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POTENTIAL ASSOCIATION BETWEEN *COXIELLA BURNETII* SEROPREVALENCE AND SELECTED RISK FACTORS AMONG VETERINARY STUDENTS IN SLOVAKIA

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Abstract: The present study investigated the prevalence of antibodies to *Coxiella burnetii* and the possible factors predisposing students of veterinary medicine to *C. burnetii* infections. IgG antibodies to phase I and phase II *C. burnetii* antigens were determined by ELISA. Out of 77 students examined, 13 were positive for phase I and 45 were positive for phase II antibodies. The titres determined were in the range of 1 : 100–1 : 3200. Some risk factors may have contributed to the high seroprevalence found in these subjects. For example, there were positive associations with rural life and exposure to the breeding of farm animals, and in addition, work in a dusty environment, such as on fields, gardens, stables and construction sites were also connected to high seroprevalence.

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INTRODUCTION

Coxiella burnetii, the aetiological agent of Q fever, is an obligate intracellular bacterium which lives in the phagolysosomes of host cells. Its most important hosts are ruminants (cows, sheep, goats) and domestic pets (cats, dogs, rabbits) [1, 2, 22]. A unique characteristic of *C. burnetii* is its antigenic phase variation. The virulent phase I can be isolated from humans or animals naturally infected, under laboratory conditions. Phase II develops during serial passage in cell cultures or fertilized eggs [28].

Infected animals shed highly stable bacteria in urine, faeces, milk, and via placental and birth fluids [25]. Humans acquire the infection mainly by inhaling infected aerosols, often from products of conception in farm environments or abattoirs, or by ingesting contaminated raw milk or fresh dairy products. Tick transmission has been proven, but is probably rare [8, 21, 25, 30].

C. burnetii may cause acute or chronic forms of Q fever in humans [27]. The most common clinical manifestations of acute Q fever are a febrile illness, pneumonia and granulomatous hepatitis [18]. In the chronic forms, endocarditis is the principal syndrome [9, 15, 33].

Routine diagnosis of Q fever is usually made by serological tests including immunofluorescence, complement fixation and enzyme-linked immunosorbent assay (ELISA). These tests have the disadvantage of indicating only the exposure rather than the ability to detect the actual organism [25]. Serologically, anti-phase I antibodies are normally found at high levels only during the chronic form of

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the disease, whereas specific anti-phase II antibodies predominate primarily in the acute Q fever [28].

The aim of the present study was to detect the presence of antibodies to *C. burnetii* in students of veterinary medicine and to identify possible associations between *C. burnetii* seroprevalence and some risk factors to which they may have been exposed.

MATERIALS AND METHODS

Human population. Of the total number of 105 students in the 4th year of study at the University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic, venous blood samples were collected from 77 of them (73.3%); these students also filled out questionnaires related to their epidemiological history. The participants of the study signed a document stating that they agreed with the collection of blood samples and the processing of the relevant data, and understood the aims of the examination.

Serum samples. Sera were obtained and stored at –20°C until they were examined. IgG antibodies to phase I and phase II *C. burnetii* antigens were determined by the ELISA method, modified in our laboratory using whole cells of the Nine Mile *C. burnetii* strain (Dolphin, Slovak Republic). Three serum samples collected from the students of the Medical faculty and examined in our previous study were used as positive and negative controls (for phases I and II) [4]. The 1st positive control (titre 1 : 800 of phase I IgG) originated from a female student who had worked on an animal farm in Canada. The 2nd positive control (titre 1 : 1600 of phase II IgG) was obtained from a student who regularly consumed goat milk and cheese. The negative control originated from a student with no suspicious epidemiological history. The 3 control serum samples were examined also by the Institute of Virology of the Slovak Academy of Sciences in Bratislava (Slovak Republic).

ELISA. The method used has been described in detail in previous studies [4, 5, 7]. A titre of 200 or greater for IgG against phase II antibodies indicates a recent Q fever infection; an IgG titre of 800 or greater against phase I antibodies suggests chronic infection. These cut-offs vary among laboratories, and defined cut-offs for each individual test should be used [6, 11].

Questionnaire. The questionnaires contained questions related to the following:

1. Demographic data (gender; age; permanent address as a criterion for rural or urban life),
2. Epidemiological data (profession and occasional short-term work that involved contact with straw, hay, soil, manure, animal skins and fur, fleece, milk, and meat; work in dusty environments; sporting activities associated with animals; long-term stay or practice abroad in endemic regions and possible contact with animals and their products; consumption of raw minced meat, unpasteurized milk and products from this milk; pet and farm animals ownership; assistance at parturition or abortion of animals; contact with farm animals or pregnant dog or cat; tick bite),
3. Clinical anamnesis, e.g. previous history of disease or presence of symptoms that could be associated with Q-fever (diseases of the respiratory tract or liver, fever of unknown aetiology, chronic fatigue, rheumatic diseases, and spontaneous abortion).

Statistical methods. The frequency of seroprevalence between individual groups was compared by χ^2 test. If the frequency of seroprevalence was < 5, χ^2 test with Fisher's Yatson correction or Fisher's exact test was used. The significance level was set at $p<0.05$.

RESULTS

Characterization of the investigated group of students. Forty seven of the 77 students examined were females and 30 were males. Their ages ranged from 21–30 years old (mean age 23.2 years). Fifty lived in towns and 27 in rural areas.

Since all of the examined subjects were veterinary students, they commonly and almost daily came into contact with farm animals (cattle, goat, sheep, swine, horse, rabbit, poultry), pets (dog, cat, rodents, parrot, pigeon, snake, turtle, non-specified exotic animals), or free-living animals. Forty six of the students reported being present or assisting at parturition or abortion of one or more farm animal species or domestic pets. The most frequent presence (assistance) at parturition involved cows and bitches (31 and 18, respectively), rodents (11), and less frequently cats (8), mares (6), sows (3), goats (2) and a ewe (1).

Fifty-nine students owned one or more pets. Forty-nine of them owned dogs and 21 had cats. Two students, who lived in a family house, reared pigeons. Other students kept animals in their apartments, such as rodents (guinea pig, mouse, rat, rabbit n=14), parrots (n=5), snakes (n=3), spiders (n=2), turtles (n=1), and non-specified exotic animals (n=1). Twenty-two students kept one or more farm animals. The students living in the rural areas helped with the rearing of poultry (n=11), rabbits (n=11), pigs (n=6), horses (n=2), goats (n=2), cows (n=2) and sheep (n=1).

As a consequence of their constant contact with animals and their products in outpatient departments or stables, all of the students reported work or had contact with straw, hay, soil, manure and animal products (skin, hair, wool, feather, droppings, meat, milk, blood, urine, etc.). All except one student included hiking among their sport activities. This involved occasional or more frequent stays or living in the open or in forests where they could come into contact with wild animals and their products. Twenty-five students went hunting; and their additional hobbies included fishing (n=1) and horse-riding (n=1), which caused them to spend more time in the open compared to their

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

Antibodies	Titre and number				
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 3200
phase I	5	5	2	1	–
phase II	1	27	13	3	1

other colleagues. Work in a dusty environment, such as in the field, garden, stables or construction sites, was reported by 51 students. Tick bites (either single or occurring repeatedly every year) were reported by 52 subjects.

Consumption of raw ground meat or meat products was recorded in 14 subjects. Non-pasteurised milk and milk products in 27 subjects were recorded with 9 of the subjects of the latter group consuming both raw meat and milk.

According to the questionnaires, some students had been affected in the past by heart disease (n=6), respiratory infections (particularly atypical pneumonia, n=5), fever of unknown aetiology (n=3), liver disease (n=3), and rheumatism with chronic fatigue syndrome (n=1).

Presence of IgG antibodies to phase I *C. burnetii*. Phase I antibodies were not detected in 64 out of 77 examined serum samples. A titre of 1:800 was determined in one subject. Antibody titre of 1:400 was detected in 2 subjects, 1:200 in 5, and detectable levels of specific IgG (1:100) in 5 subjects (Tab. 1).

Presence of IgG antibodies to phase II *C. burnetii*. Phase II antibodies were present in 45 out of 77 examined subjects. The titres determined were in the range of 1:100 –1:3200. The lowest detected titre of 1:100 was found in one student and titres of 1:200 and 1:400 in 27 and 13 students, respectively. A titre of value 1:800 for IgG antibodies to phase II *C. burnetii* was determined in 3 subjects and the highest titre 1:3200 in one female student (Tab. 1).

Seroprevalence and rural or urban life. Phase I antibodies were detected in 7 (14%) urban and 6 (22.2%) rural students, and phase II antibodies in 25 (50%) urban and 20 (74%) rural students (Tab. 3). Comparison of seroprevalence of phase I antibodies in students living in urban and rural environment showed no significant difference between the 2 groups (p=0.36), while seroprevalence of phase II antibodies differed significantly (p=0.04) (Tab. 2).

Seroprevalence and contact with animals. The results obtained agree with those presented in Table 1, i.e. phase I antibodies were detected in 16.8% and phase II antibodies in 58.4% of subjects.

Seroprevalence and presence at parturition or abortion. Overall, in the group of subjects who attended parturition or abortion, phase I antibodies were detected 8 times (17.3%) and phase II antibodies 25 times (54.3%), while the group of subjects without exposure to parturition or abortion was positive in 5 (16.1%) and 20 (64.5%) cases, respectively, but the differences were insignificant (phase I p=0.88, phase II p=0.37) (Tab. 2, Tab. 3).

Seroprevalence and pet ownership. In both groups (pet ownership/pet ownership free), the phase I antibodies were found in 10 (16.9%) and 3 (16.6%) samples, respectively, and phase II antibodies in 37 (62.7%) and 8 (44.4%) samples, respectively; the differences being insignificant (phase I p=0.97, phase II p=0.16) (Tab. 2, Tab. 3).

Seroprevalence and farm animals ownership. Phase I and II antibodies were found in 5 (22.7%) and 18 (81.8%)

Table 2. Potential factors of predisposition to Q fever.

Behaviour	Exposure	+ for phase I	p	+ for phase II	p	Total No. of students
Residence	Town	7	0.3613	25	0.0408	50
	Country	6		20		27
Presence at parturition or abortion	Parturition/ abortion	8	0.88	25	0.3746	46
	–	5		20		31
Pet ownership	Pets	10	0.9777	37	0.1686	59
	–	3		8		18
Farm animals ownership	Farm animals	5	0.501	18	0.017	22
	–	8		27		55
Work in a dusty environment	Dusty environment	9	0.8021	36	0.0025	51
	–	4		10		26
Tick bite	Tick	7	0.33	32	0.426	52
	–	6		13		25
Consumption of raw milk	Raw milk	2	0.124	12	0.067	27
	–	11		33		50

animal owners, respectively, in comparison with 8 (14.5%) and 27 (49%) cases, respectively, in the group of subjects who did not keep farm animals (Tab. 3). The difference was insignificant for phase I antibodies ($p=0.5$), but significant for phase II antibodies ($p=0.017$) when the significance level was set at $p<0.05$ (Tab. 2).

Seroprevalence and contact with hay, straw, manure and animal products. The results obtained correspond to those presented in Table 1 and in the paragraph "Seroprevalence and contact with animals".

Seroprevalence and sporting activities. Because all but one student (phase I 1:100, phase II 1:800, respectively) reported involvement in some sport activities, the seroprevalence cannot be evaluated by the χ^2 test because the differences would be insignificant.

Seroprevalence and work in a dusty environment. Phase I and II antibodies in subjects working in a dusty environment were present in 9 (i.e. 17.6%) and 36 (i.e. 70.5%) cases. For subjects not exposed to a dusty environment, phase I antibodies occurred in 4 (i.e. 15.3%) and phase II antibodies in 10 cases (i.e. 38.4%) (Tab. 3). Comparison of both groups (exposed and not exposed to dusty environment) showed no significant difference in the seroprevalence of phase I antibodies ($p=0.8$), while the results for phase II antibodies differed significantly $p=0.0025$ (Tab. 2).

Seroprevalence and tick bite. Phase I antibodies in subjects with single or multiple tick bites were detected in 7 (13.4%) samples and phase II antibodies in 32 (61.5%) samples. Tick bites or a bite by some other blood sucking arthropods was not reported by 25 subjects. In this group of subjects, phase I antibodies were detected in 6 (24%) subjects and phase II antibodies in 13 (52%) subjects (Tab. 3). Comparison of the seroprevalence of phase I and II antibodies to *C. burnetii* in subjects bitten by ticks and not exposed to tick bite, using the χ^2 test, showed that the differences were insignificant ($p=0.33$ and $p=0.42$, respectively) (Tab. 2).

Seroprevalence and consumption of raw milk. Antibodies to phase I were detected only in 2 (7.4%) subjects and antibodies to phase II in 12 (44.4%) subjects who consumed raw milk. However, phase I ($n=11$, 22%) and phase II ($n=33$, 66%) antibodies to *C. burnetii* were found also in consumers of pasteurised milk (Tab. 3). When comparing the seroprevalence of phase I and II antibodies in consumers of raw and pasteurised milk we observed no significant difference ($p=0.12$), but the difference for phase II antibodies ($p=0.06$) was close to the level of significance ($p<0.05$) (Tab. 2).

Seroprevalence and intersexual differences. In the group of women we detected phase I and II antibodies in 25.5% ($n=12$) and 74.4% ($n=35$), respectively, and in men in 3.3% ($n=1$) and 33.3% ($n=10$), respectively (Tab. 3). Comparison of the seroprevalence of phase I and II antibodies in women and men showed significant differences ($p=0.01$ and $p=0.0003$).

Seroprevalence and previous history of disease. The presence of antibodies in 10 students with a history of dis-

Table 3. Presence of antibodies associated with selected risk factors.

Selected risk factors	Titre of antibodies/No. of subjects									
	1 : 100		1 : 200		1 : 400		1 : 800		1 : 3200	
	phase I	phase II	phase I	phase II	phase I	phase II	phase I	phase II	phase I	phase II
Urban life	3	1	3	13	–	10	1	1	–	–
Rural life	2	–	2	14	2	3	–	2	–	1
Parturition/abortion	2	1	4	15	1	8	1	1	–	–
Parturition/abortion free	3	–	1	12	1	5	–	2	–	1
Pet ownership	5	–	3	21	2	12	–	3	–	1
Pet ownership free	–	1	2	6	–	1	1	–	–	–
Farm animal ownership	1	1	2	12	1	4	1	–	–	1
Farm animal ownership free	4	–	3	15	1	9	–	3	–	–
Dusty environment	3	1	4	23	2	9	–	2	–	1
Dusty environment free	2	–	1	4	–	4	1	1	–	1
Tick bite	4	–	1	19	1	10	1	3	–	–
Tick bite free	1	1	4	8	1	3	–	–	–	1
Raw milk	1	–	–	6	1	5	–	1	–	–
Pasteurised milk	4	1	5	21	1	8	1	2	–	1
Male	1	–	–	8	–	1	–	1	–	–
Female	4	1	5	19	2	12	1	2	–	1

Table 4. Presence of antibodies and disease.

Disease	Titre of antibodies	
	phase I	phase II
Hepatic disease	1 : 100	1 : 800
Heart disease	1 : 100	1 : 800
Heart disease	1 : 200	1 : 200
Rheumatism/fatigue	–	1 : 200
Heart disease (n = 2)	–	1 : 200
Respiratory infections (n = 3)	–	1 : 200
Hepatic disease	–	1 : 200

ease is shown in Table 4. In the remaining 8 students (fever n=3, respiratory infections n=2, heart disease n=2, hepatic disease n=1) we failed to detect the respective antibodies. Presence of phase I and II antibodies in groups with/without previous history of disease were not significant ($p=0.062$ and $p=0.09$, respectively).

DISCUSSION

The present study focused on the occurrence of antibodies to both phase I and phase II *C. burnetii* antigens in students of veterinary medicine. Veterinary students can be considered a population at risk of being affected by zoonotic diseases because they have contact with animals and their products during their period of instruction.

It is difficult to compare our results with those of other authors involved in similar research because, according to our knowledge, the only published paper of a similar character is that written by Valencia *et al.* [34]. However, the results of the Valencia *et al.* [34] study differed considerably from those obtained during our study. For detection of antibodies to *C. burnetii*, Valencia *et al.* [34] used the complement fixation test with *C. burnetii* phase II antigen, and the sera were considered as positive when titres were ≥ 1 : 10.

Valencia *et al.* [34] detected seropositivity in 11% of their subjects. The seroprevalence for phase I and phase II antibodies to IgG reached in our group of subjects were 16.8% and 58.4% respectively. We examined a relatively small group of subjects (n=77) and the blood was sampled only once in comparison to Valencia *et al.* [34] who examined 2,000 students and took blood samples twice – at the beginning and end of the school year. We were unable to perform a second sampling which prevented us from determining any comparison of seroconversions.

In the past we carried out a similar study on the veterinary staff (n=92) and students of human medicine (n=241) [4, 5]. In the group of the veterinary staff, we detected phase I and II antibodies in 38% and 63%. The higher percentage of the occurrence of antibodies in veterinary staff compared to veterinary students (16.8% and 58.4%, respectively) could be explained by more frequent contact with animals as the potential reservoir, and also by the length of exposure. The phase I and II antibodies were present in 24.4% and 74.2% of the students of human medicine. The higher percentage of the occurrence of antibodies in medical students may be related to the 3-fold lower amount of veterinary students examined.

In agreement with the study by Valencia *et al.* [34], some risk factors undoubtedly contributed to the higher seroprevalence, such as contact with farm animals and domestic pets, work in a dusty environment, consumption of raw milk and products from this milk.

Fifty-two of the examined students reported a tick bite. Although *C. burnetii* may be isolated from various tick species (*Ixodes*, *Dermacentor*, *Haemaphysalis*), ticks are not thought to play an important role in the transmission of *C. burnetii* to humans, but may be essential in the natural maintenance cycles. The infection of a human after a tick bite is uncommon because infection with this agent, as mentioned before, develops primarily after inhalation of contaminated aerosols, consumption of raw dairy products, and contact with infected animals [23, 29]. Our study showed no significant differences between subjects bitten by ticks and those not exposed to tick bites (phase I $p=0.33$, phase II $p=0.42$).

Hay on the floor of pens and barns is often contaminated with faeces, urine, and products of conception or sick animals. Removing the bedding would generate aerosols containing *Coxiellae* resistant to environmental influences. Contaminated hay and manure removed from animal housings is often used as natural manure to fertilize pastures and fields. Inhalation of *C. burnetii* from contaminated environments is well documented and thus contaminated fields and roads often serve as reservoirs for the airborne spread of *C. burnetii* [12]. This can explain the higher seroprevalence of antibodies to *C. burnetii* in people living in rural areas. Moreover, many students reported exposure to a dusty environment while working, for example, in fields, gardens, and construction sites where the environment could be contaminated with *C. burnetii*.

In our study, seropositivity to phase I and phase II antibodies was detected in 22.2% and 74% of subjects from the rural zone and 14% and 50% of subjects from the urban zone, respectively. Pascual-Velasco *et al.* [26] observed that the prevalence of anti-phase II *Coxiella* IgG was lower among subjects living in the urban zone (32.8%) than in those living in the rural zones (54–82.3%) when using an indirect immunofluorescence test. Seropositivity in the urban population can probably be related also to transmission of resistant forms of *C. burnetii* by wind from the remote areas where farms with seropositive animals are located, and to expanding urbanisation of mountainous areas used previously for cattle grazing [13, 32].

We determined that the seropositivity in consumers of raw milk was close to the level of significance (phase II $p=0.067$). The alimentary route, namely ingestion of contaminated milk or milk products, is a less common mode of transmission [19]. The role of drinking unpasteurized milk in *C. burnetii* infection is controversial. Epidemio-

logic studies suggest that ingestion of unpasteurized milk has been a source of *Coxiella* infection for humans [12]. On the other hand, it has been assumed that ingestion of milk leads to seroconversion but without clinical disease [10, 17]. Some subjects reported the consumption of raw meat, but we have not describe the presence of antibodies in this group because, according to the above presented literary sources, only ingestion of milk is associated with the production of antibodies.

We observed considerably lower seroprevalence in men compared to women, which is not in agreement with the results of other authors. Generally, men are more frequently affected by Q fever than women. This is most likely due to the differences in occupational and exposure risks and perhaps the possible protective role of the female sex hormones. Gender is also a risk factor: at comparable levels of exposure and seroprevalence, the ratio of male to female subjects was 2.45 among adults in France [20, 33].

At present, it is difficult to judge whether the history of some diseases reported in questionnaires could be related to infections because examination for the presence of *C. burnetii*, antibodies is not a part of the standard common clinical practice in Slovakia. Antibodies can persist for long time after infection, and it is therefore impossible to determine the exact time of the infection, whether it developed during veterinary studies or before. The respective antibodies could develop after exposure to some risk factors, even without observation of any clinical symptoms.

In conclusion, Q fever is also a occupation-related disease and prevention of its spreading within the population groups at risk requires observance of basic safety rules. Prevention includes non-specific and specific measures. Specific measures include vaccination and concerns not only persons occupationally exposed to *C. burnetii* infection, but should probably be considered also for patients at risk for chronic Q fever development (cardiac valve disease, vascular aneurysms and prostheses and immunocompromised patients) [3, 16, 24, 31]. An effective, formalin – inactivated, whole-cell vaccine is licensed for use in Australia under the trade name Q-Vax [14].

Although human and animal vaccines for Q fever have been developed, none are commercially available for use in the Slovak Republic. Therefore, preventive efforts must be focused on minimizing contact with animals that may be shedding *C. burnetii*. Although it may not be practical or possible to eliminate the risk for Q fever in the students at schools of veterinary medicine, the risk of transmission can be decreased by non-specific precautions, such as: using protective clothing, gloves and surgical masks; properly decontaminating the environment (surfaces) with formalin; washing hands with disinfectant solutions (70% ethanol); appropriate disposal of placenta, birth products, foetal membranes, and aborted foetuses; use of only pasteurized milk and milk products; quarantine of imported animals; and routinely testing animals for antibodies to *C. burnetii* [12, 23].

Additional improvements in surveillance, such as increasing physician reporting, making animal infections notifiable, and conducting systematic seroprevalence studies in both human and animal populations, would provide important information on the prevention of the spreading of the pathogen and development of the disease [23].

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