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## DETECTION OF *MYCOPLASMA* SPP. IN HORSES WITH RESPIRATORY DISORDERS

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### INTRODUCTION

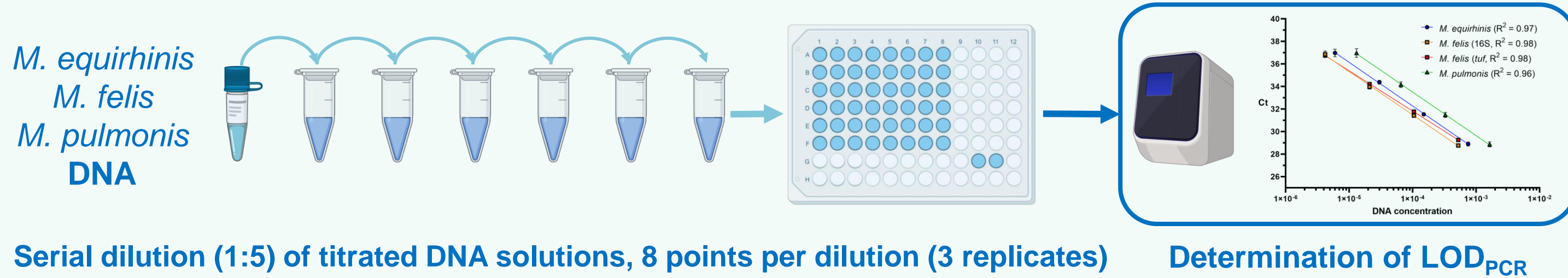
Bacteria belonging to the genus *Mycoplasma* are often involved in respiratory disorders in their animal hosts. Three species have been reported so far from the respiratory tract of horses worldwide: *Mycoplasma* (*M.*) *equirhinis*, *M. pulmonis* and *M. felis*<sup>1,2</sup>. Their clinical role remains unclear, partly owing to the lack of standardised detection tools.

### AIM OF THE STUDY

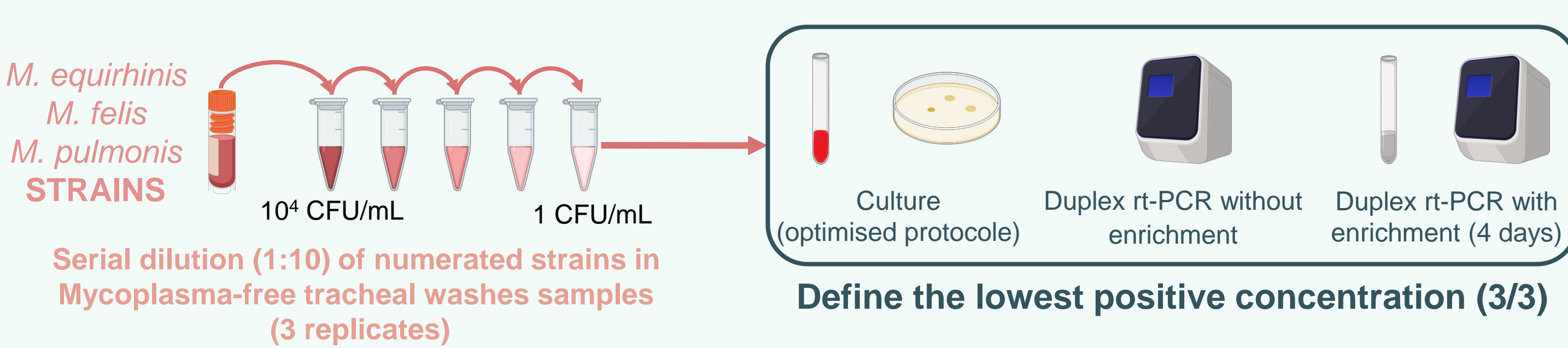
This study aimed at i) developing and validating tools to detect, isolate and identify strains from different *Mycoplasma* species in clinical respiratory equine specimens and ii) subsequently defining the three species' prevalence in France depending on the sample types and horses' characteristics (age, breed and sex).

### MATERIALS AND METHODS

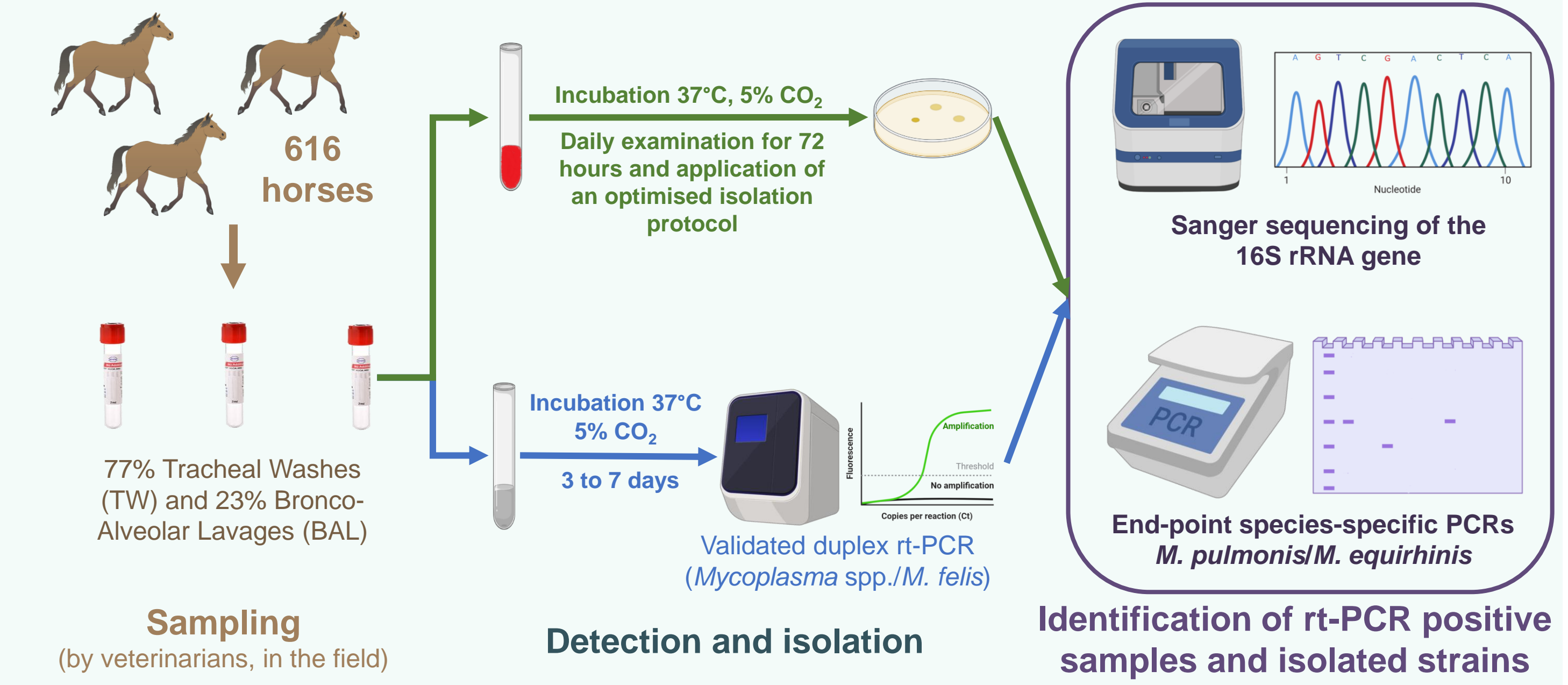
#### Validation of a duplex rt-PCR for the detection of *Mycoplasma* spp./*M. felis*



#### Determination of the analytical sensitivity of the overall method



#### Analysis of clinical samples



### RESULTS

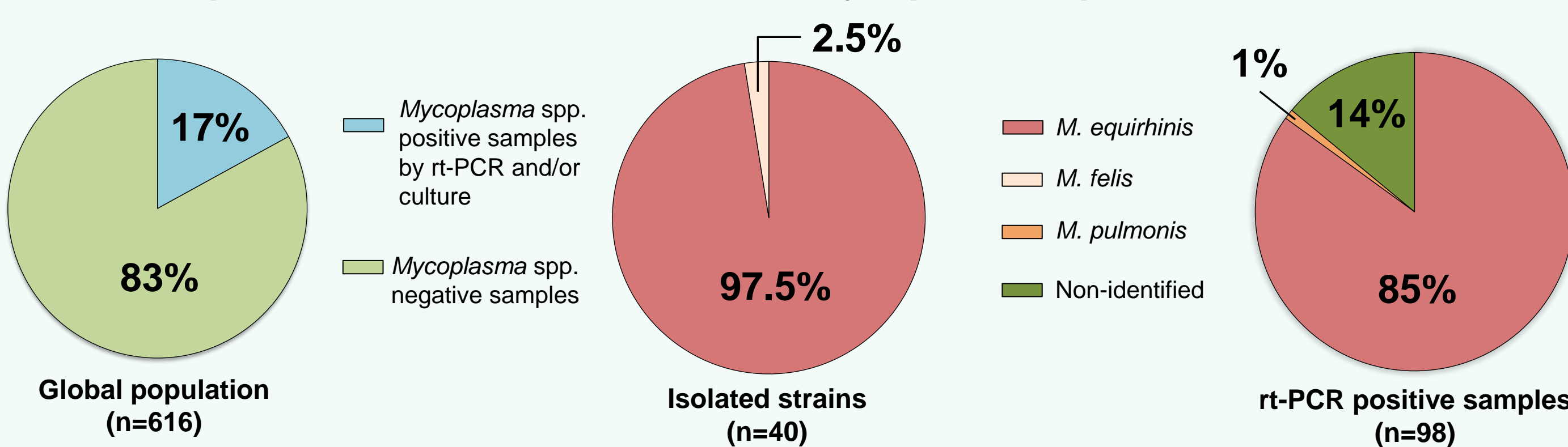
#### Analytical sensitivity of the methods

	CFU/mL		
	Culture	rt-PCR without enrichment	rt-PCR with enrichment
<i>M. equirhinis</i>	10	10000	<1
<i>M. felis</i>	10	10 and <1†	<1†
<i>M. pulmonis</i>	100	1000	100

† Using the *M. felis* specific rt-PCR, for rt-PCR with enrichment the results are identical with both systems.

→ The rt-PCR with an enrichment procedure was the most sensitive method

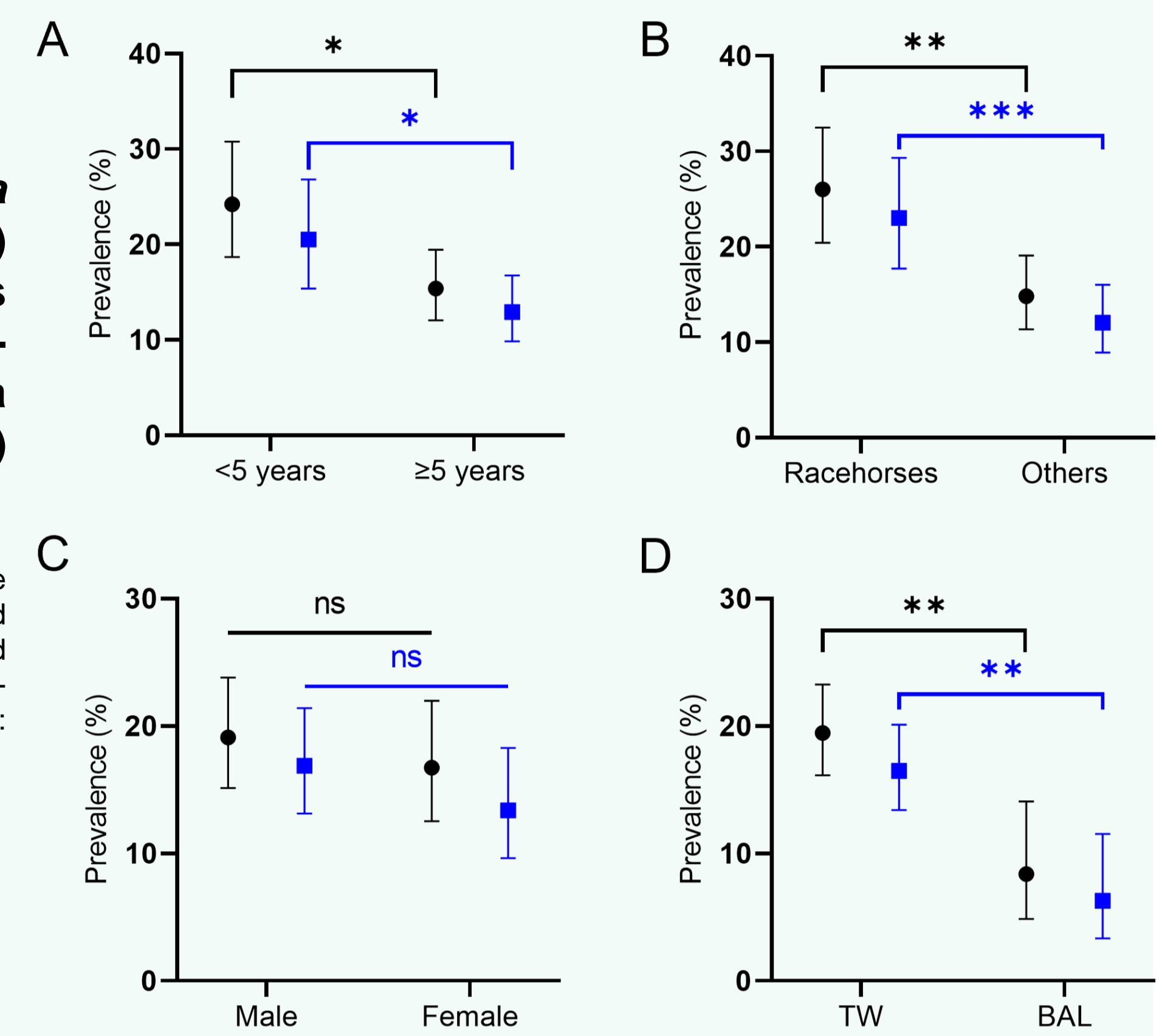
#### Overall prevalence and identification of *Mycoplasma* species



→ 104 samples were positive by at least one method. *M. equirhinis* is the predominant circulating species

#### Prevalence of *Mycoplasma* spp. (●) and *M. equirhinis* (■) in clinical specimens (positivity is defined by rt-PCR and/or culture) as a function of age (A), breed (B) sex (C) and sample types (D).

Errors bars correspond to 95% confidence interval. "ns": not significant, "\*" and "\*\*\*" refer to p-values of ≤0.05, ≤0.01 and ≤0.001, respectively, as obtained using Chi-squared test. TW: Tracheal Washes, BAL: Broncho-Alveolar Lavages.



→ The prevalence of *Mycoplasma* spp. and *M. equirhinis* varied with age, racehorse status and the type of sample

### CONCLUSION/DISCUSSION

We proposed a mixed diagnostic based on both culture and a duplex rt-PCR. The pre-enrichment step was shown to improve the sensibility of the method, but prevented an estimation of the initial mycoplasma load in the clinical samples. The commonest detected species was *M. equirhinis*, which is in agreement with a previous study<sup>2</sup>. Our results showed a significantly higher prevalence of *Mycoplasma* spp. or *M. equirhinis* in racehorses, possibly explained by the strong ventilation during exercises. Also, this prevalence was higher in TW than BAL, TW being often preferred for airway infection diagnostic. Future studies should address the actual clinical role of mycoplasmas and more specifically of the frequent *M. equirhinis* species in equine respiratory infections.

<sup>1</sup> Allam, N. M. & Lemcke, R. M. Mycoplasmas isolated from the respiratory tract of horses. *J. Hyg.* 74, 385–408 (1975).

<sup>2</sup> Uchida-Fujii, E. et al. High prevalence of *Mycoplasma equirhinis* in Thoroughbred horses with respiratory symptoms in autumn 2018. *J. Vet. Med. Sci.* 83, 1907–1912 (2021).