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Occurrence and risk factors of *Coxiella burnetii* in domestic ruminants in Lebanon

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ABSTRACT

Coxiella burnetii causes diseases in humans (Q fever) and animals, domestic ruminants playing a major role in the epidemiology of the infection. Information on *C. burnetii* infection in Lebanon is scanty. In order to assess the prevalence of *C. burnetii* infection in ruminants, a cross-sectional study was undertaken in 2014. A total of 1633 sera from ruminants (865 cattle, 384 sheep and 384 goats) from 429 farms (173 cattle, 128 sheep and 128 goats), in seven provinces of Lebanon were randomly selected and assayed for the presence of antibodies.

39.86% of farms (95% CI: 35.23–44.56) resulted positive. The seroprevalence was 30.63% in Cattle-farms, 46.88% in sheep-farms and 45.31% in goat-farms.

Milk samples collected from 282 seropositive animals (86 cows, 93 sheep and 103 goats) from 171 positive farms were tested by a high sensitive Real-Time PCR targeted to the *IS1111* transposon of *C. burnetii*. The overall prevalence in farms was estimated to be 14.04%. Cattle-, sheep- and goat farm prevalence rates were 15.09%, 10% and 17.24%, respectively.

The findings of the study show that *C. burnetii* prevalence in Lebanese domestic ruminants is related to animal species and farming practices. Indeed, the mixed herds with sheep ($p < 0.01$), the presence of common lambing/kidding areas ($p < 0.001$) in farms where the use of disinfectants was not a routine practice ($p < 0.05$) were identified as important risk factors.

The results of the study provide baseline information for setting up herd management and public health measures for the prevention and control of Q fever in Lebanon.

1. Introduction

Q (Query) fever is a zoonosis widely spreaded throughout the world with the exception of New Zealand [1]. *Coxiella (C.) burnetii* is the causal agent [2], a strict intracellular microorganism belonging to *Coxiellaceae* family, order *Legionellales* of the gamma subdivision of Proteobacteria which displays three different morphological forms in its developmental cycle [3]. Some forms can survive extracellular and even accumulate in the environment [4].

C. burnetii is found in association with arthropods (mainly ticks) [5,6] and vertebrate hosts. In humans the disease may be asymptomatic or appear as atypical pneumonia, granulomatous hepatitis, or self-limited febrile illness. Chronic Q fever can also occur with symptoms of endocarditis, hepatitis and osteomyelitis [7].

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C. burnetii infection of livestock is termed as coxiellosis and it occurs mainly as a chronic but often asymptomatic disease [8], even if reproductive failures such as abortion and stillbirth in small ruminants, and infertility in cattle can be observed [9].

Cattle, sheep and goats [10] may play a major role in human infection as they shed bacteria through milk, birth fluids, placenta, foetal membranes, urine and feces [11]. Humans are mainly infected through inhalation of infected aerosols, through direct contact with infected tissues or fluids of ruminant, or through consumption of unpasteurized milk or dairy [12].

C. burnetii circulation has been reported in several Middle-East countries. Previous studies recorded the presence of infection in east Turkey at rates of 5.8% in cattle and 10.5% in sheep [13]. On the other hand, a study performed in Jordan investigating animals with history of abortion revealed a prevalence of 12.1% in sheep and 10.7% in goats [14]. In Iran the proportion reported are 27.5% in sheep, 54% in goats and 0.83% in cattle [15]. The disease has been described in humans in Cyprus [16], Syria [17] and Iraq [18]. Lebanon, located on the eastern coast of the Mediterranean Sea, has a Mediterranean climate that makes it suitable environment for Q disease, as revealed by recent studies recording a sero-prevalence of 16.9% in goat farms [19] and a sero-prevalence of 37% in humans attending hospitals with suspected clinical symptoms [20]. Information on *C. burnetii* infection in ruminant species different from goats in Lebanon is scanty. In order to determine the prevalence of *C. burnetii* infection in cattle, sheep and goat farms serological and molecular surveys in seven provinces of Lebanon were performed from January to September 2014. Furthermore, the association between possible risk factors and the seropositivity to *C. burnetii* was examined.

2. Materials and methods

2.1. Ethics statement

Verbal consent was taken from all farmers included in the study. Due to the Lebanon cultural settings especially in agricultural areas and for field studies, written consent was not available because the participants were not convinced that they had to sign such type of consent even if they agreed on the validity of the research. The animals were handled according to the Lebanon University regulatory rules for animal research. The Ministry of Agriculture of Lebanon approved the study.

2.2. Study area

Lebanon (35°0'N 35°0'E) is located on the eastern shores of the Mediterranean Sea covering a total area of 10452 km^2 most of it being mountainous. The Mount-Lebanon and the Anti-Lebanon chains run parallel to the sea from north to south bordering a central plateau known as the Bekaa Valley. Lebanon is divided into seven provinces: Akkar and North-Lebanon in the northwest, Baalback-El Hermel in the Northeast, Mount Lebanon and Bekaa in the Middle West and East respectively and South Lebanon and Nabatieh in the South of the country.

The Lebanon climate is determined by its geography and physiography. There is a Mediterranean climate along the coastal and the middle mountain range, whilst there are sub-alpine or mountain climates on the highest slopes, covered by snow during most of the year. Furthermore, the climate becomes arid in some of the northern plains [21].

2.3. Study animals

According to the Ministry of Agriculture of Lebanon on January 2014, the regional population of cattle (N1) was composed of around 71,100 cattle and the population size of goats and sheep (N2) was 910,000 (Tables 1 and 2). Cattle are mainly raised for milk production with the majority of the livestock being in large farms of the Holstein breed in the Akkar province. The rest consists of smallholders with a few (4–5) head of local (Baladi) breeds or Baladi Friesian crossbreds. The animals never leave the farm for grazing and are kept inside all year round, even in traditional farms. Sheep are mainly of the local extremely hardy Awassi breed, and goats are mainly of the local Jabali breed, and the Damascus breed also known as Shami breed native of Syria and their crossbreed. Both sheep and goats are managed under nomadic and semi-nomadic systems, feeding on native pastures and crop residues [21].

Table 1
Density and descriptive statistics of cattle per province according to Ministry of Agriculture of Lebanon.

Province	Number of Cattle	Proportions	Proportional Repartition of cattle	Number of selected cattle Farms
Akkar	11537	0.161	140	28
North Lebanon	6801	0.092	80	16
Mount Lebanon	7065	0.099	85	17
Bekaa	20127	0.283	245	49
Baalback-ElHermel	12835	0.180	155	31
South Lebanon	5177	0.075	65	13
Nabatieh	7558	0.110	95	19
TOTAL	71100	1	865	173

2.4. Study design

This cross-sectional study was performed along all provinces of Lebanon from January to September 2014. The strategy was a simple random sample collection covering the majority of the Lebanese national farms (representative method of population), followed by a stratified random study proportional distribution in the population [22]. This allocation follows the principle of drawing lots (Random Sample) from a population where each individual has the same probability of being drawn. The number of bovine, ovine and goat herds to be sampled was set according to a stratified random sampling study [22], considering an expected prevalence of 10% in cattle and of 20% in small ruminants, according to a previous survey [19] with 5% precision at the 95% confidence level. The following relationship was used for the sample size estimation:

$$n = z^2pq/d^2$$

Where:
- n: sample size
- z: standard error (1.96 for a 95% confidence level)
- p: expected prevalence
- d: level of desired precision (set at 0.05)

2.5. Sampling and sample size

The sample size "n" is represented by 865 Cattle and 768 small ruminants (384 sheep and 384 goats because their number is equal) to estimated prevalence Pa1 = 10% and Pa2 = 20% at a desired allowable error (e = 20%) over the Lebanese territory.

The distribution of samples in seven Lebanese departments, according to the Ministry of Agriculture of Lebanon in 2014, is summarized in Table 1 and in Table 2. At the time of sampling, 5 samples of both milk and serum were collected from each cattle farm, and 3 specimens from each sheep and goats farm. When, the farms number of each species was performed by dividing the sample size into the specimens number collected from each farm.

From January 2014 until September 2014, nr. 1633 blood samples were randomly collected (865, 384, 384) from 173 cattle farms, 256 small ruminants farms (128 goats farms and 128 sheep farms) from

Table 2
Descriptive statistics of sheep and goats per province according to Ministry of Agriculture of Lebanon in 2014.

Department	Number of sheep and goats*	Proportions	Proportional Repartition of sheep and goats	Number of selected goats Farms	Number of selected sheep Farms
Akkar	250.000	0.275	212	35	35
North Lebanon	50.000	0.055	42	7	7
Mount Lebanon	50.000	0.055	42	7	7
Bekaa	200.000	0.22	168	28	28
Baalback-ElHermel	250.000	0.275	212	35	35
South Lebanon	60.000	0.066	50	9	9
Nabatieh	50.000	0.055	42	7	7
TOTAL	910.000	1	768	128	128

* No discriminate between sheep and goats according to the Ministry of Agriculture of Lebanon.

seven Lebanese provinces (Tables 1 and 2).

2.6. Blood samples and serological assay

The blood samples were collected, via jugular vein by disposable needles and vacutainer tubes. Blood samples were centrifuged (2000 *g*, 10 min, 4 °C) and the serum aliquots into sterile cryovials were stored at −20 °C until analysis. IgG phases I and II antibodies against *C. burnetii* were assayed using a commercial Indirect ELISA kit (ID Screen® Q Fever Indirect Multi-species, ID. Vet, Montpellier, France, Kit cat. No. FQS-MS-2 P). Sensitivity and specificity of the ELISA test reach 100% (according to the manufacturer internal validation report). The plates were read at 405 nm using a microplate reader (ELx808, BioTek Instruments Inc., Winooski, VT, USA). The diagnostic relevance of the result was obtained by comparing the OD of the tested sera with the OD of the positive control, and by taking a negative reference serum as the zero value according to approved standardization methods. Optical density lower than 40% was classified as a negative result, density between 40% and 50% as suspicious, while density higher than 50% was considered as positive, according to manufacturer's guidelines. A farm was considered as positive when at least one animal resulted seropositive by Elisa test.

2.7. Milk samples preparation and real time PCR analysis

Milk samples were collected and were stored (−70 °C) to preserve the bacteria, only samples from seropositive ruminants were subsequently examined. The sampling frame for each province is showed in Tables 3 and 4. Milk samples from seropositive animals (86 cows, 93 sheep and 103 goats) from 171 farms were tested for the presence of the *IS1111* transposon of *C. burnetii* by using a Real-Time polymerase chain reaction (RT-PCR) [23]. The total DNA was extracted from 200µl of milk sample by using Invitrogen Pure link Genomic DNA kit according to manufacturer's instruction; the final volume of elute was 100µl. The forward primer, Cox-F (5'-GT CTTA AGG TGG GCT GCG TG) and the reverse primer, Cox-R (5'-CCC CGA ATC TCA TTG ATC AGC) and the TaqMan probe (FAM-AGC GAACCA TTG GTA TCG GAC GTT TAT GG-TAMRA) were used. PCR amplifications were performed using a Biorad CFX96 Real Time System. The Real Time PCR reactions were performed in a final volume of 25 µl using a mixture containing: 1X Advanced Universal Probe Supermix (Biorad), 0.4µM of each primer, 0.5 µM of probe, 2 µl buffer of amplification internal control 10X (Applied biosystems by life Technologies), 0.5 µl internal control of DNA amplification 50X (Applied by life Technologies), DNA extract, H_2O.

PCR parameters were as follows: incubation at 50 °C for 2 min, incubation at 95 °C for 5 min, following 45 denaturation cycles at 95 °C for 15 s then annealing and extension at 60 °C for 1 min. Each sample was examined in duplicate. The sample was considered positive if the Ct was < 40.

2.8. Data collection

A checklist was filled out at the time of sampling to study the risk factors for *C. burnetii* at the farm level, requiring general information including: location (province), farm type (single species or cohabitation with other ruminant species), consistence of animals for farm (range [10–400] for sheep, [5–400] for goat, [3–300] for cattle) source of water (well, river and potable), presence of dogs, ticks infestation on animal at the time of sampling and use of acaricides. The checklist included also items related to the likelihood transmission of infection like existence of a parturition place, the methods of carcasses disposal, the use of disinfectants, the manure management, the movements of animals, proximity to other farms and access to common pasture.

2.9. Data analysis

Descriptive statistic analysis was applied to determine the frequency of both seropositive farms and animals for antibodies against *C. burnetii*. A farm was considered positive when at least one animal resulted positive to the ELISA test. Uncertainty of the estimates was evaluated by calculating the confidence interval at 95% for each proportion, as $C.I_{95\%} = \pm 1.96*\sqrt{P*(1-P)/n}$.

Univariable analysis was carried out by chi-square (χ^2), with the Yates' correction when appropriate, and Odd Ratio (OR) analysis for all risks. The level of significance was set at $p < 0.05$. Statistical analyses were performed based on the analysis provided by the online tool Medcalc® (https://www.medcalc.org/calc/odds_ratio.php).

3. Results

3.1. Seroprevalence

In total, 1633 ruminants (865 cattle, 384 goat and 384 sheep) from 429 farms (173 cattle, 128 goat and 128 sheep) in seven provinces were sampled (Tables 1 and 2). Antibodies specific for *C. burnetii* were detected in animals from all localities.

3.1.1. Seroprevalence at farm level

Considering farms, the overall seroprevalence was estimated to be 39.86% (95% CI 35.22–44.49). Seroprevalence for cattle, sheep and goat farms was 30.63% (95% CI 23.76–37.49), 46.88% (95% CI 38.23–55.52) and 45.31% (95% CI 36.69–53.93) ($\chi^2 = 10.366$, $p = 0.0056$), respectively (Tables 3 and 6).

According to the sampling frame, the high seroprevalence among the seven Lebanese provinces was: 58.1% (95% CI 40.69–75.44) in South Lebanon, 57.6% (95% CI 40. 71–74.44) in Nabatieh, 50.5% (95% CI 40.74–60.25) in Baalback-El Hermel, and 56.67% (95% CI 38.93–74.4) in North Lebanon (Table 3).

Considering the area of origin (Tables 3 and 6), in cattle, the highest seroprevalences at the farm level, 56.25% (9/16) was recorded in North Lebanon compared to the total of other provinces equal to 44/157

Table 3
Descriptive characteristics and estimation of *C. burnetii* seroprevalence by ELISA Test, at farm level and animal level, expressed as total and per ruminant species in different Lebanese provinces.

Variable	Frequency (n)	Positive	Prevalence with C.I (95%)	Akkar	Bekaa	South Lebaanon	Nabatieh	Baalback-El Hermel	North Lebanon	Mount Lebanon
Farm level	–	–	–	–	–	–	–	–	–	–
Cattle	173	53	30.63% (23.77-37.5%)	7/28* (25%)	12/49 (24.49%)	5/13 (38.46%)	7/19 (36.84%)	10/31 (32.26%)	9/16 (56.25%)	3/17 (17.64%)
Sheep	128	60	46.88% (38.32-55.52%)	13/35 (37.14%)	11/28 (22.91%)	7/9 (77.78%)	5/7 (71.43%)	16/35 (45.71%)	5/7 (71.43%)	3/7 (42.86%)
Goats	128	58	45.31% (36.7-54%)	4/35 (11.43%)	12/28 (42.86%)	6/9 (66.67%)	7/7 (100%)	25/35 (71.43%)	3/7 (42.86%)	1/7 (14.3%)
Total	429	171	39.86% (35.23-44.56%)	24/98 (24.49%) (15.98-33%)	35/105 (33.33%) (24.32-42.35%)	18/31 (58.1%) (40.69-75.44%)	19/33 (57.6%) (40.71-74.44%)	51/101 (50.5%) (40.74-60.25%)	17/30 (56.67%) (38.93-74.4%)	7/31 (22.6%) (7.86-37.30%)
Animal level	–	–	–	–	–	–	–	–	–	–
Cattle	865	86	9.94% (7.95-11.93%)	10/140** (7.14%)	24/245 (9.8%)	6/65 (9.23%)	7/95 (7.37%)	23/155 (14.84%)	12/80 (15%)	4/85 (4.7%)
Sheep	384	93	24.2% (19.92-28.48%)	17/105 (16.2%)	15/84 (17.85%)	10/27 (37.03%)	9/21 (42.86%)	31/105 (29.52%)	7/21 (33.33%)	4/21 (19%)
Goats	384	103	26.8% (22.37-31.23%)	4/105 (3.8%)	17/84 (20.24%)	12/27 (44.44%)	8/21 (38.1%)	54/105 (51.43%)	5/21 (23.8%)	3/21 (14.3%)
Total	1633	282	17.27% (15.44-19.10%)	31/350 (8.86%) (8.71-9.01)	56/413 (13,56%) (10.26-16.86)	28/119 (23.52%) (15.91-31.15)	24/137 (17.52%) (11.15-23.88)	108/365 (29.59.%) (24.91-34.27)	24/122 (19.83%) (12.73-26.94)	11/127 (8.67%) (3.77-13.55)

* positive farm on tested farms within province.
** positive animals on tested animals within province.

(28%; 95% CI 21–35) $\chi^2 = 4.43$, $p < 0.035$ (OR 3.39; 95% CI 1.18–9.66). In sheep, the highest seroprevalence: 77.78% in South Lebanon and 71.43 in both Nabatieh, and North Lebanon, was observed, being the total of other Lebanese provinces equal to 43/105 (40.95%; 95% CI 31.5–50.4) $\chi^2 = 6.96$, $p < 0.05$, (OR 4.08, 95% CI 1.49–11.2). In goats the highest seroprevalence of 7/7 (100%) was recorded in Nabatieh, Baalback El-Hermel 25/35 (71.43%) and South Lebanon 6/9 (66.67%) compared to 20/77 (25.97%) of the other provinces $\chi^2 = 27.23$, $p < 0.0001$ (OR 8.33, 95% CI 3.70–18.73) (Tables 3 and 6).

3.1.2. Seroprevalence at the animal level

Considering the entire population sampled (on the animal level), there were 86/865 seropositive cattle (9.94%; 95% CI: 7.95–11.93) according to the expected prevalence (10%, 95% CI 8–12), 93/384 seropositive sheep (24.2%; 95% CI 19.92–28.48) according to the expected prevalence (20%; 95% CI 15.9–24.1) and 103/384 seropositive goats (26.8%; 95% CI 22.37–31. 23) close to the expected prevalence (20%; 95% CI 15.9–24.1). No significant difference was observed between sheep and goats when the results were analysed at the species level $P > 0.1$ (OR 0.8, 95% CI 0.63–1.2), while high significant differences were detected between cattle and both sheep and goats $\chi^2 = 69.1$, $P < 0.0001$ (OR 3.1, 95% CI 2.3–6) (Tables 3 and 6).

Considering the area of origin, in cattle to animal level, the highest seroprevalence of 15% (12/80) and 14.84% (23/155) were recorded in North Lebanon and Baalback- El Hermel respectively, compared to the total of other provinces equal to 8% (51/630) $\chi^2 = 8.9$, $p < 0.05$ (OR 1.99, 95% CI 1.25–3.15). In sheep, the highest seroprevalences of 42.86% (9/21), 37.03% (10/27) and 33.33% (7/21) were detected respectively in Nabatieh, South Lebanon and in North Lebanon being the total of other Lebanese provinces equal to 21.27% (67/315) $\chi^2 = 7.43$, $p < 0.05$ (OR 2.24, 95% CI 1.28–3.9). Finally, in goats the

Table 4
Estimation of *C. burnetii* DNA prevalence by PCR in milk at farm level and animal level, expressed as total and separate for each ruminant species in different Lebanese provinces.

Variable	Frequency (n)	Positive	Prevalence with C.I (95%)	Akkar	Bekaa	South Lebaanon	Nabatieh	Baalback-El Hermel	North Lebanon	Mount Lebanon
Farm level	–			–	–	–	–	–	–	–
Cattle	53	8/53	15.09% (5.46-24.73%)	0/7 (0%)	1/12 (8.33%)	2/5 (40%)	0/7 (0%)	1/10 (10%)	4/9 (44.44%)	0/3 (0%)
Sheep	60	6/60	10% (2.41-17.59%)	0/13 (0%)	1/11 (9.1%)	2/7 (28.6%)	1/5 (20%)	0/16 (0%)	2/5 (40%)	0/3 (0%)
Goats	58	10/58	17.24% (7.52-26.96%)	0/4 (0%)	4/12 (3.33%)	0/6 (0%)	0/7 (0%)	3/25 (12%)	3/3 (100%)	0/1 (0%)
Total	171	24/171	14.04% (8.83-19.24%)	0/24 (0%)	6/35 (17.14%)	4/18 (22.22%)	1/19 (5.26%)	4/51 (7.84%)	9/17 (53%)	0/7 (0%)
Animal level	–	–	–	–	–	–	–	–	–	–
Cattle	86	9/86	10.47% (4-16.93%)	0/10 (0%)	1/24 (4.17%)	2/6 (33.34%)	0/7 (0%)	1/23 (4.35%)	5/12 (41.7%)	0/4 (0%)
Sheep	93	6/93	6.45% (1.46-11.44%)	0/17 (0%)	1/14 (7.14%)	2/10 (20%)	1/9 (11.11%)	0/31 (0%)	2/7 (28.6%)	0/4 (0%)
Goats	103	12/103	11.65% (5.45-17.85%)	0/4 (0%)	4/17 (23.53%)	0/12 (0%)	0/8 (0%)	3/54 (5.6%)	5/5 (100%)	0/3 (0%)
Total	282	27/282	9.57% (6.14-13.01%)	0/31 (0%)	6/55 (10.9%)	4/28 (14.28%)	1/24 (4.17%)	4/108 (3.7%)	12/24 (50%)	0/11 (0%)

Table 5
IS1111 gene of *C. burnetii* detection in milk samples from cattle, sheep and goats in Lebanon.

Number (#) of sample	Provinces	IS 1111 Cycle threshold: Ct	Descriptive statistic of Ct in each animal species
1	Baalback-El Hermel	39.35	Cattle Average:36
2	Bekaa	39.2	ModeValue : 39
3	North Lebanon	32.68	Median:37
4	North Lebanon	33.0	
5	North Lebanon	36.29	
6	North Lebanon	37.47	
7	North Lebanon	37.7	
8	South Lebanon	34.4	
9	South Lebanon	37.94	
10	Bekaa	38.76	Sheep
11	Nabatieh	37.37	Average :35
12	North Lebanon	32.36	Mode Value: 36
13	North Lebanon	33.44	Median:36
14	South Lebanon	37.26	
15	South Lebanon	37.39	
16	Baalback-El Hermel	39.4	Goats Average:37
17	Baalback-El Hermel	39.45	ModeValue : 39 Median:38
18	Baalback-El Hermel	39.3	
19	Bekaa	37.74	
20	Bekaa	38.22	
21	Bekaa	38.22	
22	Bekaa	39.3	
23	North Lebanon	32.0	
24	North Lebanon	33.27	
25	North Lebanon	33.5	
26	North Lebanon	36.0	
27	North Lebanon	36.0	

1→9:Cattle samples; 10→15:Sheep samples; 16→27:Goat samples.

highest seroprevalences of 51.43% (54/105) and 44.44% (12/27) were found in Baalback-El Hermel and South Lebanon, compared to 14.68% (37/252) of the other provinces $\chi^2 = 53.26$, $p < 0.001$ [OR 5.8, 95% CI 3.6–9.5] (Tables 3 and 6).

3.2. C. burnetii DNA detection in milk samples

Among 282 milk samples from seropositive ruminants, DNAs of *C. burnetii* were detected in 9 of 86 (10.47%) cattle, in 6 of 93 (6.45%) sheep and in 12 of 103 (11.65%) goats specimens (Tables 4 and 5). The mean value of the bacteria shedding, as revealed by the threshold cycle (C_t) for each positive sample, was higher in sheep ($C_t = 35$), compared to cattle ($C_t = 36$) and finally, to goats ($C_t = 37$).

Based on the area of origin, the highest shedding of *C. burnetii* DNA via milk from seropositive animals was observed in cattle (41.7%), sheep (28.6%) and goats (100%) from the North Lebanon province. The lowest estimation was observed in Bekaa province for cattle (4.17%) and sheep (7.14%), and in Baalback- El Hermel province for goats (5.6%) (Tables 4 and 5).

3.3. Risk factors analysis

The study of the possible risk variables, performed in 105/429 (25%) farms, detected three factors associated with *C. burnetii* seropositivity in the Lebanese farms (Table 7). In details, by logistic regression analysis, *C. burnetii* infection was mainly found be associated with the presence of ovine in farms ($p < 0.001$). The likelihood (OR) of infection in ovine herds compared to cattle herds was 3.28 (95% CI 1.43–7.5).

In addition, farms where the presence of cattle in farm decrease the infection ($\chi 2 = 4.3$; $p < 0.05$; OR 0.335, 95% CI 0.13-0.87), the use of disinfectants was not a routine practice ($\chi^2 = 5.78$; $p < 0.05$; OR 2.7, 95% CI 1.9–6.15) and farms with the presence of common parturition areas as compared to their absence ($\chi^2 = 16.53$; $p < 0.0001$; OR 5.94, 95% CI 2.48–12.25) had increased the likelihood of the infection. No correlations were found for other investigated variables (Table 7).

A multivariable logistic regression analysis (results not shown in table) identified the presence of lambing and kidding at the same areas as risk factors with $p = 0.024$ [OR 3.16: 95% CI1.5–6.4].

4. Discussion

As *C. burnetii* is a bacterium with unique characteristics in terms of persistence in the environment and hosts [3], gathering information on the impact of *C. burnetii* on ruminants is pivotal. In Lebanon, a few studies have investigated the diffusion of Q fever in goat herds [19] and humans [20]. Depicting a portrait of the disease status in neglected areas, such as Lebanon, is important. In order to fill this gap, in this study we monitored the presence of *C. burnetii* infection in herds of different ruminant species from all Lebanese provinces.

In order to investigate the prevalence of *C. burnetii* we used an indirect ELISA assay able to detect specific antibodies. Also, a Real-Time PCR assay was used for detection of *C. burnetii* DNA in milk samples obtained from seropositive animals. Serological methods are able to reveal previous exposure to *C. burnetii*, but they cannot demonstrate nor be related to the active shedding of this pathogen [24]. In contrast, PCR assays are able to detect *C. burnetii* in body fluids, thus unveiling the shedding patterns of this pathogen among the various herds. Accordingly, milk samples from all the seropositive animals were further tested by Real-Time PCR.

Considering the sampled population at the individual level, the overall seroprevalence of *C. burnetii*-specific IgG antibodies was 9.94%, 24.2% and 26.8% in cattle, sheep and goats, respectively. The seroprevalence rate detected in cattle population (9.94%), fell within the ranges reported in other studies elsewhere, such as in South-Eastern Iran (10.75%) [25] and in some European countries, such as the Basque region in Northern Spain (6.7%) [26] and Albania (7.9%) [27]. However, this rate was lower than those reported in other countries, i.e. 16.8% in Queensland in Australia [28], 28.3% in rural Western Kenya [29] and 38% in Hungary [9].

The seroprevalence in sheep (24.2%) fell in the same range as the rates reported in Middle-East countries, including Southern Marmara in Turkey (20%) [30], and South-Eastern Iran (29.42%) [31], but it was higher than the prevalence rates reported in other European countries (6–15.9%) [9,26,27,32,33] and rural Western Kenya (18.2%) [29]. The prevalence rate was lower than that reported in Sardinia, Italy (38%) [34].

The seroprevalence of *C. burnetii* infection in goats (26.82%) was higher than reported previously elsewhere in Basque region in Northern Spain (8.7%) [26], Albania (9.8%) [27] and in a study from Lebanon (16.90%) [19], but lower than reported in rural Western Kenya (32%) [29] and in South-eastern Iran (65.78%) [25].

These data highlight the temporal/geographical variations of *C. burnetii* seroprevalence in livestock animals and, thus, the changes in exposure risks to *C. burnetii* across different geographical regions [35]. The lack of information on the influence of environmental, socio-economic and behavioural factors on environmental contamination by *C. burnetii*, and on the ability of the pathogen to survive in the environment hampers an exact understanding of the spatio-temporal differences observed in *C. burnetii* seroprevalence. Correct interpretation of the data is also hindered by the use of different serological assays and sampling methods/plans across the various studies.

The overall seroprevalence among herds was estimated to be 39.86% with sheep (46.88%) and goat (45.31%) farms at higher risk of infection than cattle (30.63%) ($\chi^2 = 10.366$, $p = 0.0056$). The highest prevalence was detected in caprine herds as observed in previous studies [35,36], although no significant difference was observed between

Table 6
Association between variables (animal species and spatial distribution) at farm and animal level (based on 429 farms and 1633 animals) and *Coxiella* serological status by corresponding chi square, p-value, odds ratio (OR) and confidence interval (CI).

Factors	Category	Seropositivity percentage (n°seropositive/total)	χ2	P-value χ2	OR	95% CI of OR
Farm Level species	cattle	30.63% (53/173)	**10.366**	**0.0056**		
	sheep	46.88% (60/128)				
	goats	45.31% (58/128)				
	Cattle	30.63% (53/173)	**10.29**	**0.0014**	**1.94**	**1.29-2.91**
	Sheep and Goats	46.09% (118/256)				
	Cattle	30.63% (53/173)	**8.27**	**0.004**	**1.99**	**1.24-3.21**
	sheep	46.88% (60/128)				
	Cattle	30.63% (53/173)	**6.81**	**0.009**	**1.88**	**1.17-3.02**
	goats	45.31% (58/128)				
	sheep	46.88% (60/128)	0.06	0.80	1.06	0.65-1.74
	goats	45.31% (58/128)				
Animal level species	cattle	9.94% (86/865)	**70**	**0.00001**		
	sheep	24.21% (93/384)				
	goats	26.82% (103/384)				
	Cattle	9.94% (86/865)	**69.1**	**1.6-E16**	**3.1**	**2.36-4**
	Sheep and Goats	25.52% (156/768)				
	Cattle	9.94% (86/865)	**44.14**	**5.49E-11**	**2.89**	**2.09-4**
	Sheep	24.21% (93/384)				
	Cattle	9.94% (86/865)	**59.0**	**3.0E-14**	**3.32**	**2.42-4.6**
	Goats	26.82% (103/384)				
	Sheep	24.21% (93/384)	0.68	0.46	0.87	0.63-1.2
	Goats	26.82% (103/384)				
Provinces at farm level						
Cattle	North Lebanon	56.25% (7/16)	**4.43**	**0.035**	**3.39**	**1.18-9.66**
	Total other provinces	28.02% (44/157)				
Sheep	South Lebanon	77.78% (7/9)*	**6.96**	**0.008**	**4.08**	**1.49-11.2**
	Nabatieh	71.42% (5/7)*				
	North Lebanon	71.42% (5/7)*				
	Total other provinces	40.95% (43/105)				
Goats	Nabatieh	100% (7/7)*	**27.23**	**1.8-E07**	**8.33**	**3.70-18.73**
	Baalbak-El Hermel	71.43% (25/35)*				
	South Lebanon	66.67% (6/9)*				
	Total other provinces	25.97% (20/77)				
Provinces at animal level						
Cattle	Nabatieh	15% (12/80)*	**8.83**	**0.0034**	**1.99**	**1.25-3.15**
	Baalback-El Hermel	14.84% (23/155)*				
	Other provinces	8% (51/630)				
Sheep	Nabatieh	42.86% (9/21)*	**7.43**	**0.006**	**2.24**	**1.28-3.9**
	South Lebanon	37.03% (10/27)*				
	North Lebanon	33.33% (7/21)*				
	Other provinces	21.26% (67/315)				
Goats	Baalback-El Hermel	51.42% (54/105)*	**61.98**	**P < 0.0001**	**6.52**	**3.95-10.78**
	South Lebanon	44.44% (12/27)*				
	NabatieH	38.1% (8/21)*				
	Other provinces	12.54% (29/231)				

* the data for the provinces (indicated with the symbol "*"), inside each species, were summarized together and compared with the remaining data from the other provinces.

sheep and goat farms ($p > 0.1$). High significant differences were observed between cattle and sheep ($\chi^2 = 8.27$, $p = 0.004$) and cattle and goats ($\chi^2 = 6.81$, $p = 0.009$). In our study, sheep and goat farms had a nearly two-fold higher risk of infection by *C. burnetii* than bovine farms ($p < 0.001$). The reason for the lower seroprevalence rates monitored in cattle herds could be accounted by a higher susceptibility of small ruminants [24]. Furthermore, the possible observed differences in seroprevalence could be related to differences in animal management. For instance, cattle breeding is mainly based on intensive management, and the animals cannot leave the farm for grazing. On the opposite, nomadic semi-extensive management for sheep and goats is predominant throughout Lebanon [21], thus making small ruminants more exposed than cattle to the risk of infection by *C. burnetii*.

When dissecting the data, different prevalence rates were recorded among the different provinces of Lebanon. Very high rate of seroprevalence was observed in cattle and sheep farms from North Lebanon (56.25% and 71.43%, respectively) ($p < 0.05$) with a nearly three-fold higher risk of infection with respect to the rest of Lebanon. North Lebanon is characterized by subsistence agriculture, with a small bovine population and with a large population of small ruminants either in sedentary or semi-nomadic flocks [21]. This livestock economy can play an important role for transmission of the infection among small ruminants and from small to large ruminants, since in North Lebanon sheep often share pastures with different sheep flocks and with cows. On the opposite, a lower seroprevalence was detected in cattle farms from the province of Akkar, where modern intensive dairies are starting to expand (Asmar, 2011). A high prevalence of *C. burnetii*, was also monitored in sheep in South Lebanon and in Nabatieh ($p < 0.01$) and in goats in Baalback El-Hermel, Nabatieh, and in South Lebanon ($p < 0.0001$), with the risk of infection being nearly eight-fold higher than in the other areas (Table 6). Baalback-El Hermel, in Northern Bekaa, is characterized by aridity and un-cultivated lands and small ruminants are present here in semi-nomadic and nomadic flocks, moving from this province to the coastal plains between late autumn and early of spring. Animal transhumance could play a major role on *C. burnetii* spreading across the country.

In the present study, about 9.6% of the seropositive ruminants were found in active status of infection, with the milk samples testing positive by Real-Time PCR. The rates of shedding of *C. burnetii* in milk varied among the species, with the highest prevalence (11.5%) being

Table 7
Association between variables and *C. burnetii* serological *status* at farm (105) level, with corresponding chi square (Yates), p-value, odds ratio (OR) and 95% confidence interval (CI). Significant values are in bold.

Factor	Category	Frequency (N)	Seroprevalence (%)	χ2 (Yates)	P value χ2	OR	95% CI OR
Cattle in farm	Present	71	56.33	4.348	0.037	0.335	0.129-0.869
	Not present	34	79.41				
Size of cattle farms	3-100	66	60.61	4.695	0.06	15.19	0.80-289
	> 101	5	0				
Sheep in farm	yes	58	75.86	**7.02**	**0.008**	**3.28**	**1.43-7.5**
	Not present	47	49				
Size of sheep farms	10-100	34	70.59	1.247	0.26	0.48	0.13-1.77
	101-400	24	83.33				
Goats in farm	present	44	72.72	1.98	0.16	1.98	0.86-4.6
	Not present	61	57.37				
Size of goat farms	5-100	34	73.53	0.034	0.85	1.19	0.25-5.62
	101-400	10	70				
Source of water	river	15	73.33	2.22	0.33		
	well	44	70.45				
	potable	46	54.35				
Presence of dogs	yes	71	66.19	0.27	0.60	1.37	0.59-3.18
	No	34	58.82				
Presence of ticks	yes	81	69.13	3.4	0.065	2.6	1.04-6.7
	No	24	45.83				
Carcass disposal	Outdoor	40	77.5	4.85	0.182		
	Burial	32	62.5				
	burning	9	44.44				
	landfill	24	50				
Disinfectant use	No	63	73.01	**5.78**	**0.028**	**2.7**	**1.19-6.15**
	yes	42	50				
Manure management	yes	84	63.09	0	1	0.85	0.32-2.35
	No	21	66.66				
Animal movements	yes	38	57.89	0.55	0.46	0.672	0.2957-1.528
	No	67	67.16				
Closed farms	yes	74	60.8	0.59	0.44	0.635	0.257-1.57
	No	31	70.96				
Common pasture	yes	20	75	0.81	0.37	1.9	0.6-5.7
	No	85	61.2				
Lambing/kidding Areas	Present	66	78.79	**16.53**	**0.000048**	**5.94**	**2.48-14.25**
	Not present	39	41				

detected in goats. Shedding of *C. burnetii* in milk in ruminants is intermittent; it can last for several months in goats and cattle [24,37–39], whilst in sheep shedding of *C. burnetii* occurs for a shorter period, 1 to 8 days after the abortion [12]. In our study, *C. burnetii* was detected only in 6.45% of milk samples collected from seropositive sheep, although shedding of *C. burnetii* in ovine milk occurred at higher titles than in bovine and caprine. The anamnestic information and clinical history of the animals at the time of sampling were not collected. Also, the diagnostic tools used in our screening were not intended to assess if the ruminants were in acute or past phase of infection. However, we were successful to observe a correlation between the seropositive status and shedding of *C. burnetii* in milk.

Logistic regression analysis performed on a proportional number of farms, indicated that the presence of sheep in farms was a factor able to increase the risk of positivity ($p < 0.05$). The odd of *C. burnetii* infection in farms with either sheep or both sheep and other ruminant species was significantly higher than in farms where sheep were not present (OR 3.28, 95% CI 1.43–7.5). Also, the higher prevalence of infection observed in farms where common lambing and/or kidding areas were present ($\chi^2 = 16.53$; $p < 0.0001$; OR 5.94, 95% CI 2.48–12.25), may suggest the major role of small ruminants in the epidemiology of infection, likely due to spreading of bacteria with abortions or infected births [12].

There are different studies describing vector-borne transmission of *C. burnetii*. In our study, logistic regression indicated ticks as a risk factor although this was not significant ($p = 0.065$, OR 2.7), due to the relatively low numbers of cases considered. Previous studies [40] have identified in Lebanon tick species that are able to transmit *C. burnetii* [4,5,41]. In this study, *Rhipicephalus sanguineus* was detected from sheep and goats from Nabatieh and Bekaa provinces whilst *Dermacentor marginatus*, able to infect both ruminants and humans, was identified from sheep and goats from Baalback-El Hermel and Mount Lebanon. Interestingly the two species of ticks were not detected from cattle, where we monitored the lowest prevalence rate of *C. burnetii*.

An association was also identified between the herd size and the infection rate. Bovine farms with less than 100 animals were more at risk of infection. This finding mirrors previous data gathered in Southern Iran, where the highest prevalence was found in herds with less than 40 animals [42]. Notably, in Lebanon, bovine herds of small size frequently include also small ruminants [21].

A significant association was also observed between the infection rate and prophylaxis measures and not using disinfectant. In Denmark, adoption of hygienic precautions in herds has been found to decrease the risk of exposure to infection [43]. The absence of hygiene precautions before visiting farms seems to increase the risk of infection by *C. burnetii*. Both farmers and visitors may act as mechanical vectors and transfer pathogens from infected to uninfected animals [44]. Furthermore farmers and veterinary practitioners are at greater risk to be infected with *C. burnetii* being Q fever an occupational disease [45].

5. Conclusions

Our results demonstrated that *C. burnetii* infection is endemic in Lebanese domestic ruminants although with different prevalence rates across the various animal species and on the basis of the economic characteristics of the provinces, chiefly in terms of management system. This study could be a useful piece of information for improving the management of Q fever outbreaks in the future and, possibly, also for

enacting specific control measures in ruminants.

Declarations of interest

None.

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