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# SEROPREVALENCE OF ANTIBODIES TO *COXIELLA BURNETII* AMONG EMPLOYEES OF THE VETERINARY UNIVERSITY IN KOŠICE, EASTERN SLOVAKIA

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**Abstract:** *Coxiella burnetii* is an obligate intracellular pathogen known to be the causative agent of Q fever, a zoonosis with worldwide occurrence. The organism has been found in many wild and domestic animals. Infected animals shed highly stable bacteria in urine, faeces, milk, and through placental and birth fluids. Humans acquire the infection mainly by inhaling infected aerosols, or by ingesting contaminated raw milk or fresh dairy products; tick transmission has been proven but is probably rare. The aim of the present study was to determine the titres of immunoglobulin IgG against phase I and II of *C. burnetii*, and to evaluate the risk factors that might be associated with exposure to *C. burnetii* among employees of the Veterinary University. Venous blood was obtained from 92 employees. IgG antibodies were determined by ELISA method modified in our laboratory using whole cells of the Nine Mile *C. burnetii* strain. The questionare was filled out by every subject to obtain epidemiological and clinical date. Phase I antibodies were detected in 35 subjects, i.e. in 38%, and phase II antibodies in 58 subjects, i.e. in 63%. When using the titre ≥ 1:800 as a cut-off level, 2 samples were positive for phase I antibodies (2.1%) and 12 for phase II antibodies (13%). Factors predisposing to infection or exposure to *C. burnetii* included professional orientation and regular contact with farm animals and pets. Clinical history of some seropositive subjects revealed substantial problems, such as fever of unknown origin, rheumatic disease, disease of heart, liver, respiratory tract (particularly atypical pneumonia), chronic fatigue syndrome and spontaneous abortion in females. Q fever is a profession-related disease and prevention of its spreading within the risk population groups requires observation of basic safety rules.

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## INTRODUCTION

*Coxiella burnetii* is an obligate intracellular rickettsia-like bacterium that lives and proliferates within acid phagolysosome vacuoles of monocyte-macrophages and is responsible for Q fever [12]. Unique to *Coxiella* is its antigenic phase variation that appears to involve changes on lipopolysaccharide. Virulent phase I bacteria are isolated from natural infection while avirulent phase II occurs after serial passages [6].

As a disease, Q fever is characterized by its polymorphism, and can be associated with the vast majority of infectious syndromes. The disease is commonly divided into acute and chronic infections. Acute disease can manifest as a relatively mild, self-limited febrile illness, or more moderately severe disease characterized by hepatitis or pneumonia. It manifests less commonly as myocarditis, pericarditis, and meningoencephalitis. Chronic Q fever occurs in <1% of infected patients, months or years after initial infection. Chronic disease manifests most commonly

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as a culture-negative endocarditis in patients with valvular heart disease [25].

Human infection most frequently follows inhalation, but also occurs via ingestion of contaminated milk and direct contact with infected material. Human-to-human transmission (following childbirth/autopsy) has been reported, as has infection following tick bites [27]. Usually infected aerosols are generated by farm animals when they give birth or abort as the bacteria multiply and reach high concentrations in the placenta of mammals [25].

The most commonly identified sources of human infection are farm animals, such as cattle, goats and sheep, but pet cats, rabbits and dogs are also known to be potential sources of urban outbreaks [1].

Q fever diagnosis is confirmed most often by serologic tests that demonstrate significant increase in antibody titres to phase I and phase II *C. burnetii* antigens. The most reliable and commonly used serologic methods are indirect immunofluorescence, complement fixation, ELISA, and microagglutination [21]. Increasing titres of phase II antibodies are most characteristic of acute Q fever, whereas the presence and persistence of elevated phase I and phase II antibody IgG, IgA, and IgM titres most strongly suggest chronic Q fever [8].

Veterinarians are one of the highest risk groups for occupational infection because of their potential contact with infected animals [1]. The aims of our study were: (a) to determine the seroprevalence of *C. burnetii* antibodies among employees of the Veterinary University, (b) to evaluate the epidemiology of the infection in this population (identification of risk factors associated with the infection of Q fever in employees).

## MATERIALS AND METHODS

Blood samples and questionnaires were collected from persons, included in this study. Venous blood was obtained from 92 employees (veterinarians n=75, animal attendants n=8, laboratory personnel n=7, technical and administrative personnel n=2) of the University of Veterinary Medicine in Košice (Slovak Republic).

Blood samples were centrifuged at 2,500 r.p.m. for 10-15 minutes and the serum was collected and stored at -20°C until serologic examination for antibodies to *C. burnetii* was performed. The presence of IgG antibodies to phase I and phase II *C. burnetii* antigens in the baseline serum specimen was detected by ELISA method developed in our laboratory using whole cells of the Nine Mile strain *C. burnetii* (fy Dolphin, Slovak Republic).

Serum samples from 3 medical students were used as a negative and positive control. The first positive control (titre 1:800 of phase I IgG) originated from a female student who had worked on animal farm in Canada, consumed raw milk and steaks, was in contact with farm and free-living animals, and frequently suffered tick bite. The second positive control (titre 1:1600 of phase II IgG) was obtained from a student who regularly consumed goat milk and cheese because he lived in the rural zone and his family kept goats. The negative control originated from a student with no significant epidemiological history (no contact with animals or their products, no consumption of raw milk or meat, no tick bite). The 3 control serum samples were also examined by the Virology Institute of the Slovak Academy of Sciences in Bratislava (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, antihuman IgG peroxidase conjugate (1:200) in 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 was incubated at room temperature for 20–30 minutes in a humid chamber. The reaction was stopped by adding 50 μl of 3 N $H_2SO_4$ to all wells. The microplates were read by means of an automatic ELISA reader at 492 nm. The IgG titre ≥ 1:800 was used as a cut-off level.

In order to obtain epidemiological and clinical data, every person included in the experiment was asked to fill out a questionnaire. The questionnaire provided the following information: sex; age; permanent address, which determined a rural or urban life; profession and occasional temporary work during which the individual came into contact with straw, hay, soil, manure, animal skins and furs, wool, milk, meat and similar, or work in a dusty environment; sporting activities, particularly those associated with animals (e.g. tourism, hunting, horse riding, falconry and similar); stay abroad and potential contact with animals and their products; consumption of raw minced meat, non-pasteurised milk and dairy products; pet ownership; contact with farm animals or pregnant dog or cat; tick bite. The questionnaire related to clinical history included fever of unknown origin, rheumatic disease, diseases of heart, liver, respiratory tract (atypical pneumonia), chronic fatigue syndrome and in females also spontaneous abortion.

## RESULTS

**The presence of IgG antibodies to phase I *C. burnetii*.** Phase I antibodies were not detected in 57 out of 92 examined serum samples. The titre 1:800, which is considered diagnostic of Q fever endocarditis, was determined only in one subject similar to the titre 1:1600. Antibody titre 1:400 was detected in 2 subjects, 1:200 in 19 subjects and detectable level of specific IgG (1:100) in 12 subjects (Tab. 1).

**The presence of IgG antibodies to phase II *C. burnetii*.** Phase II antibodies were detected in 58 out of 92 examined subjects. Thirty-four serum samples tested negative. Antibody titres were in the range 1:100–1:1600. The lowest antibody titre 1:100 was detected in 15 subjects and titres 1:200 and 1:400 in 15 and 16 subjects, resp. In ELISA the

**Table 1.** Presence of IgG antibodies to phase I and phase II *C. burnetii*.

| Antibodies | Titre and number of subjects | | | | |
|---|---|---|---|---|---|
| | 1:100 | 1:200 | 1:400 | 1:800 | 1:1600 |
| phase I | 12 | 19 | 2 | 1 | 1 |
| phase II | 15 | 15 | 16 | 9 | 3 |

proposed cut-off value 1:800 for IgG antibodies to phase II *C. burnetii* was determined in 9 subjects and the highest antibody titre 1:1600 in 3 subjects (Tab. 1).

**Characteristic of seropositive group.** The 58-membered group consisted of 27 women and 31 men with mean age of 43.9 years. Forty of them lived in a town, 16 in the country, and two in both urban and rural areas. The group consisted of 41 veterinarians, 8 animal attendants, 7 laboratory personnel and one technical and one administrative worker.

With the exception of technical worker, all of them came into contact with hay, manure, soil, straw, and animal products (skin, hair, wool, feathers, excrements, meat, milk, blood, urine, placenta, amniotic fluid, etc.). All employees mentioned tourism as one of their frequent sport activities, namely, occasional or more frequent walking in nature or forest where they could come into contact with wild animals and their products. In addition, 8 of them mentioned hunting as their favourite hobby and therefore spending more time in the forest in comparison to the remaining colleagues. Tick bite frequency, either single or repeated, was reported by 29 subjects.

Consumption of fresh meat or meat products, non-pasteurised milk and milk products was not infrequent. With regard to the fact that all of them were on the staff of the Veterinary University, their contact with farm animals (cattle, goat, sheep, swine, horse, rabbit, poultry, etc.) and pets (dog, cat, hamster, guinea pig, pigeon, parrot, reptiles, etc.) was common and occurred almost on a daily basis. Twenty three of them owned dogs and 7 subjects kept cats. Some of them had in their homes also hamsters (n=3), parrots (n=2) and a rabbit (n=1). Those who lived in the country kept poultry (n=6), pigs (n=5), goats (n=3), sheep, cow and a horse (n=1).

Ten veterinarians participated in long-term specialised practice abroad or worked in a foreign country and were in contact with animals. In the following text we give the country of their stay and the respective levels of phase I and phase II antibodies: (1) Poland – 1:200 and 1:800; (2) Kenya – 1:200 and 1:100; (3) Hungary – 1:800 and 1:400; (4) Belgium – 1:100 and 1:400; (5) the USA and Spain – 1:100 and 1:100; (6) Germany – 1:200 and 1:400; (7) Libya and Malta – negative and 1:100; (8) Switzerland – 1:100 and 1:200; (9, 10) Great Britain – negative, 1:100 and 1:200 (Tab. 2).

**Seroprevalence and clinical picture.** Fever of unknown aetiology occurred in 5, rheumatic disease in 4, non-specified heart disease in 4, disease of liver in 8, respiratory disease in 6, chronic fatigue syndrome in 4 and spontaneous abortion in 7 examined individuals. The diseases mentioned occurred in individual persons alone or in combinations.

**Table 2.** Factors predisposing to Q fever.

| Factors | | No. of subjects |
|---|---|---|
| Place of residence | urban | 40 |
| | rural | 16 |
| | urban/rural | 2 |
| Profession | veterinarian | 41 |
| | animal attendant | 8 |
| | laboratory personnel | 7 |
| | other | 2 |
| Contact | hay, manure, soil, straw, animal products | 57 |
| Sporting activities | tourism | 58 |
| | hunting | 8 |
| Tick bite | | 29 |
| Consumption | raw meat | 17 |
| | raw milk | 20 |
| Contact with animals | farm animals, pets | 57 |
| Pet ownership | dog | 23 |
| | cat | 7 |
| | hamster | 3 |
| | parrot | 2 |
| | rabbit | 1 |
| Farm animals ownership | poultry | 6 |
| | pig | 5 |
| | goat | 3 |
| | sheep, cow, horse | 1 |
| Stay abroad | Poland, Kenya, Hungary, Belgium, USA, Spain, Germany, Libya, Malta, Switzerland, UK | 10 |

The highest titre 1:1600 of phase II antibodies was detected in 3 subjects who reported in the questionnaire no clinically important symptoms related to Q fever (with the exception of one person with fever of unknown etiology, No. 1). Phase I antibodies were detected in these persons only at low titres (1:100 n=2, 1:200 n =1).

The 1:800 titre of phase II antibodies was detected in 9 subjects. In individual members of this group, phase I antibodies reached titres 1:100 (n=3), 1:200 (n=3), 1:400 (n=1) and in 2 subjects the result was negative. The person (No. 2) with 1:400 titre of phase I antibodies overcame in the past rheumatic fever. An additional 2 persons (No. 3, 4) with 1:200 titre of phase I antibodies had hepatitis and suffered from chronic fatigue (most likely caused by hypothyreosis) and non-specified heart disease. No clinically important symptoms were detected in the remaining 6 persons.

The 1:400 titre of phase II antibodies was detected in 16 subjects. In these subjects phase I antibodies reached titre 1:100 in 7 cases, 1:200 also in 7 cases and 1:800 in one case (one tested negative). The problems that occurred in these subjects included fever (n=2), spontaneous abortion (n=3), rheumatic disease (n=2), disease of liver (n=3) and mononucleosis (n=1), respiratory disease of chlamydia etiology (n=1) and fatigue (n=1), either separately or in combination. The woman (No. 6) with 1:200 titre of phase I antibodies suffered from fever of unknown etiology, rheumatic disease, liver damage, respiratory disease of chlamydia etiology and spontaneous abortion. An employee (No. 8) with 1:800 titre of phase I antibodies had an attack of hepatitis B. In the past, Q fever and mononucleosis was diagnosed in one subject (No. 9) with 1:100 titre of phase I antibodies and this subject also suffered from chronic fatigue. Others outputs (No. 5, 7, 10, 11) are summarized in Table 2.

The 1:200 titre of phase II antibodies was detected in 15 subjects and phase I antibody titre reached 1:200 in 6 of them. An interesting result was observed in one person, an animal attendant, as titre of phase I antibodies in this person reached 1:1600, but he was free of any health problems. Eight persons tested negative for the presence of phase I antibodies.

Similar to the previous group, the 1:100 titre of phase II antibodies was detected in 15 subjects. In this group the 1:200 titre of phase I antibodies was detected in 2 cases (without health problems) and 1:400 titre in a woman (No. 22) who had spontaneous abortion. No phase I antibodies were detected in the remaining 12 subjects. With regard to occurrence of clinically insignificant titres of phase I and phase II antibodies, we do not describe in detail the related clinical symptoms and results are summarized in Table 3. In members of both groups (1:100 and 1:200 titres of phase II antibodies) we recorded the occurrence of rheumatic disease (n=1), fever of unknown origin (n=2), chronic fatigue (n=2), disease of heart (n=3) and liver (n=3), abortion (n=4) and respiratory disease (n=5) (Tab. 3).

**Seroprevalence and profession.** In the most numerous group, the veterinarians (n=41), the following phase II antibody titres were detected: 1:100 in 15, 1:200 in 8, 1:400 in 9, 1:800 in 6, and 1:1600 in 3 subjects. Phase I antibody titres were detected with the following frequency: 1:100 in 9, 1:200 in 12 and 1:400 in 2 subjects. Phase I antibody titre 1:800 was detected only in one veterinarian in combination with 1:400 titre of phase II antibodies. In 17 subjects no phase I antibodies were detected.

In the group of animal attendants (n=8) phase I and phase II antibodies occurred with the following frequency: (1) 1:1600 and 1:200; (2) 1:100 and 1:800; (3, 4) 1:100 and 1:400; (5) negative and 1:800; (6) negative and 1:400; (7, 8) negative and 1:200.

**Table 3.** Seroprevalence and disease.

| No. | Titre of antibodies | | Disease |
|---|---|---|---|
| | phase I | phase II | |
| 1. | 1:100 | 1:1600 | FV |
| 2. | 1:400 | 1:800 | RM |
| 3. | 1:200 | 1:800 | HP, FT (hypothyreosis) |
| 4. | 1:200 | 1:800 | HR |
| 5. | 1:200 | 1:400 | AB |
| 6. | 1:200 | 1:400 | FV, RM, HP, RS, AB |
| 7. | 1:200 | 1:400 | RM |
| 8. | 1:800 | 1:400 | HP |
| 9. | 1:100 | 1:400 | FV (Q fever), HP (mononucleosis), FT |
| 10. | 1:100 | 1:400 | HP |
| 11. | 1:200 | 1:400 | AB |
| 12. | – | 1:200 | HR |
| 13. | 1:200 | 1:200 | FV |
| 14. | – | 1:200 | FV, HP |
| 15. | 1:200 | 1:200 | HP, AB |
| 16. | – | 1:200 | HR |
| 17. | 1:100 | 1:200 | HR, HP, RS |
| 18. | 1:200 | 1:200 | RS |
| 19. | – | 1:100 | FT (hypothyreosis), AB |
| 20. | – | 1:100 | RS, AB |
| 21. | – | 1:100 | RM, RS, FT |
| 22. | 1:400 | 1:100 | AB |
| 23. | – | 1:100 | RS |

HP – hepatic disease, FT – fatigue, AB – abortion, FV – fever, RS – respiratory infections, RM – rheumatism, HR – heart disease.

**Table 4.** Seroprevalence and occupation.

| Occupation | Number of subjects | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Titre | 1:100 | | 1:200 | | 1:400 | | 1:800 | | 1:1600 | |
| Antibodies | phase I | phase II | phase I | phase II | phase I | phase II | phase I | phase II | phase I | phase II |
| veterinarian | 9 | 15 | 12 | 8 | 2 | 9 | 1 | 6 | – | 3 |
| animal attendant | 3 | – | – | 3 | – | 3 | – | 2 | 1 | – |
| laboratory personnel | – | – | 6 | 4 | – | 2 | – | 1 | – | – |
| technical and administrative personnel | 1 | – | 1 | – | – | 2 | – | – | – | – |

**Table 5.** Seroprevalence and rural or urban life.

| Residence | Number of subjects | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Titre | 1:100 | | 1:200 | | 1:400 | | 1:800 | | 1:1600 | |
| Antibodies | phase I | phase II | phase I | phase II | phase I | phase II | phase I | phase II | phase I | phase II |
| town | 7 | 10 | 17 | 10 | 2 | 14 | 1 | 3 | – | 3 |
| country | 4 | 4 | 1 | 5 | – | 2 | – | 5 | 1 | – |
| town and country | 1 | 1 | 1 | – | – | – | – | 1 | – | – |

Detectable phase I and phase II antibody titres in laboratory personnel (n=7) were as follows: (1) 1:200 and 1:800; (2, 3) 1:200 and 1:400; (4, 5, 6) 1:200 and 1:200; (7) negative and 1:200.

Examination of serum of one technical and one administrative employee showed presence of phase II antibodies reaching titre 1:400 and of phase I antibodies in titres 1:100 and 1:200, respectively (Tab. 4).

**Seroprevalence and rural or urban life.** Sixteen examined subjects lived in rural area and 2 in both town and rural areas. A significant titre (1:800) of phase II antibodies was detected in as many as 6 of them in comparison with 3 subjects with such a titre in the group of 40 people living in a town. The 1:800 titre of phase I antibodies was detected in one subject with permanent residence in a town, and 1:1600 titre of phase II antibodies only in 3 town inhabitants. On the contrary, 1:1600 titre of phase I antibodies was detected only in one subject living in a rural area (Tab. 5).

**Conclusion of results.** The results presented indicate that phase I antibodies were detected in 35 subjects, i.e. in 38%, and phase II antibodies in 58 subjects, i.e. in 63%. When using the titre ≥1:800 as a cut-off level, 2 samples were positive for phase I antibodies (2.1%) and 12 for phase II antibodies (13%).

## DISCUSSION

Q fever is considered to be primarily an occupational disease of workers in close contact with farm animals, pets or animal products, including veterinarians, sheep and dairy workers, meat processing plant workers, laboratory workers, hide handlers, wool spinners, taxidermists, and butchers, and occurs more commonly in agrarian communities [7, 13, 15, 18, 19, 27]. Wade *et al.* described an outbreak in a factory processing sheep placenta and foetal tissue to be used in anti-ageing products in the cosmetics industry [30]. Q fever outbreaks have also been described among soldiers. These outbreaks occurred when the troops were stationed or training in close proximity to sheep or goats, particularly parturient animals [28]. Students of veterinary medicine are also a risk group [29].

The present paper focused on the presence of specific IgG antibodies to *C. burnetii* phase I and phase II antigens in personnel of the Veterinary University. It is generally known that occupational groups with animal contact (livestock handlers, such as veterinarians, farmers, and slaughterhouse workers) have a higher prevalence of antibody to *C. burnetii* than populations without defined risk. It has been reported that the antibody-positivity rate was significantly higher in veterinarians who frequently had contact with farm animals and pets than with healthy subjects [1, 16]. However, we determined antibodies to *C. burnetii* also in students of human medicine. The IgG antibody titre 1:1600 to *C. burnetii* phase II antigen was observed only in 4 subjects (1.9%) out of the total number of 210 examined students in comparison with the group of 58 employees of the Veterinary University in which this titre was detected in 3 subjects (5.1%). The IgG antibody titres 1:1600 and 1:800 to *C. burnetii* phase I antigen were not detected in medical students (unpublished data).

The questionnaires indicated a frequent contact with hay, manure, soil, and straw, which is natural regarding the respective profession. Hay on the floor of animal housings is contaminated by *C. burnetii* in faeces, urine and birth products. Removing the bedding would generate aerosols containing *C. burnetii*. The agent is very hardy and resists desiccation, remaining viable in soil for several years [10].

The role of drinking unpasteurized milk in *C. burnetii* infection is controversial. *C. burnetii* has been recovered from milk from infected cows and goats [3, 4, 5, 9, 14, 15]. Epidemiological studies suggested that ingestion of unpasteurized milk has been a source of *C. burnetii* infection for humans. Pasteurization will effectively kill *Coxiella* in raw milk. However, Hatchette *et al.* showed that ingestion of cheese made from pasteurized goat milk was identified as a risk factor for infection [10].

Tick bite was reported by 29 subjects. The original prototype strain of *C. burnetii* was isolated from a *Dermacentor andersoni* tick collected from Nine Mile Creek in Montana, USA. The agent was found to infect more than 40 species of ticks and may be transmitted among animals via tick bite, but their role in direct transmission of pathogen to humans is not important. Excreta (faeces, saliva) from infected ticks persist in animal fur as a highly infectious dust, permitting aerosol transmission within the flock as well as to humans [11, 17, 22, 23].

Our study indicated more frequent occurrence of significant phase II antibody titres in subjects living in a rural area. In agreement with our results, many seroprevalence studies revealed lower seroprevalence in urban zones as

compared to rural ones. The differences can be attributed to the presence of livestock and farming activities (i.e. application of manure to soil) [2, 24].

The clinical data showed that 7 women had spontaneous abortion. It is highly problematic to associate these abortions with *C. burnetii* infection, also with regard to considerable time delay between blood sampling or determination of specific antibodies and the abortion which occurred a long time ago. Studies of Raoult *et al.* and Langley *et al.* showed that Q fever, when contracted during pregnancy, can result in abortions or neonatal deaths, premature births, low birth weight, or no abnormalities. In some cases, pregnancy was found to be associated with the development of chronic infections and relapses [20, 26].

It is also difficult to prove in our study group any relevant association between *C. burnetii* infection and other diseases. Frequently, the subjects were unable to give an accurate diagnosis.

In conclusion, with regard to the occurrence of bigger or smaller outbreaks of Q fever in Slovakia, it appears necessary to comply with the relevant basic preventive measures and to consider vaccination within selected professional groups associated with increased exposure to *C. burnetii* infection (i.e. veterinary personnel).

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