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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▶ **To cite this version:**

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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qPCR- and genomic-based determination of the number of IS*1111* elements suggests an impact of *C. burnetii* genotypic diversity on the diagnosis of Q fever in ruminants

Poster #160

International intracellular bacteria meeting 2022

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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 IS*1111* element** because commercial firms selected this target to develop diagnostic kits with the objective to obtain a highly sensitive method.

Because **the number of IS*1111* 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 IS*1111* 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 IS*1111* copies** in a **panel of representative animal strains** prevailing in France [4].

Methods

1. We estimated the number of IS*1111* copies using **qPCR and *in silico* approaches** depending on the nature and source of the considered data (**figure A**)
2. We constructed a **phylogenetic tree** from MLVA data considering 17 markers [5]

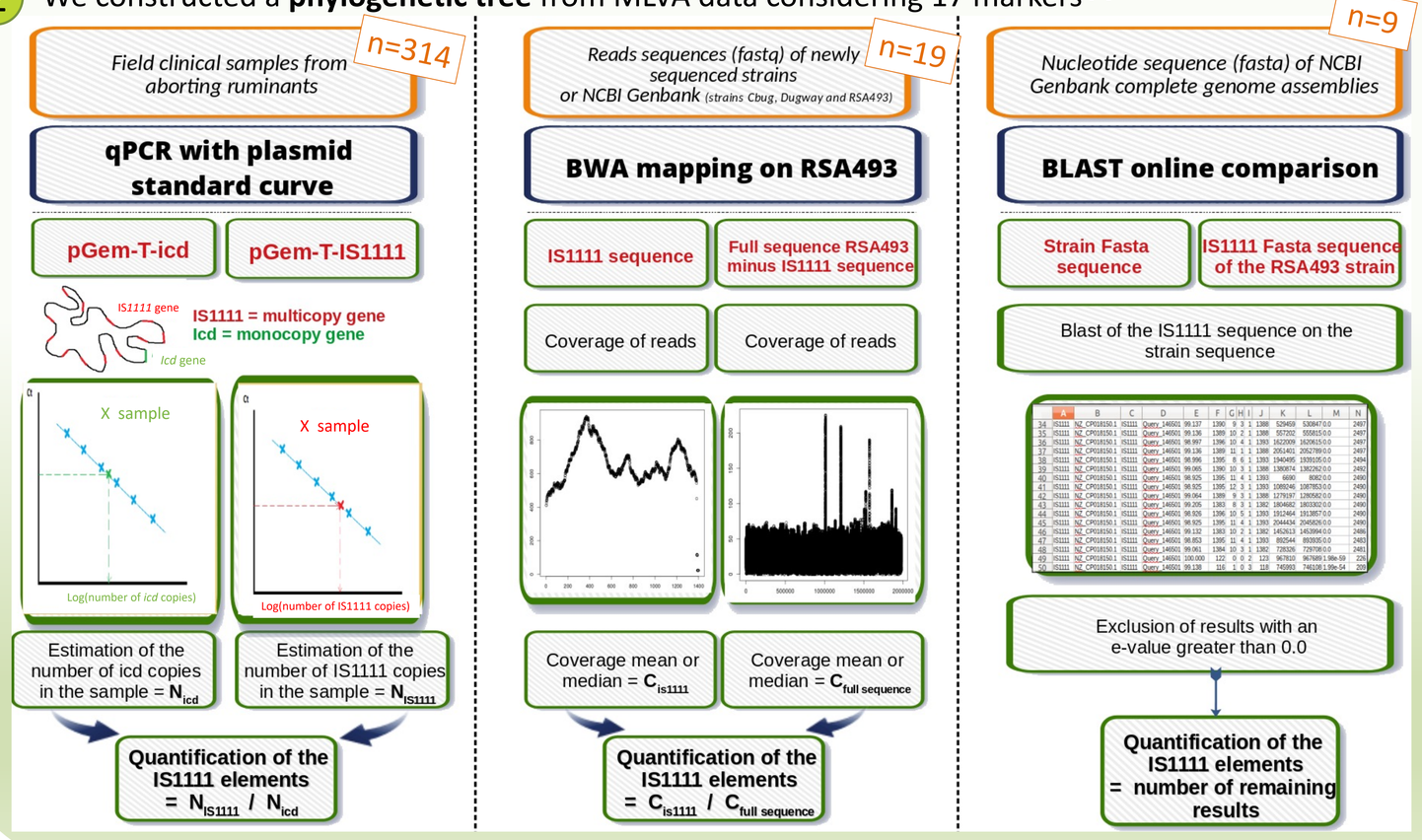

***Figure A**: the 3 methods used to count the number of IS1111 copies*

Results

1. The number of IS*1111* 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*

2. The **median** and **mean** of IS*1111* copy numbers were significantly **associated with genetic divergence** and **differed significantly among bacterial genomic groups** (**figures B, C and D**).

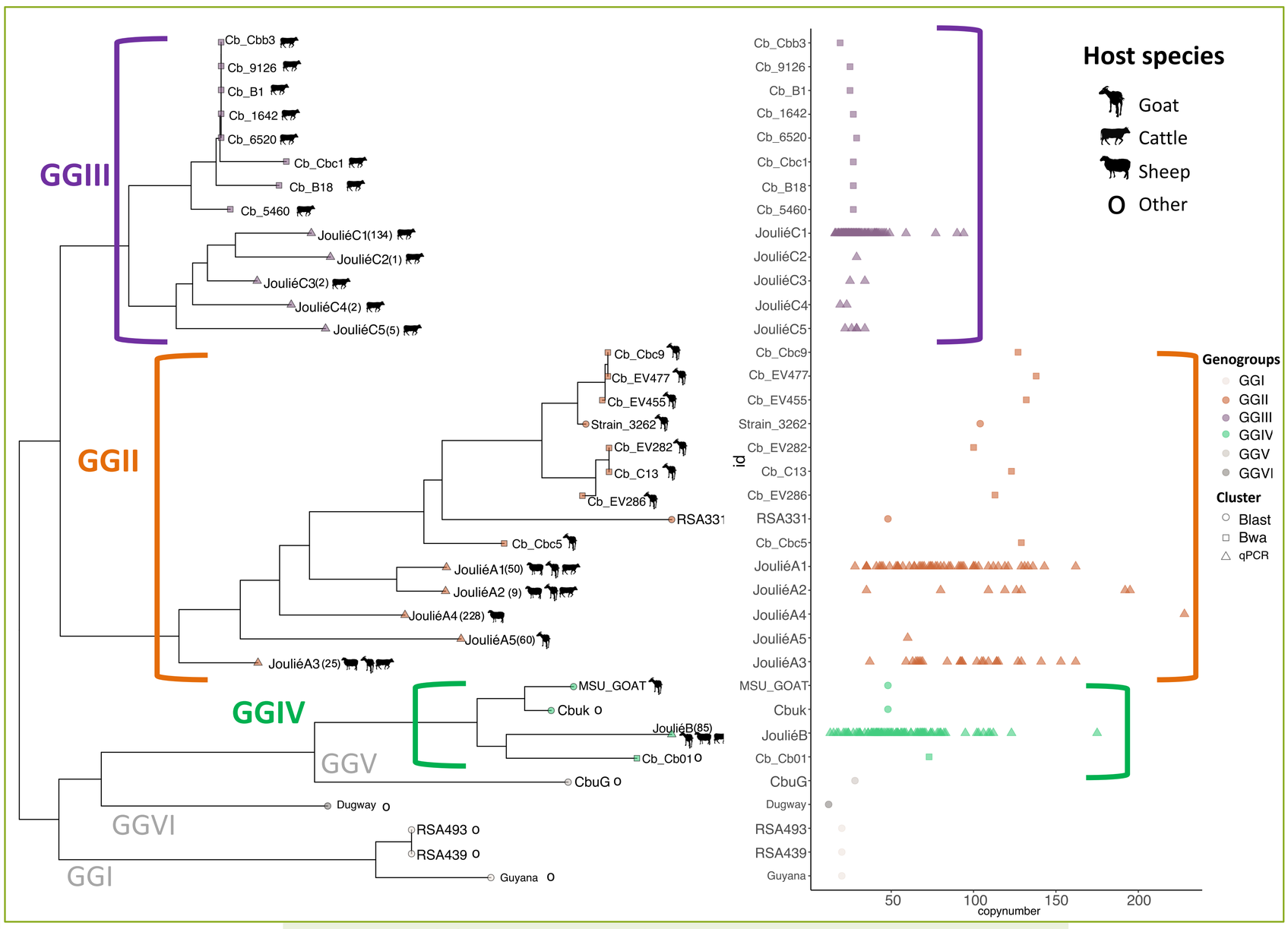

***Figure B**: MLVA tree (B1) and associated number of IS1111 copies of the corresponding samples or strains (B2)*

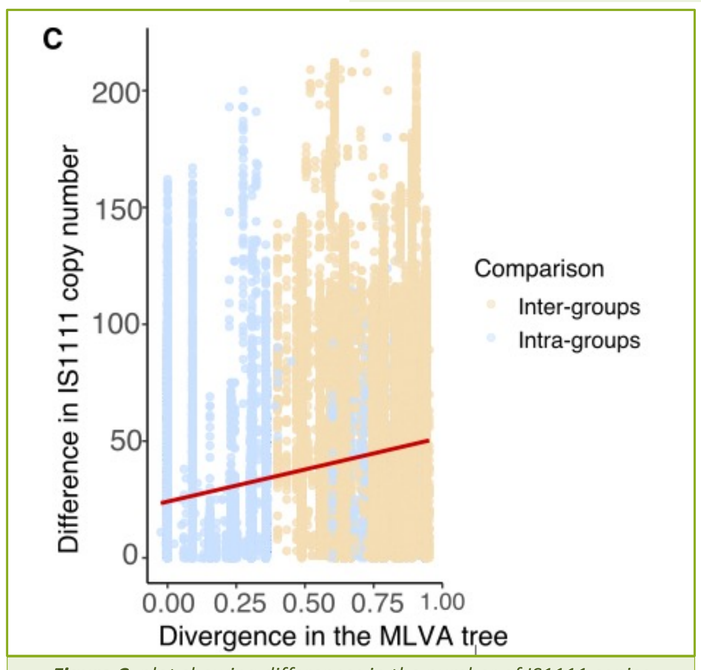

***Figure C**: plot showing differences in the number of IS1111 copies depending on divergence on the MLVA tree*

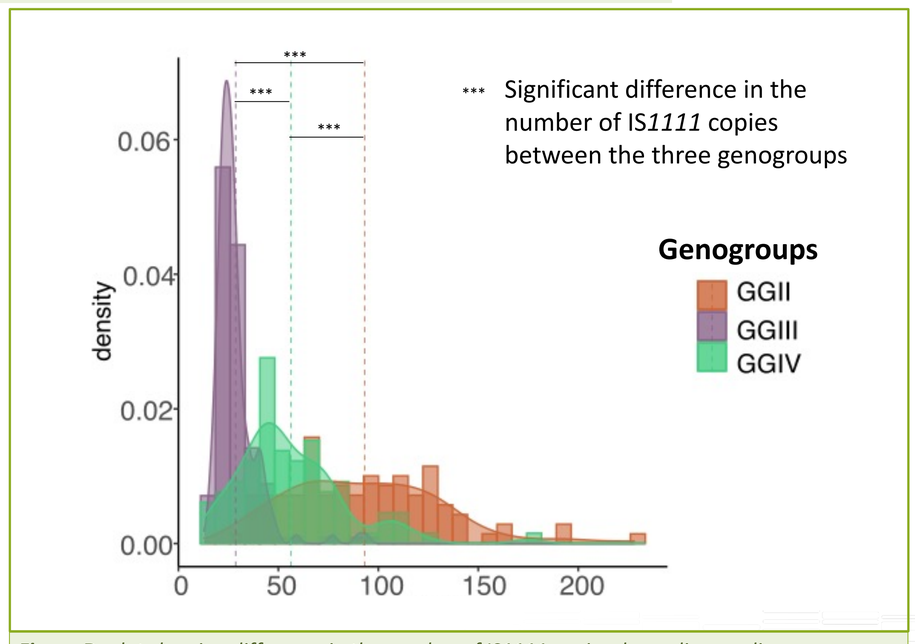

***Figure D**: plot showing difference in the number of IS1111 copies depending on divergence on the MLVA tree*

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 IS*1111* copies in a broad panel of *C. burnetii* samples and isolates.

Significant difference in the number of IS*1111* 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 **IS*1111* 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

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Acknowlegments

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.

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