Multispacer sequence typing of Coxiella burnetii *from milk and hard tick samples from ruminant farms in Lebanon*

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Summary
This study was carried out to detect and characterize *Coxiella burnetii* in ruminant milk samples and in different tick species from seropositive farms in four Lebanese regions. Milk and tick samples were screened for *C. burnetii* presence by quantitative real-time PCR (qPCR) targeting IS1111 region followed by multispacer sequence typing (MST). The overall positive percentages of 9.6% (27/282) and 95.45% (84/88) for *C. burnetii* were recorded in ruminant milk and tick samples, respectively. In detail, the *C. burnetii* DNA was recorded in 52/54 (96.3%) of *Rhipicephalus annulatus*, 20/21 (95.24%) of *Rhipicephalus turanicus*, 6/6 (100%) of *Hyalomma anatolicum*, 5/6 (83.3%) of *Rhipicephalus sanguineus* and 1/1 of *Rhipicephalus bursa*. After genotyping of some IS1111-positive samples (17/111), different MST genotypes were identified. Out of 15 positive ticks, 10 were infected with MST2 genotype, 4 were infected with MST7 genotype and 1 was infected with MST57. Moreover, genotypes MST20 and MST58 were found in one cow and one goat milk samples, respectively. The present study confirmed the high genetic diversity of *C. burnetii* in Lebanon.

Introduction

Q Fever is a zoonotic disease caused by *Coxiella burnetii* worldwide distributed except in New Zealand (Cutler *et al.* 2007). This pathogen is a small Gram negative, intracellular bacterium (Maurin *et al.* 1999, Péter *et al.* 1988) that multiplies in the phagolysosomes of eukaryotic host cells (Hackstadt *et al.* 1981, Arricau-Bouvery *et al.* 2005). This bacterium evolves in highly infective spore-like forms that are able to survive in the environment for several months (Evstigneeva *et al.* 2007). *C. burnetii* is classified as a category 'B' agent by the Centers for Disease Control (Atlanta, USA) and is considered as potential weapon for bioterrorism (Alibek *et al.* 1999).

C. burnetii reservoirs include many wild and domestic mammals (Fernández-Aguilar *et al.* 2016), with ruminants being the main source for humans (Berri *et al.* 2001, Fournier *et al.* 1998). The bacterium may also occasionally be detected in arthropods including ticks (Szymańska-Czerwińska *et al.* 2013). Hard and soft ticks are one of the most important arthropods that are known to be naturally infected with *C. burnetii* (Cutler *et al.* 2007, Maurin *et al.* 1999, Angelakis *et al.* 2010). Ticks get infected with *C. burnetii* during feeding on their animal host and over 40 tick species can be naturally infected by this bacterium (Maurin *et al.* 1999). *C. burnetii* can multiply to very high titer levels in the mid-gut and stomach cells of the infected ticks, which can excrete bacteria via saliva and feces. Infected ticks can transmit *C. burnetii* transtadially and transovarially (Dorko *et al.* 2012). The transmission of *C. burnetii* to mammal hosts might occur via tick bites or by feces contamination of their wool and skin (NASPHV and NASAHO 2013, Marrie *et al.* 1990).

The Middle East is the epicenter of the disease, and outbreaks have being reported in Jordan (Fuad *et al.* 1998), Syria (Bottieau *et al.* 2000), Turkey (Cetinkaya *et al.* 2000), Iraq (Faix *et al.* 2005), Cyprus (Cantas *et al.* 2011) and Iran (Esmaeili *et al.* 2016). Lebanon has Mediterranean climate that makes it suitable environment for Q fever disease (Dabaja *et al.* 2019) and arthropods (Dabaja *et al.* 2017). Genotyping characterization of *C. burnetii* strains detected in animals, humans and in ticks is useful for epidemiological purpose. Multispacer sequence typing (MST) is a suitable tool to genotype *C. burnetii* strains because of its high discriminatory power (Glazunova *et al.* 2005, Walker *et al.* 2014).

In the present study, the detection and the genotyping of *C. burnetii* DNA in milk and hard tick samples from ruminant farms in Lebanon was performed, as no information is available in the area.

Materials and methods

Sampling

The analyzed samples were collected under the frame of a previous cross-sectional study performed to evaluate both seroprevalence and via milk shedding of *C. burnetii* in 1,633 animals from 429 ruminant farms distributed in 7 Lebanese provinces (Akkar, Baalback-ElHermel, Bekaa, Mount Lebanon, Nabatieh, North Lebanon and South-Lebanon) in 2014 (Dabaja *et al.* 2019). Breafly, in that study 39.86% of farms (95% CI: 35.23-44.56) and 17.27% (95% CI: 15.43-19.1) of ruminants resulted seropositive (Dabaja *et al.* 2019). Moreover, 27/282 (14.08%) milk samples from *C. burnetii* seropositive animals were positive for the IS1111 target of *C. burnetii* (Dabaja *et al.* 2019). Furthermore, 219 adult hard ticks from 30 seropositive farms were collected in June 2014, as previously described (Dabaja *et al.* 2017, Dabaja *et al.* 2019). Collected ticks belonged to the genera *Rhipicephalus* and *Hyalomma* distributed in 5 species *R. annulatus, R. turanicus, R. bursa* and *R. sanguineus* and *H. anatolicum* (Dabaja *et al.* 2017).

In the present study, 2 or 3 ticks were selected from each farm. A total of 88 out of the 219 collected ticks were individually investigated by using qPCR targeted to the IS1111 region (Klee *et al.* 2006) (Table I).

Additionally, to investigate for *C. burnetii* genotypic diversity in Lebanon and to exclude cross-reactions with *Coxiella*-like endosymbionts (Duron *et al.* 2015, Elsa *et al.* 2015), some of IS1111 PCR-positive milk (n. 2) and tick (n. 15) samples from *C. burnetii* seropositive animals (Dabaja *et al.* 2017, Dabaja *et al.* 2019) were investigated by using MST (Glazunova *et al.* 2005).

Preparation of samples and PCR analysis

Total DNA was extracted from milk and tick samples by using the Pure link Genomic DNA kit (Thermo Fisher™ Applied Biosystems™, Waltham, MA USA) as described by the manufacturer's instruction. Briefly, in order to extract DNA, 200 µl of milk samples and/or an hemilateral salivary gland of tick were mixed with 180 µl PureLink Genomic Digestion buffer and 20 µl proteinase K, followed by an incubation at 55 °C with occasional vortexing until lysis is complete over 30 minutes for milk sample and from 4 hours until overnight for tick samples .

The detection of the IS1111 target (Klee *et al.* 2006) of *C. burnetii* in milk and tick samples was carried out by using a high sensitive qPCR (Biorad CFX96 Real Time System). The IS1111 was selected as a target because it is present in multiple copies in the genome of this bacterium (Klee *et al.* 2006). 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) amplifies a 295 bp fragment that was revealed by a TaqMan probe (FAM-AGC GAACCA TTG GTA TCG GAC GTT TAT GG-TAMRA). The qPCR reactions were performed in a final volume of 25 µl using a mixture containing: 1X SsoAdvanced Universal Probe Supermix (Bio-rad), 0.4 µM 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), 10 µl of DNA extract, and H_2O to volume.

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 sec then annealing and extension at 60 °C for 1 min. Each sample was determined in duplicate. The sample was considered as positive if the Ct was < 40.

Genotyping of *C. burnetii* DNA detected in tick and milk samples

A partial number of tick- milk- IS1111 positive samples were submitted to the genotyping step by using the MST assay (Glazunova *et al.* 2005). The limited sample size used in this step was due to the

Table I. *Prevalence of* C. burnetii *in ticks collected in June 2014 from ruminants in Lebanon detected by real-time PCR.*

ID farm	Locality town or village of origin	Province	Kind of farm	ID and species of ticks	Sex of ticks	IS 1111 Cycle threshold: Ct
1	Barich *33°16'22''N **35°21'9''E ***358 m	South Lebanon	Bovine	1 H. a	F	33.56
				2 R. a	F	26.6
				3 R. a	F	31.62
2	Qinarit *33°30'17''N **35°22'44''E ***233 m	South Lebanon	Bovine	10 R. a	F	25.0
				11 R. a	F	32.7
				12 R. a	F	25.87
3	AynEdelbe *33°32'40.87''N **35°24'25.834''E ***41 m	South Lebanon	Bovine	13 R. a	F	34.15
				14 R. a	F	22.32
				15 R. a	F	33.23
4	Maaroub *33°17'6''N **35°20'49.2''E ***270 m	South Lebanon	Ovine	16 R. t	F	26.15
				17 R. t	F	36.26
				18 R. t	F	23.19
5	Zayta *33°30'29''N **35°23'03''E ***300 m	South Lebanon	Bovine	49 R. a	F	28.08
				50 R. a	F	32.86
6	Bourghlieh *33°18'36''N **35°14'24''E ***19 m	South Lebanon	Bovine	56 R. a	F	31.76
				57 R. a	F	37.18
				58 R. a	F	33.06
7	Hasbaya *33°23'N ***35°41'E ***750 m	Nabatieh	Bovine	4 R. a	F	29.32
				5 R. a	F	30.66
				6 R. a	F	25.14
8	El Koulayaa *33°19'48''N **35°34'12''E ***650 m	Nabatieh	Caprine + Ovine	7 R.s	F	37.19
				8 R.b	F	25.32
				9 H.a	F	34.43
9	Zawtar El Charkiyi *33°19' 33''N **35°28'34''E ***475 m	Nabatieh	Bovine	19 R. a	F	Negative
				20 R. a	F	21.12
				21 R. a	F	34.25
10	Mayfadoun *33°20'9.6''N **35°27'43.2''E ***470 m	Nabatieh	Bovine	22 R. a	F	24.26
				23 R. a	F	30.86
				24 R. a	F	21.12
11	Marjiiyoun *33°30'N **35°30'E ***860 m	Nabatieh	Bovine	25 H. a	M	30.3
				26 H .a	M	34.0
				27 H. a	M	35.0
12	Wata El Khiyam *33°19'37.8''N **35°36'40''E ***700 m	Nabatieh	Caprine	28 H.a	M	26.35
				29 R.s	M	32.49
				30 R.s	M	27.29
13	Ibel El Saki *33°12'36''N **35°22'48''E ***800 m	Nabatieh	Ovine	31 R. t	F	34.5
				32 R. s	F	Negative
				33 R. t	F	Negative
14	El Wazani *33°16'32''N **35°37'22''E ***297 m	Nabatieh	Bovine	34 R. a	F	31.0
				35 R. a	F	32.8
				36 R. a	F	35.4
15	El Wazani *33°16'32''N **35°37'22''E ***279 m	Nabatieh	Bovine	37 R. a	F	30.2
				38 R. a	F	27.48
				39 R. a	F	34.49
16	El Wazani *33°16'32''N **35°37'22''E ***279 m	Nabatieh	Caprine	40 R. t	F	28.25
				41 R. s	F	37.7
				42 R. t	F	30.5
17	AynEbel *33°00'42''N **35°14'24''E ***800 m	Nabatieh	Bovine+ Ovine	43 R. t	F	35.35
				44 R. t	F	30.32
				45 R. t	F	34.36
18	Kfarkila *33°10'12''N **35°19'48''E ***700 m	Nabatieh	Bovine	46 R. a	F	33.2
				47 R. a	F	31.4
				48 R. t	F	35.0
19	Rmeich *33°00'54''N **35°24''E ***690 m	Nabatieh	Ovine	59 R. t	F	32.82
				60 R. t	F	28.84
				61 R. t	M	33.78
20	AynEbel *33°00'42''N **35°14'24''E ***800 m	Nabatieh	Caprine	62 R. t	F	30.85
				63 R. t	F	35.0
				64 R. t	F	30.8
21	AynEbel *33°00'42''N **35°14'24''E ***800 m	Nabatieh	Bovine	65 R. a	F	35.86
				66 R. a	F	34.14
				67 R. a	F	37.63
22	Zahleh *33°50'48''N **35°4'07''E ***963 m	Bekaa	Ovine	51 R. t	F	39.4
				52 R. t	F	30.7
23	Zahleh *33°50'48''N **35°54'07''E ***963 m	Bekaa	Ovine	53 R. t	F	33.44
				54 R. s	F	30.0
				55 R. t	F	32.54
24	Machha *34°32'25''N **36°7'56''E ***349 m	Bekaa	Ovine	68 R. a	F	30.89
				69 R. a	F	33.98
				70 R. a	F	31.6
25	Adbel *34°32'6''N **36°57'50.4''E ***300 m	Akkar	Bovine	71 R. a	F	Negative
				72 R. a	F	33.95
				73 R. a	F	30.93
26	Al Kantara *34°31'33.078''N **36°00'3.0711''E ***375 m	Akkar	Ovine	74 R. a	F	36.24
				75 R. a	F	32.1
				76 R. a	F	36.55
27	Machha *34°32'25"N **367'56"E ***349 m	Akkar	Bovine	77 R. a	F	33.0
				78 R. a	F	35.2
				79 R. a	F	33.41
28	Michmich *34°21'24.0012''N **35°55'51''E ***1,100 m	Akkar	Bovine	80 R. a	F	36.0
				81 R. a	F	31.47
				82 R. a	F	35.33
29	Bazbina *34°31' 0'' N **36°12'0''E ***955 m	Akkar	Bovine	83 R. a	F	32.84
				84 R. a	F	36.23
				85 R. a	F	34.65
30	Sahel Halba *34°33'2'' N **36°4'41''E ***120 m	Akkar	Bovine	86 R. a	F	34.37
				87 R. a	F	32.13
				88 R. a	F	33.41
Positivity percentage averall				84/88(+)		
				(95.45%)		
CT Average:						32

*Latitude; **Longitude ***Altitude; F = Female; M = Male.
R. a = *Rhipicephalus annulatus*; H. a = *Hyalomma anatolicum*; R. b = *Rhipicephalus bursa*; R. s = *Rhipicephalus sanguineus*; R. t = *Rhipicephalus turanicus*.

Table II. *Percentage of ticks positive for IS1111 detection by real-time PCR.*

Tick species	No. of positive ticks	Percentage of positive ticks (95%CI)
Rhipicephalus annulatus	52/54	96.3% (91.1-100)
Rhipicephalus turanicus	20/21	95.3% (94.88-95.72)
Rhipicephalus sanguineus	5/6	83 % (53-100)
Rhipicephalus bursa	1/1	-
Hyalomma anatolicum	6/6	100%

CI = Confidence interval

low amount DNA remaining from each specimen to perform the MST.

Multi-spacer typing was performed on IS1111 positive specimens using a set of primers targeting 10 variable spacers (Cox: 2; 5; 18; 20; 22; 37; 51; 56; 57; 61) according to previous study (Glazunova *et al.* 2005). Five µL of DNA preparation was amplified in a 50 µL reaction mixture containing 0.2 µM of each primer, 0.05 mM (each) dATP, dTTP, dCTP and dGTP; 1.25 U Taq Polymerase; $MgCl_2$ 2.5 mM and 1X Taq buffer. DNA from Nine Mile strain of *C. burnetii* was used as positive control. Amplifications were carried out using a 2720 thermal cycler (Applied Biosystems) according to the following conditions: an hot start step of 15 min at 95 °C, followed by 40 cycles of denaturation for 1 min at 95 °C, annealing for 30 sec at 59 °C, elongation for 1 min at 72 °C and final extension for 7 min at 72 °C.

PCR amplicons were visualized by electrophoresis of 6 µL of the PCR product with 2 µL of blue loading buffer on 1.5% agarose gel (0.5xTBE) with SyberSafe under UV light. PCR products were purified via vacuum filtration through the NucleoFast 96 PCR Plate (Thomas Scientific, Dueren, Germany), as described by the manufacturer.

Sequencing reactions were carried out using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems). Four µL of purified PCR were added to a 10 µL reaction containing 0.5 µL primers, 1.5 µL Big Dye buffer, and 1 µL Big Dye. The sequencing reaction was run in a thermal cycler as follows: an initial denaturation step of 1 min at 96 °C followed by 25 cycles of denaturation for 10 sec at 96 °C, annealing for 5 sec at 50 °C and elongation for 5 min at 60 °C, followed by a final step at 15 °C. Sequencing reactions were purified using Millipore Sephadex plates (Millipore, Billerica, Massachusetts) as per the manufacturer's instructions, and stored at 4 °C until analyzed. Sequencing reactions were analyzed on an ABI 3130X Genetic Analyser (Applied Biosystems) and sequence assembly performed using the multisequence align software Chromaspro (v.2.1.1).

The obtained sequences were further compared with the sequences included in the MST reference database containing *C. burnetii* genotypes from countries in Europe and other parts of the world using BLAST; the new sequences were deposited in the available database on the website: http://ifr48.timone.univ-mrs.fr/MST_Coxiella/mst/group_detail.

Results

Arthropods

IS1111 target was detected in 84 of the 88 (95.45%) investigated ticks from *C. burnetii* seropositive farms with Ct value being between 21.12 and 39. Positive samples included 52 of 54 (96.3%) *R. annulatus*, 20 of 21 (95.24%) *R. turanicus*, 6 of 6 (100%) *H. anatolicum*, 5 of 6 (83.3%) *R. sanguineus* and 1 of 1 *R. bursa* (Table III).

Milk

Among 282 milk samples from seropositive ruminants, IS1111 DNA had been detected in 9 of 86 (10.47%) cattle, in 6 of 93 (6.45%) sheep and in 12 of 103 (11.65%) goat specimens, as indicated in previous study (Dabaja *et al.* 2019).

MST

Because of the low amount of DNA, it was possible to perform MST genotyping in 84 IS111 positive ticks and 2 IS111 positive milk samples only (Tables III and IV). As result of this study, three previously described genotypes and two incomplete ones were identified (Tables III and IV). Of the 15 positive ticks, 10 hosted the MST 2 genotype, 4 the MST 7 genotype and 1 the MST 57 incomplete genotype. Of the 2 positive milk samples, one was infected with the known MST 20 and the other one with the incompletely characterized MST 58 (Tables III and IV).

MST 2 genotype was widely found in different genera and species of ticks from South Lebanon and Nabatieh; MST 7 was identified in *R. annulatum* and *R. turanicus* from Bekaa and Akkar whereas MST 20 was detected in the cow milk samples in Bekaa region (Table III).

MST 57 and MST 58 genotypes, incompletely characterized probably due to the low DNA concentration, were found in one tick and one goat milk sample from Nabatieh and Bekaa, respectively (Table III).

Discussion

Almost all the collected ticks (95.45%) from *C. burnetii* seropositive ruminant farms in Lebanon were positive for IS1111 target. Since IS1111-based

Table III. *Multispacer sequence typing (MST) genotypes of* C. burnetii *in ticks and milk specimens from ruminant farms in Lebanon.*

Sample ID	Source & host species	Town or village of origin: GPS coordinates, elevation	Province	Date	Cycle threshold: Ct	MST genotype
2	R. a (F) Bovine	Barich: *33°16'22''N; **35°21'9''E; ***358 m	South Lebanon	6/2014	26.6	2
6	R. a (F) Bovine	Hasbaya: *33°23'N;**35°41'E; ***750 m	Nabatieh	6/2014	25.14	2
8	R. b (F) Ovine	El Koulayaa: *33°19'48''N; **35°34'12''E; ***650 m	Nabatieh	6/2014	25.32	2
10	R. a (F) Bovine	Qinarit: *33°30'17''N;**35°22'44''E; ***233 m	South Lebanon	6/2014	25	2
14	R. a (F) Bovine	Ayn Eldeleb: *33°32'40.87''N; **35°24'25.834''E; ***41 m	South Lebanon	6/2014	22.32	2
20	R. a (F) Bovine	Mayfadoun: *33°20'9.6''N; **35°27'43.2''E; ***470 m	Nabatieh	6/2014	21.12	2
24	R. a (F) Bovine	ZawtarCharkiyi: *33°19'33''N; **35°28'34''E; *** 475 m	Nabatieh	6/2014	21.12	2
28	Ha. a (M) Caprine	WataElkhiyam: *33°19'37.8''N; **35°36'40''E; ***700 m	Nabatieh	6/2014	26.35	2
38	R. a (F) Bovine	El Wizani: *33°16'32''N; **35°37'22''E; ***279 m	Nabatieh	6/2014	27.48	2
40	R. t (F) Caprine	El Wizani: *33°16'32''N; **35°37'22''E; ***279 m	Nabatieh	6/2014	28.25	2
44	R. t (F) Ovine	Ayn Ebel: *33°00'42''N; **35°14'24''E; ***800 m	Nabatieh	6/2014	30.2	57
52	R. t (F) Ovine	Zahle: *33°50'48''N; **35°54'07''E; ***963 m	Bekaa	6/2014	30.7	7
53	R. t (F) Ovine	Zahle: *33°50'48''N; **35°54'07''E; ***963 m	Bekaa	6/2014	33.44	7
70	R. a (F) Bovine	Machha: *34°32'25''N; **36°7'56''E; ***349 m	Akkar	6/2014	31.6	7
75	R. a (F) Ovine	AlKantara: *34°31'33.078''N; **36°00'3.0711''E; ***375 m	Akkar	6/2014	32.1	7
19	Milk Goat	Rayak: *33°51'3''N; **36°00'42''E; ***929 m	Bekaa	5/2014	37.74	58
3038	Milk Bovine	Alkarak: *33°51'N; **35°55'35''E; ***1000 m	Bekaa	3/2014	39	20

*Latitude; **Longitude ***Altitude; F = Female; M = Male.
R. a = *Rhipicephalus annulatus*; H. a = *Hyalomma anatolicum*; R. b = *Rhipicephalus bursa*; R. s = *Rhipicephalus sanguineus*; R. t = *Rhipicephalus turanicus*.

Table IV. *Genotyping details of the detected strains according to Multispacer sequence typing (MST).*

MST genotype	Cox2	Cox5	Cox18	Cox20	Cox22	Cox37	Cox51	Cox56	Cox57	Cox61
2	5	6	3	5	x	5	8	1	5	6
7	4	6	3	5	6	5	8	x	5	x
20	3	2	6	x	5	4	x	10	6	5
57	4	6	3	5	5	5	x	x	5	6
58	4	8	2	x	x	x	x	x	x	x

PCR-assays designed to detect *C. burnetii* cross react with *Coxiella*-like bacteria (Elsa *et al.* 2015), the results of surveys carried out on ticks and based only on IS1111 PCR assay should be interpreted with caution since ticks can harbor either *C. burnetii* or *Coxiella*-like bacteria. In the present study, in order to exclude misinterpretations, some of the IS1111-positive samples were genotyped by using MST that is based on the characterization of a set of targets (Glazunowa *et al.* 2005).

Based on results obtained using this combined approach, different MST genotypes in tick and a few milk samples from different *C. burnetii* seropositive ruminant farms of different provinces of Lebanon were detected. *C. burnetii* infection had already been recorded in *R. bursa* in Turkey (Capin *et al.* 2013), in *R. annulatus* in Senegal (Mediannikov *et al.* 2010), in *R. sanguineus* in Iran, Cyprus, Italy and Switzerland (Bernasconi *et al.* 2002, Nourollahi Fard *et al.* 2011, Satta *et al.* 2011, Spyridaki *et al.* 2002), in *R. turanicus* in Turkey, Italy, Switzerland and Greece (Capin *et al.* 2013, Bernasconi *et al.* 2002, Satta *et al.* 2011, Psaroulaki *et al.* 2006), in *H. anatolicum* in Cyprus (Spyridaki *et al.* 2002), thus supporting the evidence that *C. burnetii* seems to be endemic in ticks in more areas.

In our study several MST genotypes of *C. burnetii* were found in tick and milk samples from ruminant farms in Lebanon. The most frequently detected MST 2 genotype was found in *R. annulatus, R. bursa, R. turanicus* and *H. anatolicum* ticks from the South Lebanon and Nabatieh regions. Indeed, the same MST 2 genotype had already been detected in blood samples of human beings affected with acute Q fever from France, Ukraine and Kyrgyzstan (Glazunova *et al.* 2005). MST 7 genotype, detected in both *R. annulatus* and *R. turanicus* ticks from the Bekaa and the Akkar regions, had already been found in France and Russia in human blood samples and cardiac valves (Glazunova *et al.* 2005).

Conversely, the sequence type detected in the cow milk sample from Bekaa region was similar to MST 20. This genotype had been found in animal, human and tick samples from Germany, France, Spain, Italy, Hungary, the United Kingdom, United States and Netherlands and central Africa. In particular, MST 20 was most frequently associated with cattle but rarely with goats. MST 20 was found in a single goat sample in the Netherlands (Tilburg *et al.* 2012), in two milk samples in the United States (Pearson *et al.* 2014), and in a large goat herd with abortion problems in the United Kingdom (Reichel *et al.* 2012). However, in most cases, the genotype MST 20 was associated with cattle and cow's milk (Glazunova *et al.* 2005, Astobiza *et al.* 2012, Pearson *et al.* 2014, Sulyok *et al.* 2014, Mediannikov *et al.* 2010). These findings provide further evidence for the presumed host-specific adaptation of this agent.

Finally, although incompletely characterized for technical reasons, other two genotypes, MST 57 and MST 58 were detected in a *R. turanicus* tick and a goat milk sample, respectively, thus confirming the high genetic diversity of *C. burnetii* in Lebanon.

Conclusions

This study provides the first molecular evidence of *C. burnetii* as well as a preliminary picture of the genetic diversity of the Q fever agent in different tick species and milk samples from ruminant farms in Lebanon. Although, based on the results of our study, there is no evidence of a role of ticks in the transmission of infection to the ruminants, appropriate biosecurity measures should be taken to prevent zoonotic risk since different genotypes were found in ticks and milk samples.

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