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The Frequency of Q Fever in the Human Population of Western Macedonia

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Abstract
Q fever is an acute and rarely chronic, zoonotic disease. It is caused by Coxiella burnetii, an obligate intracellular, pleomorphic coccobacillus possessing a prototypic gram-negative bacterial cell wall structure. Our study is aimed at determining the epidemiological situation of this infection in the human population of Western Macedonia. The serological test was conducted using the ELISA test kit by the German SERION Company. The serum isolated from the blood was kept at -30°C until testing. The sera were properly diluted based on the respective protocol, in the ratio of 1: 400, in two phases (dilution 1: 100 and 1: 4). A total of 520 serums were checked of all ages from people with different epidemiological conditions, of which 114 resulted positive, with positivity rate of about 21.90%. The positivity was based on the Cut-off value, which in positive cases was over 0,5. According to our study, the spreading of the infection was affected by lack of knowledge about the preventive measures, including: lack of education on the source and mode of transmission of the infection, the methods of disinfection and disposal of animal products containing C. burnetii, animal treatments, the level of living conditions, etc. Yet, based on the findings of foreign authors, we think that the infection of the people comes as a result of the presence of the infection in animals which plays an important role in spreading the cause in the environment, as well as through its airborne distribution.
Keywords: Q fever, zoonotic disease, Elisa test, human, seropositive.

Introduction

Coxiella burnetii, is a highly contagious bacterium, a pathogen that is the cause of Q fever. Q fever is a zoonotic disease that affects not only animals but also humans and is widespread throughout the world. C. burnetti is isolated by a considerable number of animals, including the farm animals (such as cattle, sheep and goats), wild animals and arthropods [7]

The epidemiological connection between animals and people has been identified later and the infection has been found in a considerable number of hosts [1] and [7]. At first, it was thought that Q fever was a professional risk (for people who worked very closely with the animals), but over time, it was found that the infection risk was related to other groups and individuals who had a specific status (pregnant women, those with heart disease, people with compromised immune systems). In Europe, cases of Q fever in humans were first seen in soldiers in the Balkan region, including Bulgaria [4] and then in Germany, right after World War II [2], and in the Netherlands in 1956 [6]. The development of the infection in humans ranges from asymptomatic, up to quite heavy, but typically it is a mild infection, self-limited and similar to the influenza infection (acute infection). Despite this, in some people it develops more as a chronic infection involving endocarditics and is complicated with other infections (such as vascular and ostheoarticular infections). In pregnant women, Q fever infection can cause a spontaneous miscarriage, premature birth, low birth weight and the development of a chronic infection of C. burnetii [5]. Q fever infection is generally associated with occasional outbreaks in animals and in humans as well as sporadic cases in humans. The assessment of the prevalence of C. burnetii infection is based on the performance of serological studies. The data from the literature suggest that this prevalence varies in different countries. It should be noted that this is related to different serological cut-offs used. So as a consequence, Q fever infection in some European countries is as follows: 2.4% in the Netherlands in blood donors, 1-4% in France, 12.2up to 24% in the Netherlands, 22% in Germany and 38% in Bulgaria. 83.8% in veterinarians who deal with animals in the Netherlands. 7.7% among pregnant women in Bulgaria and 9.1% in pregnant women in the Netherlands. [3]

Material and methods

The study includes data concerning the frequency of Q fever in the human population in both sexes in western Macedonia. For the epidemiological study of this infection, a total of 520 samples of sera were collected from people with various pathologies without any special preference. The serum was separated from blood based on known laboratory methods by centrifugation of 600 rpms for 20 minutes and were preserved in plastic ampoules at -30°C until testing. The check of serum was conducted using the ELISA-SERION test imported from Germany, highly sensitive for these tests. The result is based on the identification of IgG. Positivity is based on the cut-off value, which in this case is about 0.5 OD which is considered as positive. Prior to the check, the sera are diluted in the ratio of 1: 400 in two steps, first in the ratio of 1: 100 and then diluted in the ratio of 1: 4, in conformity with the protocol of the Serion Company.

In our study, as mentioned above, 520 persons were included. Details of the sample with male and female persons are divided based on regions (Tetova, Gostivar, Dibra and Struga) and in different age groups (0-20, 20-40 and over 40 years of age).

Data are grouped and processed to determine the percentage of the spreading of (Q fever) infection in different age groups and in different regions. The coefficient of correlation between two variables: age and percentage of Q fever disease is calculated as well. The study was conducted in the Laboratory of Virology at the Veterinary Medicine Faculty in Tirana, Albania.

Results and Discussion

Preliminary processing of the data obtained from the initial sample consisted of 520 male and female persons shows that the average Q fever in the human population sample of western Macedonia, which has been subject to serological testing, results near 21.9%. Hence, 520 sera of people were tested, of which 114 of them tested positive and are presented in the tables below by region and age groups.

- The initial sample of female and male persons

The initial sample data: Name of region – i; range interval of age groups of regions – Xmi(vj); the total number of persons tested by regions – Ni; numerical frequency of persons positive with Q fever (EQ) – Yoi(num) and the relative frequency of persons positive with Q fever –Yoi(%) are shown in Table 1.

From the data of Table 1 and Chart 1, we conclude that the observed relative frequency of people with Q fever **–y_{oi}-(%)** (Infection) in the five analyzed regions divided into age groups, shows a different frequency. It is evident that this phenomenon in the regions of Tetova, Gostivar and Dibra shows a higher frequency within the age group of over 40 years of age, while in Kercova and Struga regions it belongs to the age group of 20-40 year olds. While analyzing the phenomenon as a whole, it can be said that the infection has affected less the people in the Gostivar area, and more the people from the region of Kercova. Finally, we can conclude that as factors that have affected these results is the contact of people with infected animals, especially with sheep, particularly because in this type of animals in these regions, the infection was significantly higher compared with the infection of goats and cattle and the main unavoidable factor is believed to have been the aerosol infected with C. burnetii.

- Variation of the frequency of people with Q fever by age groups

The Table 2 shows data extracted from Table 1, for y_{oi} (%) referring to x_{mi} (year) values of the mid-intervals of age groups in the five respective regions, as well as results obtained in their processing based on a linear model function.

Table 1. Data of the initial sample divided into five working regions with men and women of different age groups

I	Xmi(vj)		Ni	Yoi(num)	Yoi(%)
Region	Range interval of age groups by regions		Total number	Numerical frequency	Relative frequency
			of tested persons	of persons positive with Q fever	of persons positive with Q fever
Tetova	0-20	0,05 – 20,05	46	0	0,00%
	20-40	20,05 – 40,05	34	8	23,52%
	> 40	>40,5	75	21	28,00%
	Total:		**155**	**29**	**18,7**
Gostivar	0-20	0,05 – 20,05	36	1	2,77%
	20-40	20,05 – 40,05	34	2	5,88%
	> 40	>40,5	70	7	10,00%
	Total:		**140**	**10**	**7,14**
Dibra	0-20	0,05 – 20,05	6	0	0,00%
	20-40	20,05 – 40,05	8	2	25,00%
	> 40	>40,5	50	20	40,00%
	Total:		**64**	**22**	**34,37**
Kercova	0-20	0,05 – 20,05	7	1	14,20%
	20-40	20,05 – 40,05	14	8	57,10%
	> 40	>40,5	53	23	43,30%
	Total:		**74**	**32**	**43,2**
Struga	0-20	0,05 – 20,05	47	10	21,20%
	20-40	20,05 – 40,05	14	5	35,70%
	> 40	>40,5	26	6	23,00%
	Total:		**87**	**21**	**24,13%**
	Total amount:		**520**	**114**	**21,90%**

Progression of the relative frequency observed of heads with Q fever (infection) from region to region is illustrated in the following diagram:

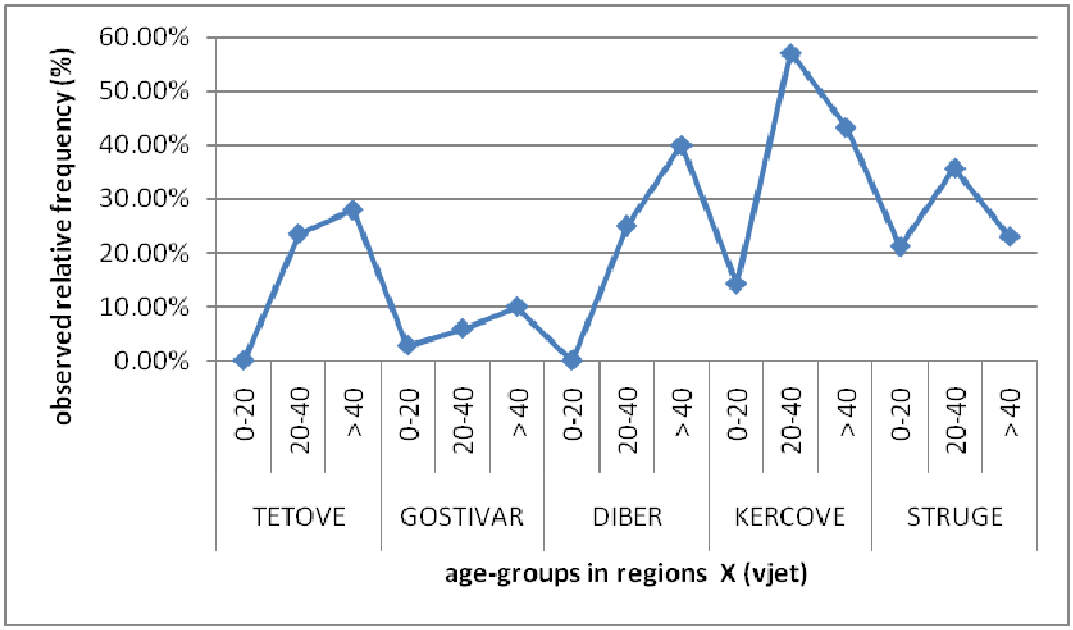

Chart 1. Polygonal line of relative frequency observed in persons with fever-Q in the initial sample.

Table 2.

I REGION	Xmi(year) Middle range of age-groups of the region		Yoi(%) Relative frequency of persons positive with Q fever	The equation of linear regression line (y) Correlation of coefficient (r) and their siginificance (p)
TETOVA	0-20	0,05	0,00%	y=0,007x + 0,0314
	20-40	20,05	23,52%	r=0,93
	> 40	40,05	28,00%	0,025<p<0,050
GOSTIVAR	0-20	0,05	2,77%	y=0,0018x + 0,0259
	20-40	20,05	5,88%	r=0,99
	> 40	40,05	10,00%	0,025<p<0,050
DIBRA	0-20	0,05	0,00%	y=0,01x + 0,0162
	20-40	20,05	25,00%	r=0,98
	> 40	40,05	40,00%	0,025<p<0,050
KERCOVA	0-20	0,05	14,20%	y=0,0073x + 0,2361
	20-40	20,05	57,10%	r=0,66
	> 40	40,05	43,30%	0,025<p<0,050
STRUGA	0-20	0,05	21,20%	y=0,0005x + 0,2573
	20-40	20,05	35,70%	r=0,11
	> 40	40,05	23,00%	0,025<p<0,050

Based on the method of smaller squares, the equation of the line of linear regression for the interval of age groups is also determined (0-20; 20-40; > 40) for all regions, and it resulted as follows:

$$Y_{e(TETOVA)} = 0{,}007x + 0{,}0314 \quad (1)$$
$$Y_{e(GOSTIVAR)} = 0{,}0018x + 0{,}0259 \quad (2)$$
$$Y_{e(DIBRA} = 0{,}01x + 0{,}0162 \quad (3)$$
$$Y_{e(KERCOVA)} = 0{,}0073x + 0{,}2361 \quad (4)$$
$$Y_{e(STRUGA)} = 0{,}0005x + 0{,}2573 \quad (5)$$

Also, the correlation coefficients between variables: $r_{(Tetova)}=0{,}93$; $r_{(Gostivar)}=0{,}99$; $r_{(Dibra)}=0{,}98$; $r_{(Kercova)}=0{,}66$ and $r_{(Struga)}=0{,}11$. The significance level $0.05 > p > 0.025$ is determined by respective statistical formulas and tables of critical values for correlation coefficients, data in the literature [Crawsh],[Fowler],[Koni].

Chart 2 illustrates the position points of the sample distribution (X_{mi}, Y_{oi}) extracted from Table 2, including the respective lines known as polygonal frequency lines as well as, the positions of the straight lines (1), (2), (3), (4) and (5).

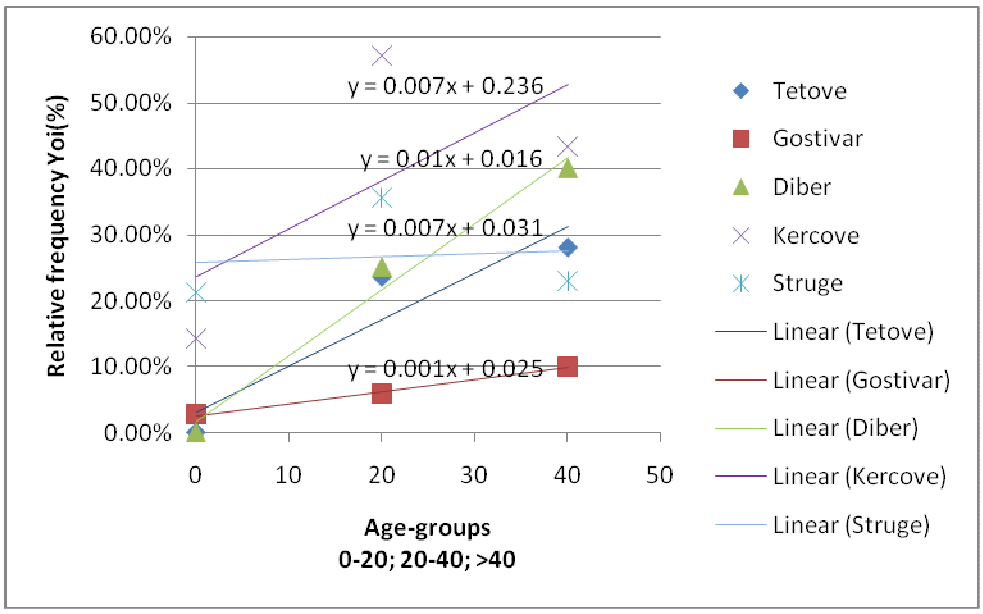

Chart 2. Polygonal line frequency; linear regression lines of samples by age groups

In Table 2 (Chart 2) is shown that:
- In the age interval of 0-20 year olds in the Tetova region (1) and Dibra (3) there is no infection, the infection is much lower in the Gostivar region (2) about 2.77, the highest is in the region of Struga, for about 7% higher than in the Kercova one, and for 18.43% higher than in the Gostivar region, while the expected data of the infection from the Kercova region (4) are around 11.43% higher than those of the Gostivar region;
- In the age interval of 20-40 year olds, we also notice that the data from Gostivar region are lower, while the highest are in the Kercova region, for about 21% higher than in the Struga region, around 32% higher than the Dibra region, around 33.5% in Tetova region and much higher, about 51% than the Gostivar region. So for this age range, the lower infection is in the Gostivar region, about 5.88%, and the highest, in the Kercova region, for about 57.10%;
- In the age interval of over 40, the lowest percentage of infection was also observed in Gostivar region with 10%, while the highest in Kercova region with 43.30%. When comparing the percentage of infection in the Kercova region with that of the other regions, we can see that they are similar to Dibra region, with a difference of only around 3.3%. With Gostivar region this difference is much higher for 33.3%, with that of Tetova for about 15.3% and for almost two times as high, with the percentage of infection with Struga region, resulting at about 20.3% higher.

Conclusions

Based on our serological research, we can conclude that we have identified for the first time in western Macedonia the presence of serological infection in humans. The percentage of this infection varies in different

areas and in different ages. In Kercova area the infection is 43.2%, in Gostivar 7.14%, in Tetova 18.7%, in Struga 24.13% and Dibra 34.37%. As it can be seen, despite the fact that in this study we have used a serological cut-off, the infection ranges from a lower level of 7.14 in Gostivar, to the highest level of 43.2% in Kercova. Moreover, in age groups, the infection shows a different frequency as well. The highest frequency of this infection is in the age group of over 40, with 28% in the Tetova region, 10% in Gostivar and 40% in Dibra, while in the age group of 20-40, the higher frequency is in the Kercova region, with 57.10% and Struga region with 35.70%. While analyzing the phenomenon as a whole within age groups, it can be said that the infection has affected less the people in Gostivar area, in the age group of 0-20 year olds, with 2.77% and more the people of the region of Kercova in the age group of 20-40 year olds, with 57.10%.

We think that our study should call attention to the human medical service, which must take into consideration the presence of Q fever infection, which should take into consideration the presence of Q fever infection in determining the diagnosis.

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