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REGULAR ARTICLES

A comprehensive molecular survey of vector-borne blood parasites in cattle in Kyrgyzstan with a note of the first molecular detection of *Anaplasma bovis* and *Candidatus* Anaplasma Camelii

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Abstract

Vector-borne pathogens continue to increase their impact on the livestock industry worldwide. To protect animals against these pathogens, it is very important to identify the species that cause the disease and understand their prevalence. This study aimed to investigate the presence and prevalence of vector-borne pathogens in apparently healthy cattle in different parts of Kyrgyzstan using molecular diagnostic techniques. For this purpose, 531 blood samples were collected from the Osh, Jalal-Abad, and Batken oblasts of Kyrgyzstan. The blood samples were investigated for vector-borne pathogens using PCR, RLB, and RFLP. Moreover, DNA sequence analyses were used to confirm the results of molecular techniques and phylogenetic analyses of these pathogens. 359 (67.61%) out of 531 samples were found to be infected with at least one pathogen, whereas 172 (32.39%) were detected to be negative. Thirteen vector-borne pathogens were detected in cattle blood samples, and the prevalence of these pathogens was as follows: *Theileria orientalis* (47.83%), *T. annulata* (25.61%), *Babesia major* (0.19%), *B. occultans* (0.38%), *Anaplasma phagocytophilum*-like 1 (3.20%), *A. capra* (3.01%), *A. centrale* (2.82%), *A. bovis* (1.13%), *(A) ovis* (0.19%), *Candidatus* Anaplasma camelii (0.94%), *Trypanosoma theileri* (19.21%), *Mycoplasma wenyonii* (6.03%), and *Ca.* Mycoplasma haemobos (2.64%). Among the positive samples, one pathogen was identified in 189 cattle (35.59%), and co-infections (two or more pathogens) were determined in 170 (32.01%) animals. *Theileria parva*, *T. mutans*, *(B) bigemina*, *B. bovis*, *B. divergens*, and *A. marginale* could not be detected in the study. *Anaplasma bovis* and *Ca.* Anaplasma camelii were detected for the first time in the country. This molecular survey provides important epidemiological and genetic data for the vector-borne pathogens in cattle. The results of the study showed that vector-borne pathogens have a significant spread and distribution in cattle in Kyrgyzstan.

Keywords Vector-borne pathogens · PCR · RLB · RFLP · Cattle · Kyrgyzstan · Phylogeny

Introduction

Vectors and vector-borne diseases have an important negative impact on animal and human health. Every year, millions of animals and hundreds of thousands of people get infected with vector-borne pathogens almost all over the world (Suarez and Noh 2011; Dantas-Torres et al. 2012; Narladkar 2018; Shaw and Catteruccia 2019). Many more animals and humans have lived in areas at risk for these pathogens (Suarez and Noh 2011; Dantas-Torres et al. 2012). Furthermore, the importance of vector-borne pathogens is increasing day by day due to global warming, increased trade between countries, human movement, and deforestation (Dantas-Torres et al. 2012; Narladkar 2018; Shaw and Catteruccia 2019; Getange et al. 2021).

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Vector-borne pathogens attract the close attention of the scientific community and are studied intensively, and microscopic, serological, and molecular identification tools have been used in these studies (Suarez and Noh 2011; Ganguly et al. 2020; Galon et al. 2022). The method of microscopic examination of Giemsa-stained blood smears is frequently preferred to the diagnosis of vector-borne pathogens because it is cost-effective and provides rapid results (Suarez and Noh 2011; Ganguly et al. 2020). This method gives successful results for the determination of acutely infected animals; yet, it is not adequate for the detection of carrier animals or species identification of pathogens in co-infected animals (Aktas et al. 2006; Altay et al. 2008; Suarez and Noh 2011). Serological methods, like indirect fluorescent antibody test (IFAT), complement fixation test (CFT), and enzyme-linked immunosorbent assay (ELISA), have been employed in parts of eradication programs and epidemiological studies; however, these methods give false positive and negative results due to cross antigenic reactions between species or poor immune responses, respectively (Aktas et al. 2006; Suarez and Noh 2011; Ganguly et al. 2020). In recent studies, molecular techniques like PCR, RFLP, RLB, and DNA sequence analyses have been preferred by researchers to identify vector-borne pathogens due to their high analytical sensitivity and specificity. Molecular techniques are also used for the detection of novel genotypes, strains, or species of pathogens, and, therefore, these techniques also contribute to understanding the biology of pathogens by enabling the discovery of new host species (Schouls et al. 1999; Ganguly et al. 2020; Altay et al. 2020, 2022a, b; Galon et al. 2022; Sahin et al. 2022).

Vector-borne diseases are one of the most important barriers to the development of cattle breeding (Suarez and Noh 2011; Galon et al. 2022). Furthermore, vectors and vector-borne diseases may cause economic losses, approximately US$22–30 billion per year, among cattle herds (Lew-Tabor and Valle 2016). The researchers report that novel and detailed information on the prevalence and distribution of VDBs is needed to reduce their harmful effects and economic losses on cattle farms (Narladkar 2018; Shaw and Catteruccia 2019; Getange et al. 2021; Galon et al. 2022).

The economy of Kyrgyzstan mostly depends on agricultural activities, and significant parts of the country's population work on livestock and related jobs (Akramov and Omuraliev 2009; Frenken 2013; Broka et al. 2016). Animal husbandry occupies an important part of a country's economy, and livestock is generally practiced in small family enterprises. In these farms, livestock activities are performed depending on the pastureland from spring to autumn (Akramov and Omuraliev 2009; Broka et al. 2016). During this period, animals have been exposed to various vector and vector-borne pathogens (Suarez and Noh 2011; Schnittger et al. 2012; Pfäffle et al. 2013; Galon et al. 2022). However, there is a lack of data on the distribution and prevalence of vector-borne pathogens in animals in the country, and limited studies have been done for this purpose in Kyrgyzstan to date (Aktaş et al. 2019; Altay et al. 2022a, c, 2023; Ozubek et al. 2022; Zhyldyz et al. 2023). This study aimed to (i) investigate the distribution and prevalence of vector-borne pathogens using conventional PCR, nested PCR, RLB, and RFLP in Kyrgyzstan, (ii) phylogenetic analyses of pathogens identified with DNA sequence analyses.

Materials and methods

Studied area and collection of samples

Kyrgyzstan, officially the Kyrgyz Republic, is a Central Asian country, and the country is bordered by China to the southeast, Tajikistan to the southwest, Uzbekistan to the west, and Kazakhstan to the north. The country is landlocked and has a continental climate. Kyrgyzstan has seven administrative oblasts: Osh, Talas, Batken, Jalal-Abad, Chuy, Naryn, and Issyk-Kul (Frenken 2013) (Fig. 1).

According to 2022 data, 1, 783, 469 cattle were present in Kyrgyzstan, and almost half of these animals were present in the Osh, Batken, and Jalal-Abad oblasts with the number of cattle heads, 397,288, 144,851, and 371,686, respectively. (https://www.stat.kg/en/opendata/category/96/). Simple random sampling size was calculated using OpenEpi Version 3 according to the following equation; with an expected disease prevalence of 5% (p), accepted absolute error of ±2% (d), confidence level 95%, design effect (for cluster surveys-DEFF) 1 (https://www.openepi.com/SampleSize/SSPropor.htm). According to the formula, a minimum of 456 samples were required with 95% confidence level for cattle. However, a total of 531 cattle blood samples were collected from Osh, Batken, and Jalal-Abad oblasts (Fig. 1). The blood samples were taken into tubes with EDTA and placed at -20ºC until DNA extraction. All cattle were seen as clinically healthy and grazing on pastureland.

Genomic DNA extraction from blood samples

Total genomic DNA extraction was done following the method previously described by Altay et al. (2005). Before the DNA extraction process, the blood samples were vigorously vortexed. 250 µL of blood was placed in a microcentrifuge tube, and 500 µL of lysis solution (0.01 M Tris, 0.005 M $MgCl_2$, 0.32 M sucrose, 1% Triton X-100, pH 7.5) was added and mixed with vortex. The mixture was centrifuged at 11.600 × g for 1 min, and the supernatant was removed. The pellet was washed three times by centrifugation with 250 µL of the lysis solution. The supernatants were discarded, and the final pellets were re-suspended in 200

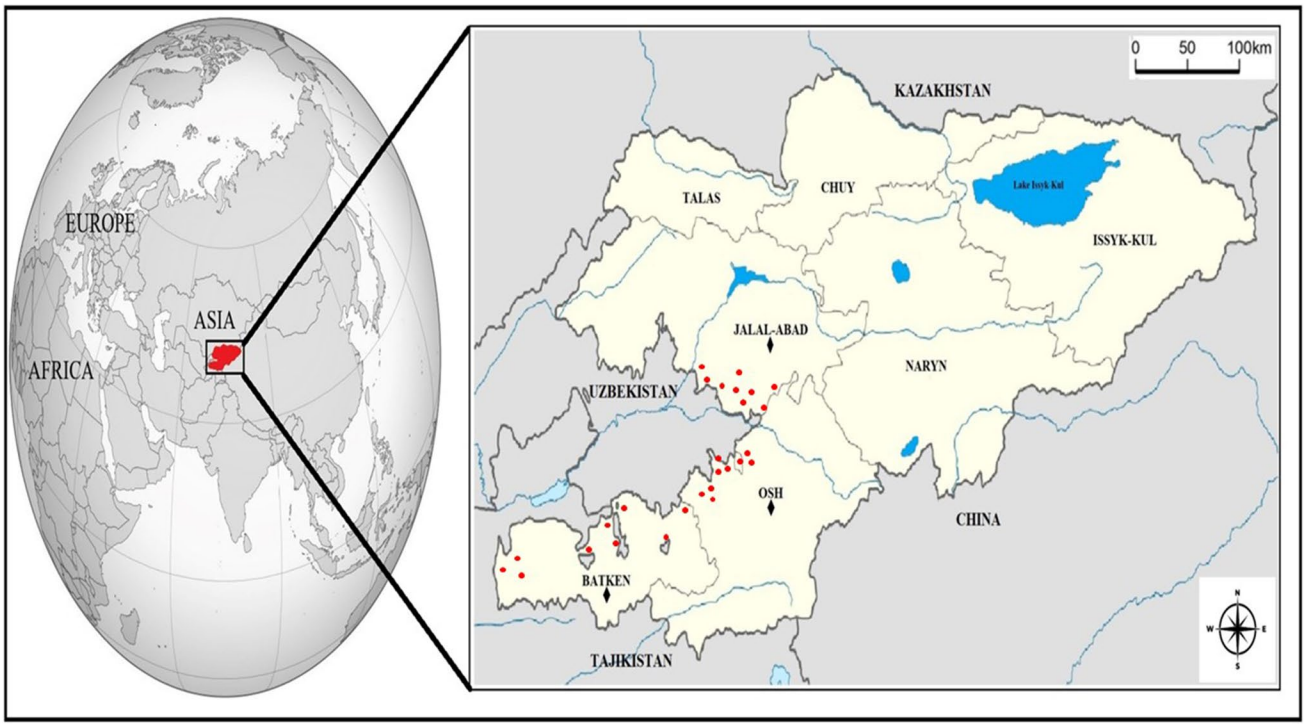

Fig. 1 The location of Kyrgyzstan in the world map and oblast were showed with diamonds sampling areas were indicated with red dots on the Kyrgyzstan map

µL of PCR buffer (10 mM Tris–HCl (pH 8), 50 mM KCl, 0.1% TritonX-100, pH 8.3). Proteinase K (50 µg/mL) was added to the pellet suspension, and the mixture was then incubated at 56 °C for 1 h. At the end of these applications, the obtained mixture was boiled for 10 min for the inactivation of proteinase K.

Molecular survey of vector-borne pathogens

In this study, different molecular methods were used to identify vector-borne pathogens in cattle blood samples, including conventional PCR, nested-PCR, RLB, RFLP, and DNA sequence analysis.

Theileria and *Babesia* species were screened with PCR and RLB. Before RLB, PCR assay was done using forward [RLB-F2 (5′-GACACAGGGAGGTAGTGACAAG-3′)] and reverse [RLB-R2 (Biotin-5′-CTAAGAATTTCACCTCTG ACAGT-3′)] primers that were amplified hypervariable V4 region of *18 S rRNA* gene (Georges et al. 2001). Preparation of the PCR mixture and PCR conduction were performed as described by Altay et al. (2007)d *orientalis* (GenBank accession number: OR140730) positive sample obtained from water buffalo was used as a positive control. The PCR products were hybridized with genus- and species-specific probes listed in Table 1. Preparation, hybridization, and stripping of RLB membrane were done as defined by Altay et al. (2007).

The species-specific PCR assay was used for the identification of *A. capra*, *A. marginale*, *A. centrale*, *A. bovis*, *A. ovis*, *A. phagocytophilum*, and *A. phagocytophilum*-related strains. In the species-specific PCR assay, positive samples for *A. capra* (OK267267), *A. marginale* (KJ183083), *A. centrale* (KJ183082), *A. bovis* (KJ183084), and *A. phagocytophilum* (MW672121) were used as positive control, these positive samples were identified in cattle in different studies. Moreover, all gDNA samples were also checked in terms of a novel *Anaplasma* species genus-specific PCR assay. *Trypanosoma theileri*, *Mycoplasma wenyonii*, and *Candidatus* Mycoplasma haemobos were also researched. Positive samples for *M. wenyonii* (OM891795), *Ca.* Mycoplasma haemobos (OM891818), *Try. theileri* (PP565860) were used as positive control in PCR assay, and these samples were detected in cattle. Detailed data about primers used for the determination of the abovementioned pathogens are listed in Table 2.

PCR assays were performed in a total volume of 25 µL, and the mixture was prepared as described by Erol et al. (2022). PCR products were loaded onto a 1% agarose gel, and electrophoresis was carried out for 60 min at 95 volts. After this, the agarose gel was stained with ethidium bromide for 20 min, the visualization was done with a UV-transilluminator, and positive samples were recorded.

Table 1 *Theileria* and *Babesia* genus and species-specific probes

Prob name	Sequence (5'-3')	References
Catchall (*Theileria* + *Babesia*)	TAATGGTTAATAGGA(AG)C(AG)GTTG	Gubbels et al. 1999
Theileria spp.	TGATGGGAATTTAAACC(CT)CTTCCA	Schnittger et al. 2004
Theileria annulata	CCTCTGGGGTCTGTGCA	Georges et al. 2001
Theileria sergenti/buffeli/orientalis	GGCTTATTTCGGWTTGATTTT	Gubbels et al. 1999
Theileria mutans	CTTGCGTCTCCGAATGTT	Gubbels et al. 1999
Theileria taurotragi	TCTTGGCACGTGGCTTTT	Gubbels et al. 1999
Theileria velifera	CCTATTCTCCTTTACGAGT	Gubbels et al. 1999
Babesia spp.	CCT(GT)GGTAATGGTTAATAGGAA	Schnittger et al. 2004
Babesia bigemina	CGTTTTTTCCCTTTTGTTGG	Gubbels et al. 1999
Babesia bovis	CAGGTTTCGCCTGTATAATTGAG	Georges et al. 2001
Babesia major	TCCGACTTTGGTTGGTGT	Georges et al. 2001
Babesia divergens	GTTAATATTGACTAATGTCGAG	Gubbels et al. 1999

Table 2 Primers information was used in this study

Pathogens	Target gene	Primer name	Sequence (5'-3')	Amplicon size (bp)	Annealing temperature (°C)	References
Anaplasma spp.	*16 S rRNA*	AnaplsppF AnaplR3	AGAAGAAGTCCCGGCAAACT GAGACGACTTTTACGGATTAGCTC	800	53	Zobba et al. 2014
A. capra	*gltA*	Outer-f Outer-r	GCGATTTTAGAGTGYGGAGATTG TACAATACCGGAGTAAAAGTCAA	1031	58	Li et al. 2015
		Inner-f Inner-r	TCATCTCCTGTTGCACGGTGCCC CTCTGAATGAACATGCCCACCCT	594	55	Yang et al. 2016
A. phagocytophilum and related strains	*16 S rRNA*	SSAP2f SSAP2r	GCTGAATGTGGGGATAATTTAT ATGGCTGCTTCCTTTCGGTTA	641	54	Kawahara et al. 2006
A. bovis	*16 S rRNA*	AB1f AB1r	CTCGTAGCTTGCTATGAGAAC TCTCCCGGACTCCAGTCTG	551	54	Kawahara et al. 2006
A. centrale	*16 S rRNA*	AC1f AC1r	CTGCTTTTAATACTGCAGGACTA ATGCAGCACCTGTGTGAGGT	426	50	Kawahara et al. 2006
A. ovis	*groEL*	JH0011 JH0012	TAAAAGCCAAGGAGGCTGTG TTGCTCTCCTCGACCGTTAT	181	55	Haigh et al. 2008
A. marginale	*Msp5*	AMF AMR	ACAGGCGAAGAAGCAGACAT ATAAATGGGAACACGGTGGA	382	54	Ganguly et al. 2020
M. wenyonii	*16 S rRNA*	F2 MW-R	ACGAAAGTCTGATGGAGCAATA AGCTTYGCARTAGATTRCAAGCC	627	53	Jensen et al. 2001 Erol et al. 2023
Ca. Mycoplasma haemobos	*16 S rRNA*	F2 CMH-R	ACGAAAGTCTGATGGAGCAATA CTACAGCACTGAGGCTCAAAC	457	53	Jensen et al. 2001 Erol et al. 2023
Trypanosoma theileri	*catl*	F R	CGTCTCTGGCTCCGGTCAAAC TTAAAGCTTCCACGAGTTCTTGATGATCCAGTA	289	59	Rodrigues et al. 2010

Table 3 The expected band size of *A. phagocytophilum* and related strains

Pathogens	XcmI	BsaI
A. phagocytophilum	297 and 344 bp	641 bp
A. phagocytophilum-like 1	641 bp	641 bp
A. phagocytophilum-like 2	641 bp	219 and 422/423 bp

Differentiation of *Anaplasma phagocytophilum* and related strains by RFLP

The discrimination of *A. phagocytophilum* and related strains was performed with RFLP analyses following the protocol delineated by Ben Said et al. (2017). For this purpose, the positive amplicon was digested with restriction enzymes XcmI (New England Biolabs®, UK) and BsaI (New England Biolabs®, UK). The XcmI enzyme was used for differentiation of *A. phagocytophilum* and *A. phagocytophilum*-related strains, whereas BsaI *A. phagocytophilum*-like 1 and like 2. Detailed data on the expected band profile after restriction using XcmI and BsaI are listed in Table 3.

DNA sequence and phylogenetic analyses of vector-borne pathogens

DNA sequence analyses were performed in this study for different purposes; (i) for the verification of PCR, RLB, and RFLP assays, (ii) for the species identifications of positive samples that gave positive results at the genus level in the PCR and RLB assay, and (iii) for the phylogenetic analyses of vector-borne pathogens identified in the study. For these objectives, randomly selected PCR-positive products were sequenced by BM Labosis (Ankara, Türkiye) using primers listed in Supplementary Table S1. ABI 3730XL analyzer (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA) were used for DNA sequence analyses. Before DNA sequence analyses, all PCR products were purified with the HighPrepTM PCR Clean-up System (Cat. No.: AC-60005, MagBio) following the producer's instructions.

The sequence files were opened with FinchTV (version 1.4.0) software (Geospiza Inc., Seattle, Washington, USA), and chromatogram qualities were controlled. The consensus sequences were determined using Mega-11 software (Tamura et al. 2021). After that, the BLASTn algorithm within NCBI was used for the determination of nucleotide similarities between our vector-borne pathogens and sequences belonging to pathogens present in the GenBank.

Phylogenetic trees were generated using maximum likelihood analysis in Mega-11 (Tamura et al. 2021) for the determination of the genetic connections between our vector-borne pathogens and those present in the GenBank. Before phylogenetic trees were constructed, the best-fit model for maximum likelihood for each pathogen was determined using the Find Best-Fit Substitution Model feature of Mega-11, and these models were listed as follows; T92 for *T. orientalis*, *Try. theileri*, *M. wenyoni*, and *Ca*. Mycoplasma haemobos (Tamura 1992), TN93 (Tamura and Nei 1993) for *T. annulata*, *B. major*, and *B. occultans*, HKY for *Anaplasma* species, except *A. capra* and *A. phagocytophilum* (Hasegawa et al. 1985), Kimura-2 for *A. capra* and *A. phagocytophilum* and related strains (Kimura 1980).

Results

The general prevalence of pathogens

Out of 531 cattle blood samples were researched with molecular methods in terms of vector-borne pathogens. 359 (67.61%) out of 531 cattle blood samples were found to be infected with at least one pathogen, whereas no pathogen was detected in 172 (32.39%) blood samples. Thirteen vector-borne pathogens (*T. orientalis*, *T. annulata*, *B. major*, *B. occultans*, *A. phagocytophilum*-like 1, *A. capra*, *A. centrale*, *A. bovis*, *A. ovis*, *Ca.* Anaplasma camelii, *Try. theileri*, *M. wenyonii*, and *Ca.* Mycoplasma haemobos) were identified in this work with a different prevalence (Table 4). While a single pathogen was detected in 189 (35.59%) cattle blood samples, co-infections were detected in 170 (32.01%) cattle blood samples, of which 119 had two pathogens, 36 had three pathogens, 11 had four pathogens, two had five and

Table 4 Detailed information on the prevalence of pathogens identified in this work

Pathogens	Number of positive samples (*n*)	Prevalence (%)
Theileria orientalis	254	47.83%
Theileria annulata	136	25.61%
Babesia major	1	0.19%
Babesia occultans	2	0.38%
Anaplasma phagocytophilum-like 1	17	3.20%
Anaplasma capra	16	3.01%
Anaplasma centrale	15	2.82%
Anaplasma bovis	6	1.13%
Anaplasma ovis	1	0.19%
Candidatus Anaplasma camelii	5	0.94%
Trypanosoma theileri	102	19.21%
Mycoplasma wenyonii	32	6.03%
Candidatus Mycoplasma haemobos	14	2.64%

six kinds of pathogens. Detailed information about mixed infection is listed in Table 5.

Theileria and *Babesia* species

Theileria orientalis, *T. annulata*, *B. major*, and *B. occultans* were detected using RLB and DNA sequence analyses (Table 4).

Theileria orientalis emerged as the predominant vector-borne pathogen in the current work, being detected in 254 out of the 531 cattle samples subjected to RLB assay, thereby constituting a prevalence rate of 47.83%. Subsequently, three randomly selected PCR products underwent sequencing procedures for the *MPSP* gene, leading to the establishment of consensus sequences. These sequences were deposited in the GenBank under the accession numbers: PP565854-PP565856. The *MPSP* gene sequence analyses showed 98.82–100% nucleotide similarities between *T. orientalis* isolates obtained in this study and *T. orientalis* genotype-3 identified in different parts of the world, like Türkiye (KT220521), Australia (KM624621), Kyrgyzstan (LC768171), China (ON462019), and Thailand (AB562534). The phylogenetic tree revealed that our *T. orientalis* isolates were grouped with *T. orientalis* type-3 (buffeli group) and clustered in different clades with other *T. orientalis* genotypes (Fig. 2).

Theileria annulata was detected in 136 cattle, representing a prevalence rate of 25.61%. After the identification of PCR-positive samples, three representative samples underwent sequencing procedures for the partial part of the *18 S rRNA* gene, leading to the determination of consensus sequences. These consensus sequences were deposited in the GenBank database under the accession numbers: PP555861-PP555863. The DNA sequence results of the *18 S rRNA* gene for *T. annulata* identified in the study showed 100% nucleotide identities with *T. annulata* present in the GenBank detected from cattle in Italy (MT341858), Türkiye (OQ179583), Algeria (ON239758), Pakistan (OQ253515), and India (ON724327). The phylogenetic tree showed that our *T. annulata* isolates were grouped with *T. annulata* isolates reported from different parts of the world and clustered in different clades with other *Theileria* species (Fig. 3).

Babesia major was detected in one cattle sample (0.19%) with RLB. In two samples (0.38%), the RLB assay gave positive signals with the *Babesia* spp. probe. DNA sequence analysis of these two samples showed that they were infected with *B. occultans*. The consensus sequence of *B. major* and *B. occultans* was deposited to the GenBank under accession numbers: PP555867 and PP555868-PP555869, respectively. BLAST analyses showed *B. major 18 S rRNA* gene sequence was 99.68–100% similar to the *B. major* isolate detected in different hosts. Moreover, our *B. major* isolate had 100% nucleotide identities with *B. major* detected in cattle (GU194290 and EU622907) from France and identified from *Haemaphysalis punctata* (KF791206) in Türkiye. *Babesia occultans 18 S rRNA* gene sequence results were compared to the determination of nucleotide similarities, and 98.82–100% identities were seen between our *B. occultans* isolates and those present in the GenBank identified from cattle in Egypt (MN227675), Kyrgyzstan (LC768120), Türkiye (OR211415), and Italy (KC157568). *Babesia major* and *B. occultans* isolates were clustered within *B. major* and *B. occultans* isolates identified in various parts of the world, and these isolates were grouped in different clusters with other *Babesia* species in the phylogenetic tree (Fig. 3).

Anaplasma species

Anaplasma phagocytophilum-like 1, *A. capra*, *A. centrale*, *A. bovis*, *A. ovis*, and *Ca.* Anaplasma camelii were identified with PCR, RFLP, and DNA sequence analyses (Table 4).

As a result of PCR assay for *A. phagocytophilum* and related strains, 17 (3.20%) samples were found positive. These positive samples were included in the RFLP assay to differentiate *A. phagocytophilum* and related strains. The RFLP assay revealed that *A. phagocytophilum*-like 1 DNA was detected in all positive samples, whereas *A. phagocytophilum* and *A. phagocytophilum*-like 2 DNA were not detected. Subsequent to the identification of PCR-positive samples, three representative samples undertook sequencing analyses, resulting in the determination of consensus sequences. These consensus sequences, derived from the current study, were uploaded to the GenBank database under the accession numbers: PP555844-PP555846. Partial parts of the *16 S rRNA* gene sequence of *A. phagocytophilum*-like 1 obtained were compared with *A. phagocytophilum*-like 1 sequence present in the GenBank, and 99.64–100% nucleotide identities were seen between our sequence and *A. phagocytophilum*-like 1 isolate identified in different hosts from various countries, such as Italy (MN462996), Türkiye (OR807551), China (OL678408), Japan (AB588974), and South Africa (OQ909490). The phylogenetic tree demonstrated that our *A. phagocytophilum*-like 1 isolates were grouped with *A. phagocytophilum*-like 1 isolates, and these isolates were clustered in different branches with *A. phagocytophilum* and *A. phagocytophilum*-like 2 (Fig. 4). Further phylogenetic analyses of the *16 S rRNA* gene of *A. phagocytophilum* and related strains revealed that there was high nucleotide differentiation among the above-mentioned pathogens. Moreover, nucleotide differentiations were seen between *A. phagocytophilum*-like 1 isolates reported in different countries (Fig. 5).

Anaplasma capra was detected in 16 (3.01%) cattle blood samples using nested-PCR. After the determination of *A. capra* PCR positive samples, three randomly selected positive samples were sequenced, and obtained consensus

Table 5 Co-infection of pathogens identified in cattle in Kyrgyzstan

Type of the co-infection	Number of positive samples
T. orientalis + *Try. theileri*	44 (8.29%)
T. orientalis + *T. annulata*	32 (6.03%)
T. annulata + *Try. theileri*	14 (2.64%)
T. orientalis + *M. wenyonii*	7 (1.32%)
T. orientalis + *Ca.* Mycoplasma haemobos	4 (0.75%)
A. centrale + *T. annulata*	2 (0.38%)
Ca. Mycoplasma haemobos + *Try. theileri*	2 (0.38%)
A. phagocytophilum-like 1 + *T. orientalis*	2 (0.38%)
T. annulata + *M. wenyonii*	2 (0.38%)
A. capra + *T. orientalis*	2 (0.38%)
M. wenyonii + *Try. theileri*	1 (0.19%)
A. bovis + *A. centrale*	1 (0.19%)
A. phagocytophilum-like 1 + *Ca.* Mycoplasma haemobos	1 (0.19%)
A. capra + *A. phagocytophilum*-like 1	1 (0.19%)
T. annulata + *Ca.* Mycoplasma haemobos	1 (0.19%)
A. centrale + *A. ovis*	1 (0.19%)
A. centrale + *T. orientalis*	1 (0.19%)
T. orientalis + *Ca.* Anaplasma camelii	1 (0.19%)
T. orientalis + *T. annulata* + *Try. theileri*	19 (3.58%)
T. orientalis + *T. annulata* + *M. wenyonii*	3 (0.56%)
A. capra + *A. phagocytophilum*-like 1 + *T. orientalis*	2 (0.38%)
T. orientalis + *M. wenyonii* + *Try. theileri*	1 (0.19%)
A. phagocytophilum-like 1 + *A. centrale* + *T. orientalis*	1 (0.19%)
A. capra + *A. centrale* + *T. orientalis*	1 (0.19%)
A. bovis + *A. centrale* + *Try. theileri*	1 (0.19%)
A. bovis + *B. major* + *M. wenyonii*	1 (0.19%)
T. annulata + *Ca.* Mycoplasma haemobos + *M. wenyonii*	1 (0.19%)
T. annulata + *Ca.* Mycoplasma haemobos + *Try. theileri*	1 (0.19%)
Ca. Mycoplasma haemobos + *M. wenyonii* + *Try. theileri*	1 (0.19%)
T. orientalis + *T. annulata* + *Ca.* Mycoplasma haemobos	1 (0.19%)
T. orientalis + *T. annulata* + *B. occultans*	1 (0.19%)
A. capra + *T. orientalis* + *T. annulata*	1 (0.19%)
T. orientalis + *T. annulata* + *Ca.* Anaplasma camelii	1 (0.19%)
A. capra + *A. phagocytophilum*-like 1 + *T. orientalis* + *M. wenyonii*	2 (0.38%)
T. orientalis + *T. annulata* + *Ca.* Anaplasma camelii + *Try. theileri*	1 (0.19%)
A. phagocytophilum-like 1 + *A. centrale* + *T. orientalis* + *Try. theileri*	1 (0.19%)
A. centrale + *T. orientalis* + *T. annulata* + *Try. theileri*	1 (0.19%)
A. capra + *A. phagocytophilum*-like 1 + *A. centrale* + *T. orientalis*	1 (0.19%)
A. capra + *A. phagocytophilum*-like 1 + *T. orientalis* + *T. annulata*	1 (0.19%)
T. orientalis + *T. annulata* + *M. wenyonii* + *Try. theileri*	1 (0.19%)
T. orientalis + *T. annulata* + *Ca.* Mycoplasma haemobos + *Try. theileri*	1 (0.19%)
A. bovis + *T. orientalis* + *T. annulata* + *B. occultans*	1 (0.19%)
T. orientalis + *T. annulata* + *M. wenyonii* + *Ca.* Anaplasma camelii	1 (0.19%)
A. capra + *A. phagocytophilum*-like 1 + *A. centrale* + *T. orientalis* + *Try. theileri*	2 (0.38%)
A. capra + *A. phagocytophilum*-like 1 + *A. centrale* + *T. orientalis* + *Try.theileri* + *T. annulata*	2 (0.38%)
Total	**170 (32.01%)**

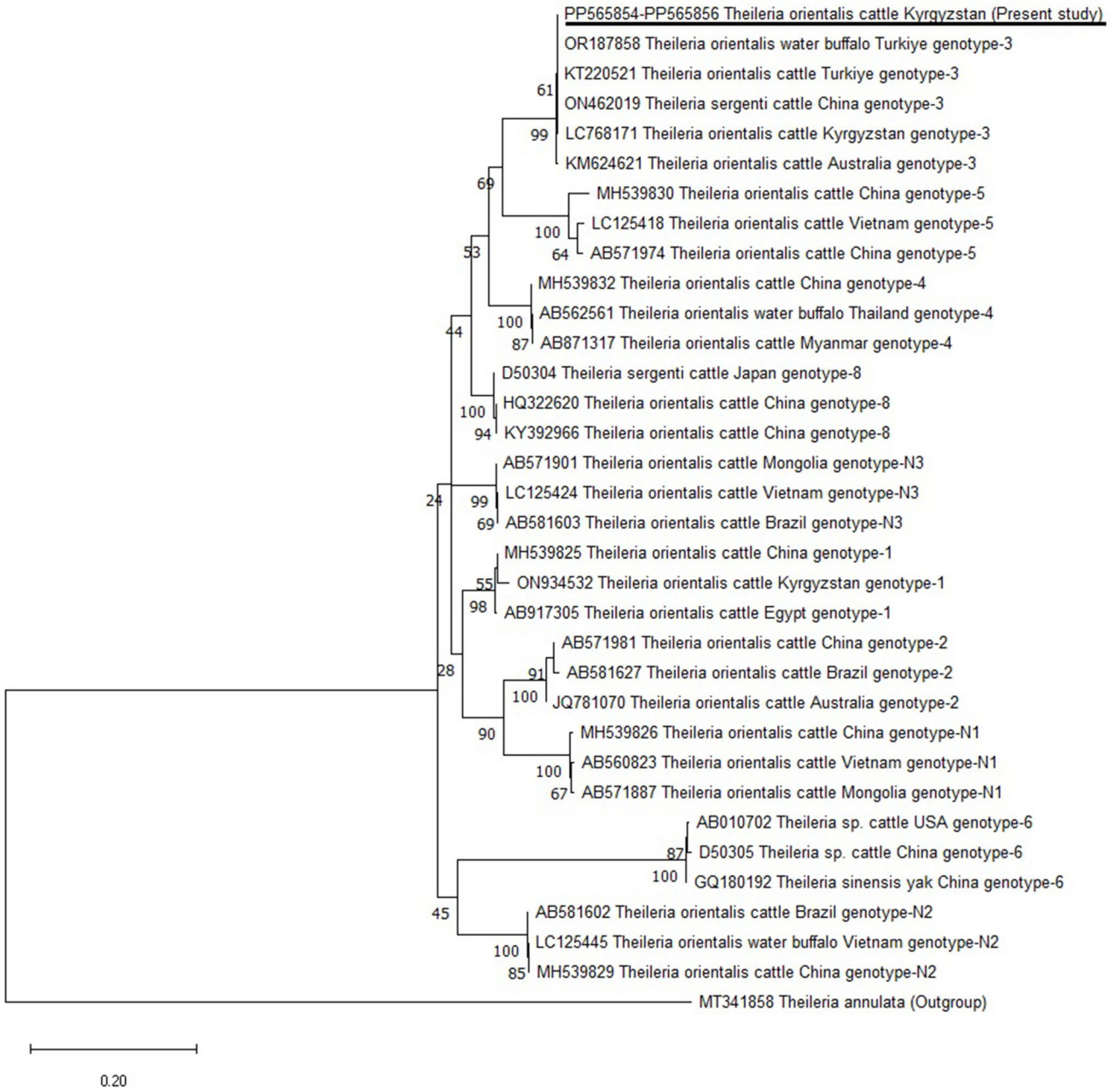

Fig. 2 Phylogenetic tree of *T. orientalis MPSP* gene sequence. *Theileria orientalis* identified in the study is underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the T92 model (Tamura 1992). The scale bar represents 0.20 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

sequences were deposited to the GenBank under accession numbers: PP565857-PP565859. The *gltA* gene sequence of three samples had 87.77–100% nucleotide identities with *A. capra* isolates present in GenBank identified in several hosts. Moreover, 100% nucleotide similarities were seen between our *A. capra* isolates and *A. capra* identified in sheep (MW930533-MW930534), goat (MW930535), red deer (MH084720), and swap deer (MH084719) from France, water buffalo (ON783817-ON783819) and human (OQ819441-OQ819446) from Türkiye, and sheep (OM100820-OM100840) from Kyrgyzstan. The phylogenetic tree showed that *A. capra* isolates were grouped in different clusters (Fig. 6). Further phylogenetic analyses of the *gltA* gene sequence demonstrated that there were several single nucleotide polymorphisms (SNPs) seen within and between *A. capra* genotypes (Fig. 7). When considering both

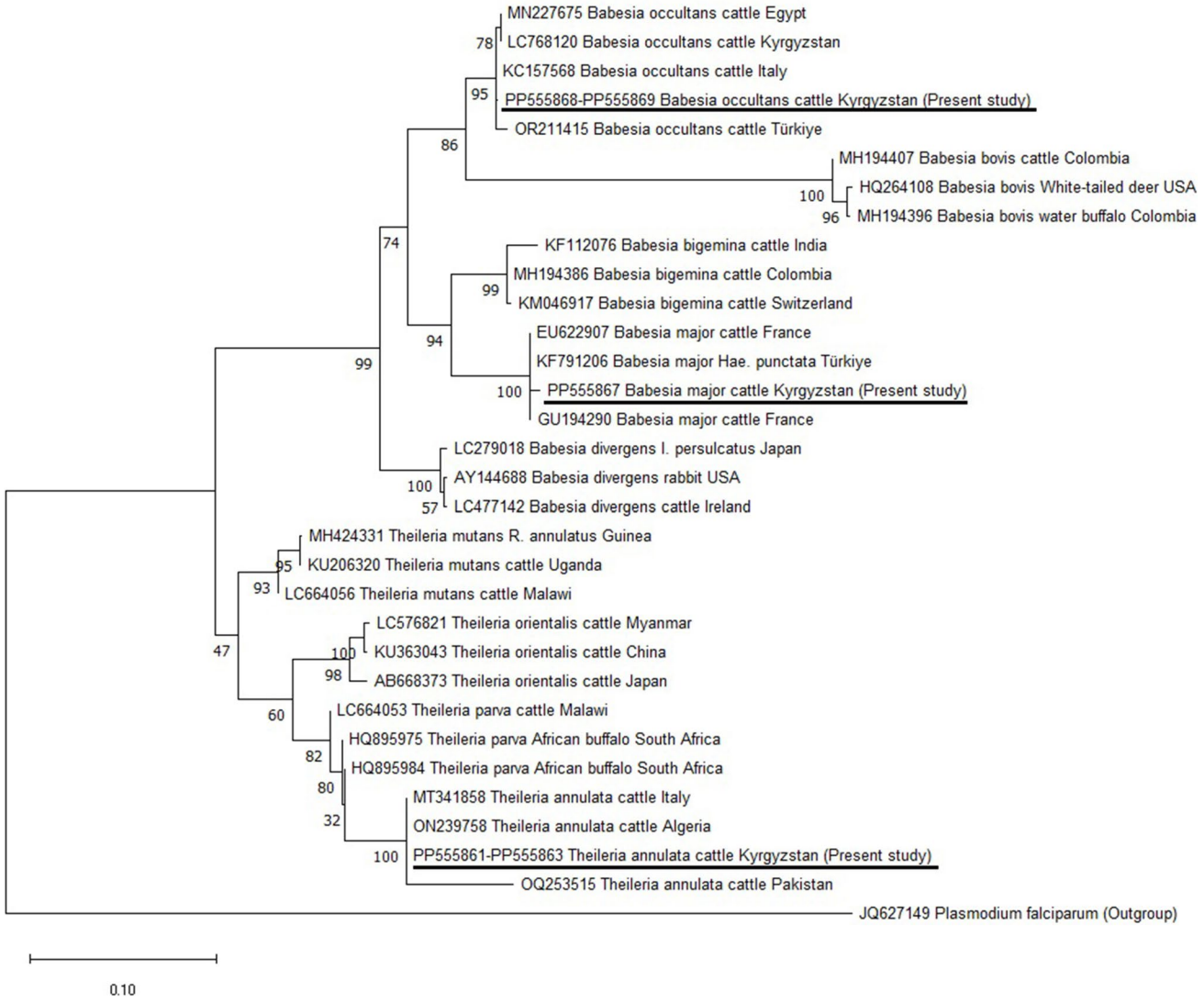

Fig. 3 Phylogenetic tree of *Theileria* and *Babesia 18 S rRNA* gene sequence. *Theileria annulata*, *B. major*, and *B. occultans* identified in the study are underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the TN93 model (Tamura and Nei 1993). The scale bar represents 0.10 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

data, it was seen that at least two *A. capra* genotypes were circulating among hosts.

The 15 (2.82%) out of 531 cattle samples were found to be infected with *A. centrale*. Following the specification of *A. centrale* positive samples, three representative samples were sent to the sequence of the *16 S rRNA* gene. After that consensus sequences were determined and deposited to the GenBank under accession numbers: PP555850-PP555852. The 98.57–100% nucleotide similarities were determined between our *A. centrale* isolates and other *A. centrale* isolates present in the GenBank. The 100% nucleotide identities were also seen between our *A. centrale* isolates and *A. centrale* reported in cattle from Italy (EF520686), Tunisia (KY362540), and Uganda (KU686784), and also Israel vaccine strain (AF309869). It was seen that our *A. centrale* isolates were grouped within the same cluster other *A. centrale* those determined in different parts of the world (Fig. 8).

Anaplasma bovis was detected in six (1.13%) samples. Afterward the detection of *A. bovis*-positive samples, randomly selected three samples were sequenced, and after the determination of the consensus sequence, these were uploaded to GenBank under accession numbers: PP555847-PP555849. BLAST search of *16 S rRNA* gene sequence of our *A. bovis* isolates demonstrated that 100% nucleotide similarities were seen between our *A. bovis* isolates and *A. bovis* isolates identified several hosts such as in sheep (MT036513) from Russia, in horse (MK028574) and cattle (MT754860) from South Korea, in goat (MH255939), *R.*

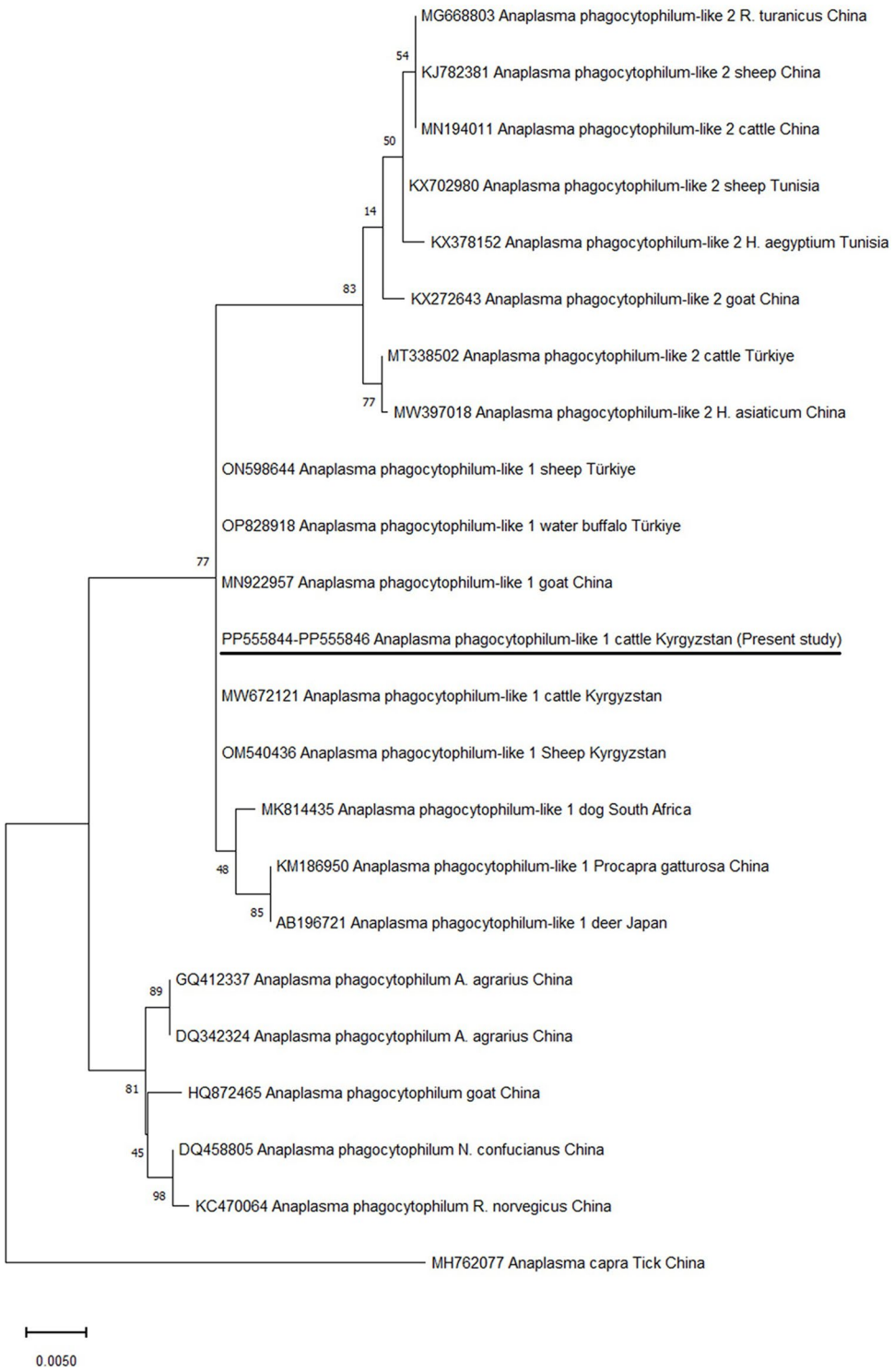

Fig. 4 Phylogenetic tree of *A. phagocytophilum* and related strains *16 S rRNA* gene sequence. *Anaplasma phagocytophilum*-like 1 identified in the study is underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the Kimura-2 model (Kimura 1980). The scale bar represents 0.005 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

microplus (MN044716), and *Hae. longicornis* (OQ132533) from China. The phylogenetic tree revealed that our *A. bovis* isolates were clustered with other *A. bovis* isolates (Fig. 8).

Anaplasma ovis, which is normally identified in small ruminants, was researched, and this pathogen was detected in one sample (0.19%) with species-specific PCR. To verify the PCR result, DNA sequencing was done on this sample, after the determination of the consensus sequence this was deposited to the GenBank with accession numbers: PP555853. The *16 S rRNA* gene sequence of this sample had 98.82–100% nucleotide identities with *A. ovis* isolates identified in different parts of the world. Furthermore, our *A. ovis* isolate showed 100% nucleotide similarities with *A. ovis* isolates obtained from goat (MG869525) and sheep

	GenBank Accession Numbers	Country	Host	Nucleotide Positions													
				997	1095	1097	1099	1106	1124	1134	1223	1225	1226	1228	1237	1245	1277
	PP555844-PP555846	**Kyrgyzstan**	**Cattle**	**A**	**G**	**A**	**–**	**C**	**G**	**C**	**T**	**T**	**C**	**C**	**G**	**G**	**C**
A. phagocytophilum - like 1	OQ909490	South Africa	Zebra	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	MW008791	China	Tian Shan wapiti	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	OR807551	Türkiye	Sheep	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	AB196720	Japan	Deer	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	OM540436	Kyrgyzstan	Sheep	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	KM285232	Tunisia	Sheep	*	*	*	–	*	*	*	*	*	*	*	A	*	*
	MN462996	Italy	Sheep	*	*	*	–	*	*	*	*	*	*	*	*	*	*
	MG869523	China	Goat	*	*	*	T	*	*	T	*	*	*	*	*	*	*
	OQ152552	China	*Hae. concinna*	*	*	*	T	*	*	T	*	*	*	*	*	*	*
	KJ872386	China	Sheep	*	*	*	T	*	*	T	*	*	*	*	*	*	*
	OQ179909	Türkiye	Cattle	*	*	*	T	*	*	T	*	*	*	*	*	*	*
	OP828918	Türkiye	Water buffalo	*	*	*	T	*	*	T	*	*	*	*	*	*	*
	ON598644	Türkiye	Sheep	*	*	*	T	*	*	T	*	*	*	*	*	*	*
A. phagocytophilum - like 2	MH292917	Tunisia	*R. turanicus*	G	*	*	T	T	*	C	C	C	T	*	*	*	T
	MG668803	China	*R. turanicus*	G	*	*	T	T	*	C	C	C	T	*	*	A	T
	KX378152	Tunisia	*Hya. aegyptium*	G	*	*	T	T	*	C	C	C	T	*	*	*	T
	KX702980	Tunisia	Sheep	G	*	*	T	T	*	C	C	C	T	*	*	A	T
	MT338502	Türkiye	Cattle	G	A	*	T	T	*	C	C	C	*	*	*	A	T
A. phagocytophilum	DQ458805	China	*N. confucianus*	*	*	T	A	*	A	T	*	*	*	T	*	*	*
	KC470064	China	*R. norvegicus*	*	*	T	A	*	A	T	*	*	*	T	*	*	*
	GQ412337	China	*A.agrarius*	*	A	T	A	*	A	T	*	*	*	*	*	*	*
	DQ342324	China	*A.agrarius*	*	A	T	A	*	A	T	*	*	*	*	*	*	*

Abbreviations: Nucleotides C, Cytosine; T, Thymine; G, Guanine; A, Adenine. * Asterisks show the conserved nucleotide positions.

Fig. 5 Nucleotide differences according to partial sequences *A. phagocytophilum* and related strains *16 S rRNA* gene

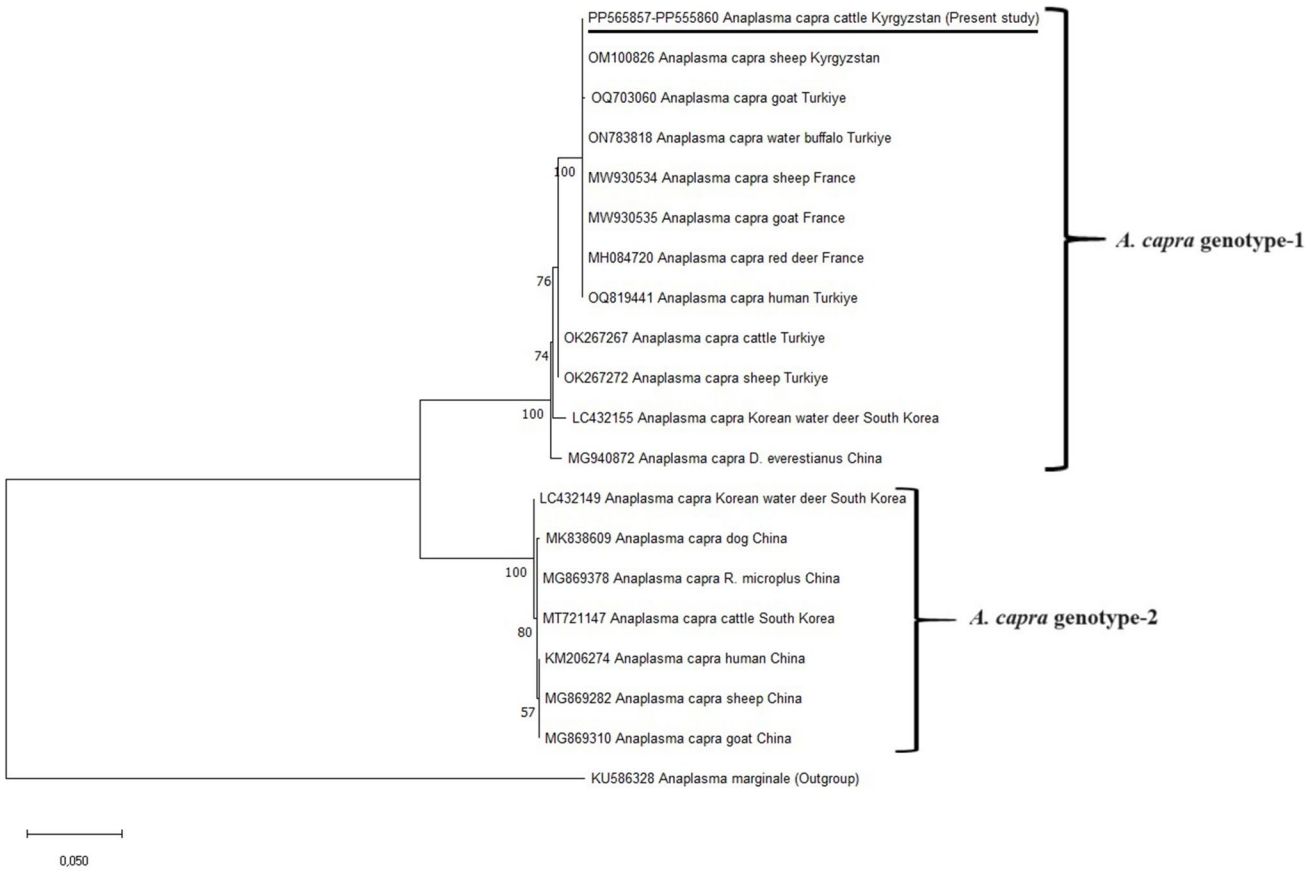

Fig. 6 Phylogenetic tree of *A. capra gltA* gene sequence. *Anaplasma capra* identified in the study is underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the Kimura-2 model (Kimura 1980). The scale bar represents 0.050 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

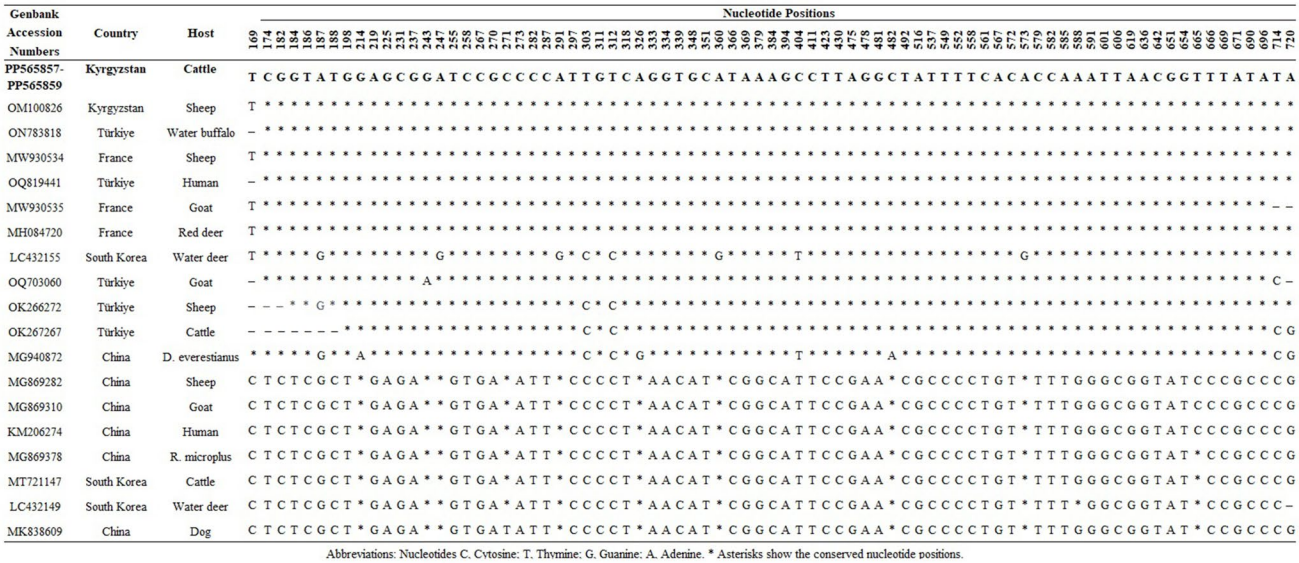

Fig. 7 Nucleotide differences according to partial sequences *A. capra gltA* gene

(KX579073) in China, sheep in Kyrgyzstan (OM453953), *R. sanguineus* (MH292896) in Tunisia, goat (MW600409) in Russia, and sheep (KF293717) in Italy. The phylogenetic tree also revealed that *A. ovis* isolate identified in this work was clustered with *A. ovis* isolates present in GenBank deposited from various parts of the world (Fig. 8).

Anaplasma genus-specific PCR was also performed to determine the presence of *Anaplasma*-associated pathogens, which were not previously reported in Kyrgyzstan. Five samples showed amplicons as a result of genus-specific PCR, while these samples did not give bands in *Anaplasma* species-specific PCR using primers listed in Table 2. All five samples were sequenced and obtained consensus sequences were uploaded to the GenBank under accession numbers: PP555856-PP555860. The BLASTn analyses revealed that our isolates had high nucleotide similarities (99.70-99.85%) with *Candidatus* Anaplasma camelii isolates reported in camels from Kenya (MT510533), Iran (KX765882), Saudi Arabia (KF843825) and tick species such as *H. rufipes* (MT929199), *H. dromedarii* (MT929200), and *R. camicasi* (MW690202) from Kenya. Moreover, our isolates were grouped within the same cluster of *Candidatus* Anaplasma camelii isolates in the phylogenetic trees and placed in different branches with other *Anaplasma* species (Fig. 8).

Trypanosoma Theileri

Trypanosoma theileri DNA was found in 102 (19.21%) cattle samples, and this was the third most prevalent pathogen. Subsequent to the identification of PCR-positive samples, randomly selected two samples were sequenced. The consensus sequences were determined, and deposited to the GenBank under accession numbers: PP565860-PP565862. The *cathepsin L* gene (*catl*) sequence of our two samples had 91.53–100% nucleotide similarities with *Try. theileri* isolates detected in various hosts and deposited to the GenBank. In the phylogenetic analysis of *Try. theileri* isolates, many nucleotide differences were determined in the *catl* gene; therefore, it was thought that two different *Try. theileri* strains (TthI and TthII) were circulating in the study area. Similar results were seen in the phylogenetic tree and our *Try. theileri* isolates were grouped in divergent clusters (Fig. 9). *Trypanosoma theileri* TthI isolates had 100% nucleotide identities with *Try. theileri* cattle isolates identified in Sri Lanka (LC438508), Iran (MK393794), and Vietnam (LC125447), whereas our *Try. theileri* TthII isolates had 100% similarities with *Try. theileri* water buffalo isolates in Vietnam (LC125455), cattle isolates reported in Sri Lanka (AB930159), and Ecuador (ON063530).

Mycoplasma wenyonii and *Candidatus* Mycoplasma haemobos

Bovine hemoplasma species were also researched, and *M. wenyonii* was detected in 32 (6.03%) samples, whereas *Ca.* Mycoplasma haemobos was identified in 14 (2.64%) samples. After the detection of bovine hemoplasma-positive samples, three representative samples from each pathogen were sent for sequencing analyses, resulting in the determination of consensus sequences. These consensus sequences, obtained in the present study, were uploaded to the GenBank database under the accession numbers: PP621034-PP621036 for *M. wenyonii* and PP621031-PP621033 for *Ca.* Mycoplasma haemobos. Partial parts of the *16 S rRNA*

Fig. 8 Phylogenetic tree of *Anaplasma* species *16 S rRNA* gene sequence. *Anaplasma centrale*, *A. bovis*, *A. ovis*, and *Ca.* Anaplasma camelii identified in the study are underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the HKY model (Hasegawa et al. 1985). The scale bar represents 0.050 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

gene sequence of *M. wenyonii* isolates identified in the present study showed 95.71–100% nucleotide identities with *M. wenyonii* isolates present in the GenBank. The 100% nucleotide similarities were seen between our *M. wenyonii* isolates and *M. wenyonii* isolates identified in cattle from Kyrgyzstan (OM891795), Germany (FN392885), and Bosnia and Herzegovina (MK608707). BLASTn analyses of *16 S rRNA* gene sequences belonging to *Ca.* Mycoplasma haemobos isolates determined in the current work revealed 97.18–100% nucleotide similarities with *Ca.* Mycoplasma haemobos isolates that were reported in various parts of the world. Furthermore, 100% of nucleotide identities were seen between our *Ca.* Mycoplasma haemobos and *Ca.* Mycoplasma haemobos identified in cattle from Kyrgyzstan (OM891818), Japan (EU367965), Nigeria (ON346533), and Türkiye (OM468184). In the phylogenetic tree, our *M. wenyonii* and *Ca.* Mycoplasma haemobos isolates were grouped with *M. wenyonii* and *Ca.* Mycoplasma haemobos isolates, respectively, and those isolates were placed in different clusters from other hemotropic mycoplasma species (Fig. 10).

Discussion

Cattle can host various vector-borne pathogens, especially *Theileria*, *Babesia*, *Anaplasma*, *Trypanosoma*, and hemotropic mycoplasmas (Altay et al. 2008, 2022a, 2023; Schnittger et al. 2012; Galon et al. 2022; Liu et al. 2022a). These pathogens can cause mild to severe infections in cattle, resulting in reduced profitability of cattle farms.

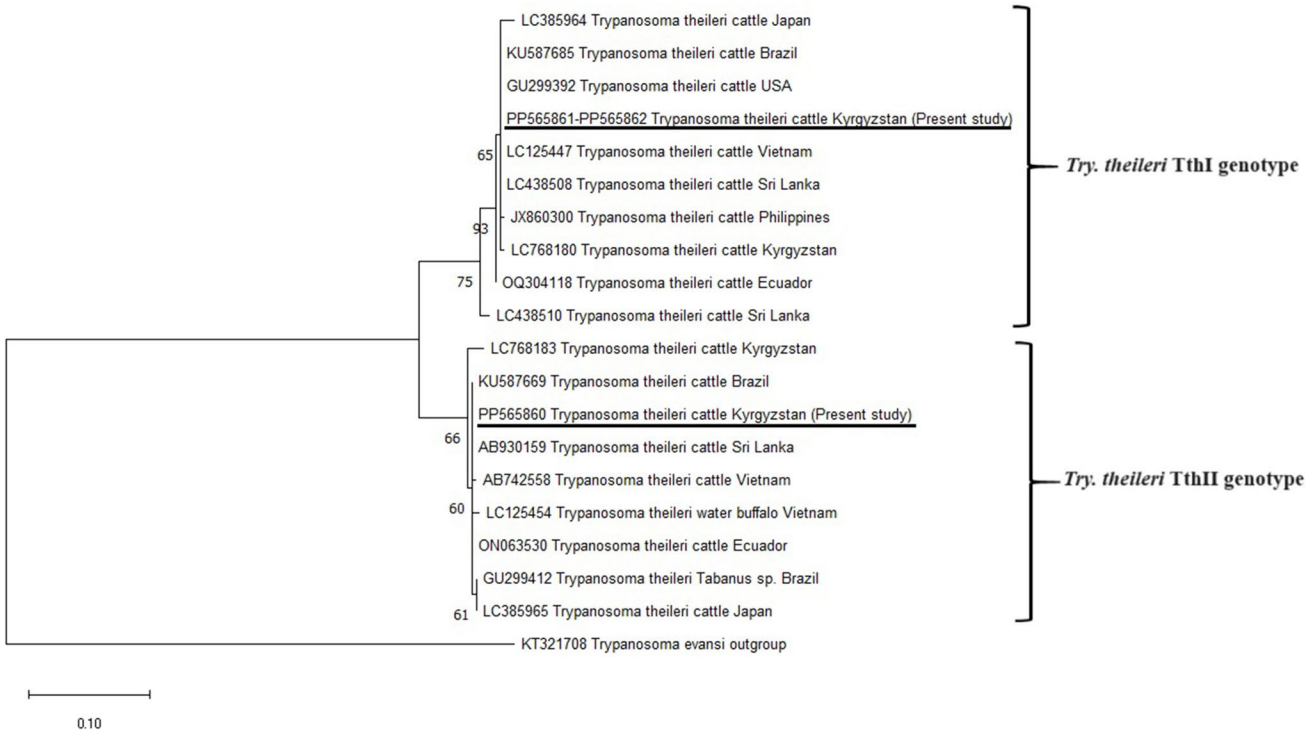

Fig. 9 Phylogenetic tree of *Try. theileri catl* gene sequence. *Trypanosoma theileri* TthI and TthII isolates identified in the study are underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the T92 model (Tamura 1992). The scale bar represents 0.10 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

Several vector-borne pathogens were researched in cattle blood samples obtained from different parts of Kyrgyzstan, and *T. orientalis*, *T. annulata*, *B. major*, *B. occultans*, *A. phagocytophilum*-like 1, *A. capra*, *A. centrale*, *A. bovis*, *A. ovis*, *Ca.* Anaplasma camelii, *Try. theileri*, *M. wenyonii*, and *Ca.* Mycoplasma haemobos were identified. The 67.61% (359/531) of researched animals were found to be infected with at least one pathogen. In studies conducted in Asian countries to investigate vector-borne pathogens, it has been determined that the prevalence and distribution of these pathogens in cattle are quite different from each other (Aktaş et al. 2019; Debbarma et al. 2020; Altay et al. 2022a; Galon et al. 2022; Kuibagarov et al. 2023). Among these studies, 87.8% in Iran (Fathi et al. 2024), 34.84% in India (Debbarma et al. 2020), 57.94% in China (Li et al. 2020), 1.7–36.1% in Kyrgyzstan (Aktaş et al. 2019; Altay et al. 2022a), 88.6% in Kazakhstan (Kuibagarov et al. 2023), 100% in Sri Lanka (Gunasekara et al. 2019), and 92.3% in Malawi (Chatanga et al. 2022) were found to be vector-borne pathogens in cattle. The prevalence of vector-borne pathogens may influence various factors, such as the number of animals, specificity and sensitivity of the methods used, geographical features of sampling areas, species, distribution, and abundance of vectors present in the studied areas, and the number of the pathogens researched within the scope of the studies (Suarez and Noh 2011; Schnittger et al. 2012; Pfäffle et al. 2013; Aktaş et al. 2019; Altay et al. 2022a; Chatanga et al. 2022; Kuibagarov et al. 2023; Fathi et al. 2024). The differences in prevalence between studies, including ours, might be related to one or more of the reasons listed above.

Vector-borne pathogens have a wide distribution, and these pathogens have been reported in almost all parts of the world. Moreover, studies have shown that hosts are exposed to several vector-borne pathogens at the same time (Aktaş et al. 2019; Gunesekara et al. 2019; Altay et al. 2020, 2023; Erol et al. 2023; Kuibagarov et al. 2023; Fathi et al. 2024). Co-infections were also determined in the surveyed cattle, and 32.01% (170/531) of the animals were infected with two or more pathogens (Table 5). Studies have shown that the co-infection with vector-borne pathogens may be because of several reasons, such as vector species can carry and transmit more than one pathogen, cattle can be exposed to more than one vector species at the same time, especially on the pastureland, and some pathogens can cause persistently infected (Suarez and Noh 2011; Aubry and Geale 2011; Schnittger et al. 2012; Pfäffle et al. 2013; Altay et al. 2020; Chatanga et al. 2022; Kuibagarov et al. 2023; Fathi et al. 2024). There is no information on the presence of ticks in the animals examined in this study. However, since cattle grazing on pasture were used in the study, it is considered

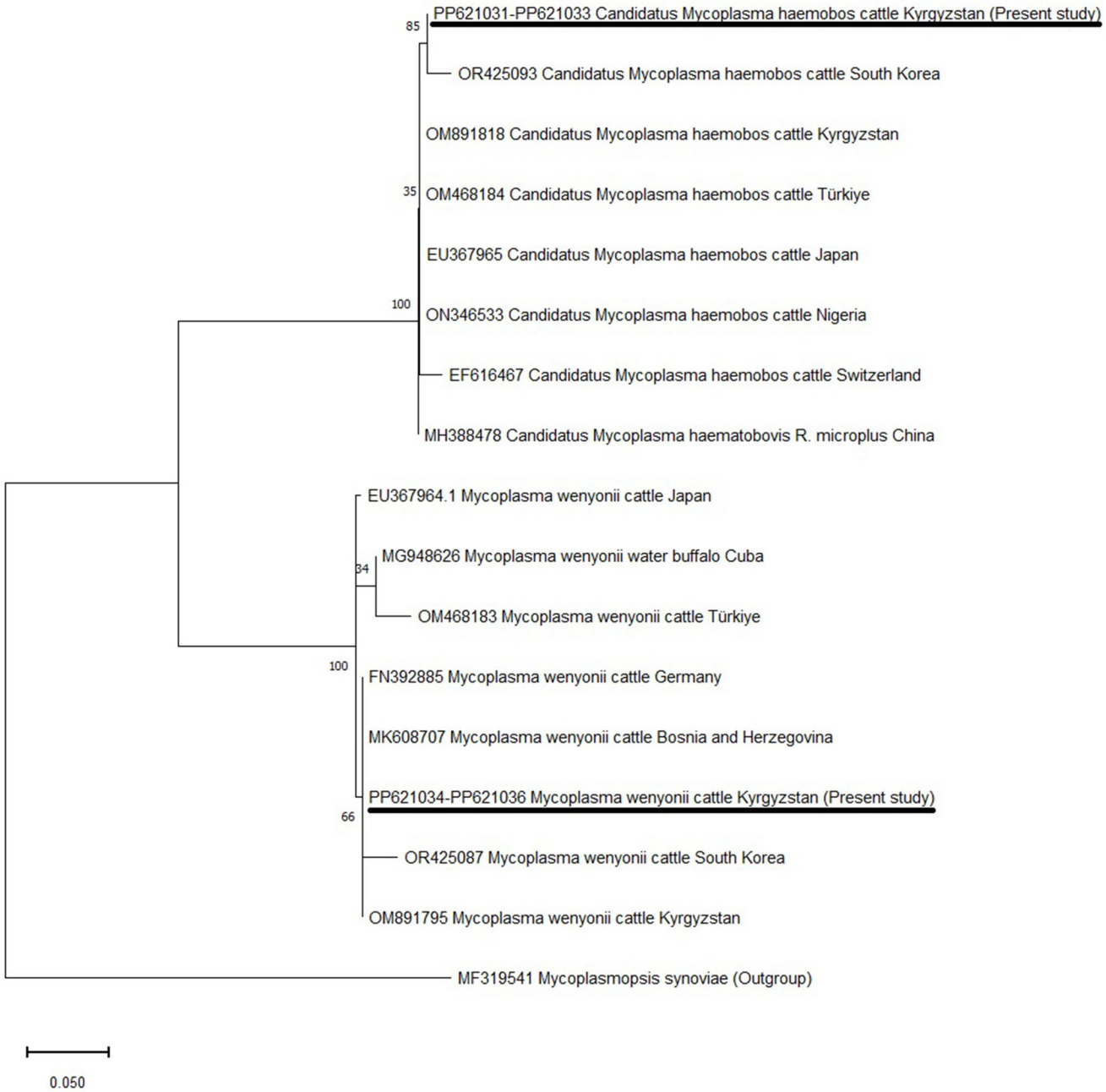

Fig. 10 Phylogenetic tree of *M. wenyonii* and *Ca.* Mycoplasma haemobos *16 S rRNA* gene sequence. *Mycoplasma wenyonii* and *Ca.* Mycoplasma haemobos identified in the study are underlined. Numbers at the nodes represent the bootstrap values with 1,000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method and the TN93 model (Tamura and Nei 1993). The scale bar represents 0.050 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA-11 (Tamura et al. 2021)

that these animals were exposed to many vector arthropods during their stay in the pasture, and mixed infections were detected for this reason.

Theileria orientalis is mostly linked to benign or non-transforming theileriosis in cattle (Cufos et al. 2012; Bogema et al. 2015; Watts et al. 2016; Aktaş et al. 2019). This parasite has a worldwide distribution and has been reported with a different prevalence in Türkiye (Altay et al. 2008), Australia (Kamau et al. 2011), Japan (Ota et al. 2009), China (Liu et al. 2022b), and USA (Oakes et al. 2019). *Theileria orientalis* was the most common pathogen circulated in cattle herds and was detected in 47.83% (254/531) of researched animals. This parasite was identified by Aktaş et al. (2019) and Zhyldyz et al. (2023)

in Kyrgyzstan, with a prevalence of 32.8% and 84.3%, respectively. Molecular studies based on the *MPSP* gene sequence of *T. orientalis* demonstrated that 11 *T. orientalis* genotypes, type 1 (chitose), type 2 (ikeda), type 3 (buffeli), type 4–8, and N1-N3, were circulated in cattle herds in different parts of the world (Ota et al. 2009; Cufos et al. 2012; Bogema et al. 2015; Watts et al. 2016; Ozubek et al. 2022). The chitose and ikeda genotypes have a high pathogenic effect on cattle herds, whereas others are considered low pathogenic. Studies revealed that pathogenic genotypes may lead to outbreaks in cattle herds with clinical manifestations such as lethargy, icterus, depression, abortion, and death (Kamau et al. 2011; Watts et al. 2016; Liu et al. 2022b). The DNA sequence of the *MPSP* gene of *T. orientalis* had high nucleotide similarities with the *T. orientalis* type-3 (buffeli). In recent studies conducted by Ozubek et al. (2022) and Zhyldyz et al. (2023) in Kyrgyzstan, it was reported that *T. orientalis* type-1 (chitose) and type-3 (buffeli) genotypes were commonly found in cattle. The fact that only *T. orientalis* type-3 (buffeli) was detected in the study may be related to the fact that this study was conducted in different geographical regions than the studies conducted by Ozubek et al. (2022) and Zhyldyz et al. (2023). The samples collected in the studies conducted by Ozubek et al. (2022) and Zhyldyz et al. (2023) were mostly from the northern regions of Kyrgyzstan. The blood samples of the present work were collected from the southern regions of the country. The features of geographic regions may affect the distribution of both tick species and pathogens carried by these ticks (Schnittger et al. 2012; Pfäffle et al. 2013).

Theileria annulata, causes lymphoproliferative infection with high mortality and morbidity in cattle, and approximately 250 million animals live in areas at risk of this parasite (Liu et al. 2022a). *Theileria annulata* has been reported in many countries in Asia, north Africa, and southern Europe (Aktas et al. 2006; Altay and Aktas 2004; Altay et al. 2008; Gunasekara et al. 2019; Aktaş et al. 2019; Liu et al. 2022a; Zhyldyz et al. 2023). The 136 (25.61%) out of 531 animals were found to be infected with *T. annulata*. Before this study, *T. annulata* was detected in cattle in Kyrgyzstan at a prevalence of 1.9–16.6% (Aktaş et al. 2019; Zhyldyz et al. 2023). In endemic regions, *T. annulata* may cause subclinical infection, and infected animals do not show clinical symptoms (Altay and Aktas 2004; Aktas et al. 2006). Persistently infected animals are important for the epidemiology of *T. annulata*. These animals, which do not show clinical symptoms, remain latently infected for a long time and cause the transmission of the agent to different cattle through tick vectors (Aktas et al. 2006; Altay and Aktas 2004;Altay et al. 2008; Aktaş et al. 2019; Liu et al. 2022a). In this study, no clinical manifestations were seen in infected animals. Therefore, detecting latent animals is considered important in preventing economic loss due to *T. annulata*, and veterinarians in the region should keep cattle coming from outside the region under observation for *T. annulata*.

Babesiosis is known as one of the most common vector-borne diseases in cattle farms, and bovine babesiosis has an enormous negative effect on the cattle industry (Suarez and Noh 2011; Schnittger et al. 2012; Galon et al. 2022). *Babesia bigemina*, *B. bovis*, and *B. divergens* are associated with severe babesiosis in cattle herds. Meanwhile, *B. occultans*, *B. major*, and *B. ovata* are considered relatively low-pathogenic species compared to the above-mentioned *Babesia* species (Altay et al. 2008; Suarez and Noh 2011; Schnittger et al. 2012). One sample (0.19%) was found to be infected with *B. major* using RLB assay and *B. occultans* was identified in two samples (0.38%) via RLB and DNA sequence analyses in the study. Before this report, *B. major* (1.3%) (Aktaş et al. 2019), B. *bovis* (2.5%), *B. bigemina* (47.6%), and *B. occultans* (0.31%) (Zhyldyz et al. 2023) were reported in Kyrgyzstan. *Babesia bovis* and *B. bigemina* were not detected in the present study. This result may be related to the fact that these studies, including ours, were conducted in different geographical regions in Kyrgyzstan, and these areas have different ecological features. Ecological differences in the sampling areas cause fractionation of tick species in the region and also affect the prevalence and distribution of ticks in these areas. The differentiation of tick species can also change the species of pathogens to which animals in the region are exposed and their prevalence (Suarez and Noh 2011; Schnittger et al. 2012; Pfäffle et al. 2013).

Anaplasma species are known as one of the important vector-borne pathogens, and these species can infect various hosts, including cattle. *Anaplasma* species cause mild to severe infections in cattle and even death can occur in untreated cases (Aubry and Geale 2011; Kolo 2023). *Anaplasma marginale*, *A. centrale*, *A. bovis*, *A. capra*, *A. phagocytophilum*, and *A. phagocytophilum*-related strains species have been reported in cattle (Aktas and Çolak 2021; Rar et al. 2021; Altay et al. 2022a, b). *Anaplasma* species were researched using PCR, RFLP, and DNA sequence analyses. *Anaplasma phagocytophilum* infects various wide range of hosts, and the pathogen has zoonotic importance (Chen et al. 1994; Zobba et al. 2014; Ben Said et al. 2017). Molecular-based studies on *A. phagocytophilum* revealed that there are several strains genetically related to *A. phagocytophilum*, and these strains are named *A. phagocytophilum*-like 1 and -like 2 (Ben Said et al. 2017). *Anaplasma phagocytophilum* and related strains were researched in cattle samples via PCR, and positive amplicons were seen in 17 (3.20%) cattle samples. For the differentiation of *A. phagocytophilum* and related strains, an RFLP assay was performed, and *A. phagocytophilum*-like 1 was detected in all positive samples. *Anaplasma phagocytophilum*-like 1 was reported

in cattle (0.3%) and sheep (6.9%) by Altay et al. (2022a, c) in the country. The combination of PCR and RLB is a useful method for wide epidemiological studies. DNA sequences and phylogenetic analyses confirmed the presence of *A. phagocytophilum*- like 1 (Figs. 4 and 5). There is still limited information on the hosts, prevalence, distribution, vectors, and clinical symptoms of *A. phagocytophilum*-related strains in the world. However, studies on different host species showed that *A. phagocytophilum*-like 1 has been more prevalent than both *A. phagocytophilum* and *A. phagocytophilum*-like 2 (Ben Said et al. 2017; Aktas and Çolak 2021; Altay et al. 2022a, c; Erol et al. 2022; Sahin et al. 2023). Our results were consistent with the results of other studies (Aktas and Çolak 2021; Altay et al. 2022a, c; Erol et al. 2022; Sahin et al. 2023)d *phagocytophilum* and *A. phagocytophilum*-like 2 were not identified in the researched animals. It is speculated that this may be associated with the vector species of *A. phagocytophilum*-like 1 being more widespread than others or that *A. phagocytophilum*-like 1 has a higher host adaptability than *A. phagocytophilum* and *A. phagocytophilum*-like 2.

Anaplasma capra was reported in goats for the first time in China in 2012 (Liu et al. 2012); after that, this pathogen was detected in humans in the same country (Li et al. 2015). Understanding the zoonotic importance of *A. capra*, lots of studies have been conducted for the determination of host species and geographic distributions for this pathogen (Seo et al. 2018; Altay et al. 2022a, b, c, 2024; Sahin et al. 2022), and in these studies, *A. capra* has been reported in domestic and wild animals in 18 countries present on three continents, Asia, Europe, and Africa (Altay et al. 2024). *Anaplasma capra* was detected in 16 (3.01%) cattle samples. Before this work, *A. capra* had been reported in cattle (0.3%) (Altay et al. 2022a) and sheep (5.3%) (Altay et al. 2022c) in Kyrgyzstan. These reports revealed that *A. capra* is circulated among domestic ruminants, and therefore, farmers should consider this pathogen to protect their health. Molecular studies based on phylogenetic analyses of *gltA* and *groEL* gene sequences demonstrated that at least two *A. capra* genotypes (*A. capra* genotype-1 and *A. capra* genotype-2) circulate among hosts in the world (Altay et al. 2022b, c, 2024; Sahin et al. 2022). However, it has also been shown in these studies that these genotypes do not have a species or geographical differentiation (Altay et al. 2024). In the present work, the *gltA* gene of randomly selected three *A. capra*-positive samples was sequenced, and our *A. capra* isolates had high nucleotide similarities with *A. capra* genotype-1 circulated in Türkiye, France, South Korea, Kyrgyzstan, and China. Although many studies have been conducted to understand the host species and geographical distribution of *A. capra*, it is thought that studies are still needed to understand the clinical symptoms in animals and vector species, responsible for transmissions, of this pathogen. Studies to determine the epidemiology of *A. capra*, a relatively new species, are important as they will contribute to understanding the effects of this species on human and animal health.

Anaplasma bovis can lead to clinical disease in cattle with symptoms of high body temperature, decreased milk production, weight loss, and rarely abortion or death in cattle (Rar and Golovljova 2011; Kolo 2023). *Anaplasma bovis* has a worldwide distribution and has been reported in various domestic and wild animals to date (Rar and Golovljova 2011; Altay et al. 2020; Kolo 2023). *Anaplasma bovis* was identified in six (1.13%) cattle samples, and this is the first molecular report of this pathogen in Kyrgyzstan. Recent studies conducted in China and the USA revealed that *A. bovis* can infect humans (Lu et al. 2022; Karpathy et al. 2023). In China, high fever, chills, thrombocytopenia, rash, loss of appetite, asthenia, muscular pain, and gastrointestinal disorders were seen in patients (Lu et al. 2022). Considering that *A. bovis* can cause infection in both cattle and humans, veterinarians and clinicians should take into account the pathogen to protect animals and humans in Kyrgyzstan or any other country.

A study conducted in Saudi Arabia by Bastos et al. (2015) identified a pathogen in dromedary camels genetically related to *Anaplasma* species, and the pathogen was provisionally named *Candidatus* Anaplasma camelii. After this discovery, the pathogen was reported in camels and tick species in the genus *Hyalomma*, *Amblyomma*, and *Rhipicephalus*, which were obtained from camels in Kenya (Getange et al. 2021), Morocco (Lbacha et al. 2017), and Iran (Sharifiyazdi et al. 2017). *Candidatus* Anaplasma camelii was detected in five cattle samples (0.94%). This is the first molecular report of the pathogen in Kyrgyzstan, and this result supplies important epidemiological data for the understanding of the distribution of *Ca.* Anaplasma camelii. To date, *Ca.* Anaplasma camelii has been reported in apparently healthy camels, and therefore researchers have considered that this bacterium has a low pathogenic effect (Getange et al. 2021). However, in recent years, it has been reported that *Anaplasma* species, like *A. capra*, *A. ovis*, and *A. bovis*, that do not cause non-pathogenic or mild clinical symptoms in animals can cause serious diseases in humans (Chochlakis et al. 2010; Hosseini-Vasoukolaei et al. 2014; Li et al. 2015; Lu et al. 2022; Karpathy et al. 2023; Altay et al. 2024). Considering this information, it is thought that the zoonotic potential of *Ca.* Anaplasma camelii should be investigated.

Additionally, two *Anaplasma* species, *A. centrale* and *A. ovis*, were also detected in cattle samples. *Anaplasma centrale* is considered a less pathogenic *Anaplasma* species (Aubry and Geale 2011) and has been reported in different parts of the world (Kawahara et al. 2006; Aktas et al. 2011; Aubry and Geale 2011; Altay et al. 2022a). *Anaplasma centrale* was detected in 2.82% (15/531) of researched animals.

Before this detection, *A. centrale* was reported at a detection rate of 1.1% in cattle in Kyrgyzstan by Altay et al. (2022a). *Anaplasma centrale* is genetically related to *A. marginale*, a highly pathogenic *Anaplasma* species in cattle, and studies have demonstrated that *A. centrale* infection supplies important and long-term protection against *A. marginale*. Therefore, *A. centrale* has been used as a live vaccine against *A. marginale* infection in South America, Israel, South Africa, and Australia (Kocan et al. 2010; Aubry and Geale 2011; Rar and Golovljova 2011). *Anaplasma ovis* is known to be a pathogen associated with small ruminants but, this pathogen was also identified in humans (Chochlakis et al. 2010; Hosseini-Vasoukolaei et al. 2014). *Anaplasma ovis* mostly has a low pathogenic effect on animal hosts, however, in the case of immunosuppression in infected animals, this pathogen leads to severe clinical infection (Renneker et al. 2013). *Anaplasma ovis* was found in one sample (0.19%) in the present work. The positive sample was sequenced for the confirmation of PCR results, and the obtained sequence data showed that high nucleotide similarities were present between our *A. ovis* isolates and *A. ovis* isolates identified in small ruminants and tick species from Italy, Tunisia, Russia, Kyrgyzstan, and China. This is the first molecular report on the detection of *A. ovis* in cattle. Before this, *A. ovis* was identified in sheep (22.5%) in Kyrgyzstan (Altay et al. 2022c). This may be related to the tick origin of the pathogen and the transmission of *A. ovis* to cattle by infected ticks when cattle and sheep graze on the same pasture. In addition, given that *A. ovis* can cause infection in small ruminants and humans, different hosts, like cattle, should be considered when protecting against this pathogen.

Trypanosoma theileri is a highly prevalent parasitic pathogen among cattle herds. This parasite has been detected in various blood-sucking arthropods, such as tabanids, mosquitoes, deer keds, tsetse flies, and tick species (Villa et al. 2008; Brotánková et al. 2022). There is limited data available on the prevalence and distribution of *Try. theileri* compared to other vector-borne pathogens. Studies demonstrated that *Try. theileri* can mostly lead to asymptomatic or chronic infection in cattle (Villa et al. 2008; Garcia et al. 2011). However, this parasite may cause severe clinical symptoms, like anemia, fever, abortion, reproductive problems, and also death in the case of the distribution of parasites to various organs and the central nervous system in immunosuppressed cattle (Villa et al. 2008; Garcia et al. 2011; Amato et al. 2019; Suganuma et al. 2022). To date, this parasite has been reported in Italy (Amato et al. 2019), Spain (Villa et al. 2008), Ecuador (De la Cadena et al. 2023), Brazil (Pacheco et al. 2018), Sri Lanka (Gunasekara et al. 2019), Vietnam (Weerasooriya et al. 2016), Japan (Suganuma et al. 2022), and Philippines (Ybañez et al. 2013). *Trypanosoma theileri* was detected in 19.21% (102/531) of the researched animals. This is the second molecular report of the presence of *Try. theileri* in Kyrgyzstan, and this pathogen was detected at a prevalence of 7.2% in the country (Zhyldyz et al. 2023). Studies revealed high genetic diversity present between *Try. theileri* isolates according to *catl* gene sequences, and two *Try. theileri* isolates (TthI and TthII) circulate among cattle herds (Pacheco et al. 2018). In the present work, the DNA sequence of the *catl* gene was derived from three representative PCR-positive samples for the determination of *Try. theileri* isolates in the country, and it was seen that *Try. theileri* TthI and TthII isolates were circulated among cattle in Kyrgyzstan (Fig. 9).

Mycoplasma wenyonii and *Ca.* Mycoplasma haemobos are known to be etiological agents of hemoplasma infection in cattle. Bovine hemoplasma species are transmitted via hematophagous arthropods, like flies, mosquitoes, lice, and ticks (Ybañez et al. 2019; Shi et al. 2019). Studies revealed that hemoplasma species may lead to unspecific clinical symptoms among cattle herds such as anemia, fever, icterus, lack of appetite, a drop in milk production, and decreased body weight in calves at birth (Meli et al. 2010; Genova et al. 2011; Tagawa et al. 2013; McFadden et al. 2016), and these results in economic losses in cattle farms. Bovine hemoplasma species have a cosmopolitan distribution, and these pathogens have been reported in Switzerland (Meli et al. 2010), Germany (Niethammer et al. 2018), Türkiye (Erol et al. 2023), Cuba (Díaz-Sánchez et al. 2019), Brazil (de Mello et al. 2019), Philippines (Ybañez et al. 2019), South Korea (Kim et al. 2024), Japan (Tagawa et al. 2013), and New Zealand (McFadden et al. 2016). *Mycoplasma wenyonii* and *Ca.* Mycoplasma haemobos were detected in 32 (6.03%) and 14 (2.64%) cattle blood samples, respectively. Only one study was conducted to understand the prevalence and distribution of bovine hemoplasma species in Kyrgyzstan (Altay et al. 2023), and in the study, *M. wenyonii* was identified in 15.08% whereas, *Ca.* Mycoplasma haemobos 9.21%. Bovine hemoplasma species can persist in hosts for long periods without causing any infection. These animals pose a risk to other animals in the area (Genova et al. 2011; Díaz-Sánchez et al. 2019). Therefore, it is considered very important to identify infected animals to prevent economic loss caused by hemoplasma species.

Conclusion

Vector-borne pathogens may lead to emerging and re-emerging diseases in cattle, and these pathogens may cause significant damage to cattle herds almost all over the world. In this study, 13 vector-borne pathogens (*T. orientalis*, *T. annulata*, *B. major*, *B. occultans*, *A. phagocytophilum*-like 1, *A. capra*, *A. centrale*, *A. bovis*, *A. ovis*, *Ca.* Anaplasma camelii, *Try. theileri*, *M. wenyonii*, and *Ca.* Mycoplasma haemobos) were detected in cattle in

Kyrgyzstan, with a high prevalence. *Anaplasma bovis* and *Ca.* Anaplasma camelii were identified for the first time in the country. The epidemiological data obtained will form the basis for the determination and implementation of effective control methods against vector-borne pathogens. In addition, zoonotic species, especially *A. capra*, should be taken into consideration by animal breeders, veterinarians, and persecutors living in the region. The results of the DNA sequence analysis of the pathogens revealed that there was a high nucleotide similarity between the pathogens detected in the study and those reported in different parts of the world. This high nucleotide similarity between pathogens from different continents may be due to the import or export of animals between countries today and in the past decades, as well as the migration of blood-sucking vector arthropods between continents through migratory birds. Large-scale molecular studies are needed to understand the high genetic similarity of pathogens detected in different countries.

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Declarations

Ethics approval All procedures performed in studies involving animals were in accordance with the ethical standards approved by the Kyrgyz-Turkish Manas University Animal Researches Local Ethic Committee (decision number: 2023-08).

Consent for publication All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, has not received prior publication, and is not under consideration for publication elsewhere.

Consent to participate The consent of all animal owners was sorted before this study was carried out.

Conflict of interest The authors declare that they have no conflict of interest.

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