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Molecular Survey of Selected Vector-Borne Pathogens in Algerian Horses

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Abstract

Vector-borne pathogens (VBPs) can affect equine health, welfare, and productivity, with potential implications for animal management and trade. However, data on their occurrence in North Africa remain limited. The present study aimed to estimate the molecular occurrence of selected equine VBPs in clinically healthy horses from Algeria. Specifically, a cross-sectional molecular survey was conducted between May and November 2024 on 241 clinically healthy horses selected using convenience sampling from three ecologically distinct regions of Algeria (Tiaret, Laghouat, and Tlemcen). Demographic and management data were collected at sampling, and blood samples were spotted onto filter paper for DNA preservation. Genomic DNA was extracted using a modified Chelex/InstaGene Matrix protocol, followed by conventional and nested PCR assays targeting piroplasms, Anaplasmataceae, filarioid helminths, *Rickettsia* spp., Trypanosomatidae, haemotropic *Mycoplasma* spp., and *Bartonella* spp. Positive amplicons were subjected to sequence and phylogenetic analysis. *Theileria equi* (*T. equi*) DNA was detected in 4.98% (12/241) of examined horses, and one animal (0.41%; 1/241) tested positive for *Theileria capreoli* (*T. capreoli*). *T. equi* genotypes A and B were identified via molecular characterisation, but no amplification was obtained for Anaplasmataceae, *Babesia caballi*, *Rickettsia* spp., haemotropic *Mycoplasma* spp., *Bartonella* spp., Trypanosomatidae, or filarioid helminths. These findings should be interpreted cautiously, given the convenience sampling design, the clinically healthy status of the sampled animals, and the known limitations of PCR-based detection in low-parasitaemia infections. Nevertheless, this study provides preliminary molecular data on equine VBPs in Algeria and supports the need for broader epidemiological investigations using complementary molecular and serological approaches.

Keywords: Algeria; equine piroplasmosis; *Theileria capreoli*; *Theileria equi*; vector-borne diseases; zoonosis

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1. Introduction

Vector-borne diseases (VBDs) are increasingly recognised as important infectious threats affecting equine health, welfare, and productivity worldwide, and horses are exposed to a wide range of arthropod-borne pathogens transmitted by ticks, flies, mosquitoes,

fleas, and lice, some of which also have zoonotic significance. Among equine VBPs, equine piroplasmosis (EP) is considered one of the most important VBPs of horses and is caused by apicomplexan parasites including *Theileria equi*, *Theileria haneyi*, and *Babesia caballi* [1]. *Theileria equi* is regarded as the most pathogenic among these parasites and may induce acute or chronic disease characterised by fever, anaemia, inappetence, spleno- and hepatomegaly, and in severe cases death, with vertical transmission from mares to fetuses also documented [2]. The international movement and trade of horses further contribute to the spread of equine piroplasmosis into non-endemic regions [3]. Transmission occurs primarily through ixodid ticks, especially species belonging to the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus* [4]. Additionally, *Babesia caballi* is another important intraerythrocytic protozoan of equids, capable of causing haemolytic anaemia, fever, icterus, and occasionally death [5]. More recently, *Theileria haneyi* has been recognised as an additional equine piroplasm with a broad geographic distribution and is generally associated with milder clinical signs and frequent co-infections with *T. equi* [6,7]. Occasional detections of atypical cervid-associated piroplasms, including *T. capreoli*, outside their usual wildlife hosts suggest the possibility of incidental spillover into atypical hosts, although their epidemiological significance in horses remains unclear [8]. In addition to piroplasms, horses may also be exposed to bacterial and parasitic vector-borne agents such as *Anaplasma phagocytophilum*, *Rickettsia* spp., *Trypanosoma evansi*, haemotropic *Mycoplasma* spp., *Bartonella* spp., and filarioid nematodes, all of which may influence equine health and disease ecology [9–14].

The epidemiology of equine VBPs is strongly influenced by ecological conditions, vector abundance, and animal management practices. In North Africa and neighboring Mediterranean regions, equine piroplasmosis is considered endemic, with variable prevalence reported in Tunisia, Egypt, and southern Europe [15–18]. Considerable genetic diversity has been described within *T. equi* populations worldwide, with multiple genotypes circulating among equids from different geographic areas [1]. Additionally, other VBPs have also been reported in equids from Africa and surrounding regions. *Anaplasma phagocytophilum* has been detected in horses and ticks in Tunisia [19], while *Trypanosoma evansi* infection has been documented in equids from Egypt [20]. Haemotropic *Mycoplasma* infections, although rarely reported, have also been described in horses in Africa [21]. Likewise, molecular evidence indicates occasional equine exposure to *Rickettsia* spp. and *Bartonella* spp., although their epidemiological significance in equine hosts remains insufficiently understood [13,22].

In Algeria, available studies on equine VBPs have mainly focused on equine piroplasmosis, and previous investigations confirmed the endemic presence of *T. equi* in horses from different regions of the country, with prevalence varying according to ecological conditions, horse management systems, and diagnostic methods used [23]. *T. equi* circulation in northeastern Algeria has also been recently confirmed via molecular characterisation [24]. However, information regarding other equine vector-borne bacterial and parasitic pathogens remains scarce, and comprehensive molecular screening studies in Algerian horses are limited. Consequently, the diversity and circulation patterns of VBPs in Algerian equine populations remain incompletely understood. We hypothesized that horses from ecologically distinct Algerian regions are exposed to a broader spectrum of VBPs than currently documented, including pathogens beyond equine piroplasms. Therefore, this study aimed to estimate the molecular occurrence of selected equine VBPs in convenience-sampled clinically healthy horses from ecologically distinct regions of Algeria using PCR-based screening, followed by sequencing and phylogenetically characterising the detected piroplasm isolates.

2. Materials and Methods

2.1. Study Population

Overall, 241 horses sampled across three regions in Algeria were included in this study (Figure 1). The three study regions (Tiaret, Laghouat, and Tlemcen) were selected to represent ecologically distinct Algerian areas differing in climate, altitude, vegetation cover, and livestock management systems, factors that may influence vector abundance and pathogen circulation. The demographic characteristics of the sampled horses, including age group, sex, and geographic origin, are summarized in Table 1. All horses were selected based on availability, owner consent, and veterinary visits, and all of the selected horses appeared to be in good health and had no clinical signs potentially indicating VBDs. Clinically healthy horses were included to investigate the occurrence of subclinical or asymptomatic carriage of VBPs in the general equine population, as chronically infected animals may act as reservoirs despite the absence of overt clinical signs. The sample size was determined based on logistical constraints and animal accessibility during the study period, rather than a formal sample size calculation. This study therefore used a convenience sampling design, and the sampled population cannot be considered representative of the wider Algerian equine population. Therefore, the results should be interpreted as exploratory.

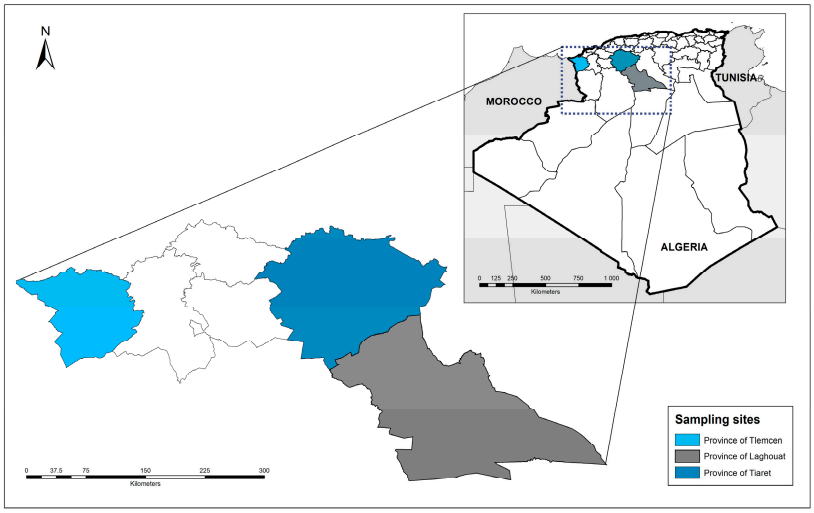


Figure 1. Geographical distribution of sampling sites in Algeria.

Table 1. Demographic characteristics of sampled horses.

Variable	Category	Number (*n*)	Percentage (%)
Age	<5 years	12	5
	5–10 years	110	45.6
	>10 years	119	49.4
Sex	Male	72	29.9
	Female	169	70.1
Region	Tiaret	150	62.2
	Laghouat	42	17.4
	Tlemcen	49	20.3

Information regarding management practices, including vector-control and acaricide treatment history, was obtained directly from horse owners during sampling through brief verbal interviews conducted by the attending veterinarians. According to owner-reported histories, most horses did not receive regular vector-control or acaricide treatments.

A cross-sectional study was conducted between May and November 2024, corresponding to the season of increased tick activity in Algeria. Under good veterinary practice and with owner consent, experienced veterinarians collected blood samples from horses from three ecologically distinct regions that were screened for selected equine VBPs. Sampling was performed by experienced veterinarians under good veterinary practice and with owner consent.

2.2. DNA Extraction

Blood samples were collected from the jugular vein by experienced veterinarians using sterile needles and EDTA tubes during routine veterinary handling procedures. Horses were manually restrained by their handlers to minimise stress during sampling. For DNA extraction, 50 µL of blood was spotted on individual pieces of Whatman R© filter paper (VWR International GmBH, Vienna, Austria), dried at room temperature, and stored individually in envelopes. The samples were then forwarded to the University of Veterinary Medicine in Vienna for further molecular analysis. Using sterile blades, approximately 4 mm sections were excised from the center of the blood spots on the filter paper. A modified Chelex-based method employing the InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA) was used for DNA extraction, following previously established procedures [16], and DNA samples were stored at −20 °C prior to analysis.

2.3. PCR Amplification

Serological assays were not included in the present study because the primary objective was the molecular screening of selected pathogens for current molecular detection, followed by sequence characterisation of the detected isolates. A panel of PCR assays targeting piroplasms, Anaplasmataceae, filarioid helminths, *Rickettsia* spp., *Bartonella* spp., haemotropic Mycoplasmas, and Trypanosomatidae was conducted according to protocols listed in Table 2. The Trypanosomatidae PCR assay detects a broad range of trypanosomatid parasites at the family level and does not allow species-level identification; therefore, positive results cannot be directly attributed to *Trypanosoma evansi* without subsequent sequence confirmation. Conventional PCR assays targeting specific genes were used based on previously validated protocols. Although more recent techniques such as multiplex qPCR offer higher sensitivity, the selected methods remain widely used and suitable for pathogen detection.

All PCR amplifications were performed using GoTaq® G2 Flexi DNA Polymerase (Promega Corporation, Madison, WI, USA) with the manufacturer's supplied Green GoTaq® Reaction Buffer (containing $MgCl_2$ at a final concentration of 1.5 mM), 200 µM of each dNTP (New England Biolabs, Ipswich, MA, USA), 0.4 µM of each primer, and approximately 50 ng of the template DNA in a final volume of 25 µL. PCR cycling conditions are detailed in Table 2, and each PCR run included a negative control (no DNA template) and target-specific positive controls consisting of previously confirmed positive DNA samples for the respective pathogen groups whenever available, including a *T. equi*-positive control obtained from the Institute of Parasitology, University of Veterinary Medicine Vienna. Amplifications were performed using a Biometra TOne thermal cycler (Analytik Jena GmbH, Jena, Germany), and PCR products were separated by electrophoresis on 2% agarose gels (Carl Roth GmbH, Karlsruhe, Germany), stained with Midori Green Advance DNA stain (Nippon Genetics Europe GmbH, Düren, Germany), and visualized under UV light.

Table 2. PCR protocols and sequences used for molecular detection of VBP in Algerian horses.

Organism	Gene/Locus	Primer Sequences (5 → 3)	Amplification Protocol	Product Size (bp)	References
Piroplasms	18S rRNA	Nest 1: BTH-1F: CCTGAGAAAGGCTACACATCT BTH-1R: TTGCGACCATACTCCCCCCA Nest 2: GF2: GTCTTGTAATTGGAATGATGG GR2: CCAAAGACTTTGATTTCTCTC	94 °C: 2 min, 40 cycles– 95 °C: 30 s, 68 °C: 1 min, 72 °C: 1 min, 72 °C: 10 min	Nest 1: 700 Nest 2: 561	[25]
Rickettsia spp.	23S/5S rRNA	ITS-F: GATAGGTCGGGTGTGGAAG ITS-R: TCGGGATGGGATCGTGTG	96 °C: 4 min, 35 cycles– 94 °C: 1 min, 52 °C: 1 min, 72 °C: 2 min, 72 °C: 3 min	350–550	[26]
Filarioid nematodes	mt COI	H14FilaCO1Fw: GCCTATTTTGATTGGTGGTTTTGG H14FilaCO1Rv: AGCAATAATCATAGTAGCAGCACTAA	95 °C: 2 min, 30–35 cycles– 95 °C: 1 min, 53 °C: 1 min, 72 °C: 1 min, 72 °C: 5 min	724	[27]
Trypanosomatidae	18S rRNA	Nest 1: Tryp18SF1: GTGGACTGCCATGGCGTTGA Tryp18SR1: CAGCTGGATCTCGTCCGTTA Nest 2: Tryp18SF2: CGATGAGGCAGCGAAAAGAAATAGAG Tryp18SR2: GACTGTAACCTCAAAGCTTTCGC	94 °C: 5 min, 35 cycles– 94 °C: 1 min, 56 °C: 1 min, 72 °C: 1 min, 56 °C: 1 min, 72 °C: 5 min	Nest 1: 1320 Nest 2: 960	[28]
Haemotropic mycoplasmas	16S rRNA	HBT-F: ATACGGCCCATATTCCTACG HBT-R: TGCTCCACCACTTGTTCA	94 °C: 2 min, 40 cycles– 95 °C: 1 min, 60 °C: 1 min, 72 °C: 1 min, 72 °C: 7 min	600	[29]
Anaplasmataceae	groEL	Apgroe02for: CGAAAGCTGCTGGATCTGA Apgroe02rev: TCCTTGAAGCCTTTGCTTTC	94 °C: 5 min, 40 cycles– 94 °C: 30 s, 58 °C: 30 s, 72 °C: 30 s, 72 °C: 7 min	235	[30]
Bartonella spp.	gltA	BhCS.781p: GGGGACCAGCTCATGGTGG BhCS.1137n: AATGCAAAAAGAACAGTAAACA	94 °C: 5 min, 40 cycles– 94 °C: 1 min, 54 °C: 1 min, 72 °C: 1 min, 72 °C: 10 min	379	[31]

2.4. Sequencing and Molecular Analysis

Positive samples were sent to Microsynth AG (Vienna, Austria) for Sanger sequencing, and the resulting sequences were assembled using BioEdit® version 7.7.1 (North Carolina State University, Raleigh, NC, USA) and compared with sequences available in the GenBank® database using the NCBI Nucleotide BLAST tool (National Center for Biotechnology Information, Bethesda, MD, USA). Phylogenetic analyses were performed using MEGA11 software 11.0.13 (Molecular Evolutionary Genetics Analysis, Pennsylvania State University, University Park, PA, USA) to construct maximum likelihood trees.

For molecular analysis, dried blood spots (DBSs) were used for sample preservation. This method was selected because it is practical under field conditions. DBS samples are easy to collect and store at ambient temperature. They also simplify transport from remote sampling sites where cold-chain maintenance is difficult. The PCR assays used in this study have previously been validated for the detection of *Theileria* and *Babesia* species in

equine whole-blood samples. However, these assays were not formally validated on DBS matrices in the present study. No comparison with whole-blood samples was performed for DNA yield or diagnostic sensitivity. Therefore, potential matrix-related effects cannot be excluded and should be considered when interpreting the results [32].

2.5. Statistical Analysis

Associations between infection status and categorical host variables (age group and sex) were evaluated using Fisher's exact test due to the low number of positive cases and small expected cell counts, with statistical significance set at $p < 0.05$.

3. Results

3.1. Geographical Distribution of Detected Pathogens

The distribution of piroplasm infections varied among the sampled regions. *Theileria equi* was detected in all three investigated regions, with the highest prevalence observed in Tiaret (9/150; 6.0%), followed by Laghouat (2/42; 4.8%) and Tlemcen (1/49; 2.0%). Conversely, a single horse from Tiaret (1/150; 0.67%) tested positive for *T. capreoli*. Overall, *T. equi* and *T. capreoli* were detected in 12 (4.98%) and 1 (0.41%) horses, respectively (Table 3).

Table 3. Geographic distribution of *T. equi*-positive horses in the sampled regions of Algeria.

Region	Positive/Tested	Prevalence (%)	95% CI
Tiaret	9/150	6.0	2.8–11.0
Laghouat	2/42	4.8	0.6–16.2
Tlemcen	1/49	2.0	0.05–10.9

3.2. Molecular Detection of Vector-Borne Pathogens

A total of 241 horses were examined, including 72 males and 169 females. Overall, 12 horses (4.98%; 95% CI: 2.6–8.5) tested positive for *T. equi*, while one horse (0.41%; 95% CI: 0.01–2.29) was positive for *T. capreoli*.

No amplification was obtained for *Anaplasma* spp., *Rickettsia* spp., *Bartonella* spp., haemotropic *Mycoplasma* spp., *Babesia caballi*, *Trypanosoma* spp., or filarioid helminths.

3.3. Association with Host Factors and Epidemiological Variables

Associations between *T. equi* infection status and the investigated variables, including sex, age group, geographic region, and sampling month, were evaluated.

Positive horses were identified in all three sampled regions. Prevalence ranged from 2.0% to 6.0% (Table 3). The highest prevalence was observed in Tiaret (9/150; 6.0%), followed by Laghouat (2/42; 4.8%) and Tlemcen (1/49; 2.0%). Geographic comparisons were interpreted cautiously because of the limited number of positive animals.

The prevalence of *T. equi* infection was 6.5% (11/169; 95% CI: 3.3–11.3) in females and 1.4% (1/72; 95% CI: 0.03–7.5) in males. According to age group, prevalence was 0.0% (0/31) in horses younger than 5 years, 4.0% (4/99; 95% CI: 1.1–10.0) in horses aged 5–10 years, and 7.2% (8/111; 95% CI: 3.2–13.7) in horses older than 10 years (Table 4).

The distribution of *T. equi* infection according to sampling month is presented in Table 5. Positive horses were detected in June, September, October, and November, whereas no positive animals were identified in May, July, or August. The highest prevalence values were observed in September (8.3%) and November (9.8%).

Table 4. Association between *T. equi* infection and host variables in sampled horses.

Variable	Category	Positive/Tested (%)	95% CI
Age Group	<5 years	0/31 (0.0)	0.0–11.2
	5–10 years	4/99 (4.0)	1.1–10.0
	>10 years	8/111 (7.2)	3.2–13.7
Sex	Male	1/72 (1.4)	0.03–7.5
	Female	11/169 (6.5)	3.3–11.3

Table 5. Association between *T. equi* infection and sampling month.

Region	Positive/Tested	Prevalence (%)
May	0/10	0.0
June	1/45	2.2
July	0/15	0.0
August	0/31	0.0
September	4/48	8.3
October	3/51	5.9
November	4/41	9.8

No statistically significant association was detected between *T. equi* infection status and sex ($p = 0.28$), age group ($p = 0.36$), or sampling month ($p = 0.33$).

3.4. Association with Sampling Month

The distribution of *T. equi* infection according to sampling month is presented in Table 5. Positive horses were detected in June, September, October, and November, whereas no positive animals were identified in May, July, or August. The highest prevalence values were observed in September (8.3%) and November (9.8%). However, no statistically significant association was detected between *T. equi* infection status and sampling month ($p = 0.33$).

3.5. Molecular Characterisation and Phylogenetic Analysis

The *T. equi* sequences obtained in this study (GenBank: PX670377–PX670386 and PZ345192) were detected in horses from Tiaret, Laghouat, and Tlemcen. Most isolates from Tiaret and Laghouat showed 100% similarity with a reference sequence previously reported from equids on São Luís Island, northeastern Brazil (GenBank: KY952226).

One isolate showed 100% similarity with reference sequences reported from Mongolia (GenBank: LC781893) and the USA (GenBank: JX177673). In contrast, the isolate obtained from Tlemcen (GenBank: PX670387) showed 100% similarity with another Brazilian reference sequence (GenBank: KY952228).

The single *T. capreoli* isolate (GenBank: PX670376) showed 100% sequence identity with previously reported *T. capreoli* sequences from roe deer in China (GenBank: MH085202) and from a dog in Croatia (GenBank: MK089570), supporting its molecular assignment to this species despite the different host origins of the reference isolates.

Phylogenetic analysis of the 18S rRNA gene sequences revealed the presence of two distinct *T. equi* genotypes circulating among the sampled equine population. The first and largest cluster (Genotype A) comprised the majority of isolates, including GenBank: KY952228, PX670382, PX670387, JX177673, MT767149, PX670381, PX670379, KY952226, PX670380, PX670383, and MG052902. These sequences exhibited minimal genetic divergence and grouped closely with previously characterised reference isolates from different

geographic regions, indicating that Genotype A represents the predominant lineage detected in the study area.

A second well-supported clade (Genotype B), comprising GenBank: PX670378, PX670377, PQ044777, and LC781893, showed moderate divergence from Genotype A and corresponded to a less frequently detected lineage, highlighting the genetic diversity of *T. equi* isolates circulating in the studied equine population. The phylogenetic relationships are presented in Figure 2.

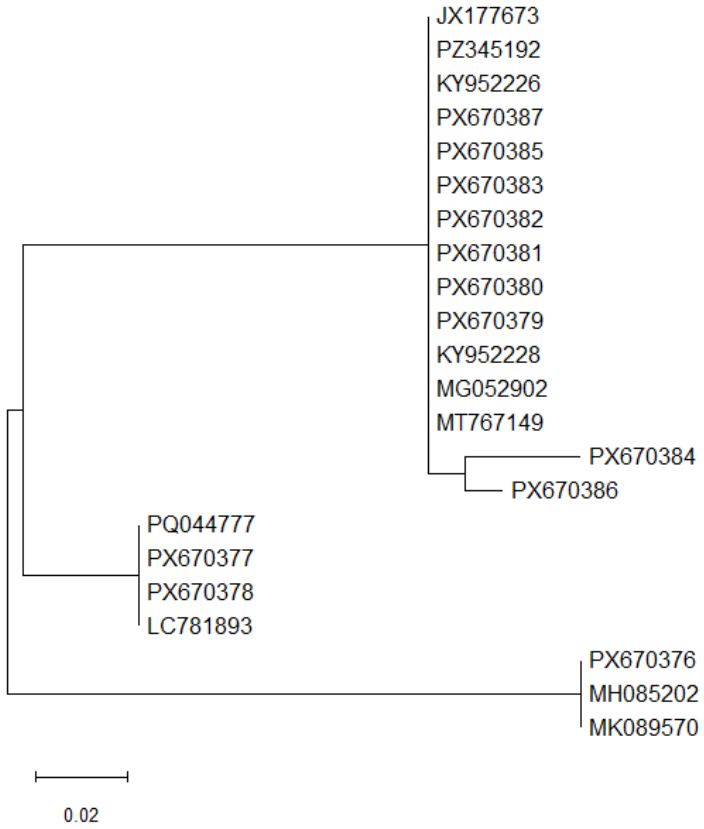


Figure 2. Maximum likelihood phylogenetic tree based on partial 18S rRNA gene sequences of *T. equi* and *T. capreoli* detected in horses from Algeria. Phylogenetic analysis was performed using MEGA11 software with the maximum likelihood method based on the Kimura 2-parameter model. Bootstrap support values were calculated using 1000 replicates, and only values above 50% are shown at the branch nodes. Sequences obtained in the present study are indicated by GenBank accession numbers PX670376–PX670387 and PZ345192. Two major *T. equi* genotypes (A and B) were identified among the Algerian isolates. The *T. capreoli* isolate (PX670376) clustered with previously reported reference sequences from non-equine hosts. The scale bar represents the number of nucleotide substitutions per site.

4. Discussion

The present study investigated the occurrence of selected VBPs in horses from different regions of Algeria using PCR-based methods. The overall detection rate of *T. equi* (4.98%) was relatively low compared to reports from other regions of North Africa and the Mediterranean basin, where higher prevalence values have often been described. For example, a previous study from Northeastern Algeria reported a rate of 11.11% [24], which is relatively low compared to the findings from Tunisia (22.1%) and Egypt (20.3%) [15,16] and those from France (36.9%) and Spain (42%) [17,18]. Such variation may be influenced by differences in ecological conditions, vector distribution, host management practices, and the diagnostic methods employed [4].

The detection of *T. equi* confirms the pathogen's presence in Algerian horses and is consistent with previous reports indicating its widespread distribution in endemic areas. However, the relatively low prevalence observed in the present study should be interpreted with caution. Several methodological factors may have contributed to an underestimation of infection rates. The use of dried blood spot (DBS) samples may reduce DNA yield and PCR sensitivity, particularly in cases of low parasitaemia [33]. Furthermore, because the PCR assays used in this study were not formally validated on DBS matrices, potential matrix-related effects on diagnostic sensitivity cannot be excluded.

The absence of serological testing represents an important limitation of the present study. Because antibodies may persist beyond detectable parasitaemia, serological assays would provide complementary information on prior exposure and may be more suitable for broader epidemiological assessment, particularly for chronic or low-parasitaemia infections such as those by *T. equi*. Consequently, the prevalence reported here likely reflects molecular detection rather than total exposure within the studied population.

Genetic characterisation of the *T. equi* 18S rRNA gene showed diversity among the obtained isolates, which clustered into two genotypes, A and B, according to phylogenetic analysis. This finding is consistent with a previous study that identified two major *T. equi* genotypes [34]. However, previous studies have classified the genotypes of *T. equi* sequences into three [35] and four groups [36], with Genotype B previously identified in Brazil [37] and Portugal [38].

In contrast, *Babesia caballi* was not detected in the analyzed samples. Although this finding may reflect a lower circulation of the parasite in the studied population, it should not be interpreted as evidence of absence, given the known limitations of PCR-based detection and the potential for low-level or chronic infections.

The detection of *Theileria capreoli* DNA in a single horse from Tiaret represents an unusual finding, as this piroplasm is primarily associated with wild cervids [8]. Reports of *T. capreoli* detection in equids remain extremely limited in the available literature, and the epidemiological significance of this finding in horses is currently unclear. No clinical signs were observed in the positive horse, and the parasite was not isolated. In addition, follow-up sampling was not performed. Therefore, it remains unclear whether this finding reflects active infection, transient parasitaemia following an infected tick bite, or passive contamination. Regardless, this finding does not suggest that *T. capreoli* is an established equine pathogen, but rather highlights the value of broad-spectrum molecular screening for detecting rare spillover events. Although the closest reference sequences originated from non-equine hosts, such comparisons are consistent with standard molecular identification practices and reflect the limited availability of *T. capreoli* sequences from equids in public databases. No known proximity or direct contact between the positive horse and wild cervid populations was identified in the present study, making a wildlife-associated transmission pathway uncertain. Nevertheless, occasional host switching (spillover) through shared tick vectors cannot be completely excluded. Tiaret is characterised by semi-arid high plains and extensive grazing systems that may favor exposure to ixodid ticks potentially contributing to piroplasm transmission. Because only one positive animal was detected, the epidemiological significance of this finding remains unclear and should be interpreted cautiously. Thus, further studies involving vector surveillance and broader molecular screening are needed to clarify the origin and transmission dynamics of this parasite in Algerian equine populations.

The absence of PCR amplification for *Anaplasma* spp., *Rickettsia* spp., *Bartonella* spp., haemotropic *Mycoplasma* spp., *Trypanosoma* spp., and filarioid helminths in the present study should not be interpreted as evidence of absence of infection or absence of pathogen circulation in Algerian equine populations. Previous studies from Algeria and neighboring

countries have documented equid exposure to some VBPs using serological and molecular approaches. The negative findings observed here may reflect factors such as low pathogen load, asymptomatic carrier status, temporal variation in parasitaemia, or methodological limitations associated with PCR-based detection and dried blood spot samples. Overall, the findings of this study provide preliminary molecular data on the presence of selected VBPs in Algerian horses. While the detection of *T. equi* contributes to existing knowledge, the low number of positive cases, methodological constraints, and limited epidemiological data restrict the strength of the conclusions.

Future studies should incorporate larger and more representative sample sizes and employ more sensitive diagnostic approaches such as quantitative or multiplex PCR. Additionally, integrating serological methods and investigating tick vectors would also improve our understanding of pathogen circulation and transmission dynamics.

5. Conclusions

This survey provides an updated overview of vector-borne pathogens circulating among horses in Algeria. The molecular detection of *T. equi* confirms the continued presence of equine piroplasmosis in the studied regions, including northwestern Algeria (Tiaret and Tlemcen) and the Saharan Atlas region (Laghouat), with an overall prevalence of 4.98% (12/241). In addition, the identification of a single *T. capreoli* case highlights the possibility that atypical piroplasm species may be incidentally detected in equine hosts, although its epidemiological significance remains unclear and warrants further investigation. These findings support the need for sustained epidemiological monitoring and vector control. No molecular evidence of the other investigated vector-borne agents was detected in the examined population. However, these findings should not be interpreted as evidence of epidemiological absence. Several factors may have influenced pathogen detection, including the clinically healthy status of the sampled animals and the methodological limitations associated with PCR-based detection. In particular, DBS samples were used without formal assay validation on this sample matrix. Overall, these findings contribute valuable baseline data for future surveillance and control strategies targeting equine vector-borne diseases in Algeria.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BLAST	Basic Local Alignment Search Tool
bp	Base pairs
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
EP	Equine Piroplasmosis
ITS	Internal Transcribed Spacer
mt COI	Mitochondrial Cytochrome Oxidase I
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
p-value	Probability value
qPCR	Quantitative PCR
rRNA	Ribosomal RNA
VBD(s)	Vector-Borne Disease(s)
VBP	Vector-borne pathogen
µL	Microliter

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