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Molecular typing of *Coxiella burnetii* from sheep in Egypt

Abdelfattah Selim[a,*], Amir Abdelrahman[b], Richard Thiéry[c], Karim Sidi-Boumedine[c]

[a] Animal Medicine Department (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Egypt
[b] Animal Medicine Department (Infectious Diseases), Faculty of Veterinary Medicine, Alexandria University, Egypt
[c] Anses, Sophia Antipolis Laboratory, Animal Q fever Unit, Sophia Antipolis, France

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ABSTRACT

Coxiella burnetii, the etiological agent of Q fever, is a globally distributed zoonotic disease. The disease was reported serologically in different animal species and humans in Egypt but the genetic information about circulating *Coxiella* strains is limited. The present study aimed to genetically characterize *Coxiella* positive samples, identified in abortive sheep, based on a 17-loci Multiple Locus Variable number tandem repeat analysis (MLVA) panel and Multispacer Sequence Typing (MST). Four MLVA types were found among six examined samples. While all three samples examined by MST were identified as novel sequence type (ST) closely related to human heart valve isolates from France, Saudi Arabia, USA and United Kingdom. This study provides the first genetic information about circulating *Coxiella* strains in Egypt and improves epidemiological data of Q fever in the country.

1. Introduction

Coxiella burnetii is an obligate intracellular bacterium, which causes Q fever disease, and is widely distributed throughout the world. The main reservoir for the pathogen is domestic animals such as cattle, sheep and goats which have a main role in the disease cycle [1].

The diseased animals suffer from several reproductive disorders such as abortion, still birth, and delivery of weak offspring. Q fever is mainly transmitted by inhalation; it could also occur through the consumption of milk or dairy products [2].

According to published data, Q fever seroprevalence, using ELISA, among sheep and goats in Egypt was 22.7% and 12.5%, respectively [3]. While the prevalence of antibodies against *C. burnetii* in humans who had contacted with animals was 19% [4].

Genetic characterization of *C. burnetii* is required for epidemiological investigation in Q fever outbreaks and for surveillance purposes. A number of different molecular typing methods have been described to analyze the genetic variability of *C. burnetii*. Recently, PCR-based methods were developed including multi-locus variable number of tandem repeats analysis (MLVA) and multispacer sequence typing (MST) [5].

While MLVA is based on variable copy number in tandemly repeated DNA on multiple loci in *C. burnetii* genome, MST is based on DNA sequence variation in 10 short intergenic regions. Both methods can be performed directly on extracted DNA from clinical samples or on *C. burnetii* isolates [6–8].

As, there is evidence of the presence of Q fever in domestic animals in Egypt [4]. Therefore, the identification of the source of infection and molecular characterization of the circulating *C. burnetii* strains in Egypt is very important to enhance the wealth of molecular epidemiology data of Q fever in the country.

The aim of this study was to characterize strains of *C. burnetii* circulating in domestic animals in Egypt, using MLVA and MST genotyping. In addition, the profiles obtained were subsequently compared with other reported genotypes.

2. Material and methods

2.1. Samples

The study comprised one sheep herd which suffered from abortion and located at Alexandria governorate, Egypt. A total of 21 vaginal swabs were collected from aborted cases during the first week after abortion. All samples were transferred to Anses Laboratory, Animal Q fever Unit for molecular identification and characterization.

2.2. DNA extraction and PCR assay

Total DNA was extracted from vaginal swabs using the QIAamp Tissue and Blood Kit (Qiagen GmbH, Hilden, Germany) according to the

* Corresponding author.
E-mail address: Abdelfattah.selim@fvtm.bu.edu.eg (A. Selim).

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manufacturer's instructions. To detect the positive samples and quantify the bacterial load, all samples were examined with Taqman real-time PCR targeting IS1111 as previously described by Joulie et al. [9].

2.3. Multiple-locus variable-number tandem repeat analysis (MLVA)

MLVA typing was performed directly on DNA from the samples with Ct value below 34 in the real time PCR using 17 loci (ms01, ms03, ms07, ms12, ms20, ms21, ms26, ms30, ms36, ms23, ms24, ms27, ms28, ms31, ms33 and ms34) as described by Arricau-Bouvery et al. [10]. The Nine Mile strain, for which the expected MLVA pattern is known, was used as a reference to determine the number of repeat in each marker and was included in all the experiments [11]. Comparison analysis and cluster identification of the obtained numerical typing data were performed using the Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

MLVA genotypes, from different species and identified genotypes, available in the MLVA database were used. The unweighted pair group method with arithmetic mean clustering (UPGMA) were generated using the MLVA numbers of repetition and the predefined Bionumerics template for categorical data.

2.4. Multi-spacer sequence typing MST

MST typing was performed only for the three highly concentrated samples. Briefly, the spacers were first amplified, then purified and finally sequenced.

The primer sequences and PCR conditions were described elsewhere [12]. The amplification was followed by sequencing of the different spacer regions of the *Coxiella* genome (Cox2, 5, 18, 21, 22, 37, 51, 56, 57 and 71) according to Glazunova et al. [12].

The obtained sequences of the PCR products (chromatograms) were manually inspected, cleaned and analyzed using BioEdit Sequence Alignment Editor v. 7.0.5.3 [13]. Sequence types (ST) were determined using the MST database (http://ifr48.timone.univ-mrs.fr/mst/ coxiella_burnetii/) and/or with BLAST software by comparison with sequences available in GenBank. Similarly, to MLVA, the MST obtained allele numbers were used to generate the UPGMA tree using the predefined Bionumerics template for categorical data (Applied Maths, Sint-Martens-Latem, Belgium).

3. Results

All 21 vaginal swabs were positive with *Coxiella* specific real-time PCR but only six samples with Ct-value below 34 were analyzed for MLVA genotyping as in Table 1 and only three samples were analyzed using MST genotyping.

The six *C. burnetii* samples revealed different MLVA pattern in comparison with NM as reference strain based on the 17 MLVA loci used as in Table 1.

Interestingly, all six samples revealed no alleles with ms23 and ms24 markers in comparison to NM. In addition, Eg4, Eg14 and Eg18 samples showed no alleles with ms01 marker. Finally, the six Egyptian samples were separated in two groups by the ms02, ms20, ms28 and ms34 markers as shown in Table 1.

The obtained MLVA genotypes were compared with those from human, sheep, goat, cattle and antelope from USA, Poland, Italy, France, Spain, Portugal, Netherlands, Dubai, Qatar, Saudi Arabia, Germany and United Kingdom based on the available information in the " http://mlva.u-psud.fr/MLVAnet/spip.php?rubrique50 database. The clustering analysis of the six *C. burnetii* samples revealed three main clusters where the Eg4, Eg14 and Eg18 grouped together in one cluster, Eg10 and Eg11 in another cluster and Eg15 in a third cluster as shown in (Fig. 1). The *C. burnetii* samples identified from sheep in Egypt were closely related to human *Coxiella* strains isolated from Poland and Italy as shown in (Fig. 1).

The results of three examined samples (Eg10, Eg11 and Eg15) with MST was identified as novel ST. The allele's codes for the newly identified ST are described in Table 2.

One of the newly discovered ST (Eg10) was closely related to ST51 (strain CB 196) and ST42 (Strain CB162) which have been reported from human heart valve in Saudi Arabia and USA, respectively. While ST (Eg11) was closely related to ST9 (strain CB32) which has been reported from human heart valve in France and also to two others from Kazakhstan. Finally, the ST (Eg15) was identical to ST55 (strain CB155) which was identified from human heart valve in the United Kingdom (Fig. 2).

4. Discussion

Molecular characterization of *C. burnetii* is a powerful tool to explore genetic diversity of strains and determine the relationships between isolates causing disease. Typing data can provide information about the source of infection and the risk of transmission of the infections between animals and human [14,15].

In the case of Q fever, MLVA and MST methods have been used for the typing of *C. burnetii* strains, both methods have high discriminatory power and can be applied directly on clinical samples without previous cultivation of the bacteria [10,16,17].

In the current study, three MST (sequences types) and four MLVA profiles (identified as A, B, C, D) were identified among the examined *Coxiella* samples from aborted sheep. The four identified MLVA profiles revealed slight heterogeneity among them and have not been previously detected in human or animals. Some of these new genotypes differed only by one marker from other previously identified genotypes and thus represented microvariants. The comparison between the novel MLVA genotypes and others described elsewhere revealed high similarity with genotypes NM from host tick (USA) and other isolated from human in Poland.

Looking at MLVA genotypes results, we found highly similar *C. burnetii* genotype profiles (A, B, C and D) in one farm which may indicate a highly wide spread dissemination of *Coxiella* strains [9]

The analysis of MST results revealed that Eg10 is closer to ST51 (strain CB 196) which was previously isolated and cultured from the valve of human [18]. Another new ST for Eg11 was detected which was closely related to ST9 (strain CB32) which had been detected in human heart valve in France [12].

Finally, the MST genotype detected in Eg15 sample had not been

Table 1
MLVA typing results for *C. burnetii* strains isolated from small ruminants in Egypt.

ID	Host	Ct value	ms01	ms03	ms07	ms12	ms20	ms21	ms22	ms26	ms30	ms36	ms23	ms24	ms27	ms28	ms31	ms33	ms34	MLVA type*
NM	Tick	30	4	7	8	8	15	6	6	4	6	4	8	27	4	6	5	9	5	O
Eg10	sheep	30	4	7	6	4	14	7	6	3	6	2	0	0	4	5	3	6	3	A
Eg11	sheep	22.9	4	7	6	4	14	7	6	3	6	2	0	0	4	5	3	6	3	A
Eg15	sheep	28.22	4	7	6	4	14	7	6	3	6	2	0	0	4	6	3	6	3	B
Eg4	sheep	33.28	0	6	6	4	15	7	6	3	6	2	0	0	4	5	3	6	2	C
Eg18	sheep	33.75	0	6	6	4	15	7	6	3	6	2	0	0	4	5	3	6	2	C
Eg14	sheep	32.46	0	6	6	4	14	7	6	3	6	2	0	0	4	5	3	6	2	D

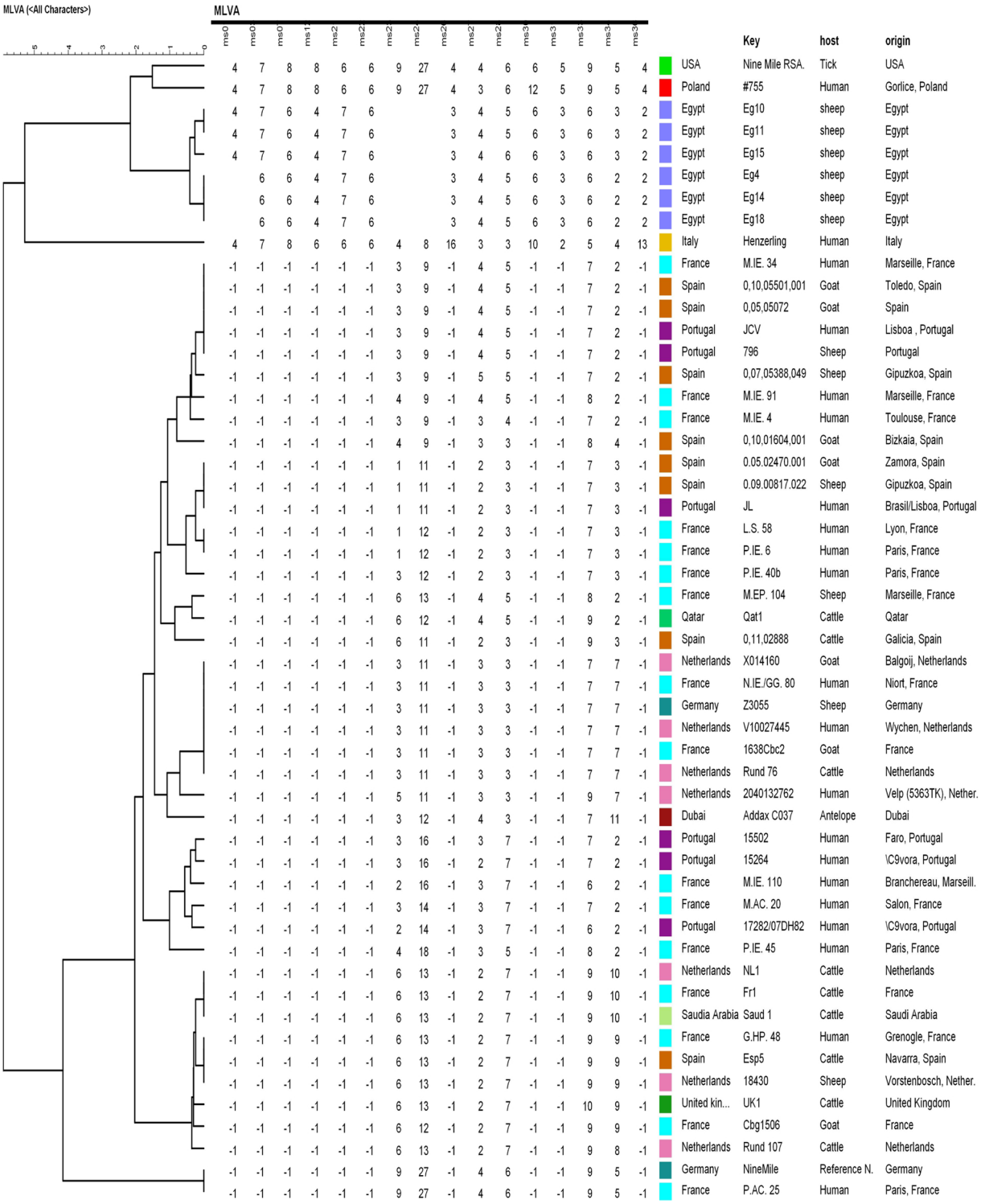

Fig. 1. Phylogenetic tree with genotypes of *C. burnetii* of isolated positive samples on the basis of 16 multilocus variable-number tandem-repeat analyses (MLVA).

Table 2
MST typing analysis for *C. burnetii* strains isolated from Sheep in Egypt.

ID	Host	Cox2	Cox5	Cox18	Cox20	Cox22	Cox37	Cox51	Cox56	Cox57	Cox61	MST type
Eg10	Sheep	5	4	5	5	8	5	2	ND	ND	ND	New
Eg11	Sheep	10	4	9	5	1	5	2	3	4	6	New
Eg15	Sheep	5	4	9	5	8	5	2	3	ND	ND	New

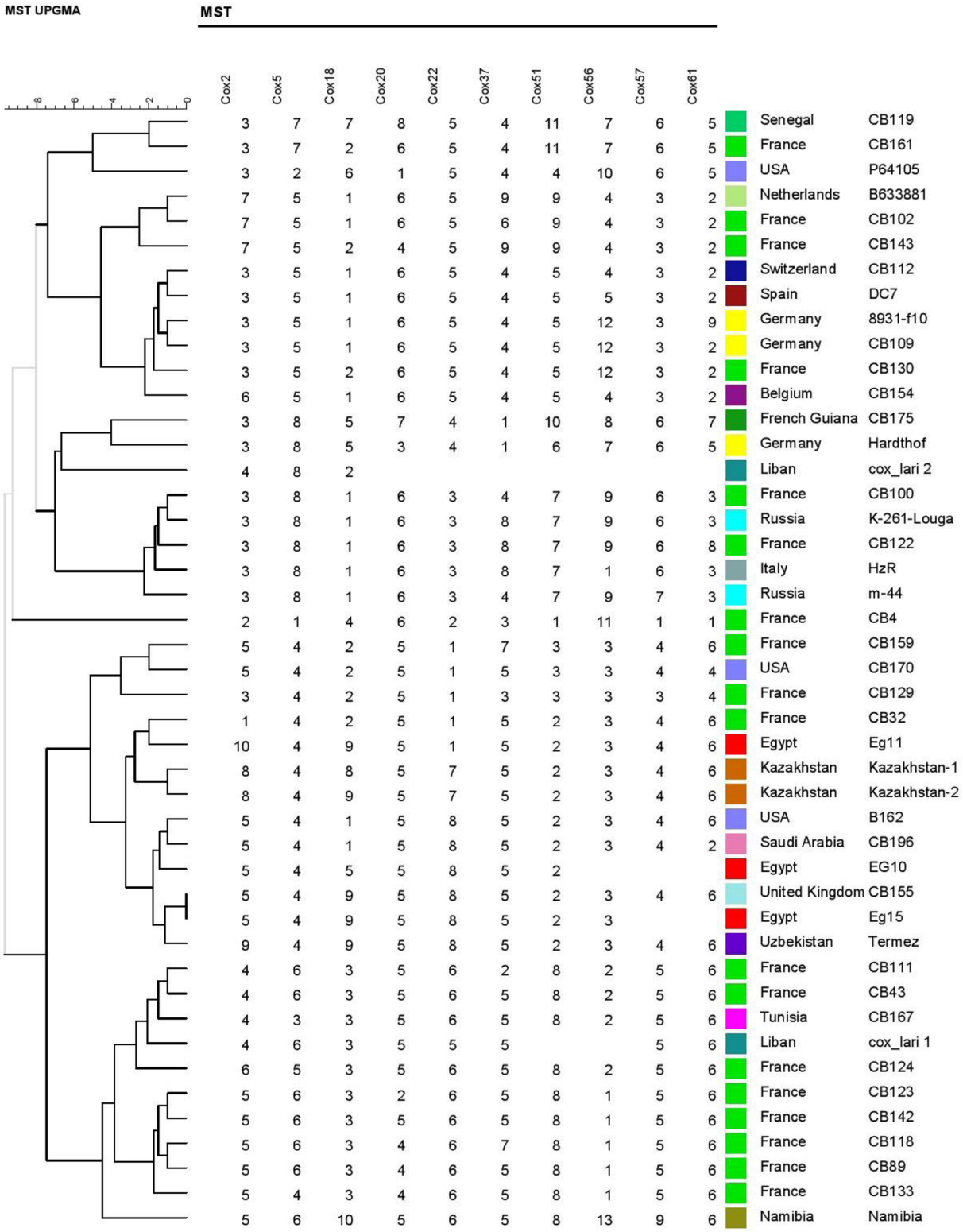

Fig. 2. Parsimony tree showing the placement and phylogenetic relationships of the sequence types (ST) from this study with known STs.

previously reported. This genotype (partial profile) is also probably similar to a human sample ST55 (strain CB155) from United Kingdom [12]

The relationship between animal and human strains of *C. burnetii*, was previously reported in other studies, using both MLVA and MST typing [16,19]. The MLVA and MST genotypes detected in sheep from Egypt in the present study showed genetic relation with human isolates.

5. Conclusion

The present work supports epidemiological information about genetic diversity for *C. burnetii* occurring in Egypt. Examination of six *C. burnetii* samples originating from sheep revealed three MST types (completely novel profiles) and four MLVA types. The detected genotypes revealed close relation to *C. burnetii* isolated from humans.

Additional analyses using larger samples from different livestock species and locations are needed to confirm these findings and to construct a reference database of genotypes in Egypt in order to identify the source of infection when human cases emerge.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest associated with this publication.

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