



RETROSPECTIVE STUDY REGARDING THE MOLECULAR DETECTION OF PATHOGENS FROM CANINE BLOOD SAMPLES

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Abstract: More zoonotic infections are carried by ticks than by any other kind of vector, and they are prevalent on all continents. Since all vector-borne diseases species have been affected by climate change detected in recent years, an important number of vector species (ticks, mosquitoes, fleas, sand-flies, etc.), and the pathogens they carry are currently expanding in both distribution and incidence, with significant implications for human and animal health and welfare. Therefore, the aim of this retrospective study was to determine the main vector-borne diseases identified in 45 canine patients with or without specific symptoms from Iasi County, between March 2023 and December 2025. Molecular screening was performed using multiplex quantitative real-time PCR (qPCR) instrument, being able to detect the DNA of more than 19 pathogens specific to dogs. The main species of pathogens requested by veterinarians (*Babesia* spp., *Babesia canis*, *Babesia gibsoni*, *Babesia vogeli*, *Anaplasma/Ehrlichia* spp., *Mycoplasma haemocanis*, *Hepatozoon canis*, *Dirofilaria immitis*, *Borrelia* spp., or *Leptospira* spp.) were detected in 51.12% of the samples analyzed. The molecular results showed that the most prevalent vector-borne species detected in canine blood were: *Babesia* spp. (30.43%; 7/23), followed by *Babesia gibsoni* (17.39%; 4/23), *Babesia vogeli* (8.7%; 2/23), *Babesia canis* (4.35%; 1/23), *Hepatozoon canis* (4.35%; 1/23), *Dirofilaria immitis* (4.35%; 1/23), *Anaplasma/Ehrlichia* spp. (4.35%; 1/23), and *Mycoplasma haemocanis* (4.35%; 1/23). Even though *Leptospira* is not part of the group of vector-borne diseases, it is an important zoonotic pathogen, and the high number of cases detected in dogs (21.73%; 5/23) raises numerous concerns regarding biosecurity measures in veterinary clinics, as well as insufficient prophylactic measures. These results underline the necessity of constant monitoring of vector-borne and zoonotic infections, being essential to both veterinary and human medicine within a One Health framework.

• Introduction

- Vector-borne agents consist of a collection of worldwide distributed and proliferating pathogens responsible for several zoonotic diseases transmitted by the bites of hematophagous arthropods, primarily ticks and mosquitoes.
- Humans and domestic animals are constantly at high risk of contracting vector-borne diseases due to ticks' or other vectors' capacity to spread pathogens. It is becoming more and more evident that ticks are well adapted to urban and suburban surroundings, despite the fact that tick infections are typically correlated with rural areas.

• Results and discussions

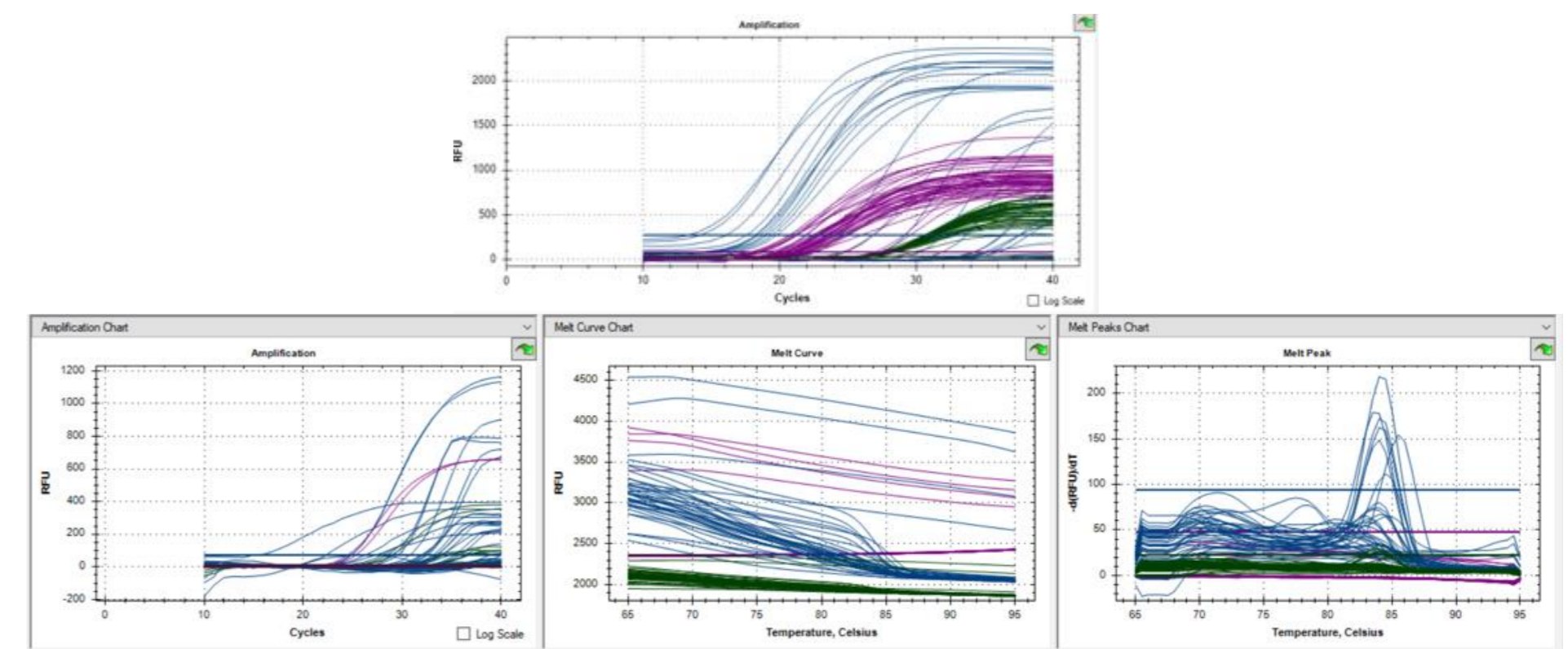


Figure 2. Amplification curves of samples through qPCR.

Retrospective study

The determination of the main vector-borne diseases identified in canine patients, using the qPCR technique

Molecular Biology Laboratory of the Faculty of Veterinary Medicine in Iasi

AIM OF THE STUDY

• Material and method

- 45 canine blood samples
- were evaluated for 1 to 9 pathogens
- average of 5 pathogens per sample

March 2023-
December 2025

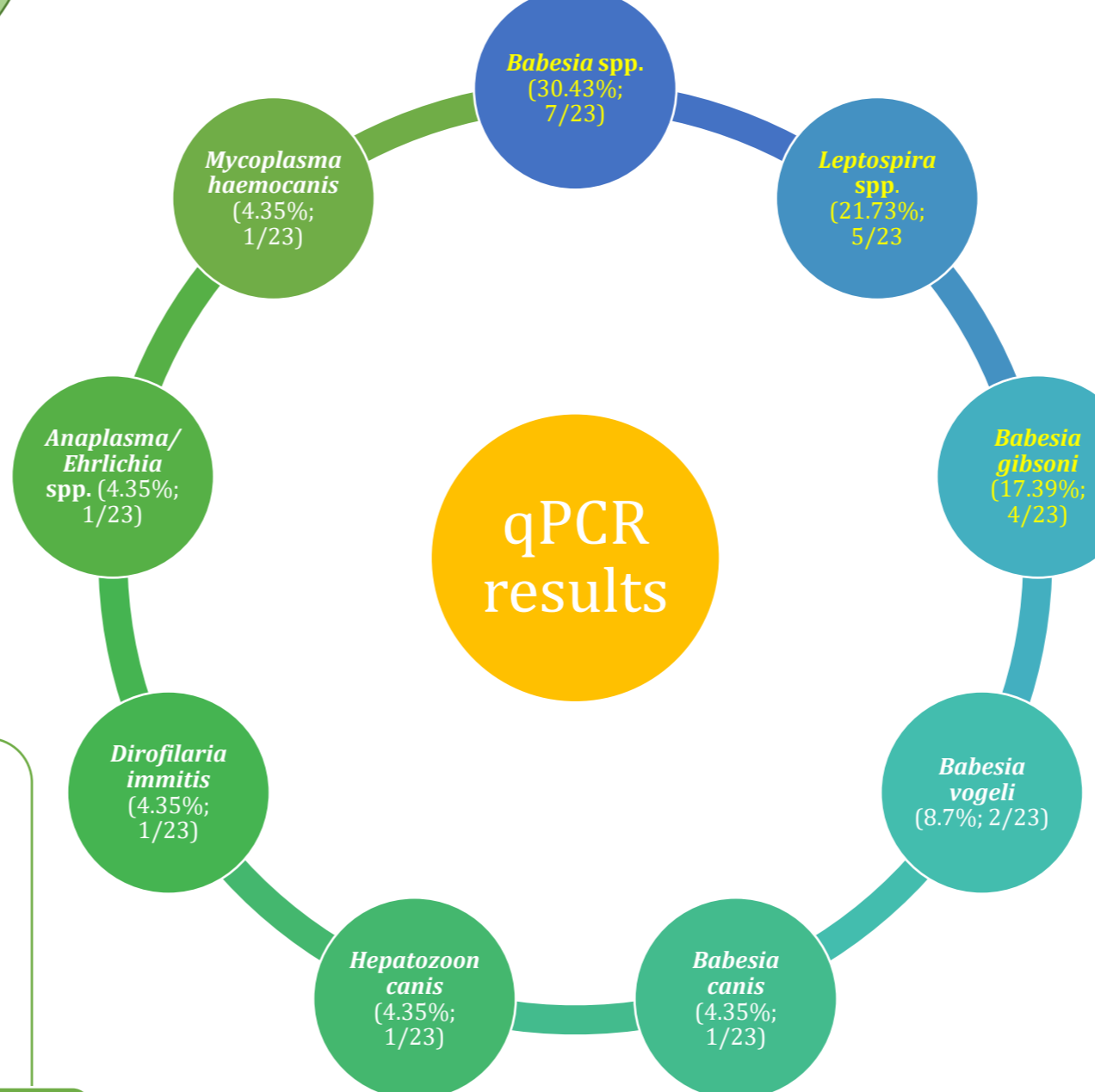
DNA extraction

- PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific)
- V=200 µL blood/100 µL of blood and 100 µL of urine as samples - only for *Leptospira* spp.

- multiplex protocol
- TaqMan and SYBR Green principles
- CFX96™ Real-Time Detection System
- CFX Manager Software Version 3.1

qPCR

qPCR results



➤ In Romania, canine babesiosis continues to be one of the most researched tick-borne diseases, with the majority of studies concentrating on pathogen identification in blood samples.

➤ This elevated frequency of *B. gibsoni* (17.39%), compared to the molecular prevalence documented in the literature, should concern veterinarians, as identifying this small species in cytological smears is challenging, and its treatment differs from that of bigger *Babesia* species.

➤ Although *Leptospira* is not classified among vector-borne diseases, it remains a significant zoonotic pathogen. Our study also highlights elevated incidence of *Leptospira* infections in symptomatic dogs (21.73%), necessitating improved preventive strategies through vaccination and biosecurity protocols in veterinary clinics, given the potential for facile transmission from hospitalized patients during handling or during biological sample collection procedures.

• Results and discussions

- ✓ The Molecular Biology Laboratory from Faculty of Veterinary Medicine - Iasi offers the possibility for veterinarians (both within the institution, and in private clinics) to test a variety of pathogens using the molecular method - qPCR.
- ✓ This method is significantly more sensitive, rapid, and cost-effective than the traditional approach, delivering precise data to vets in a brief period of time.

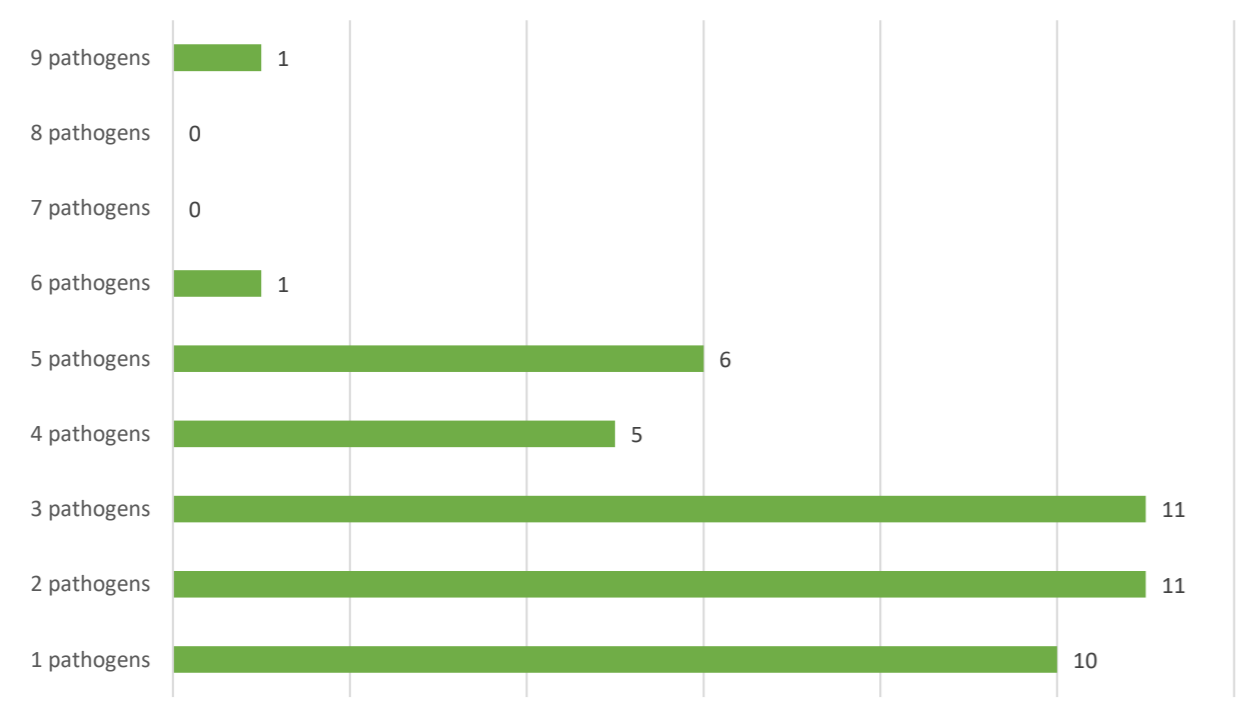


Figure 1. Number of pathogens examined per sample.

Consequently, in the period March 2023-December 2025, 45 blood samples from canine patients were received. These samples were evaluated for 1 to 9 pathogens (Fig. 1).

Pathogens predominantly wanted by veterinarians, including *Babesia* spp., *Babesia canis*, *Babesia gibsoni*, *Babesia vogeli*, *Anaplasma/Ehrlichia* spp., *Mycoplasma haemocanis*, *Hepatozoon canis*, *Dirofilaria immitis*, *Borrelia* spp., and *Leptospira* spp., were identified in 51.12% (23/45) of the tested samples.

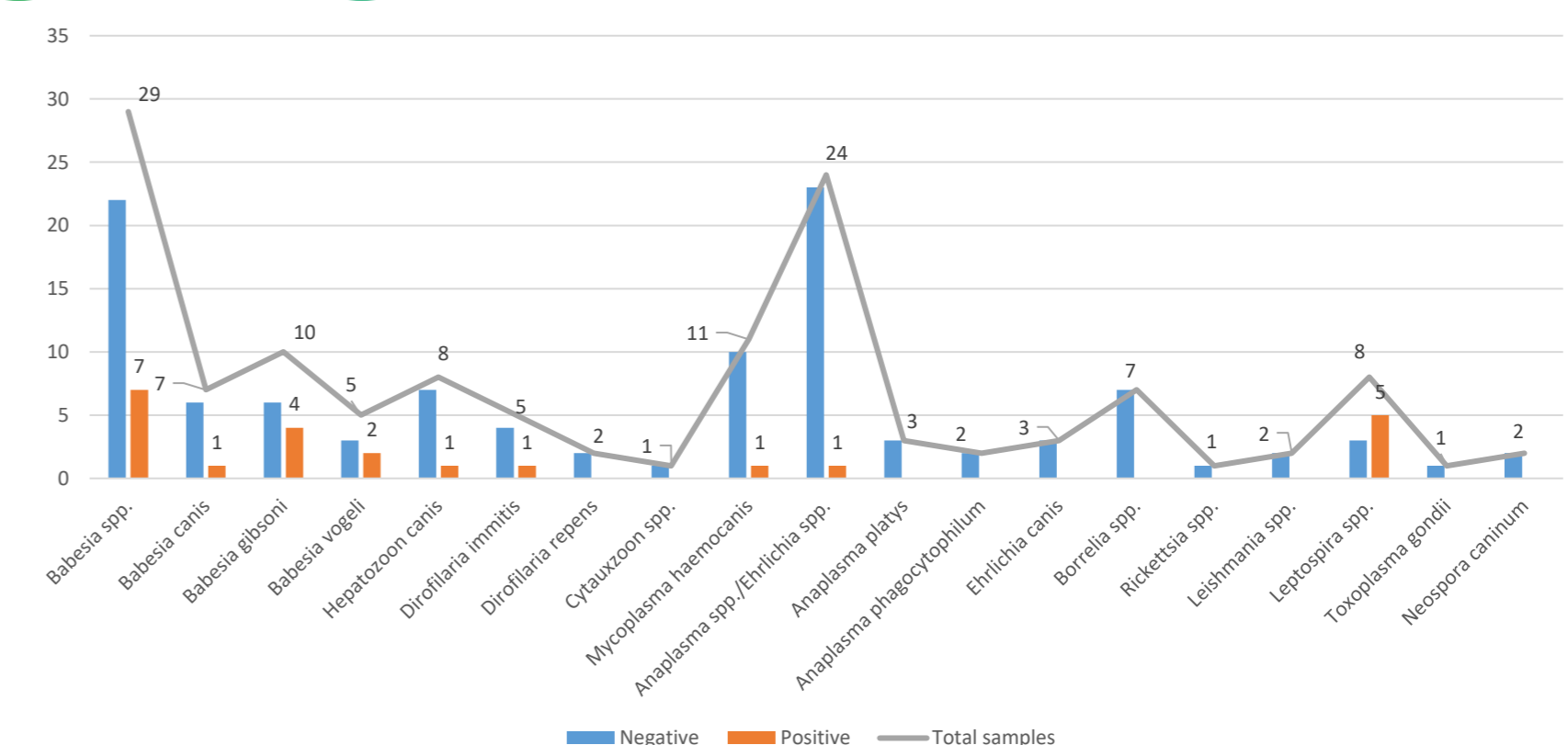


Figure 3. Canine samples examined using the qPCR molecular method.

According to the results obtained, the Ct value ranged from 18.05 to 38.45, with an average Ct value of 31.5674. The precise cycle number at which the fluorescence signal crosses a predetermined threshold, indicating significant amplification, is known as the Ct value (cycle threshold) in qPCR.

• Conclusions

- Controlling vector populations and developing innovative, workable methods to reduce vectors and the spread of vector-borne disease pathogens are necessary to protect human and companion animal health from vector-borne diseases.
- The results obtained in this study are extremely valuable for veterinarians in the Eastern region of Romania, as some findings may raise concerns about certain lesser-known vector-borne diseases, or could help improve diagnostic and treatment strategies, as well as biosecurity protocols in veterinary clinics.

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