

SEROLOGICAL STUDY OF Q FEVER IN SHEEP IN THE TERRITORY OF EASTERN SLOVAKIA

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Abstract: Q fever is a zoonosis caused by infection with *Coxiella burnetii*. Although the reservoir of *C. burnetii* consists of various species, the most common sources of human infection are farm animals, such as cattle, goats, and sheep. The agent is typically transmitted by the aerosol route, and in more than half of the cases primary infection is symptomless. Clinical outcomes of *C. burnetii* infection in domestic ruminants consist of abortion and stillbirths in sheep and goats, while in cattle it causes infertility and mastitis. A serological survey for *C. burnetii* was undertaken on a population of sheep. A total of 269 sheep serum samples were collected and tested for the detection of antibodies against *C. burnetii* phase I and II antigens using an enzyme-linked immunosorbent assay. The herd investigated was tested twice, i.e. in 2000 and 2009, to detect the changes in seroprevalence. In the first year of investigation, the prevalence of antibodies against *C. burnetii* phase II antigen was estimated at 37.22% and ten years later at 58.42%. Antibodies against phase I antigen were not detected in any examined serum samples. The difference in seroprevalence after ten years of observation was significant ($p=0.001$).

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INTRODUCTION

Coxiella burnetii is an obligate intracellular pathogen known to be causative agent of Q fever, a zoonosis with a worldwide occurrence [6, 14]. The most common animal reservoirs for this pathogen are cattle, sheep, and goats. The organism is found in placental tissues and birth fluids, and in the milk, urine, and faeces of infected animals. Human infection usually occurs by inhalation of infected dust or from exposure to amniotic fluid or placenta where they are present in high quantities. Q fever manifests in humans as acute or chronic disease. The acute disease may include an undifferentiated febrile syndrome, pneumonia, or hepatitis. The most common chronic symptom is endocarditis [5, 11]. Although *C. burnetii* infection is usually not harmful in infected animals, abortions in sheep and goats and lower

birth weight and infertility in cattle have been associated with chronic *C. burnetii* infection [15, 16, 22].

Routine diagnosis of Q fever normally relies on serological methods. *C. burnetii* can have two distinct antigenic phases. In acute stadium there typically is an initial rise in antibody against phase II antigen. In chronic infection, high titres of antibodies against phase I antigen are characteristic [12].

Isolation of *C. burnetii* is hazardous, difficult and time-consuming, and requires confined biosafety level 3 laboratories. In contrast, polymerase chain reaction (PCR) is a safe and useful method for detection of *C. burnetii* [10].

The aim of the present study was to investigate the presence of antibodies against *C. burnetii* in sheep from a village of the eastern Slovakia, and detect potential changes in seroprevalence of antibodies in these animals within a ten-year period.

Table 1. Seroprevalence in sheep.

Year of examination	Number of animals	Number of positive animals at the given titer						Positive animals		P
		1:100	1:200	1:400	1:800	1:1,600	1:3,200	n	%	
2000	180	33	17	11	5	1	-	67	37.22	0.001
2009	89	19	8	14	6	2	3	52	58.42	

MATERIAL AND METHODS

A total of 269 blood samples were collected from sheep on one farm. The samples were obtained from the jugular vein in a ten-years interval – 2000 (n=180) and 2009 (n=89). All animals were adults and their history showed no diseases, parturitions or abortions. The sera were separated after sampling by centrifugation (2,500 r.p.m.) and kept at -20°C until assayed.

Antibody titres against *C. burnetii* phase I and II antigens were determined as previously described [3, 4]. Antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) with whole cells of the Nine-Mile strain of *C. burnetii* (fy Dolphin, Slovak Republic). Positive and negative control sera were tested simultaneously with all samples.

Cut-off \geq 1:800 antibodies to *C. burnetii* is considered as significant for infection. However the cut-off values for individual tests may differ between laboratories and antigens used.

Statistical analysis. Proportions were compared using the chi-squared (χ^2) test. Value $p \leq 0.05$ was considered as significant result.

RESULTS

The first sampling involved 180 sheep that were sampled in 2000. The prevalence of antibodies against *C. burnetii* phase II antigen was estimated at 37.22%, i.e. 67 sheep tested positive and 113 negative. Antibody titres ranged between 1:100 and 1:1,600. The titre 1:1,600 was determined in one sheep, while the lowest phase II antibody titre (1:100) was detected in 33 sheep. The phase II antibody titre reached 1:800 in five samples, 1:400 in eleven and 1:200 in seventeen samples. Phase I antibodies were not detected in any tested sheep of this group.

The second sampling (2009) involved 89 sheep. Fifty-two (e.g. 58.42%) of them harboured antibodies against the phase II antigen. This sampling showed the presence of the highest titre (1:3,200) in three cases which was not the case in the year 2000. Other titres and phase II antibodies positive samples were as follows: 1:100 n=19, 1:200 n=8, 1:400 n=14, 1:800 n=6, and 1:1,600 n=2. Phase II antibodies were not detected in 37 sheep. Similar to the results from 2000, phase I antibodies were not detected in samples collected in 2009.

When comparing the seroprevalence determined in 2000 and 2009, the second examination showed 20% increase (from 37.22% in 2000 to 58.42% in 2009) which can be explained also by the different number of sheep examined in both years (year 2000 n=180 and year 2009 n=89). In 2009, the number of sheep examined for specific antibodies against *C. burnetii* was half of what it was in the previous examination.

Of the total number of examined sheep (n=269) phase II antibodies were detected in 119 sheep, i.e. 44.23%. Our results revealed high seroprevalence rate in 2000 and 2009 ($p=0.001$) (Tab. 1).

DISCUSSION

Q fever was probably introduced to Slovakia by infected sheep from Romania. The sheep were in very poor condition and most probably had *Coxiella* latent infection which were activated during pregnancy and released into the their surroundings during parturition [17]. An extensive serological survey of the whole of Slovakia towards the late 1950s, showed that sheep were the main source of infection [8].

An epidemic of Q fever involving 20 persons occurred in a sheep co-operative farm in Eastern Slovakia during sheep shearing in spring, supported by the 13% seropositivity rate in sheep [20]. A further outbreak of Q fever suggested that sheep can also contribute to the maintenance and dissemination of Q fever in this region [2]. The significance of sheep in the epidemiology of Q fever in Slovakia was confirmed also in literature resources from other authors [1, 18, 19].

It has also been observed that seroprevalence of *C. burnetii* is increasing among sheep in some parts of the world, reaching for example in Canada 3%, in Bulgaria almost 100% [7, 21]. Our outputs showed seropositivity in 37.22% of sheep in the first year of examination and in 58.42% of sheep after the elapse of ten years, which is in agreement with the results of the above mentioned authors who reported a wide range of seroprevalence.

As mentioned previously, *C. burnetii* can have two distinct antigenic presentations or phases; animals and humans develop antibody responses to both phases [6]. The importance of the development of antibody responses to phase I and phase II and stage of infection has not been sufficiently evaluated in animals. Serologic tests are not useful tools in order to determine which animal represents 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 pose a risk for infection prior to the development of antibodies by shedding the bacteria, and some infected animals never seroconvert. Seroprevalence studies do not provide information or indicate whether an animal is infectious. Instead, the results of the studies can only be interpreted in animals as evidence of previous infection (past exposure) [9].

In humans, phase II antibodies persist at moderate titres for 3 months to 1 year. A similar quantitative serologic response has been assumed in mammals. Martinov and Pandurov (2005) detected low titres of antibodies against phase I antigens in naturally infected sheep up to 1.5–2, and low titres of antibodies to phase II antigens up to 5 years [13].

Altogether, our study detected antibody titres \geq 1:800 in 17 cases, which suggests a significant confirmation of infection. The presence antibodies is not always associated with the clinical signs of *Coxiella burnetii* infection. We did not observe any abortions and parturitions as the most common manifestation of infection, as described in the section "Material and Methods". As one possible reason we suggest the fact that the obtained sera were collected from rams or from non-pregnant ewes. A rising level in paired sera is considered as an evidence of active infection. Unfortunately we did not have the possibility to investigate paired sera.

Testing sheep based on only one type of biological sample (e.g. serum) may lead to underestimation of the risk of bacterial spread within a herd. In the future, it should be used also with other biologic materials (placenta, birth fluid, aborted fetuses) investigated with new techniques – PCR or western blot.

Appropriate measures should be used in the prevention and control of Q fever in sheep herds: disposal of placenta, birth products, foetal membranes, and aborted fetuses; vaccinate animals; quarantine imported animals, animals should be routinely tested for antibodies to *C. burnetii*, and other general measures (e.g. disinfection of barns, stables, pens).

The results of this study confirmed the presence of anti-*C. burnetii* antibodies in sheep in Eastern Slovakia. Further studies involving collaboration between veterinary and medical services on *Coxiella* infection in both domestic animals and humans are needed to elucidate the epidemiology of Q fever.

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