J. Int. Environmental Application & Science, Vol. 16(3): 106-112 (2021)
Research Paper

Analysis of Q Fever Among Farm Animals with ANOVA test in the Western Part of North Macedonia

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Received July 03, 2021; Accepted August 18, 2021

Abstract: Q fever is a zoonotic disease caused by the ubiquitous pathogen *Coxiella burnetii*. The objective of this study was to investigate the comparison of the average differences of positive frequencies with Q fever in farm animals in five regions of western North Macedonia, with the statistical method of Anova and the homogeneity of the regions. From 1120 tested serums, 178 resulted positive, with deviation of the average from one region to another. The Kicevo region had the highest overall average of 0.28 compared to the Gostivar region average of 0.08. It was concluded that the difference between the groups is statistically significant for the level of reliability 0.01. Homogeneity with the Tukey method showed that the sample frequencies in the regions of Dibra and Kicevo were 0.26 and 0.28 above the general average, while in the regions of Gostivar, Tetovo and Struga were 0.15 below the general average and it was concluded that there is a connection between the regions in the spread of Q fever infection. The definite diagnosis of Q fever is made based on a significant increase in serum antibody titers. The serums were conserved in -30 °C and as a serological test ELISA from ID vet Monpelie France was used, which was carried out based on its relevant protocol using purified antigen of *C. burnetii.*
Keywords: *Q-fever, Zoonosis, Anova, Pathogen, Antibody.*

Introduction

Q fever is a zoonotic bacterial infection caused by Gram-negative intracellular bacterium *Coxiella burnetii* (Eldin et al. 2017), which causes abortion in livestock and, acute and chronic illness in humans. Cattle, sheep and goat are considered the main reservoirs of the disease, although the infection has been identified in dogs, cats, wildlife, reptiles and birds (Das *et al*. 2013; OIE, 2013).

The disease is considered as endemic in more than 51 countries (Guatteo *et al.* 2011) but remains a largely 'neglected zoonosis' (Porter et al. 2011). In addition, the disease has been ranked as the most contagious and listed as one among the 13 'global priority zoonoses' (Grace *et al*. 2012). In developing countries, the disease causes significant impact on public health as well as socio-economic structure of the animal husbandry sector. The prevalence in these countries has been reported around 25% and the infected animals are the major sources of infection to farmers and other contact groups (Grace *et al*. 2012). The Netherlands outbreak (2007–2010) of Q fever provided a clear demonstration of the serious threat posed to the public health in the absence of adequate diagnostic, therapeutic and epidemiological tools (Schimmer *et al*. 2012).

Infections caused by *C. burnetii* usually present asymptomatically in livestock although the disease has been implicated in abortion, stillbirths, endometritis, mastitis and infertility (Radolakis et al. 2007; Angelakis and Raoult 2010; OIE, 2013). Infected animals shed *C. burnetii* in urine, faeces, milk, vaginal fluids, semen, placental and birth fluids (Guatteo et al. 2011; Rad *et al*. 2014).

Humans become infected with *C. burnetii* through the inhalation of aerosolized bacteria (Ratmanov et al. 2013) and consumption of contaminated unpasteurized milk. An outbreak of human cases of Q fever reported in the Netherlands was linked to abortions in dairy goat and sheep farms (Angelakis and Raoult, 2010). Clinical cases of Q fever have been reported among the military and paramilitary deployed to Iraq (White *et al*. 2013). Clinical signs in humans include fever, fatigue, weight loss, pneumonia and hepatitis. Patients with underlying cardiac valve defects who get exposed to *C. burnetii* develop endocarditis or vascular infections (Wielders et al. 2015). Miscarriage and abortions have been reported as well (de Lange et al. 2015).

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Regarding food and farm biosafety, *C. burnetii* is the most extreme risk to humans. Due to its qualities such as: small infectious doses, resistance to the environment, as well as air transmission routes, make it capable of causing non-communicable disease forms in groups with large populations. *C. burnetii*, is currently considered a potential bioterrorism agent and is classified by the CDC as a group B biological agent (Drancourt & Raoult, 2005; Kersh *et al*. 2010).

Given the mode of transmission and risk of exposure of Q fever to humans, a neglect of the disease among livestock farming communities could endanger the lives of those who work and live-in close proximity to the livestock farms. The objective of this study was, by the statistical analysis of the data to investigate comparison of the average differences of positive frequencies with q fever among farm animals between regions of the western part of North Macedonia.

Material and Method

The study in question includes data related to the frequency of the Q-Fever in farm animals (sheep, goats and cows) in five regions of the western part of North Macedonia: Tetovo, Gostivar, Kicevo, Dibra and Struga. A total of 1,120 serums were collected. They were taken randomly without any preference. Blood samples were collected aseptically from the jugular vein directly into plain vacuum tubes. Samples were centrifuged at 1500 × g for 15 min to obtain sera. The serums after the 2ml plastic ampoule was set, they were kept at -30°C until they were used. Antibodies to *C. burnetii* were detected by a commercial indirect enzyme-linked immunosorbent assay (ELISA test using microtitre plates pre-coated with the *C. burnetii* phase I and II strains). 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 aboding by in conformity with the present 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:

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

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:

$S/P \leq 40\%$ = Negative; 40% - $\leq$ 50 % suspicious; $\geq$ 50 % positive

So, as recommended by the manufacturer, an animal was considered to be ELISA-strong positive if the optical density (OD) percent was over 80. An OD percent between 50 and 80 was considered positive. A doubtful ELISA result was noted if the OD percent was between 40 and 50, while an OD below or equal to 40 was considered a negative animal. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer (ID vet – Montpelier in France) was 99% and 98%, respectively.

The study in question was carried out at the Virology Lab of the Faculty of Veterinary Medicine in Tirana, Albania. Exploratory data analysis was performed to generate descriptive statistics. Categorical variables were compared using statistical methods Anova and Tukey. The Anova method was used with a confidence level of 0.01, to analyse the comparison of mean differences of the positive frequencies of Q fever between the regions. The homogeneity with the Tukey method was used with the reliability limits for the level of 95% of the sample in the regions grouping.

Results

In our study, are involved 1120 samples of farm animals without any visible specific clinical signs in terms of the presence of the Q fever (508 sheep, 212 goats and 400 cows). The sample data have been separated from region to region (Tetovo, Gostivar, Kicevo, Dibra and Struga) in the western part of North Macedonia. The serologic examination confirmed the presence of antibodies to *Coxiella burnetii* in almost all zones, though with a different level in different areas and in different species. In the western part of North Macedonia, apart from the epidemiological situation of the Q fever in animals, we have investigated it also in the over-40 age group in the human population, in the same regions where also animals have been investigate, and we have noticed the presence of the infection with about 28.10% positivity from a total of 274 examined human serums (female gender with positivity 26.76% and male

gender with 29.54%) (Reçi *et al.* 2020), 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. The results were processed with statistical methods Anova and Tukey, and are presented below:

Comparison of the mean difference between animals infected (in total) between regions with the statistical method Anova

By comparing the differences of the mean of positive frequencies with the Q fever between the regions, they were grouped and a comparison between them was made. For this conclusive analysis, the statistical method of Anova and the homogeneity of the regions were utilized.

Table 1. Comparison of the difference of the mean between the regions

Region	N	Mean	Std. Deviation	Std. Error	95% confidence		Min.	Max.
					Lower Bound	Upper Bound		
Tetovo	322	0.13	0.337	0.019	0.09	0.17	0	1
Gostivar	298	0.08	0.273	0.016	0.05	0.11	0	1
Dibra	151	0.26	0.443	0.036	0.19	0.34	0	1
Kicevo	166	0.28	0.449	0.035	0.21	0.35	0	1
Struga	183	0.14	0.350	0.026	0.09	0.19	0	1
Total	1120	0.16	0.366	0.011	0.14	0.18	0	1

In the first column from table 1 is shown the number of animals infected with Q fever between the regions, while in the second column is shown how the averages deviate from each other, e.g., the Kicevo region has the highest average of 0.28 compared to the Gostivar region average of 0.08 of the overall average.

Table 2. Coefficients obtained by the Anova method

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.159	4	1.540	11.959	0.000
Within Groups	143.552	1115	0.129		
Total	149.711	1119			

Table 3. Coefficients for multiple comparison

Region	Region	Std. Deviation	Std. Error	Sig.	Lower Bound	Upper Bound
Tetovo	Gostivar	0.050	0.029	0.416	-0.03	0.13
	Dibra	-0.134*	0.035	0.001	-0.23	-0.04
	Kicevo	-0.147*	0.034	0.000	-0.24	-0.05
	Struga	-0.012	0.033	0.997	-0.10	0.08
Gostivar	Tetovo	-0.050	0.029	0.416	-0.13	0.03
	Dibra	-0.184*	0.036	0.000	-0.28	-0.09
	Kicevo	-0.197*	0.035	0.000	-0.29	-0.10
	Struga	-0.062	0.034	0.359	-0.15	0.03
Dibra	Tetovo	0.134*	0.035	0.001	0.04	0.23
	Gostivar	0.184*	0.036	0.000	0.09	0.28
	Kicevo	-0.012	0.040	0.998	-0.12	0.10
	Struga	0.123*	0.039	0.016	0.02	0.23
Kicevo	Tetovo	0.147*	0.034	0.000	0.05	0.24
	Gostivar	0.197*	0.035	0.000	0.10	0.29
	Dibra	0.012	0.040	0.998	-0.10	0.12
	Struga	0.135*	0.038	0.004	0.03	0.24
Struga	Tetovo	0.012	0.033	0.997	-0.08	0.10
	Gostivar	0.062	0.034	0.359	-0.03	0.15
	Dibra	-0.123*	0.039	0.016	-0.23	-0.02
	Kicevo	-0.135*	0.038	0.004	-0.24	-0.03

From the Anova table, we see and conclude that the difference between the groups is statistically significant for the level of reliability valued 0.01. Table 3 presents the analyses with the Tukey method for detailed comparisons between one region with all other regions. Their statistical indicators and reliability limits for the 95% sample level are presented in the following Table 3

Table 4. Coefficients for ranking cities according to the mean difference

Tukey HSD			
Region	N	Subset for alpha = 0.05	
		1	2
Gostivar	298	0.08	
Tetovo	322	0.13	
Struga	183	0.14	
Dibra	151		0.26
Kicevo	166		0.28
Sig.		0.416	0.997

Table 4 of homogeneity with the Tukey method, shows that the frequencies of our sample have grouped the regions into two groups according to similarity. In the first group: the regions of Gostivar, Tetovo and Struga are similar in terms of the mean difference, while the second group includes the regions of Dibra and Kicevo, all this for 95% of the sample. So, the regions of Dibra and Kicevo are by 0.26 and 0.28 above the overall average in contrast to the regions of Gostivar, Tetovo and Struga which are below 0.15 lower than the overall average. All this is presented in the figure below:

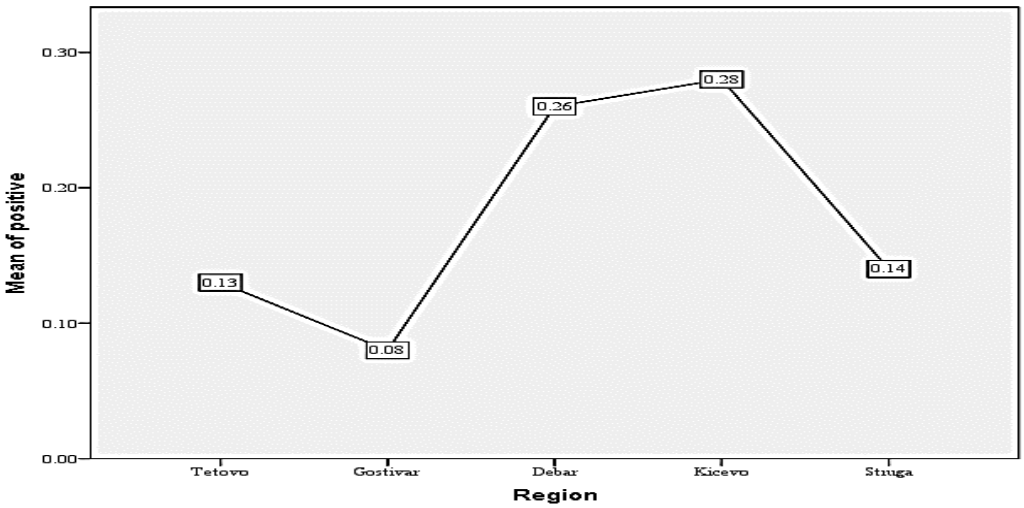

Figure 1. Presentation of regions according to the mean frequencies of Q fever

Comparison of the means differences between animal species with the statistical method Anova
To analyse the differences in means between animal species, descriptive statistics are firstly presented as follows:

Table 5. Comparison of the mean differences between animal species

	N	Mean	Std. Deviation	Std. Error	95% confidence interval for mean		Mean	Max.
					Lower Bound	Upper Bound		
Sheep	508	0.26	0.441	0.020	0.23	0.30	0	1
Goats	212	0.07	0.249	0.017	0.03	0.10	0	1
Cows	400	0.08	0.264	0.013	0.05	0.10	0	1
Total	1120	0.16	0.366	0.011	0.14	0.18	0	1

Table 5 shows that the mean of sheep moves by 0.26 above the total average, despite the low mean of cows and goats by 0.08 and 0.07 above the total average, with standard deviation from 0.249 to 0.441.

Table 6. Coefficients obtained by the Anova method

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.232	2	5.116	40.970	0.000
Within Groups	139.479	1117	0.125		
Total	149.711	1119			

The difference of the means between the groups is statistically significant for the level 0.01, ascertained from the Anova table above.

Table 7. Coefficients for multiple comparison

(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% confidence interval for mean	
					Lower Bound	Upper Bound
Sheep	Goats	0.198*	0.029	0.000	0.13	0.27
	Cows	0.189*	0.024	0.000	0.13	0.24
Goats	Sheep	-0.198*	0.029	0.000	-0.27	-0.13
	Cows	-0.009	0.030	0.952	-0.08	0.06
Cows	Sheep	-0.189*	0.024	0.000	-0.24	-0.13
	Goats	0.009	0.030	0.952	-0.06	0.08

From the multiple comparison in the table 7, can be noticed the differences between one type with other types as well as their reliability indicators.

Table 8. Coefficients for classification of species according to the mean difference

Species	N	Subset for alpha = 0.05	
		1	2
Goats	212	0.07	
Cows	400	0.08	
Sheep	508		0.26
Sig.		0.944	1.000

Based on the analysis in table 8, we can also divide the animal species into two groups according to the similarity of the positive frequencies. In the first group, goats with an average of 0.07 and cows with an average of 0.08 are distinguished, while in the second group, sheep with an average of 0.26 are distinguished from the first group. In the graph presentation, this movement of means looks like this:

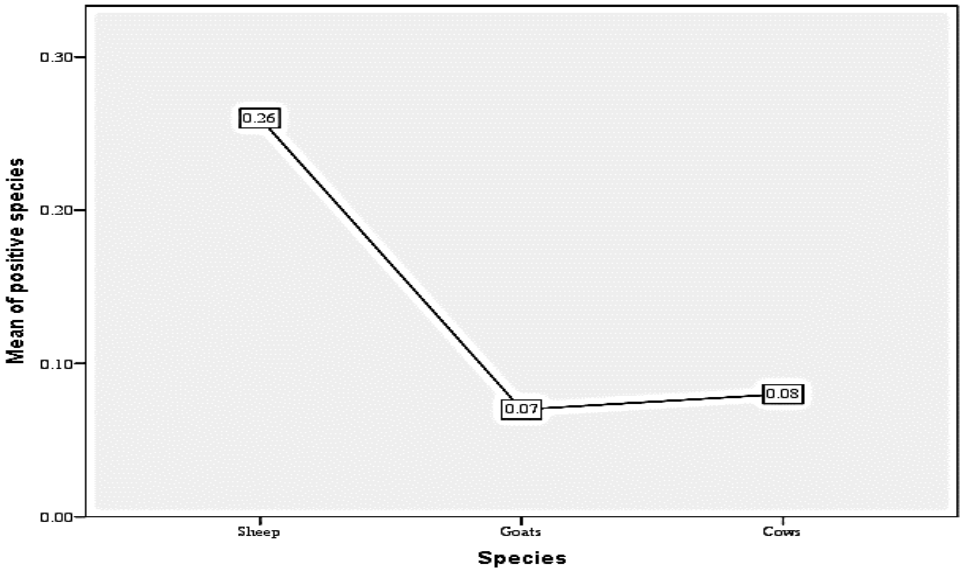

Figure 2. Presentation of animal species according to the mean frequencies of Q fever

Discussion and Conclusions

Although the chi-square method has proven that there is a relation among the five regions in the spread of Q fever infection (Reçi & Qoku, 2017), anyway, the statistical method of the mean differences between the regions has found that there is a mean difference between the regions and this difference distinguishes the regions of Dibra and Kicevo as regions with the highest prevalence of Q fever, in contrast to the regions of Gostivar, Tetovo and Struga, but with a higher standard error in the averages of Dibra and Kicevo, which indicates that this the phenomenon will not be constant with increasing sample number.

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).

In animals, measures should be implemented to control Q fever, especially for domestic ruminants. Only a combination of measures can be effective in fighting this infection. Long-term options, including vaccination, changes in farm characteristics, manure management, animal shearing management, special places for giving birth and keeping little ones, elimination of risk materials, entry ban in stables, the total slaughter of infected animals, the identification and elimination of animals that are eliminators of the microorganism and the control of animal movement, are considered the most optimal measures in case of human outbreaks.

Although in many countries of the world there is no accurate overview of the presence of this infection, in order to establish the rate of infection in other parts of the country and access the need for inclusion of Q fever among diseases under surveillance, action must be taken in order to recognize the situation and to stop outbreaks in animals and humans. Further study is required Collaboration between the Veterinary Services and Ministry of Health is the key to control diseases in the country.

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