in humans and animals. The most important reservoirs of *C. burnetii* in nature are small wild rodents, but infection was also demonstrated in other animals, including ruminants. Samples of blood were obtained from 4 mouflons, 60 fallow deer, 9 Cameroun goats, 8 Carpathian goats, and 8 Cameroun sheep living in a zoo. Antibodies to phase I and phase II *C. burnetii* antigens were determined in sera by ELISA. Antibody titres were detected in the range 1:100–1:200. The serum prevalence of phase II and phase I antibodies to *C. burnetii* antigens was 25% and 0% in mouflons, 70% and 0% in goats, 37.5% and 12.5% in sheep and 28.3% and 5% in fallow deer, resp. Serologic diagnosis of Q fever in animals can be difficult. Some animals may shed *C. burnetii* and pose a risk for infection prior to the development of antibodies, and some infected animals never seroconvert. The employed ELISA test is a very sensitive assay for *C. burnetii*, but it is also a labour intensive method and therefore not routinely available.

Address for correspondence: Prof. Erik Dorko, MD, PhD, Department of Public Health, Faculty of Medicine, Šrobárova 2, 041 80 Košice, Slovak Republic.
E-mail: erikdorco@pobox.sk

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INTRODUCTION

Coxiella burnetii is an obligate intracellular agent that causes Q fever in humans and animals [20]. This microorganism has two different antigens corresponding with different stages of the agent related to infection of animals or the adaptation to cell host. Phase I antigens are isolated from animals or from parasites. Phase II antigens are found in organisms that have been passed serially in embryonated eggs [33]. The serological response in acute infections is mainly against phase II cells, whereas in chronic infection there is a serological response to phase I and II antigens [17].

The most important reservoirs of *C. burnetii* in nature are small wild rodents, but infection was also demonstrated in insectivores, lagomorphs, carnivores, ungulates,

ruminants, marsupials, monkeys, bats, birds, and even reptiles and fish [8, 28, 29]. Antibodies against *C. burnetii* have been detected among many other wildlife species, including snowshoe hares, moose, white-tailed deer in Nova Scotia, wild Dall sheep in Alaska, black bears in Idaho and California, coyotes, foxes, skunks, raccoons, and rabbits [3, 18, 19, 22, 34]. However, cattle, sheep and goats are the main sources of human infection. Infected animals shed highly stable bacteria in urine, faeces, milk, and through placental and birth fluids [16, 23, 26, 27].

The organism is able to survive for a long period in the environment and may be spread long distances by the wind. *C. burnetii* is very resistant to heat, ultraviolet light, chemical and osmotic shock, and is therefore extremely difficult to decontaminate from environmental surfaces [11, 12, 32]. *C. burnetii* aerosol can be used in biological warfare and is

considered a potential terrorist threat. Q fever is deemed a category B biological terrorist agent [14, 17].

Human infection is acquired most often by the inhalation of contaminated aerosols, but may also occur through the digestive tract, through skin trauma, or by sexual contact. Ingestion of contaminated non-pasteurized milk has also been considered to be a possible route of infection, and the disease may also develop after tick bite [13, 14, 29, 31, 33].

C. burnetii infection is usually asymptomatic or manifests as a mild disease with spontaneous recovery. However, Q fever may lead to serious complications and even death in patients with acute disease. Predominant clinical manifestations are fever, pneumonia and granulomatous hepatitis for acute cases, and endocarditis for chronic cases. Infection in animals is mainly subclinical but has been associated with late abortions, stillbirth, delivery of weak offspring and also infertility [20].

The aim of our study was to detect the presence of antibodies to phase I and phase II *C. burnetii* antigens in selected ruminant species (sheep, goat, mouflon, fallow deer) living in a zoo. The further aim of the paper was to assess the risk of Q fever for visitors, veterinary and care staff in the zoo settings, because Q fever is considered to be primarily an occupational disease of workers in close contact with animals or processing products. On the other hand, it is deemed to be an air-borne disease, which therefore may also occur among the visitors.

MATERIALS AND METHODS

Blood samples were obtained from the jugular vein of 5 mouflons (*Ovis musimon*), 80 fallow deer (*Dama dama*), 9 Cameroun goats (*Capra hircus*), 8 Carpathian goats (*Capra aegagrus f. hircus*), and 8 Cameroun sheep (*Ovis ammon f. aries*).

Blood samples were centrifuged at 2,500 r.p.m. for 10–15 minutes, and the serum collected and stored at -20°C until serologic examination for antibodies against *C. burnetii* was performed. Sera exhibiting haemolysis, lipaemia or microbial growth were not examined. Because of that we eliminated one mouflon serum and 20 fallow deer sera.

The presence of antibodies to phase I and phase II *C. burnetii* antigens in the baseline serum specimen was

determined using ELISA method adjusted in our laboratory with whole cells of the Nine Mile strain of *C. burnetii* (fy Dolphin, Slovak Republic).

Brief description of the ELISA method. Serum was diluted in 0.4% milk (obtaining dilutions 1:100–1:102 400). The serum was incubated in a microtitration plate coated with antigen (4 µg per well) at 37°C for 2 hours in a humid chamber. After washing, the swine anti-sheep and goat globulin conjugated with horseradish peroxidase (SwASH/Go/Px, fy Sevac, Czech Republic) Tris buffer was added and incubated at 37°C for 1 hour in a humid chamber. After washing, 100 µl of chromogen solution was added to each well and the plate incubated at room temperature for 20–30 minutes in a humid chamber. The reaction was stopped by adding 50 µl of 3 N H₂SO₄ to all wells. The microplates were read at 492 nm using an automatic ELISA reader [3].

RESULTS

Mouflons (n=4) formed the smallest groups of examined ruminants. Phase II antibodies to *C. burnetii* were detected only in one animal (titre 1:200). No phase I antibodies to *C. burnetii* were detected in mouflons.

Examination of 2 Cameroun goats for phase II antibodies to *C. burnetii* provided negative results, in 6 goats the phase II antibody titre to *C. burnetii* was 1:100, and in one goat 1:200. Of the 8 Carpathian goats examined, phase II antibodies to *C. burnetii* were detected in 5 animals (titre 1:100). Similar to mouflons, no phase I antibodies to *C. burnetii* were detected in goats.

Of the 8 Cameroun sheep, only 3 showed serum positivity to phase II antibodies (titre 1:100 in 2 and 1:200 in one animal). The sheep with phase II antibody titre 1:200 was positive also for phase I antibody (titre 1:100).

No phase II antibodies were detected in 43 fallow deer. Titres 1:200 were detected in 4 serum samples and 1:100 in 13 samples. Phase I antibodies, detected in one and 2 fallow deer with phase II antibody titres reaching 1:100 and 1:200, respectively, were present at titres 1:100 (Tab. 1).

The results indicate that the serum prevalence of phase II and phase I antibodies to *C. burnetii* antigens reached 25% and 0% in mouflons, 70% and 0% in goats, 37.5% and 12.5% in sheep, and 28.3% and 5% in fallow deer.

Table 1. Seroprevalence in animal species.

Animal species	Phase I and II antibodies (titre and number)		Phase I and II antibodies (titre and number)		Negative results phase I and II antibodies (number)		Total number of animals
	1:100	1:200	1:100	1:200			
Mouflon	–	–	–	1	4	3	4
Goat	–	11	–	1	17	5	17
Sheep	1	2	–	1	7	5	8
Fallow deer	3	13	–	4	57	43	60

However, the results of examination for serum prevalence in mouflons and goats cannot be considered significant because of the relatively low number of animals in the respective groups.

DISCUSSION

It is difficult to compare the results presented in this study with those reported in other papers written on this subject. Although they present information on the presence of antibodies to *C. burnetii* in many wildlife animals (as stated in the Introduction), they fail to evaluate specifically phase I and phase II antibody titres, and frequently report only serum prevalence in percent.

This is the case with papers published previously. Riemann *et al.* observed seropositivity in 57% of black-tailed deer (*Odocoileus hemionus columbianus*) and 51% fallow deer, which is practically 50% higher than that presented in this study [25]. Hubálek *et al.* collected sera from 33 roe deer (*Capreolus capreolus*), 24 red deer (*Cervus elaphus*), 4 fallow deer, 2 mouflon, 34 wild boars (*Sus scrofa*), and 48 hares (*Lepus europaeus*), and sera contained antibodies to *C. burnetii* in 12% [10].

The literature review suggests that *C. burnetii* is enzootic among ruminants and wild animals and there is widespread human exposure to this pathogen. Sheep and goats appear to be a more important risk for human infection than cattle or wild animals.

Human outbreaks of Q fever have been associated with employees of slaughterhouses, farms, and research institutions where parturient ruminants were housed. Large outbreaks have also been reported among persons residing in cities or towns downwind from sites where infected animals are kept [5, 21].

Several examples of possible transmission of the infection from wildlife reservoir animals to man can be found in the literature. The transmission of Q fever to humans from wild rabbits was documented in the 1980s [18]. Potasman *et al.* described an outbreak of Q fever in visitors to a safari park. The investigation found that 4 (8%) of 50 safari travellers to Kenya contracted Q fever; 2 travellers developed overt infection, whereas 2 others developed asymptomatic illness [24].

Laughlin *et al.* (1991) reported the first outbreak of Q fever after exposure to a deer and an infected pregnant dog. 7 members of a family from New Brunswick became ill with headache, fever, myalgia, fatigue, sweats, and a mild non-productive cough. 6 had a greater than four-fold rise in phase II antibody titre to *C. burnetii* antigen with the indirect immunofluorescence test. A detailed history revealed that one family member shot a male deer. 6 members of the family watched while the deer was skinned. Some of the deer liver was fed to the family dog. One week later the dog gave birth to pups; one was stillborn and 2 died in the first week of life. The pups were born under a bed in the family's house in which another subject was asleep at the time. The next day, a family supper was held and the

dog was present in the vicinity of the supper table. The dog had a phase I antibody titre of 1:256 and a phase II titre of 1:8192. *C. burnetii* was isolated from the dog's uterus. The dog was almost certainly responsible for this outbreak of Q fever and became infected after eating the deer liver [15].

Gami *et al.* described the presentation and clinical course of a 40-year-old man with Q fever endocarditis. He had a history of congenital bicuspid aortic valve stenosis and valve replacement 5 years previously. He had hunted deer, bear, and pheasant but was not exposed to farm animal, and had no pets [7].

The examples mentioned in the references indicate that veterinarians, tending staff and visitors to zoos can be infected with *C. burnetii*; consequently, regular Q fever surveillance of animals living in zoos appears to be appropriate.

The zoo staff should be protected from the infection by appropriate disposal of placenta, birth products, faetal membranes, and aborted fetuses. Workers often help clean and dry newborn animals covered in amniotic fluid without the protection of masks or gloves [9, 30]. One of the possible human protection measures is Q fever vaccination. A vaccine for Q fever has been developed and has successfully protected humans in occupational settings in Australia. However, this vaccine is not commercially available [2]. Further important preventive measures include: limitations of direct contact of zoo visitors with animals, acceptance of measures implemented to prevent airflow to other occupied areas, and similar procedures for protection against air-borne transmission of the disease to the communal vicinity.

The preventive measures in animals should incorporate: the quarantine of imported animals, routinely testing of animals for antibodies to *C. burnetii* (not limited only to ruminants, because the sources of infections could also be wild-life species), tick transmitted disease prevention and maintenance of surrounding bushes. Ticks are considered to be the natural primary reservoirs of *C. burnetii* responsible for the spread of the infection in wild animals and for transmission to domestic animals [23]. In the accessible literature sources, there is no reference to the vaccination of wild-life animals; accessible and published data refer only to the vaccination of domestic animals. Despite the existence of vaccines against animal Q fever, the vaccination of animals is not widely used, because it is protective only in animals uninfected at the time of vaccination. In Slovakia, the occurrence of Q fever in domestic animals and humans has probably been dramatically reduced due a large-scale vaccination of cattle in the 1970s and 1980s [14].

CONCLUSION

Serologic diagnosis of Q fever in animals can be difficult. Phase I and phase II antibody responses have not been well evaluated in domestic animals, and IgG-based antibody tests provide only evidence of past exposure. Our detection of antibodies was based on polyvalent sera which prevented us from judging the presence of individual

classes of immunoglobulins. Serologic tests are not useful for determining which animals represent a current risk for transmission, as animals may seroconvert without shedding, or remain seropositive long after the acute infection has resolved. Conversely, some animals may shed *C. burnetii* and pose a risk for infection prior to the development of antibodies, and some infected animals never seroconvert [22].

Some measures are not yet confirmed, e.g. whether permanently seropositive animals should be treated or put down. Hatchette *et al.* recommend chemotherapy in herds with a rate of >20% seropositivity containing animals with titres $\geq 1:32$, but this measure is only applicable in domestic animals [9]. Antibodies might persist at low concentrations for many months after acute infection and does not indicate current activity of the disease, but these data are confirmed in humans [1, 17]. The putting down of zoo animals could be considered an appropriate measure only on rare occasions because these animals are usually very exceptional species. After becoming infected, female animals shed large quantities of *Coxiella* into the environment during abortion or normal delivery through birth fluids, placenta and foetal membranes. Moreover, following parturition, these infected animals excrete the bacteria via the urine, faeces, vaginal discharges, and milk for several months [4]. We assume that similar to humans, the appropriate measures in animals would be tetracycline treatment (e.g. doxycycline), and further follow-up and monitoring of the seroprevalence.

In conclusion, despite the fact that zoo animals may be potential reservoirs of infection, to our knowledge, none of the staff employees, veterinary doctors, or visitors have acquired Q fever.

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