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FRENCH ROE DEER AS A RESERVOIR OF VECTOR-BORNE HAEMOPARASITES FOR LIVESTOCK AND HUMANS? DATA FROM THE FRENCH DEPARTEMENT OF AIN.

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Introduction
While improving surveillance systems for emerging pathogens in livestock and humans requires early detection in wildlife, while hyperthermia-anemia syndromes are on the rise in livestock when in parallel tick and deer populations are booming, while in 2020 a first case of tick-borne encephalitis by consumption of goat cheese was described in the French department of Ain, there are still very few data in France on the possible role of roe deer as a reservoir for haemoparasites such as *Anaplasma phagocytophilum*, *Babesia*, *Mycoplasma wenyonii* or Tick-Borne Encephalitis Virus (TBEV).
We then decided to study on more than 150 samples of roe deer kept in the laboratory the prevalence of 11 haemoparasites of livestock ruminants and/or for humans, using in-house real-time PCR tests.

Methods
155 spleens, dried blood spot (DBS) and ears of roe deer had been collected, in a representative manner throughout the territory of our French department, during fall 2020 and 2021.
Each ear was examined to determine the carriage of hematophagous arthropods such as ticks (number, stage) and Lipoptena.
Total nucleic acid extraction and purification were performed on spleen and DBS using respectively MagMAX™ Pathogen RNA/DNA kit on KingFisher Flex device (ThermoFisher Scientific) and QIAamp DNA mini kit (Qiagen). Six in-house multiplex real-time PCR tests, each with endogenous Internal Positive Control (Gapdh) were developed:

- Test 1: *Anaplasma phagocytophilum*; *Anaplasma marginale*
- Test 2: *Babesia spp.*
- Test 3: *Mycoplasma ovis*; *Anaplasma ovis* ; *Candidatus* Mycoplasma haemovis
- Test 4: *Candidatus* Mycoplasma erythrocervae ; *Mycoplasma haemocervae*
- Test 5: *Mycoplasma wenyonii*; *Candidatus* Mycoplasma haemobos
- Test 6: TBEV

Results
56% of ears are bearing ticks belonging only to *Ixodes ricinus*, with an average of 2.3 ticks per positive ear. Overall, the number of ticks is 30% higher in 2020 than in 2021, also with more blood-soaked nymphs. *Lipoptena cervi* was found at quite the same prevalence no matter of the year of sampling.

No samples were found positive for TBEV.
Regardless of the nature of sample (spleen or DBS), the prevalence of neutrophil granulocyte-hosted *A. phagocytophilum* is consistent with other studies in Europe (over 85% including 74% spleens with an early Ct value <30).
On the other hand, all haemoparasites of erythrocytes, are either detected with a very low prevalence and weak signals, or not detected on spleens, while the results are different on DBS.

Discussion and Conclusion
We wondered if there was not a bias in the treatment of the spleens or with the spleen itself. The most likely hypothesis is that infected erythrocytes are sequestered in the spleen where they are quickly phagocytosed. Phagocytosis leads to the rapid degradation of the haemoparasites genome.
In conclusion, future studies in roe deer should be performed on circulating whole blood (DBS or even heart clot) to ensure the detection of all haemoparasites.