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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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

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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]

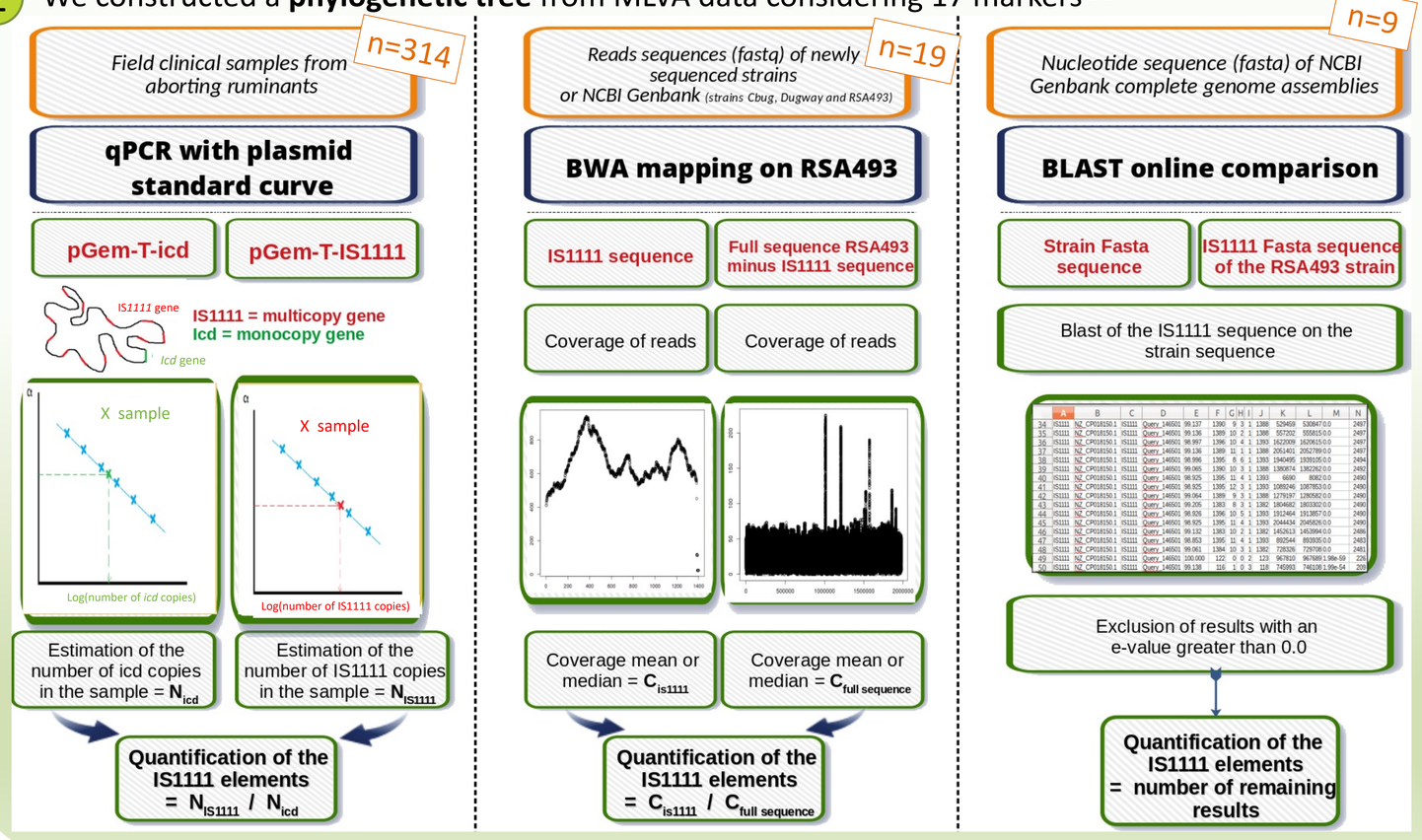

***Figure A**: the 3 methods used to count the number of IS1111 copies*

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**).

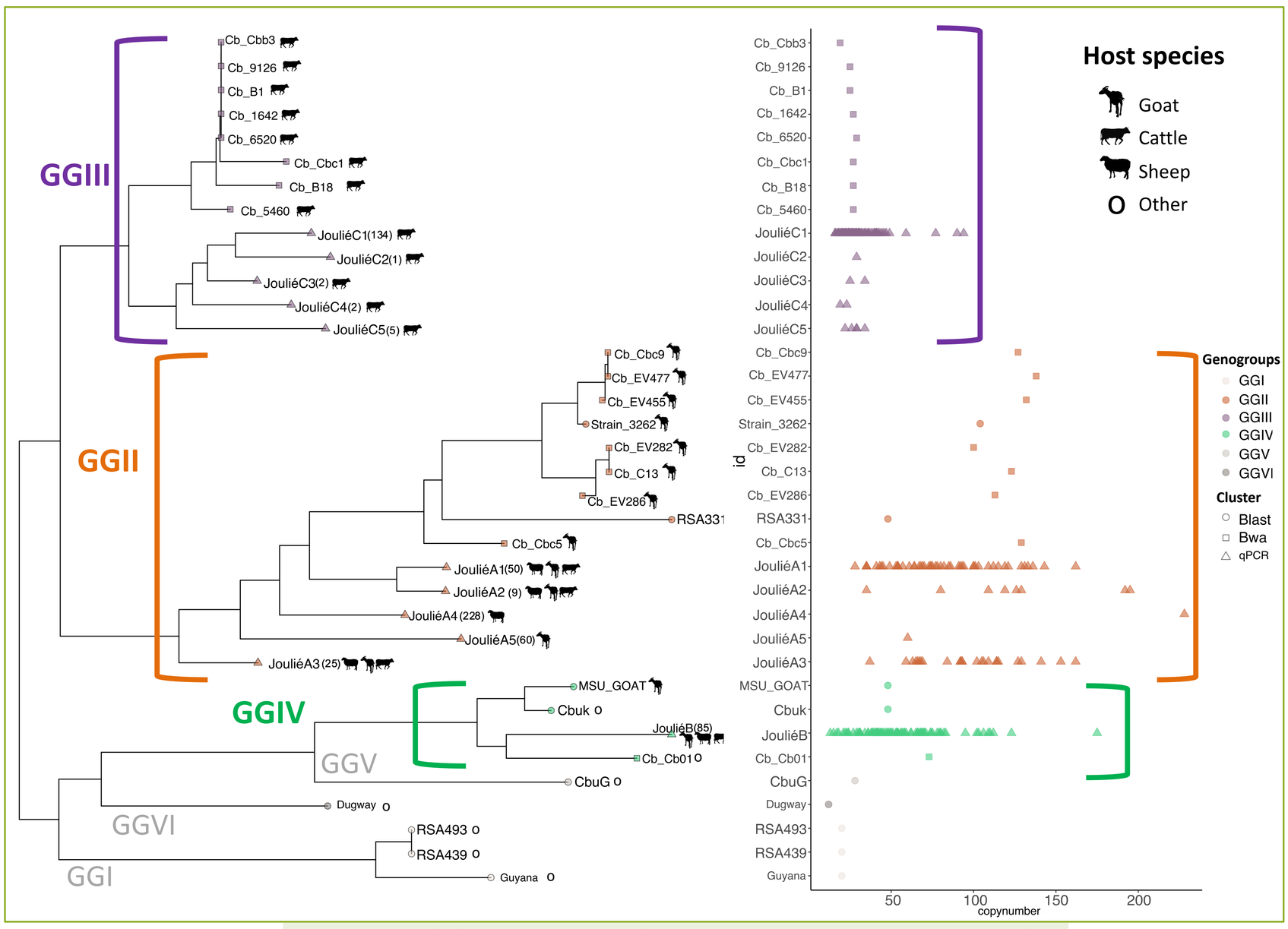

***Figure B**: MLVA tree (B1) and associated number of IS1111 copies of the corresponding samples or strains (B2)*

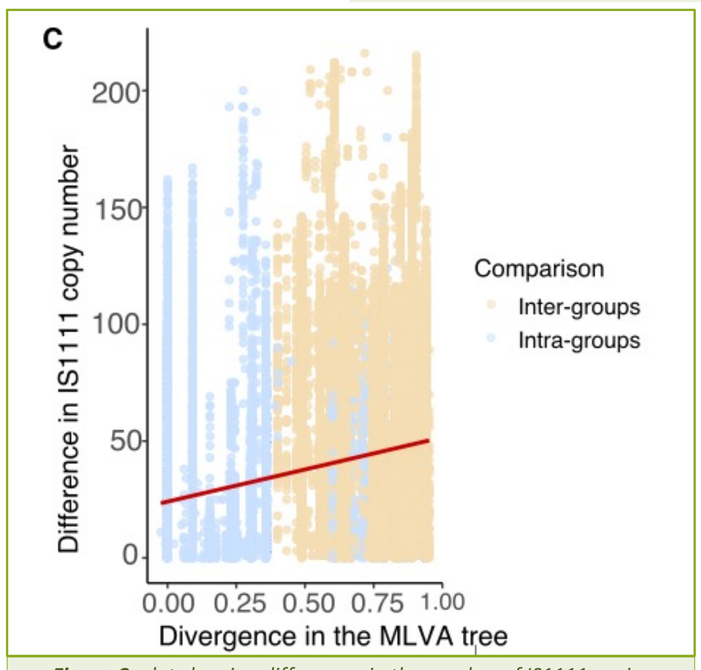

***Figure C**: plot showing differences in the number of IS1111 copies depending on divergence on the MLVA tree*

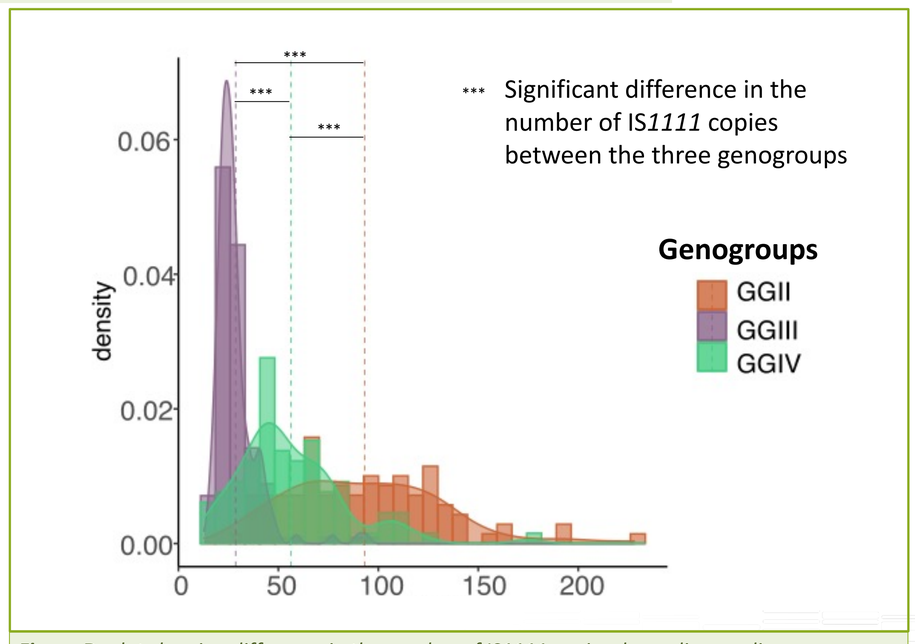

***Figure D**: plot showing difference in the number of IS1111 copies depending on divergence on the MLVA tree*

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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