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Genotyping Reveals the Presence of a Predominant Genotype of *Coxiella burnetii* in Consumer Milk Products

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Real-time PCR shows the widespread presence of *Coxiella burnetii* DNA in a broad range of commercially available milk and milk products. MLVA genotyping shows that this is the result of the presence of a predominant *C. burnetii* genotype in the dairy cattle population.

Q fever is a zoonosis caused by the pathogen *Coxiella burnetii*, which is prevalent throughout the world (1). Ruminants (sheep, goats, and cattle) are often asymptomatic carriers of *C. burnetii* and are considered to be a source of infection to humans (1). *C. burnetii* can cause abortion in small ruminants such as sheep and goats and may cause reproductive disorders in cattle (7). Huge numbers of *C. burnetii* can be released into the environment via birth products (6). Lower numbers are usually shed in milk, even in asymptomatic herds (2, 3, 4, 5, 8, 9). Although consumption of raw or insufficiently pasteurized milk is very rarely identified as a source of Q fever infection, asymptomatic cattle herds can be considered potential *C. burnetii* reservoirs capable of transmitting the disease to humans.

We applied real-time PCR, targeting the multicopy IS*1111a* insertion element of *C. burnetii* as described earlier (11), and a 6-locus multiple-locus variable number tandem repeat analysis (MLVA) panel (12) to a broad range of milk and milk products with the aim to determine the prevalence and genotypes of *C. burnetii* in milk (Table 1). The study included commercially available semi-skimmed milk samples from cows (obtained from large supermarket chains) and milk products, such as coffee creamer, obtained throughout Europe and from an additional 10 non-European countries. Samples were collected from different brands, and according to the information on the packages they were produced by the (local) dairy industry in these countries. The origin of the milk samples from Egypt, Saudi-Arabia, and Qatar could not be identified.

Eighty-eight out of 116 (76%) milk samples or milk products from 28 countries contain significant amounts of *C. burnetii* DNA (Table 1). No *C. burnetii* DNA was detected in milk obtained from Finland, Norway, Costa Rica, and New Zealand. MLVA genotypes I to O were identified in samples from France, Germany, The Netherlands, Portugal, Slovak Republic, Spain, Switzerland, United Kingdom, Qatar, and Saudi Arabia. MLVA genotypes P, Q, and R were identified in samples from Slovak Republic, Qatar, and Russia, respectively. A partial MLVA genotype (Table 1, "Part") was obtained from samples that contained insufficient DNA to obtain a full profile. In 4 samples from Slovak Republic, we observed more than one allele per locus, suggesting the presence of at least two or more different genotypes in these samples (Table 1). Clustering of the MLVA genotypes using the minimum spanning tree method showed a high degree of genetic similarity between the MLVA genotypes I to O (Fig. 1). These MLVA genotypes are interconnected by repeat number changes in only one of the six markers and may represent microvariants of one founder genotype. In contrast, MLVA genotypes P and R and the genotypes of five sequenced *C. burnetii* strains all differed in at least 3 markers from the MLVA genotypes I to O.

The MLVA genotypes were compared to an in-house database containing 57 different *C. burnetii* MLVA genotypes from 197 human, caprine, ovine, and cattle clinical samples obtained from Canada, France, Germany, The Netherlands, Portugal, Spain, and the United States. MLVA genotypes I and J have also been recognized incidentally in 8 human clinical samples (placenta and heart valve) from France and in 2 animal samples (cattle and goats) from The Netherlands. However, very different MLVA genotypes (A to H) were identified in human, ovine, and caprine clinical samples from the Q fever outbreak in The Netherlands using a 6-locus and 10-locus MLVA panel (10, 12), indicating that the Dutch Q fever outbreak is not related to the presence of *C. burnetii* in cattle.

The presence of highly similar *C. burnetii* genotypes in consumer milk products may indicate a widespread dissemination of a specific cattle-adapted strain. Alternatively, this genotype may have been introduced into different countries by transport of asymptomatic *C. burnetii*-positive cattle, as well as by export of milk and milk products from a restricted number of countries to other countries (e.g., Egypt, Saudi Arabia, Qatar) by the dairy industry. By testing bulk milk products instead of milk from individual animals, any positive milk specimen is likely to be diluted with negative milk specimens, leading to an average lower DNA concentration, resulting in higher threshold cycle (C_T) values as well as partial genotypes.

This is the first report of genotypic diversity among *C. burnetii* from cow milk throughout Europe and beyond. Integration of such data in international databases can be instrumental to understand the global epidemiology of Q fever in animals.

In conclusion, this study demonstrated the presence of *C. burnetii* DNA in a broad range of commercially available cow milk and milk products, indicating a high prevalence of *C. burnetii* among the dairy cattle population worldwide and a possible clonal

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TABLE 1 Prevalence of *C. burnetii* DNA in commercially available bulk tank cow milk and milk products from 18 countries throughout Europe and from 10 non-European countries[a]

Geographical source	Origin	C_T value	No. of PCR-positive samples/total no. of samples tested	No. of samples with the same MLVA genotype	No. of repeats						MLVA type
					Ms23	Ms24	Ms27	Ms28	Ms33	Ms34	
Austria	Semi-skimmed milk	37.4	1/1	1	6	–	2	7	4	–	Part
Belgium	Semi-skimmed milk	33.6	1/1	1	6	13	–	7	4	10	Part
Croatia	Semi-skimmed milk	34.1–35.2	4/4	4	–	9	–	–	–	–	Part
Denmark	Semi-skimmed milk	34.5	1/1	1	6	13	–	–	5	–	Part
Finland	Semi-skimmed milk	–	0/1								
France	Semi-skimmed milk	30.4–34.1	6/6	1	6	13	2	7	4	9	I
				2	6	13	2	7	4	10	J
				1	6	–	2	5	–	10	Part
				1	6	–	2	7	4	–	Part
				1	6	–	–	–	4	9	Part
Germany	Low-fat and semi-skimmed milk	32.0–37.3	6/6	1	6	13	2	7	4	–	Part
				1	6	13	2	6	4	10	K
				1	–	–	2	7	–	10	Part
				1	6	13	–	–	4	9	Part
				2	–	–	–	–	–	–	–
Ireland	Semi-skimmed milk	32.5	1/1	1	–	11	–	–	–	9	Part
Italy	Semi-skimmed milk	33.2	1/1	1	6	–	–	7	4	11	Part
Netherlands	Low-fat and semi-skimmed milk, coffee creamer, and milk powder	31.5–41.2	16/27	1	6	13	2	7	4	9	I
				1	6	13	2	–	4	9	Part
				1	6	13	–	7	4	9	Part
				2	6	13	2	7	4	10	J
				1	–	–	2	7	–	7	Part
				10	–	–	–	–	–	–	–
Norway	Semi-skimmed milk	–	0/2								
Poland	Semi-skimmed milk	35.2	1/1	1	–	13	–	–	–	–	Part
Portugal	Semi-skimmed milk	31.5–36.8	10/12	2	6	13	2	7	4	9	I
				1	6	13	2	7	4	–	Part
				1	5	13	2	–	4	–	Part
				1	6	13	–	–	4	13	Part
				1	6	–	–	7	4	9	Part
				4	–	13	2	–	–	9	Part
Slovak Republic	Semi-skimmed milk	33.0–35.5	11/11	1	6	13	2	7	5	10	L
				1	6	12	4	7	4	5	P
				1	9	–	4	4	–	5	Part
				1	5	13	–	–	4	–	Part
				1	6	–	–	–	4	–	Part
				2	–	–	–	–	–	–	–
				1	4/6	7	3	3/6	4	3/5	Mix
				1	4	27	3/4	3/6	4	3/5	Mix
				1	–	13	2/4	7	4	10/11	Mix
				1	6/9	8/13	–	3/6	4	3	Mix
Spain	Low-fat and semi-skimmed milk	31.9–35.6	7/7	2	6	13	2	7	4	9	I
				2	6	13	2	7	4	10	J
				1	6	13	2	7	4	11	M
				1	5	13	2	7	4	9	N
				1	5	–	2	8	4	–	Part
Switzerland	Semi-skimmed milk	33.3–37.7	6/6	1	6	13	2	7	4	9	I
				1	5	13	2	7	4	–	Part
				1	5	–	–	7	4	9	Part
				3	–	–	–	–	–	–	–
Sweden	Semi-skimmed milk	36.2	1/1	1	–	–	–	2	–	–	Part
United Kingdom	Semi-skimmed milk	31.6	1/1	1	6	13	2	7	5	9	O
Australia	Semi-skimmed milk and coffee creamer	33.9–36.7	4/6	1	–	1	–	5	–	–	Part
				1	–	18	–	–	–	–	Part
				2	–	–	–	–	–	–	–
Canada	Semi-skimmed milk	32.7	1/1	1	6	13	–	7	4	11	Part
Costa Rica	Milk powder	–	0/2								
Cuba	Semi-skimmed milk	38.1	1/2	1	–	–	–	–	–	–	–
Egypt	Semi-skimmed milk	34.1–36.0	2/2	2	5	13	–	–	4	–	Part
India	Coffee creamer, including milk powder	34.8–37.3	2/3	1	6	–	–	–	5	–	Part
				1	–	–	–	–	–	–	–

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TABLE 1 (Continued)

Geographical source	Origin	C_T value	No. of PCR-positive samples/total no. of samples tested	No. of samples with the same MLVA genotype	No. of repeats						MLVA type
					Ms23	Ms24	Ms27	Ms28	Ms33	Ms34	
New Zealand	Semi-skimmed milk and milk powder	–	0/5								
Qatar	Semi-skimmed milk	30.3–32.6	2/2	1	6	12	4	5	4	2	Q
				1	6	13	2	7	4	10	J
Russia	Semi-skimmed milk and milk powder	33.3	1/2	1	4	14	2	6	3	11	R
Saudi Arabia	Semi-skimmed milk	33.5	1/1	1	6	13	2	7	4	10	J
C. burnetii Dugway	DNA				?	5	4	4	3	3	
C. burnetii RSA331					4	7	3	3	−1[b]	3	
C. burnetii RSA493					9	27	4	6	4	5	
C. burnetii CbuG_Q212					?	8	3	4	2	2	
C. burnetii CbuK_Q154					?	9	4	5	2	2	
Total			88/116 (76%)								

[a] The number of repeats in each marker was determined by extrapolation using the sizes of the obtained fragments relative to those obtained using DNA from the Nine Mile strain. Furthermore, the genotypes of four additional *C. burnetii* strains, i.e., Dugway (GenBank accession number CP000733), RSA331 (CP000890), CbuG Q212 (CP001019), and CbuK Q154 (CP001020) were determined *in silico* using the published sequences. –, no result was obtained; Part, partial genotype; Mix, 2 or more genotypes; ?, the number of repeats could not be determined due to apparent sequence assembly errors.

[b] *In silico* analysis resulted in a 5-repeat-number difference compared to the Nine Mile strain, which by convention was assigned 4 repeats.

spread of *C. burnetii* among the European dairy cattle population. In addition, since this dominant genotype is only incidentally found in humans, the risk of obtaining Q fever via exposure to infected cattle may be much lower than via exposure to infected small ruminants. The incidental observation of mixed alleles does not exclude the possibility of the presence of other minority genotypes in cattle that may be relevant to humans after all.

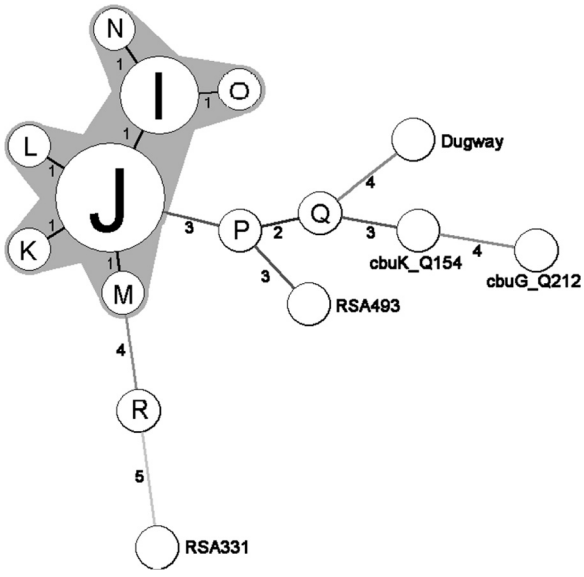

FIG 1 Minimum spanning tree showing the relationship between the obtained MLVA genotypes identified in this study and five sequenced *C. burnetii* strains, i.e., Dugway (GenBank accession number CP000733), RSA331 (CP000890), Nine Mile RSA493 (AE016828), CbuG Q212 (CP001019), and CbuK Q154 (CP001020). Each circle represents a unique genotype, and the size of the circle corresponds to the number of samples with that genotype. Only full MLVA genotypes were included in this analysis. Branch labels and connecting lines correspond to the number of different markers between the genotypes. Genotypes connected by a gray background differ in only one marker from each other and may represent microvariants of one founder genotype.

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