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qPCR- and genomic-based determination of IS1111 copy numbers suggests an impact of *Coxiella burnetii* genotypic diversity on Q fever diagnosis and epidemiological studies in ruminants

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Aminah A. Keliet, Xavier Bailly, Aurélien Joulié, Aurore Fourcot, Séverine Barry, et al.. qPCR- and genomic-based determination of IS1111 copy numbers suggests an impact of *Coxiella burnetii* genotypic diversity on Q fever diagnosis and epidemiological studies in ruminants. ESCCAR International congress on Rickettsiae and 9th Meeting of the European Society for Chlamydia Research (ESCR), Aug 2022, Lausanne, Switzerland. , pp.#160. hal-03758053

HAL Id: hal-03758053

<https://hal.inrae.fr/hal-03758053>

Submitted on 2 Sep 2022

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SCAN ME

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Background

Q fever is a zoonosis caused by *Coxiella burnetii*. Domestic ruminants are the main reservoirs and their main clinical manifestations are **abortions**.

To date, the most commonly used method for the diagnosis of Q fever abortions is a qPCR assay based on the **multicopy IS1111 element** because commercial firms selected this target to develop diagnostic kits with the objective to obtain a highly sensitive method.

Because **the number of IS1111 sequences varies between strains** [1,2,3], the estimation of bacterial burdens shed by infected females is biased with such kits. To account for the presence of several IS1111 copies in a bacterium, the French NRL recommends using a standard range based on the *Nine Mile* reference strain, which displays 20 copies, in each qPCR run. However, a **bias remains** and is not quantified.

To assess this bias, we aimed to **determine the number IS1111 copies in a panel of representative animal strains prevailing in France** [4].

Methods

- We estimated the number of IS1111 copies using **qPCR and *in silico*** approaches depending on the nature and source of the considered data (**figure A**)
- We constructed a **phylogenetic tree** from MLVA data considering 17 markers [5]

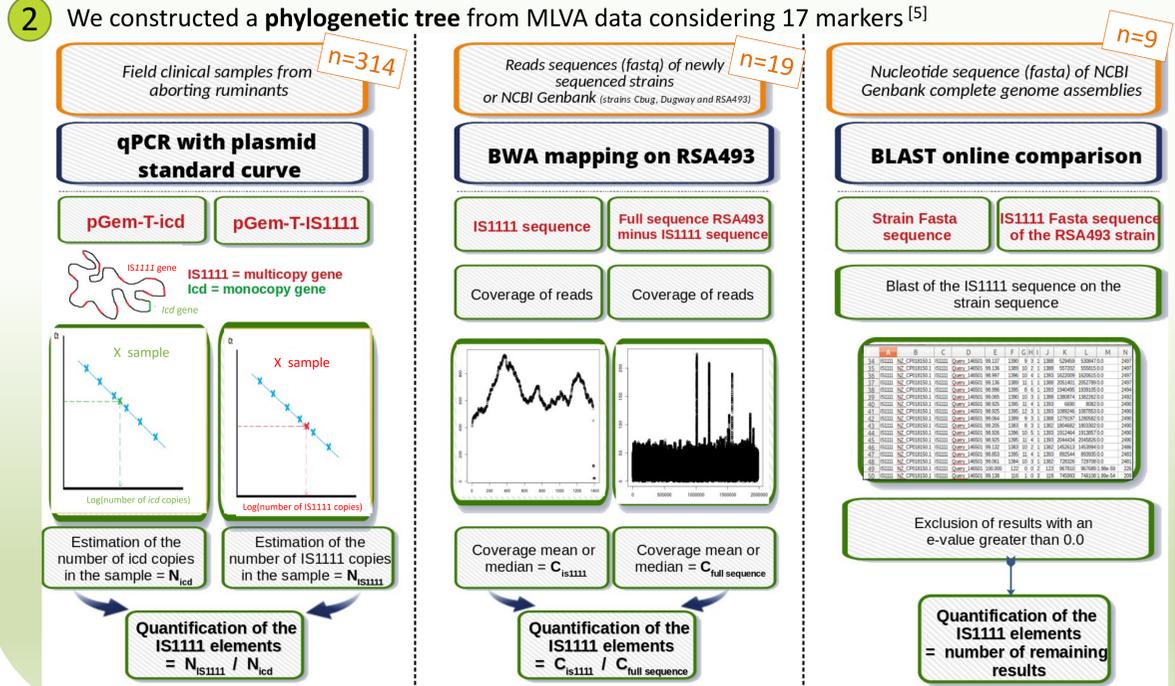


Figure A: the 3 methods used to count the number of IS1111 copies

Results

- The number of IS1111 copies varied between **12 and 228** depending on samples or strains (**figure B2, table**).

MLVA genogroup	Host species	No. of samples	No. of genotypes	Copy no. Median	Copy no. Range
GGI	Other	3	3	20	20-20
GGII	Sheep	51	4	86	35-228
GGII	Goat	38	13	100	28-195
GGII	Cattle	4	3	135	93-192
GGII	Other	1	1	48	48
GGIII	Cattle	152	13	26	16-94
GGIV	Goat	64	2	51	13-112
GGIV	Sheep	22	2	53	17-175
GGIV	Cattle	1	1	39	39
GGIV	Other	1	1	48	48
GGV	Other	1	1	28	28
GGVI	Other	1	1	12	12

Table: number of IS1111 copies according to the genogroup and host species

- The **median** and **mean** of IS1111 copy numbers were significantly associated with genetic divergence and differed significantly among bacterial genomic groups (**figures B, C and D**).

Discussion & conclusion

In this study, we took up the challenge of combining both wet lab and *in silico* data in the aim to investigate the number of IS1111 copies in a broad panel of *C. burnetii* samples and isolates.

Significant difference in the number of IS1111 copies among *C. burnetii* genomic groups

The diversity of *C. burnetii* strains has an impact on the DETECTION and QUANTIFICATION of the Q fever agent when diagnostic is based on IS1111 amplification

In the absence of genotyping data on the investigated samples, it is clearly preferable to use qPCR methods targeting a monocopy gene to perform the clinical diagnosis of *C. burnetii* abortions

We thus encourage commercial firms to develop such kits for use with diagnostic purposes

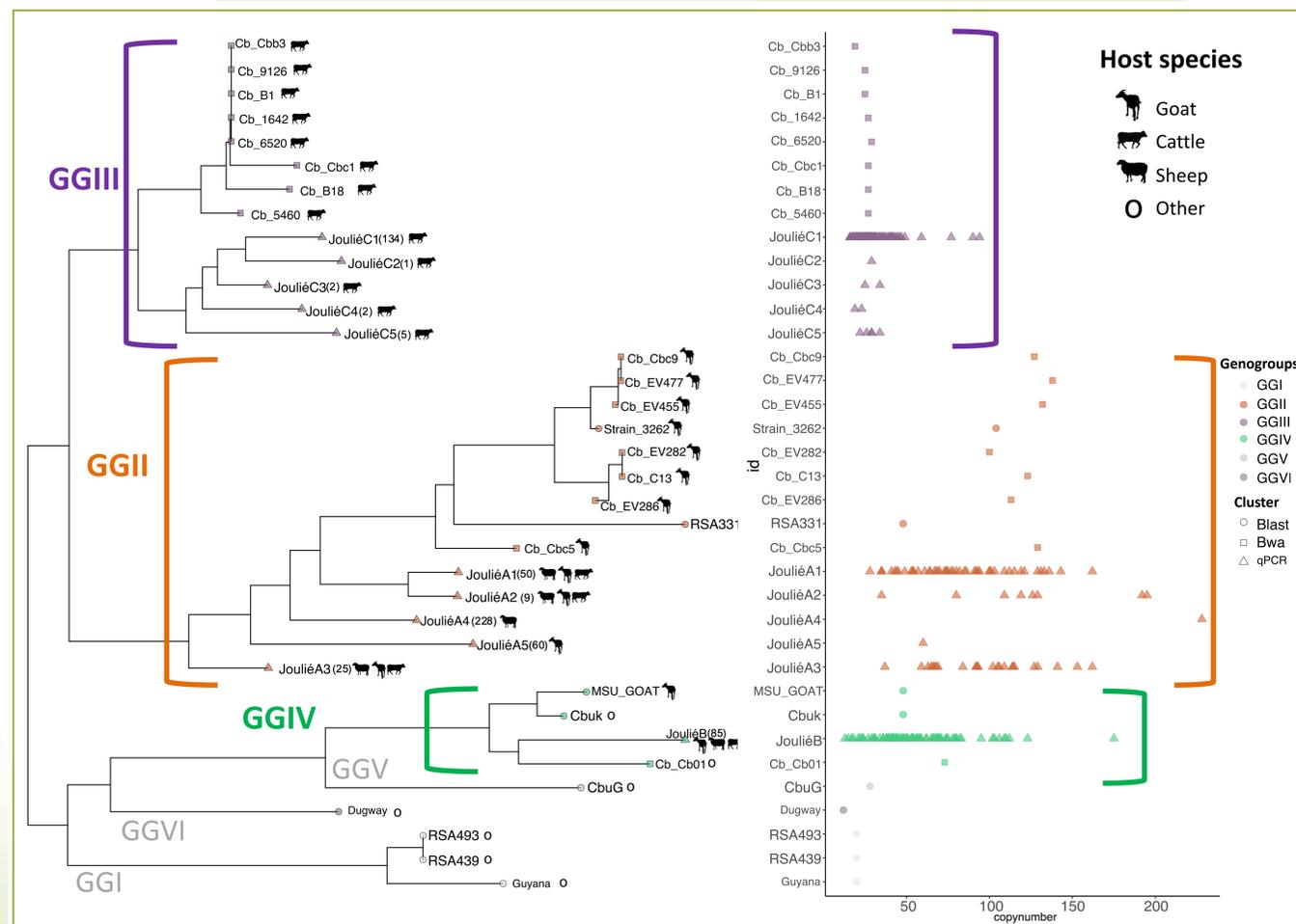


Figure B: MLVA tree (B1) and associated number of IS1111 copies of the corresponding samples or strains (B2)

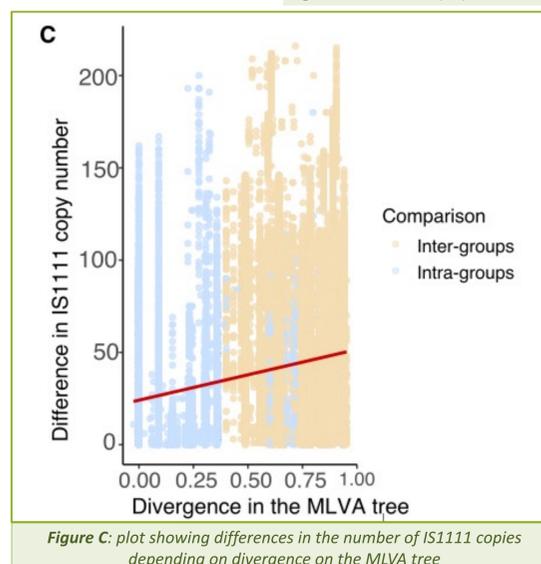


Figure C: plot showing differences in the number of IS1111 copies depending on divergence on the MLVA tree

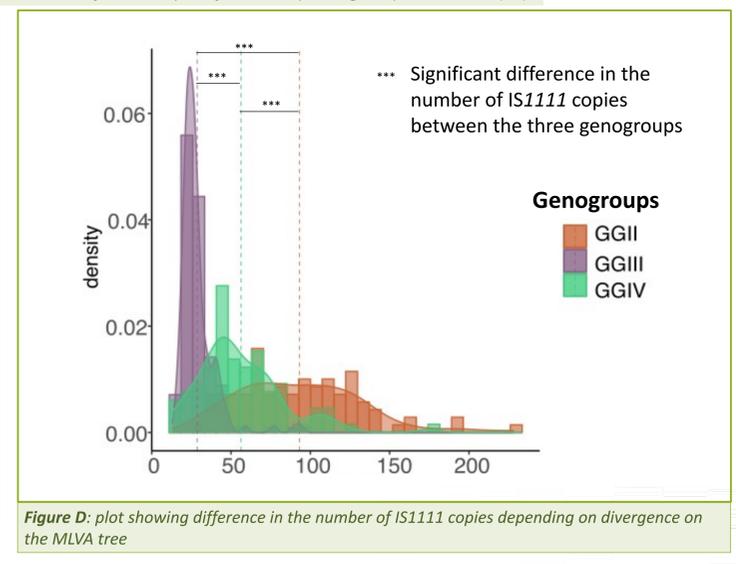


Figure D: plot showing difference in the number of IS1111 copies depending on divergence on the MLVA tree

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Acknowledgments

We thank all the partners that contributed to supplying field samples and sequenced strains as well as performing laboratory and sequencing analyses. This research did not receive any specific grant from funding agencies.