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The statistical analysis of the prevalence of Q fever antibodies in farm animals in Western Macedonia

ISMIJE SAITI[1*], KASTRIOT KORRO[2], KRISTAQ BËRXHOLI[3]

[1]Faculty of Natural Sciences and Math, Study Program of Biology, State University of Tetova, Tetovo, Macedonia,

[2]Lecturer for "Zoo & Wildlife and Medicine", Faculty of Veterinary Medicine Agricultural University of Tirana, Tirana, Albania

[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine Agricultural University of Tirana, Tirana, Albania

*Corresponding author; E-mail: ismije.saiti@unite.edu.mk

Abstract

Query or Queensland fever (Q fever) is a bacterial infection affecting a variety of animal species as well as human beings. Q fever is caused by *Coxiella burnetii*, an obligate, intracellular, rickettsial organism that can survive in a dried condition for extended periods. The aim of study was to examine the prevalence of Q fever antibodies in farm animals (sheep, goats and cows) and determining the statistical trend with descriptive and conclusive statistical methods according to species in five regions in Western Macedonia (Tetovo, Gostivar, Kicevo, Debar and Struga). A total of 1,120 farm animals were examined, of which 178 serums resulted positive, with a scale of 15.89% positivity. Based on species, the infection is widespread in all three species and in every region. The percentage of infection in sheep in the whole region of Western Macedonia is 26.37% - a very high rate compared to that in goats 6.60% or cows 7.50%. The statistical analysis of the data results, prove that there is a connection and similarity among the samples from five regions in terms of the spread of the Q fever infection in farm animals (sheep, goats and cows). The serums were conserved in -30 °C and as a serological test was used ELISA IDEXX, which is carried out based on its relevant protocol using purified antigen of C. burnetii.

Keywords: Q-fever, Elisa test, antigen, prevalence, farm animals.

1. Introduction

Q fever is a disease caused by infection with *Coxiella burnetii*, [2] a bacterium that affects humans and other animals. This organism is uncommon, but may be found in cattle, sheep, goats and other domestic mammals, including cats and dogs. The infection results from inhalation of a spore-like small cell variant, and from contact with the milk, urine, feces, vaginal mucus, or semen of infected animals. Rarely, the disease is tick borne. [1] The incubation period is 9-40 days. A human being can be infected by a single bacterium. [13] The bacterium is an obligate intracellular pathogen.

It was first described by Edward Holbrook Derrick [7] in abattoir workers in Brisbane, Queensland, Australia. The "Q" stands for "query" and was applied at time when the causative agent was unknown; it was chosen over suggestions of "abattoir fever" and "Queensland rickettsial fever", to avoid directing negative connotations at either the cattle industry or the state of Queensland. [9]

The pathogen of Q fever was discovered in 1937, when Frank Macfarlane Burnet and Mavis Freeman isolated the bacterium from one of Derrick's patients. [3] It was originally identified as a species of *Rickettsia*. H.R. Cox and Gordon Davis isolated it from ticks in Montana, USA in 1938. [6] It is azoonotic disease whose most common animal reservoirs are cattle, sheep and goats. *Coxiella burnetii* is no longer regarded as closely related to Rickettsiae, but as similar to *Legionella* and *Francisella*, and is a protobacterium.

Cattle, goats and sheep are most commonly infected, and can serve as a reservoir for the bacteria. Q fever is a well-recognized cause of abortions in ruminants and in pets. *C. burnetii* infection in dairy cattle has been well documented and its association with reproductive problems in these animals has been reported in Canada, USA, Cyprus, France, Hungary, Japan, Switzerland and West Germany. [18]For instance, in a study published in 2008, [5] a significant association has been shown between the herds' seropositivity and typical clinical signs of Q fever observed such as abortion, stillbirth, weak calves and repeat breeding. Moreover, experimental inoculation of C. burnetii in cattle induced not only respiratory disorders and cardiac failures (myocarditis) but also frequent abortions and irregular repeat breeding. [12]

The pathogenic agent is found everywhere except New Zealand. [4] The bacterium is extremely sustainable and virulent: a single organism is able to cause an infection. The common way of infection is inhalation of contaminated dust, contact with contaminated milk, meat, wool and particularly birthing products. Ticks can transfer the pathogenic agent to other animals. Transfer between humans seems extremely rare and has so far been described in very few cases.

Most cases of Q fever are diagnosed based on detection of phase I and II antibodies (between acute and convalescent paired sera); [11], [14] a 4-fold rise in complement-fixing antibody titer against phase II antigen occurs and yields the highest specificity. This requires a baseline sample and another sample in 3-4 weeks. Thus, serologic tests are not helpful acutely but may later confirm the diagnosis: seroconversion generally occurs between days 7 and 15 and is almost always present by 21 days.

The 3 serologic techniques used for diagnosis include indirect immunofluorescence (IIF) (method of choice), complement fixation, and enzyme-linked immunosorbent assay (ELISA) (comparable to IIF). As noted above, significant titers may take 2–4 weeks to appear. Laboratory values vary considerably, so clinicians must interpret results according to their local standards [17].

Q fever can cause endocarditis (infection of th e heart valves) which mayrequire transoesophageal echocardiography to diagnose. Q fever hepatitis manifests as an elevation of ALT and AST, but a definitive diagnosis is only possible on liver biopsy, which shows the characteristic fibrin ringgranulomas. [19]

Q fever has been described as a possible biolo gical weapon. [10] The United States Investigated of Q fever as a potential biological warfare agent in the 1950s, with eventual standardization as agent OU. At Fort Detrick and Dugway Proving Ground, human trials were conducted on Whiecoat volunteers to determine the median infective dose (18 MICLD 50/person i. h.) and course of infection. As standardized biological, it was manufactured in large quantities at Pine Bluff Arsenal, with 5,098 gallons in the arsenal in bulk at the time of demilitarization in 1970. Q fever is category "B" agent. [15] It can be contagious, and is very stable in aerosols in a wide range of temperatures. Q fever microorganisms may survive on surfaces up to 60 days. It is considered a good agent in part because its ID50 (number of bacilli needed to infect 50% of individuals) is considered to be 1, making it the lowest value known to man.

2. Material and Methods

The study in question includes the statistical data related to the frequency of the Q-Fever in farm animals (sheep, goats and cows) in five regions in Western Macedonia: Tetovo, Gostivar, Kicevo, Debar and Struga. A total of 1,120 serums were collected. They were taken randomly without any preference. The serums were extracted from blood through centrifugation and after the 2ml plastic ampoule was set, they were kept at -30°C until they were used. ELISA was the method that was applied in this case. The ELISA kit was imported from ID vet – Montpelier in France. The functioning principle of the kit is as follows: the serums (that are to be examined)

will be diluted in micro titration plates at 1:10. They are then incubated for 45 minutes and after rinsing, the conjugate is added and then other ingredients to finish with the stoppage solution. The incubation times have been strictly abode by in conformity with the preset criteria in the respective kit. The measurement of OD was made using a 450nm ELISA reader. The calculation of results (for every examined serum) was done based on the following formula:

$$S / P = \frac{\text{ODsample} - \text{ODNC}}{\text{ODPC} - \text{ODNC}}$$

Where upon: NC = Negative Control;PC = Positive Control; OPD sample = OD of the examined sample.

The assessment of the examined serums is based on the data taken from the above-cited formula having in consideration that:

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

Results were processed by using statistical methods, such as the line equation of linear regression and the correlation coefficient.The study in question was carried out at the Virology Lab of the Faculty of Veterinary Medicine in Tirana, Albania.

3. Results and Discussion

Preliminary processing of the data obtained from the work sample consisted of 1120 farm animals (sheep, goats and cows) shows that the average Qever in the farm animals sample of western Macedonia, which has been subject toserological testing, results near 15.89%. The serums were collected from animals without any visible specific clinical signs in terms of the presence of the Q-Fever. The serologic examination confirmed the presence of the infection in almost all zones, though with a different level in different areas and in different species. The affected animals are usually asymptomatic, which requires serologic examinations to be carried out in order to realize the real situation. In the region of Western Macedonia, there are a few or no data at all in terms of the dissemination of this infection in animals, let alone human beings. Apart from the epidemiological situation of the Q-Fever in animals, we have investigated it in the human population in the same regions where animals have been observed and for the first time in Macedonia, we have noticed the presence of the infection with about 21.9% positivity from a total of 520 examined human serums, [8] yet, based on the findings of the foreign authors, we think that the infection of the people comes as a result of the presence of the infections in animals which plays an important role in spreading the cause in the environment, as well as through its airborne distribution.Hence, 1120 sera of farm animals were tested, of which 178 of them tested positive andresults were processed with statistical methods from which emerged the following results:

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Table 1. Data of the work sample divided in five regions with farm animals

I	*Ni*	*Yoi (num)*	*Yoi (%)*
Region	Total number of examined animals	Numeric frequency of animals with positive Q-Fever	Relative frequency of animals with positive Q-Fever
TETOVO	322	42	13,04%
GOSTIVAR	298	24	8,05%
DEBAR	151	40	26,49%
KICEVO	166	46	27,71%
STRUGA	183	26	14,20%
Total:	**1120**	**178**	**15,89%**

Descriptive statistics

The work sample data of farm animals are shown in Table 1. Based on the above-presented data, we can see that the infection with the Q-Fever is present in all regions of Western Macedonia. According to the data from Table 1, we can see that the numeric frequency and relative frequency of Q-Fever is higher in the Kicevo region where 46 of 166 (27.71%) farm animals resulted positive; it is followed by the Tetovo region with 42 positive cases out of 322 examined or 13.04%; the region of Debar is next with 40 positive cases out of 151 examined (26.49%); 26 positive cases out of 183 (14.20%) were identified in Struga and finally the Gostivar region was represented with 24 positive cases out of 298 examined farm animals or 8.05%. We can therefore conclude that the observed relative frequency of farm animals with Q-Fever is higher in the Kicevo region and lower in the region of Gostivar. But statistical analysis [16] that will continue to verify this assertion

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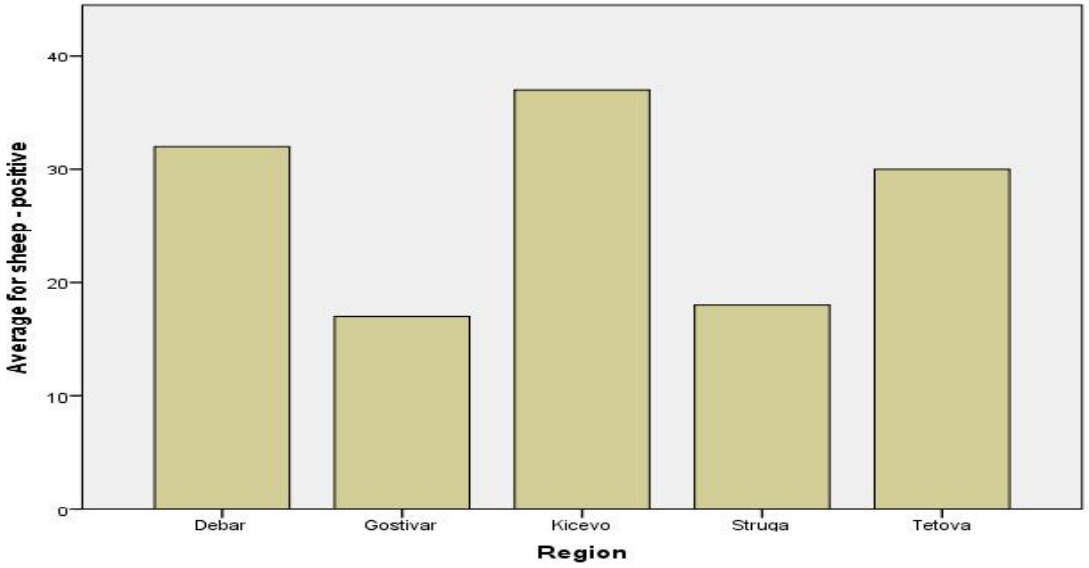

Figure 1. Frequency of average values of sheep infected with Q-Fever in regions

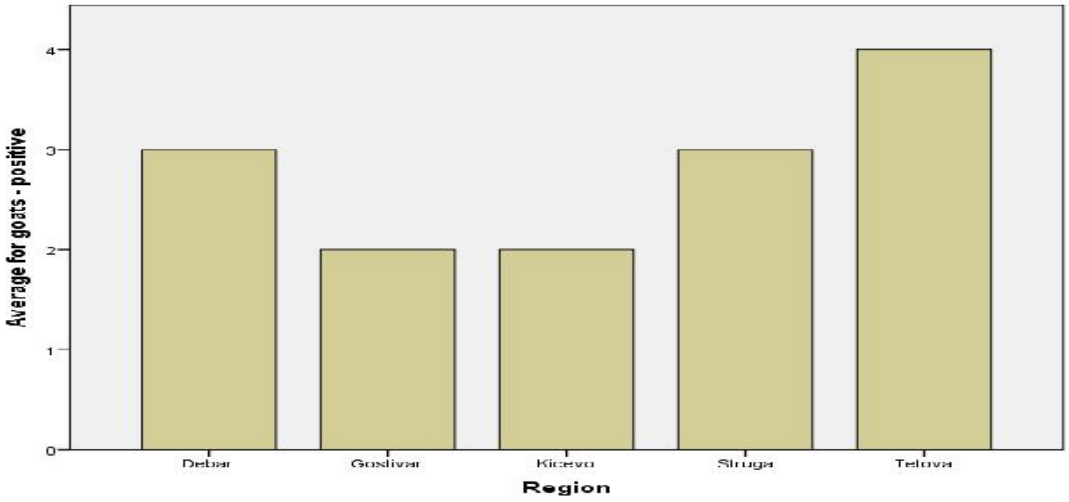

Figure 2. Frequency of average values of goats infected with Q-Fever in regions

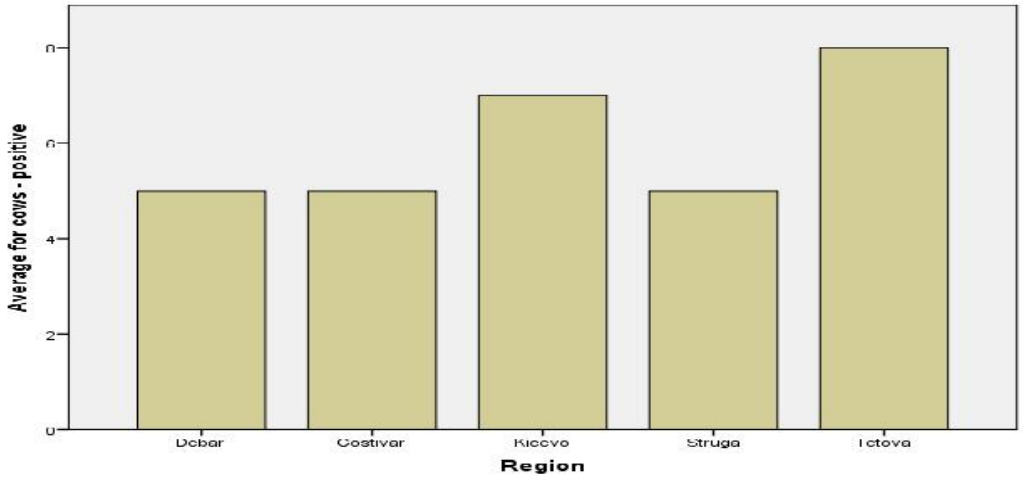

Figure 3. Frequency of average values of cows infected with Q-Fever in regions

Table 2. Descriptive statistics of the spreading of Q-fever

	Minimum	Maximum	Average	Standard Dev.
Frequency of positive cases of Q-Fever in sheep	17	37	26.80	8.871
Frequency of positive cases of Q-Fever in goats	2	4	2.80	0.837
Frequency of positive cases of Q-Fever in cows	5	8	6.00	1.414

As we can see from the table above as well as from the figure representation, we cannot ascertain the statistical trend of the spreading of the Q-Fever; therefore, we will analyze it by using descriptive statistical methods and determine the statistical trend line based on the regression line equation itself.Based on Table 2, it is a bit easier for us to compare the standard deviation coefficients, whereupon we can see that standard deviation in sheep is higher than in goats and cows; we can therefore, conclude that the spreading of Q-Fever in sheep in different regions is not proportionally distributed as in goats and cows. That is why it is difficult to foresee the statistical trend of the spreading of Q-Fever, and as a result, we will analyze it through the regression equation method which will clearly show the statistical trend as well.

Conclusive statistics on animal frequency across regions

The question that may rise here is: "Is there a relation among the regions as far as the spreading of the Q-Fever is concerned?"

In order to come to such a conclusion, we will have to analyze the Chi-Square or the binominal test of confidence of empirical and theoretical frequencies. In case we obtain same data for the five regions under observance, then we can automatically conclude that there is a similarity in the spreading of the frequency of the Q-Fever and the relation among the regions in terms of the spreading of this disease. By analyzing the binominal test (containing two categorizations, i.e. positive-negative), the probability of spreading of the frequency of the Q-Fever based on the theoretical frequency 50%-50% is very low, i.e. approximately 0.000; this shows the probability of the emergence of theoretical frequencies of our sample. In other words, there are very little chances for the theoretical hypothesis to happen in our sample. In all five regions, the indicator is the same, i.e. there is a relation among them ($0.01 > p < 0.05$).

Below you can see the confidence limits having used the Clopper-Pearson method. As regards the Tetovo region, in the first line, we can see that the average of 87% of negative cases has been obtained between the limits of 82% and 90% of the sample of

322 examined heads, which then continues in the regions of Gostivar, Debar, Kicevo and Struga.

Now, let us analyze the total number of infected animals in all regions by using the Chi square and binominal test method. The obtained coefficient should correspond with the above-stated ones, separately for each region.

This means that H0 shows that there is no relation among the regions, whereas H1 shows there is a relation with regard to the existence of the frequency if the Q-fever in said regions, where the logical expectation should be 50%-50%. These analyses will be carried out by using the confidence test and Chi Square method, in the category of dichotomy, because we only have two categories in our case, i.e. positive and/or negative.

Table 3. Clopper -Pearson confidence coefficients

Confidence Interval Summary

Confidence Interval Type	Parameter	Estimate	95% Confidence Interval	
			Lower	Upper
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability(PozitivTE=Negativ).	,870	,828	,904
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability(PoitivGV=Negativ).	,919	,883	,948
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability(PozitivDi=Negativ).	,735	,657	,804
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability(PozitivKE=Negativ).	,723	,648	,789
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability(PozitivST=Negativ).	,858	,799	,905

Table 4. Chi-square coefficients

	Observation N	Expectation N	Residual	**Test statistics**	
Negative	942	560.0	382.0		Positive
Positive	178	560.0	-382.0	Chi-Square	521.157[a]
Total	1120			df	1

By using the classical Chi-square method from Table 5, we can clearly see the total frequencies related to the positive and negative heads: 178 positive and 942 negative heads in all regions. As regards the third column, it shows our expectations for the 50-50 probability, and the fourth column shows the residual difference. Based on these data, we obtain the Chi-square coefficient which is largely greater than zero (the further it is from the zero, the highest the chances are to reject H0 and accept h1), and what interests us in this case is that the probability indicator 0.000 which is smaller than 0.05 (for 95% of the sample) sows that H0 is rejected and H1 is accepted, meaning that there is a relation among the regions in terms of the spreading of the Q-Fever. However, we continue our trials by using the binominal confidence test, for 50-50 expectations or theoretical frequencies, against the empirical frequencies of our study.

Table 5. Statistical indicator of the hypothesis

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The categories defined by Pozitiv = Negativ and Pozitiv occur with probabilities 0.5 and 0.5.	One-Sample Binomial Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Similar to the classical Chi-square result, the binominal confidence test of our study across the regions shows an important piece of information for 0.00 which is again a probability lower than 0.05. In other words, this would mean that our empirical results represent differential aspects of our theoretical results. On the other hand, in the last column, we can see that H0 has to be rejected and H1 accepted, meaning that there is a relation among the regions in terms of the spreading of the Q-Fever.

Based on the binominal confidence test, we can see that 84% are negative in our empirical outcome (0.841) and this average value of 1,120 heads is obtained mainly between the limits of 81% and 86% of our sample, with a 0.00 indicator for 0.05% of the sample.

According to the statistical analyses, we can conclude that there is a connection and similarity among the five observed regions (Tetovo, Gostivar, Debar, Kicevo and Struga) with regard to the spreading of the Q-Fever in our sample consisting of sheep, goats, and cows.

In addition, based on the method of average differences among the types of infected animals with Q-Fever in five regions, it has been concluded that sheep have been the most affected species, with a statistically important difference. With the increase of the number of examined animals, the difference trend will increase in sheep as well, compared to goats and cows, because the standard deviation in calculation does not differ a lot among the three concerned species).

4. Conclusions

The statistical analysis of the data results in the conclusions:

There is a connection and similarity among the samples from five regions (Tetovo, Gostivar, Debar, Kicevo and Struga) in terms of the spread of the Q fever infection in farm animals (sheep, goats and cows).

The difference in mean values among the different animal species affected by the Q fever in the five regions shows that sheep are the most affected compared to goats and cows, with a statistically significant mean variation. With the increase of the number of examined animals, the difference trend will also increase, i.e. the number of affected sheep in contrast to goats and cows will increase too (because

the standard deviation does not differ much among the three species).

5. Recommendations

Based on the above-mentioned results, our recommendations are as follows:

The study on the presence of the Q fever infection should continue;

The study should have a more epidemiological character, whereupon the relation of the infection among different species as well as the role of other social and ecological factors should be thoroughly analyzed;We recommend the usage of infection maps by different subjects involved in the matter.

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