BIOLOGIJA
2024. Vol. 70. No. 4. P. 219–228
ISSN 1392-0146 eISSN 2029-0578
DOI: https://doi.org/10.6001/biologija.2024.70.4.6

Serological prevalence of the *Coxiella burnetii* infection in humans in the age group of 0–20 years in some regions of North Macedonia

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The aim of this study was to identify the seroprevalence of *Coxiella burnetii* in humans and to examine the variation of infection in females and males in the age group of 0–20 years in some regions of North Macedonia. Blood samples were collected from 142 random people from five regions. These patients were tested for antibodies against *C. burnetii* phase II antigen by enzyme-linked immunosorbent assay (ELISA). Positive test was based on the cut-off value, which in this case was over 0.5 optical density (OD). Of 74 female serums examined, seven samples resulted positive (9.45%), and of 68 male serums examined, five resulted positive (7.35%). In total, the infection percentage was approximate in both sexes; the differences depended on the regions. These findings on Q fever in humans can be used to improve the visibility of this zoonotic disease especially in North Macedonia, where very little information is available on *C. burnetii* infections. These findings will help local health authorities to focus on the origin of the problem and facilitate the application of preventive measures.

Keywords: *Coxiella burnetii*, Q fever, blood samples, seroprevalence

INTRODUCTION

One of the most common zoonotic diseases at the global level is Q fever, which is caused by γ-Proteobacteria *Coxiella burnetii*, a Gram-negative bacterium (Shaw, Voth, 2019). Q fever, generally known as Query fever, is an airborne zoonotic infection with an impact on public health and initially unknown (query) cause. It is a notorious zoonotic agent due to its high physical resistance against a tough environment and its high infectivity (Klemmer et al., 2018). This resistance is based on some of its cell forms. Once the pathogen enters cells, it undergoes a morphological differentiation from the spore-like small cell variant (SCV) to the metabolically active and replicative large cell variant (Coleman et al., 2004). The transmission of infection to humans occurs through direct and indirect routes. Ruminants are considered the main reservoir for human infections (Njeru et al., 2016). In these animals, the infection is often asymptomatic but can lead to abortions and weak offspring. The bacteria are shed in urine, faeces, milk, and, in tremendously amounts, in the birth

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products (Roest et al., 2012; Roest et al., 2013). The direct routes of transmission of infection from infected animals to humans are contact with unattended birth products and body fluids (Eldin et al., 2017).

Consumption of unpasteurised milk and its products is at least associated with seroconversion (Njeru et al., 2016; Porter et al., 2011). The most common indirect source of infection are aerosols from infected farm animals because *C. burnetii* can remain in the environment over long periods of time and is transported by winds over long distances (Boden et al., 2014). Other animals, such as cats, dogs, rabbits, wild animals, and birds, have also been described as hosts (Gürtler et al., 2014; Shapiro et al., 2016). In urban settings, outbreaks of Q fever in humans were linked with serologically positive pet cats (Malo et al., 2018). Q fever is registered in the list of emerging diseases by the WHO and US Centers for Disease Control and Prevention (CDC) (Babudieri, 1959; Angelakis, Raoult, 2010).

In humans, Q fever leads to clinical disease in 40% of infected persons presenting with flu-like symptoms such as headache and fever, and atypical pneumonia or granulomatous hepatitis in 10% of patients. In 1–2% of patients, the disease becomes chronic, leading to endocarditis and chronic fatigue syndrome. Moreover, clinical symptoms of patients with acute infection vary across countries (e.g., the Netherlands, Spain, France, and Kenya) and typically include abdominal pain, cough, chest pain, diarrhoea, dyspnoea, fatigue, fever, headache, joint pain, muscle pain, night sweating, nausea, and vomiting (Njeru et al., 2016; Maurin, Raoult, 1999; Parker et al., 2006; Wielders et al., 2014; Tissot-Dupont et al., 1992; Raoult et al., 2000; Dijkstra et al., 2012). Chronic Q fever may develop from an acute infection. Possible predisposing factors are preexisting vascular grafts, cardiac valvulopathy, immunosuppression, and aneurysms. However, a combination of serological testing and clinical presentation helps in accurate identification of the cases of chronic human Q fever (Kampschreur et al., 2012; Wegdam-Blans et al., 2012).

Considering the arguments mentioned above, it is clear that *C. burnetii* is a dangerous agent for both animals and humans. The necessity of knowing the epidemiological situation of infection not only in animals but also in humans remains one of the tasks of both veterinary and human medical services services. The primary objective of the present seroepidemiological study was to determine the prevalence of infection caused by *C. burnetii* in humans, in the 0–20 age group, in five regions of North Macedonia and specifically in the areas of Tetovo, Gostivar, Kichevo, Debar, and Struga. Currently, there is no extensive study on this issue in these areas. As a study conducted many years ago does not provide complete data related to this human infection, the present study is the first of this kind.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Virology of the Faculty of Veterinary Medicine at the Agricultural University of Tirana (Albania),using the ELISA Test (ELISA Kit, Serion, Germany) in humans, a highly sensitive test for the identification of *C. burnetii* antibodies (IgG). The aim of the study was to identify the IgG (presence of Q fever antibodies). Blood was taken from random people with different symptoms from rural and urban regions, more specifically from Gostivar, Tetovo, Kichevo, Struga, and Debar areas in North Macedonia. The number of people tested was 142, of whom 68 were males and 74 females. The blood serum was separated by centrifugation at 6000 rpms in 20 min. The serum placed in plastic ampoules was kept frozen at –30°C, until testing. The sera were diluted before the test at a ratio of 1:400, in two steps. The first dilution was done at a ratio of 1:100, then the second dilution at a ratio of 1:4. They were then incubated for 45 min and, after the rinsing, the conjugation solution was added, and then other ingredients. Finally, the halting solution was added. The incubation time matched the criteria preset for the respective kit. The test was conducted in accordance with the manufacturer's protocol.

Positivity was based on the cut-off value, which in this case was over 0.5 optical density (OD). The measurement of OD was done by ELISA reader in 450 nm.

The calculation of results (for each controlled serum) was made by the following formula:

$$S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}}$$

where NC = negative control; PC = positive control; OD sample = OD of the controlled sample.

The assessment of controlled serums was based on the data taken from above formulas, having in mind that

S/P ≤ 40% = Negative; 40% – ≤ 50% suspicious; ≥ 50% positive

The aim of the study was identifying the prevalence of *C. burnetti* antibodies and not the interpretation of the diagnostic results and diagnostic outcomes. The data were pooled and processed to determine the percentage (%) of *C. burnetii* antibody presence in general, as well as in the observation and analysis of eventual differences between the sexes regarding the frequency of Q fever. The correlation coefficient between the two variables was also calculated: age and percentage (%) of Q fever by *C. burnetii*.

During data processing, correlation and regression analysis was performed, in particular: the determination of correlation coefficients between variables, regression analysis, determination of linear regression coefficients, etc.

RESULTS

Samples of work with males and females in the age group of 0–20 years. As mentioned above, 142 people were involved in our study (74 samples from females and 68 samples from males). They were aged between 0.5 and 20.5 years and not all lived in rural areas or had contact with animals. However, the presence of Q fever antibodies was detected in the residents of both rural and urban areas. The initial data of male and female samples were separated by region (Gostivar, Tetovo, Kichevo, Struga, and Debar) in North Macedonia. The serologic examination confirmed the presence of antibodies to *C. burnetii* in some areas, though with a different level in different areas and in different sexes. During the serological control of 142 samples, we obtained the results which are presented in tables.

The results of the study sample in question (Table 1): region – I, range interval of age group by regions – X_{mi} (year), total number of tested persons – Ni, numerical frequency of persons infected with *C. burnetii* – Y_{oi} (num) and relative.

Table 1. **Data on the working sample divided into five regions, females and males in the age group 0–20 years**

			Female		
I	**X_{mi} (year)**		**Ni**	**Y_{oi} (num)**	**Y_{oi} (%)**
Region	Range interval of age group by regions		Total number of tested persons	Numerical frequency of persons infected with *C. burnetii*	Relative frequency of persons infected with *C. burnetii*
Tetovo	0–20	0.05	26	0	0.00%
Gostivar		5.05	16	0	0.00%
Debar		10.05	3	0	0.00%
Kichevo		15.05	3	1	33.30%
Struga		20.05	26	6	23.00%
Total amount			74	7	9.45%

Table 1. **(Continued)**

			Male		
I	**X_{mi} (year)**		**Ni**	**Y_{oi} (num)**	**Y_{oi} (%)**
Region	Range interval of age group by regions		Total number of tested persons	Numerical frequency of persons infected with *C. burnetii*	Relative frequency of persons infected with *C. burnetii*
Tetovo	0–20	0.05	20	0	0.00%
Gostivar		5.05	20	1	5.00%
Debar		10.05	3	0	0.00%
Kichevo		15.05	4	0	0.00%
Struga		20.05	21	4	19.00%
Total amount			68	5	7.35%

Variation of the frequency of people with *C. burnetii*, females and males in the age group of 0–20 years

Table 2 shows data extracted from Table 1, for y_{oif}(%) and y_{oim}(%), referring to x_{mi}(year) values, in the five respective regions for females and males, as well as results obtained in their processing based on a linear model function.

For the pairs of variables [X_{mi}(year), Y_{oi}(%)] and [X_{mi}(year), Y_{oi}(%)], the correlation coeffi-

Table 2. **Variation of the frequency of the infection in both sexes**

			Female	
I	**X_{mi} (year)**		**Y_{oi} (%)**	**Correlation of coefficient (*r*)**
Region	Range interval of age group by regions		Relative frequency of persons infected with *C. burnetii*	Significance (*p*)
				The equation of linear regression line (*y*)
Tetovo	0–20	0.05	0.00%	$r = 0.79$
Gostivar		5.05	0.00%	
Debar		10.05	0.00%	$0.025 < p < 0.05$
Kichevo		15.05	33.30%	
Struga		20.05	23.00%	$y = 0.0159x - 0.0468$
			Male	
I	**X_{mi} (year)**		**Y_{oi} (%)**	**Correlation of coefficient (*r*)**
Region	Range interval of age group by regions		Relative frequency of persons infected with *C. burnetii*	Significance (*p*)
				Equation of linear regression line (*y*)
Tetovo	0–20	0.05	0.00%	$r = 0.63$
Gostivar		5.05	5.00%	
Debar		10.05	0.00%	$0.025 < p < 0.05$
Kichevo		15.05	0.00%	
Struga		20.05	19.00%	$y = 0.0066x - 0.0183$

cients were found to be $r_f = 0.79$ and $r_m = 0.63$ of which are statistically significant with p-values between $0.05 > p > 0.025$ (Fowler et al., 2002). The equations of linear regression lines resulted in:

$$Y_{ef} = 0.0159x - 0.0468 \quad (1)$$
$$Y_{em} = 0.0033x + 0.0183 \quad (2)$$

DISCUSSION

As we can see from Table 1, the data presented reveals the presence of antibodies in people aged 0–20 in North Macedonia, with a general relative frequency of 8.45%. This infection was present in both males and females, with a relative frequency of 9.45% for females and 7.35% for males, respectively. As can be seen, the difference between the sexes in this case was 2.1%, so the females had a higher infection rate. So, based on the study of the data, we can see that the infection has no significant difference between females and males. The data from our study is inconsistent with the statistical data of US CDC, which mentions that the rate of infection among males is twice as high as among females. This is probably due to the fact that other social or ecological factors also interfere here. Since the infection in females and males was approximately at a 1:1 ratio, the data obtained agrees with the data of the French authors Tissot-Dupont et al. (1992), whose studies show that the ratio between males and females is almost 1:1, although the authors point out that there are also cases where it is at the level of 1:2 in favour of males. In order to clarify this aspect, it is necessary to undertake other studies clearly defined from the epidemiological point of view.

The infection rate varied by sex and across different regions. It was observed that in the regions of Tetovo and Debar, the level of antibodies was zero both among males and females; in the region of Gostivar, in age group studied the level of antibodies was zero among females and 5% among males. In the region of Kichevo, the situation was opposite to that of Gostivar: the study revealed positive results among females and negative in males.

We focused our discussion on the number of people with positive results, because if we consider the percentage, the percentage of positivity is high in this age group, of course. This happens due to the small number of analysed samples: one out of three tested was positive. We must emphasise that in the region of Struga, the positivity observed in this age group is much higher than in all other regions. At present, we are not able to explain the reasons why this happens in this region, but perhaps it may have something to do with the fact that as far as we have observed, this particular age group is much closer to animals in this region than in other regions. There may be other causes, which we think should be identified in other epidemiological studies that would take many other epidemiological factors into account. However, we can say that despite the aspects that we discussed for this age group, the total presence of antibodies in all five regions in 142 people tested turns out to be about 8.45%. Compared to other age groups, this prevalence of antibodies in this age group is much lower: in the same regions, in the age group of 20–40 years the prevalence of antibodies was 27.45% (Saiti et al., 2017), and in the age group of over 40 years the prevalence of antibodies was 29.54% (Reçi et al., 2020). It is understood that in the age group of 0–20 years, the frequency of immunoglobulins G was about three times lower. With this serological control alone, we are not able to say why this happens, what the reasons for this level of infection are, but perhaps further research in this direction will give an even more accurate overview that we think will be an important contribution in this direction.

As mentioned, the Q fever infection in humans knows no age limits. We found a positive case in a 15-year-old girl in the Kichevo region. We did not conduct epidemiological research in this direction as it was not the object of our work, but in this case, we emphasise once again the fact that despite our results, we think that if the research is expanded further, this age group will also possibly have higher infection rates. We emphasise this especially in the regions where the number of people tested was

low and the level of infection was 0%. Apart from the epidemiological situation of Q fever in the human population, we also investigated it in animals in these same regions where people were observed, and we noticed the presence of the infection of about 15.89% positivity from a total of 1120 serums of examined farm animal (Saiti, Bërxholi, 2015); this value is about twice as high as in people aged 0–20 years. In support of this fact are also the values of animal immunoglobulins, which in these regions were relatively high: 27.71% in Kichevo, 26.49% in Debar, 14.20% in Struga, 13.04% in Tetovo, and 8.05% in Gostivar (Saiti, Bërxholi, 2015). Therefore, based on the findings of foreign authors, we think that the infection of people comes as a result of the presence of infections in animals, which plays an important role in the spreading of *C. burnetii* in the environment, as well as through its airborne distribution.

In the region of Tetova, the infection in the age group of 0–20 years is at zero level in both sexes, although the number of males tested was 20, while the number of females was 26. With this number of persons (total 46 samples) tested, we cannot with these data definitively to conclude about the absence of the presence of infection. This is because in our opinion we must take into consideration the fact of: (1) the presence of the infection in this region in animals (Saiti, Bërxholi, 2015) as well as (2) the approximate ecological conditions, (3) uncontrolled movements of animals from one region to another and many other epidemiological factors. For this reason, we conclude that these data can serve as a basis for undertaking other broader and deeper studies to clarify these moments. As mentioned above, in the region of Gostivar in the age group of 0–20 the level of antibodies among females was zero and among males it was 5%. Meanwhile, the infection in people in total in this region in all age groups was 7.14% (Reçi, Qoku, 2019), this digit is not high compared to other regions. In addition, in this region it was found that the infection in females and males in all age groups (Reçi, Qoku, 2019) did not have any visible difference. Thus, in males the infection in total was about 7.59 (8%), while in females it was about 6.55 (7%), i.e., approximately at the 1:1 ratio.

The infection was not present in the Debar region, although the number of people tested was not large (three people). We must, however, emphasise that although in the 20–40 age group (Saiti et al., 2017) the number of people tested was not very large compared to the first group (four people), the infection here turned out to be present in one person (about 25%). This data makes us think and conclude that in this region, this group (0–20 years of age) may not have been infected despite the small number of samples tested.

We emphasise yet again that Kichevo is the region with the highest percentage of infection both in humans and in animals (Saiti, Bërxholi, 2015). In our opinion, the high level of infection in animals also plays an important role in the infection of humans. In our opinion, this data is also supported by the data from the literature: according to Maurin and Raoult (1999), the transmission of the infection from humans does not play any important role in the spread of the infection in humans. This fact makes one think that the only way of infection remains the infection from animals, especially during the calving period, this circumstance is also emphasised by Tissot-Dupont et al. (1999).

As shown by the overall data, in the region of Struga the infection of persons tested, by sex, turns out to be at 1:1 ratio, which, as mentioned above, is also supported by the data of Tissot-Dupont et al. (1992). What stands out in this region is the fact that while in the age group of 0–20 years the percentage of infection was higher among females, with 23% (out of 26 people, six tested positive), while among the males of this group the infection fluctuated at the level of 19.05% (four out of 21 tested positive), with a difference of about 4%. In this region, the highest percentage of infection was also observed in other age groups: 37.5% among males in the 20–40 group and 33.3% among females (Saiti et al., 2017). In the age group of over 40 years (Reçi et al., 2020), it was higher among males (25%) than among females (22.2%), with

a difference of about 3%. In our opinion, this difference is also supported by foreign authors, who emphasise that the infection rate of *C. burnetii* can vary by age and sex across different regions. This variation depends on the presence of reservoir animals in the area, as these animals are the source of infection, as well as the opportunities for individuals to be exposed to them (Maurin, Raoult, 1999). Our data are almost consistent with the data of Maurin and Raoult (1999), who emphasise that Q fever appears in an unexpected form and rather in males of active age from 30–60 years.

As Table 2 shows, we analysed the age group of 0–20 years for each region by means of the equation of the regression line and the correlation coefficient, because above we did the numerical and frequency comparative analysis. In the regions of Tetovo, Gostivar, and Debar, the age group of 0–20 years was not infected with *Coxiella burnetii*, in contrast to the region of Kichevo with 33.3% and the region of Struga with 23% infected among females. Meanwhile, males were not affected by the infection in the regions of Tetovo, Debar, and Kichevo, in contrast to the region of Struga with 19% and Gostivar with 5%. Based on the regression plots, we can conclude that there is a more complete linear regression in females as opposed to the straight line in males. The line has a positive direction, which means that with the increase in one, the other also increases, but the distribution of individual cases from the average shows that these two variables are not closely related or the dependence between them, although it exists, is weak. This is also confirmed by the correlation coefficient for females 0.79 (significant, but not high) and for males 0.63 (significant, but not high), which shows that the increase in one affects the increase in the other.

Based on our serological research, *C. burnetii* infection was present in both sexes and with an approximate frequency between them. Based on regions, the infection percentage was different among the sexes. With regard to males, we point out that the infection rate was very high in the region of Struga, while in females the highest infection rate was detected in the Kichevo region. The living conditions in the zones under scrutiny are poor, which represents a predisposing factor in the spread of the infection. Our data are based on the analyses provided by other researchers who report that *C. burnetii* infection in humans is an illness mainly present in the developing countries.

The main conclusion of this introductory research of the problem of Q fever is that since in many countries of the world there is no accurate overview of the presence of this infection, actions must be taken first to recognise the outbreaks in animals and in humans and stop them. Actions to control the epidemiological situation (humans and animals) must be carried out by the veterinary authorities together with the health authorities at the local and national level.

In animals, measures should be implemented to control Q fever. This applies especially to domestic ruminants. Only a combination of measures can be effective in the fight against this infection. Long-term options including vaccination, changes in farm characteristics, manure management, management of animal shearing, special places for birth and holding of the young, elimination of risk materials, control of reservoir animals, ban on visitors in stables, thorough culling of infected animals, the identification and elimination of animals that spread the microorganism, as well as the control of the movement of animals are considered the most optimal measures in the case of outbreaks among humans.

Regarding food and farm biosecurity, *C. burnetii* is the most extreme risk to humans. Due to its qualities such as small infectious doses, resistance in the environment, as well as the routes of air transmission make it capable of causing non-explosive forms of the disease in groups with large populations of people. *C. burnetii* is currently considered a potential agent of bioterrorism and is classified by the CDC as group B biological agent (Drancourt, Raoult, 2005).

Conflict of interest

All authors declare that they have no conflicts of interest.

Author contribution statement

The authors confirm contribution to the paper as follows: study conception and design: Mije Reçi; data collection: Mije Reçi, Ismail Ferati; analysis and interpretation of results: Mije Reçi, Ismail Ferati, Hava Miftari; draft manuscript preparation: Hava Miftari, Ismail Ferati. All authors reviewed the results and approved the final version of the manuscript.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Received 4 October 2024
Accepted 24 October 2024

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COXIELLA BURNETII INFEKCIJOS PAPLITIMAS TARP 0–20 METŲ AMŽIAUS ŽMONIŲ KAI KURIUOSE ŠIAURĖS MAKEDONIJOS REGIONUOSE

Santrauka

Šio tyrimo tikslas buvo nustatyti serologinį *C. burnetii* paplitimą žmonių organizme bei ištirti 0–20 metų amžiaus moterų ir vyrų infekcijos kaitą kai kuriuose Šiaurės Makedonijos regionuose. Kraujo mėginiai buvo paimti iš 142 atsitiktinių žmonių, priklausančių penkiems regionams. Fermentiniu imunosorbento tyrimu (ELISA) šiems pacientams buvo nustatyti antikūnai prieš *C. burnetii* II fazės antigeną. Teigiamas testas buvo pagrįstas ribine verte, kuri šiuo atveju yra didesnė nei 0,5 optinio tankio (OD). Iš 74 tirtų moterų serumų mėginių 7 buvo teigiami (9,45 %), o iš 68 tirtų vyrų serumų mėginių teigiami buvo 5 (7,35 %). Abiejų lyčių infekcijos procentas buvo panašus, o skirtumus lėmė lytis ir regionai. Duomenys apie žmonių Q karštinę gali praversti siekiant plačiau sužinoti apie šią zoonozinę ligą, ypač Šiaurės Makedonijoje, kur yra labai mažai informacijos apie *C. burnetii* infekcijas. Šios išvados turėtų paskatinti sveikatos priežiūros institucijas sutelkti dėmesį į problemos kilmę ir palengvins prevencinių priemonių taikymą.

Raktažodžiai: *Coxiella burnetii*, Q karštinė, kraujo mėginiai, serologinis paplitimas