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Molecular Detection and Characterization of *Anaplasma* spp. in Domestic Cats from Veterinary Hospitals in Zhengzhou, China

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Simple Summary

Domestic cats are increasingly popular pets in Chinese households, and share close contact with humans. However, they can carry tick-borne bacteria such as *Anaplasma*, some of which can cause disease in people. In this study, we tested blood samples from 229 domestic cats in Zhengzhou, China, to check for *Anaplasma* spp. infection. We found that 20.5% of the cats were infected with at least one type of *Anaplasma*. Three species were detected: *Anaplasma ovis* (17.5%), *Anaplasma capra* (5.2%), and *Anaplasma platys* (3.1%). Some cats had mixed infections. This is the first time *A. capra* has been found in cats anywhere in the world. Because cats and humans live in such close proximity, we recommend implementing regular veterinary ectoparasite control programs and strengthening tick and flea control measures to reduce the risk of disease transmission. Our findings highlight the need for greater awareness of these infections in pet cats and their potential impact on public health.

Abstract

Anaplasma spp. are tick-borne zoonotic pathogens, but their molecular detection frequency and clinical sinificance in domestic cats in urban China remain largely unknown. To address this gap, we investigated *Anaplasma* infection in cats from Zhengzhou, Henan Province. A total of 229 feline blood samples collected from four veterinary hospitals in Zhengzhou were screened for *Anaplasma* spp. using nested PCR, and positive products were subjected to phylogenetic analysis. The overall *Anaplasma* molecular detection frequency was 20.5% (47/229). Specifically, *A. ovis*, *A. capra*, and *A. platys* were detected in 17.5% (40/229), 5.2% (12/229) and 3.1% (7/229) of samples, respectively, with co-infections accounting for 5.2% (12/229). PCR positivity was significantly higher in cats diagnosed with influenza and gastrointestinal disorders ($p = 0.041$ and $p = 0.013$, respectively), and in those not dewormed within the preceding 3 months ($p < 0.001$). This study provides the first molecular evidence of *A. capra* in domestic cats. Partial *gltA* and *msp4* sequences (MW520362 and MW520360) from feline-derived *A. capra*, together with one *A. ovis msp4* sequence (MW520355), shared 100% nucleotide identity with previously published zoonotic isolates from human, animal, and tick sources, suggesting a potential public health risk; however, full-length genome data are needed to further assess their zoonotic capacity. Dual-species co-infections of *Anaplasma* were also observed among the sampled cats. We recommend implementing regular veterinary-supervised ectoparasite control programs to reduce the risk of disease transmission.

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1. Introduction

Cats have become increasingly popular as ideal companions among urban young adults, due to their lower space requirements and no need for daily walks. Consequently, an increasing number of cats have become members of Chinese households, closely interacting with humans and sharing living spaces. However, cats can be infected with *Anaplasma* species, including *Anaplasma phagocytophilum* and *Anaplasma ovis* [1–3]. Notably, cats infected with *Anaplasma* spp. are often asymptomatic or present with nonspecific fever, posing a hidden risk of zoonotic transmission through vectors such as ticks [2,3]. Consequently, a "One Health" approach should be adopted for the prevention and control of feline anaplasmosis [4].

Anaplasma species are zoonotic pathogens transmitted by ticks, which holds significant veterinary and public health importance. Currently, seven common species have been identified based on 16S *rRNA* and *groEL* gene analysis, including *A. phagocytophilum*, *Anaplasma bovis*, *A. ovis*, *Anaplasma marginale*, *Anaplasma platys*, *Anaplasma centrale*, and *Anaplasma capra* [5]. To date, *A. phagocytophilum*, *A. ovis*, and *A. capra* have been identified as pathogenic species associated with human anaplasmosis, and *A. phagocytophilum* being the primary etiological agent [1,6,7]. *A. ovis* was once detected in a patient in Cyprus presenting with an 11-day fever following a tick bite, accompanied by clinical manifestations including thrombocytopenia and moderate anemia [8]. In 2014, *A. capra* was identified in blood samples from 28 patients with a history of tick exposure in Northeast China [1]. Nowadays, *A. capra* is regarded as a newly emerging human pathogen [7,9]. Recent reports of *A. bovis* or *A. bovis*-like infections in humans from China and the United States suggest this pathogen poses a potential zoonotic risk [10–13]. For decades, *A. platys* was considered exclusively a canine pathogen; however, recent reports have documented its presence in cats, humans, and both domestic and wild ruminants [7,14–20].

It is worth noting that the above potential zoonotic agents such as *A. phagocytophilum*, *A. ovis*, *A. platys* and *A. bovis* has been identified in domestic and wild cats [2,3,14,21]. However, to our knowledge, there have been no worldwide reports of *A. capra* infection in cats to date. In China, limited studies have investigated vector-borne zoonotic pathogens in cats from the Qinghai–Tibetan Plateau, Tianjin, and Hong Kong SAR [3,22,23]. These studies have only detected *Anaplasma*-specific IgG or IgM in stray cats from the Tibetan Plateau and *A. ovis* in companion cats from Tianjin [3,22]. The provincial capital of Henan, Zhengzhou, serves as a major central city in central China and a key national comprehensive transportation hub. Its population of over 13 million provides a substantial base that supports a significant number of domestic cat breeders. To investigate the molecular detection frequency, clinical manifestations, and risk factors of *Anaplasma* species infection in domestic cats from Zhengzhou, Henan Province, we conducted this study.

2. Materials and Methods

2.1. Sample Collection

A total of 229 feline blood samples were collected from four veterinary hospitals in Zhengzhou City during 2017–2018 and again in 2025. During the sampling periods, samples were obtained from cats that required blood collection for physical examination or disease diagnosis, provided that the owner gave verbal consent and a sufficient volume of EDTA-anticoagulated whole blood was available for DNA extraction. This approach was adopted to avoid causing direct harm to the animals, as no blood was collected specifically

for research purposes. Samples with insufficient blood volume or severe hemolysis were excluded from further molecular testing. Patient medical record numbers, cat names, and owner names were recorded to prevent duplicate sampling. After obtaining verbal consent from all owners, licensed veterinarians collected blood samples via venipuncture of the forelimb or hindlimb veins using sterile EDTA tubes. Samples were stored at −20 °C until DNA extraction. Clinical data, including age, sex, and clinical manifestations, were obtained from medical records, owner-reported histories, and veterinary clinical diagnoses.

2.2. DNA Preparation and Pathogen Detection

All samples used for DNA extraction were blood samples stored for no more than one month. Genomic DNA was extracted from 200 µL aliquots of EDTA-treated blood using the Blood DNA Kit (Omega, Norcross, GA, USA) according to the manufacturer's instructions, and DNA concentration and quality were assessed using a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples with a minimum DNA concentration of 20 ng/µL were considered suitable for subsequent PCR analysis. The extracted DNA was stored at −20 °C until further use. DNA samples were identified for *Anaplasma* species using PCR amplification. *A. phagocytophilum* and *A. bovis* were identified based on 16S *rRNA* gene using the EE1/EE2 primer pair for the first PCR round, the SSAP2f/SSAP2r and AB1f/AB1r primer pairs for the second PCR round, respectively [24]. *A. capra* was detected by amplification of the citrate synthase gene (*gltA*), heat shock protein gene (*groEL*), and major surface protein 4 gene (*msp4*) using primers acagltf1/acagltr1 and acagltf2/acagltr2, groELf/groELr, msp4f/msp4r, respectively, and each DNA sample was tested for the presence of all three *A. capra*-specific genetic markers, with successful amplification of at least one target gene considered indicative of a positive infection [1,25]. The primer pairs of AMOF/AMOR and MSP4F/MSP4R were used to amplify the *msp4* gene of *A. ovis* [26]. The primer pairs 8F/1448R and PLATYS/EHR16SR targeting the 16S *rRNA* gene were used to screen *A. platys* [27]. All the primer sequences and PCR conditions are shown in Table 1. The PCR amplification systems used in this study were prepared as follows. The first PCR reaction was set up in a 25 µL volume comprising 4 µL dNTP mixture (2.5 mmol L^{-1}), 2.5 µL 10× LA PCR Buffer (Mg^{2+} Plus), 0.5 µL each of forward and reverse primers (10 pmol each), 1.25 U LA Taq DNA Polymerase (5 UμL^{-1}) (TaKaRa, Dalian, Liaoning, China), 1 µL DNA template, and 16.25 µL distilled water. The second amplification reaction was carried out in a 25 µL volume comprising 2.0 µL PCR products, 0.75 U Taq DNA polymerase (5 UμL^{-1}) (TaKaRa, Dalian, Liaoning, China), 2.5 µL 10× PCR buffer (Mg^{2+} Plus), 2.0 µL dNTPs (2.5 mmol L^{-1}), 0.5 µL primers (10 pmol each), and 17.35 µL distilled water.

Table 1. Primer sequences and PCR conditions for the identification of *Anaplasma* species.

Pathogens	Target Gene	Primer	Primer Sequence (5′–3′)	Annealing Temperature	Amplicon Size (bp)	Cycles
A. phagocytophilum [24]	16S *rRNA*	* EE1	TCCTGGCTCAGAACGAACGCTGGCGGC	55 °C	1430	35
		* EE2	GTCACTGACCCAACCTTAAATGGCTG			
		# SSAP2f	GCTGAATGTGGGGATAATTTAT	55 °C	641	40
		# SSAP2r	ATGGCTGCTTCCTTTCGGTTA			
A.bovis [24]	16S *rRNA*	* EE1	TCCTGGCTCAGAACGAACGCTGGCGGC	55 °C	1430	35
		* EE2	GTCACTGACCCAACCTTAAATGGCTG			
		# AB1f	CTCGTAGCTTGCTATGAGAAC	55 °C	551	40
		# AB1r	TCTCCCGGACTCCAGTCTG			
A. ovis [26]	*msp4*	* AMOf	GCTCCCTACTTGTTAGTGG	58 °C	795	25
		* AMOr	TTAGCTGAACAGGAATCTTG			
		# MSP4f	CAAGCAGAGAGACCTCGTAT	58 °C	584	35
		# MSP4r	GGCTTTTGCTTCTCCGGG			

Table 1. *Cont.*

Pathogens	Target Gene	Primer	Primer Sequence (5′−3′)	Annealing Temperature	Amplicon Size (bp)	Cycles
A. capra [1,25]	*gltA*	* gltAf1	CGATTTTAGAGTGYGGAGATTG	55 °C	1031	25
		* gltAr1	ACAATACCGGAGTAAAAGTCAA			
		# gltAf2	TCATCTCCTGTTGCACGGTGCCC	60 °C	594	30
		# gltAr2	TCTGAATGAACATGCCCACCCT			
	groEL	groELf	TGAAGAGCATCAAACCCGAAG	55 °C	874	35
		groELr	CTGCTCGTGATGCTATCGG			
	msp4	msp4f	GGGTTCTGATATGGCATCTTC	53 °C	658	35
		msp4r	GGAAATGTCCTTATAGGATTCG			
A. platys [27]	16S *rRNA*	* 8F	AGTTTGATCATGGCTCAG	55 °C	1440	40
		* 1448R	CCATGGCGTGACGGGCAGTGTG			
		# PLATYS	GATTTTTGTCGTAGCTTGCTATG	55 °C	678	35
		# EHR16SR	TAGCACTCATCGTTTACAGC			

* outer primers; # inner primers.

All PCR amplifications were performed employing an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). To ensure the reliability of the results, three parallel PCR amplifications were performed for each gene locus of every DNA sample, using nuclease-free water as a negative control. Positive controls consisted of confirmed *A. phagocytophilum*, *A. bovis*, *A. capra*, *A. ovis*, and *A. platys* DNA samples, which had been previously sequenced and maintained in the laboratory. The final PCR products (6 μL) were separated by 1.0% agarose gel electrophoresis and detected by ethidium bromide fluorescence under UV illumination.

2.3. Sequencing and Phylogenetic Analysis

PCR-positive products were subjected to bidirectional sequencing by a commercial company (TSINGKE, Beijing, China) on an ABI 3730 XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were identified and analyzed using Chromas Lite v2.66 (https://technelysium.com.au/wp/chromas/) (accessed on 1 November 2026) and BLASTN (BLAST+ v2.17.0+) (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) (accessed on 1 November 2026). Multiple sequence alignments of the 16S *rRNA*, *msp4*, *groEL*, and *gltA* genes were performed using CLUSTAL W v2.0.10 (https://www.ebi.ac.uk/Tools/msa/clustalo/) (accessed on 1 November 2026). Nucleotide sequences were analyzed using BLAST tool (BLAST+ v2.17.0+) (http://www.ncbi.nlm.nih.gov/BLAST) (accessed on 1 November 2026) to determine similarity with known sequences in GenBank. When constructing the phylogenetic tree, the final sequence length used corresponded essentially to the length of the sequence submitted to NCBI for each gene locus. To make the genetic distances in the tree more closely reflect reality, intermediate gaps were not deleted. For outgroup selection, we generally chose species sequences with a relatively long evolutionary distance. Phylogenetic reconstruction was performed using the neighbor-joining algorithm with Kimura 2-parameter distance modeling in MEGA 12.0 (MEGA Software, State College, PA, USA) to determine the relationships among *Anaplasma* spp. The confidence values for each branch of the resulting tree were confirmed by bootstrap analysis with 1000 replicates.

2.4. Statistical Analysis

Categorical comparisons of *Anaplasma* spp. PCR positivity across variables including sampling site, sampling period, breed, age group, season, gender, clinical signs, and vermifuge-usage history were performed using Pearson's chi-square test. Fisher's exact test was adopted for 2 × 2 contingency tables when one or more cells had expected counts less than 5. Records marked as "Unknown" were excluded from each corresponding statistical comparison. The 95% confidence intervals for PCR-positivity proportions were calculated

using the Clopper-Pearson exact binomial method. All statistical analyses were performed in SPSS 24.0 (IBM Corp., Armonk, NY, USA). $p < 0.05$ was considered statistically significant.

2.5. Nucleotide Sequence Accession Numbers

The GenBank accession numbers for *msp4* gene sequences obtained in this study were as follows: MW520355 to MW520359 for *A. ovis*, MW520360 for *A. capra*. The *gltA* gene sequences of *A. capra* were assigned accession numbers MW520361 and MW520362. The *groEL* gene sequence of *A. capra* was assigned accession numbers MW520363. The partial 16S *rRNA* gene sequences of *A. platys* obtained in this study was PV834200.

3. Results

3.1. Infection of Anaplasma Species in Domestic Cats

A total of 229 blood samples from domestic cats were analyzed in this study, and 47 were positive for at least one *Anaplasma*, with an overall PCR positivity of 20.5% (47/229, 95% CI: 15.29–25.76). A total of three *Anaplasma* species including *A. ovis*, *A. platys* and *A. capra* were screened, with PCR positivity of 17.5% (40/229, 95% CI: 12.55–22.38), 3.1% (7/229, 95% CI: 0.83–5.29) and 5.2% (12/229, 95% CI: 2.35–8.13), respectively. Significant differences were observed between the different *Anaplasma* species. (Table 2).

Table 2. Total detections by different Anaplasma species, single-species infections, co-infections and total positive cats in domestic cats ($n = 229$).

Category	No. Positive (%)	95% CI	OR (95% CI)	*p* Value
Total detections by different *Anaplasma* species (a cat co-infected with two species contributes one detection to each species)				
A. ovis	40 (17.5)	12.55–22.38	1	<0.001
A. capra	12 (5.2)	2.35–8.13	0.261 (0.133–0.513)	<0.001
A. platys	7 (3.1)	0.83–5.29	0.149 (0.065–0.340)	<0.001
A. bovis	0 (0)	–	–	–
A. phagocytophilum	0 (0)	–	–	–
Single-species infections (only one species detected)				
A. ovis only	28 (12.2)	7.98–16.47	–	–
A. capra only	4 (1.7)	0.05–3.44	–	–
A. platys only	3 (1.3)	0.00–2.78	–	–
Co-infections				
A. ovis + *A. capra*	8 (3.5)	1.12–5.87	–	–
A. ovis + *A. platys*	4 (1.7)	0.05–3.44	–	–
A. capra + *A. platys*	0 (0)	–	–	–
A. ovis + *A. capra* + *A. platys*	0 (0)	–	–	–
Total co-infected cats	12 (5.2)	2.35–8.13	–	–
Total positive cats				
Any *Anaplasma* species detected	47 (20.5)	15.29–25.76	–	–

In this study, blood samples were collected from domestic cats during two sampling periods: 2017–2018 and 2025, and the molecular detection frequency of *Anaplasma* spp. were 20.9% (27/129, 95% CI: 13.91–27.95) and 20.0% (20/100, 95% CI: 12.16–27.84), respectively. No statistically significant difference was observed between the two periods ($p = 0.863$).

Regarding PCR positivity across sampling sites, all four sites tested positive for at least one *Anaplasma* species, with positivity rates ranging from 4% to 33.3% and marked differences among sites ($p = 0.001$) (Table 3). Additionally, further analysis of PCR positivity across breeds, seasons, and genders, revealed no statistically significant differences

($p = 0.055$, 0.077 and 0.117, respectively). For different age groups, the PCR positivity was highest in cats aged 1–5 years (34.2%, 95% CI: 19.13–49.29) and lowest in those over 5 years (15.6%, 95% CI: 3.04–28.21) ($p = 0.164$) (Table 3). Cats diagnosed with influenza or gastroenteritis had a higher PCR positivity than those without these diseases ($p = 0.041$ and $p = 0.013$, respectively; Table 3). Likewise, the PCR positivity among cats that had not received vermifuge in the past three months was significantly higher than that among cats that underwent regular deworming ($p < 0.001$; Table 3).

Table 3. Univariable of risk factors associated with *Anaplasma* spp. in cats.

Factors	(Positive No./ Total No.)	PCR Positivity (%) 95% CI	OR 95% CI	*p*-Value
Sampling sites [a]				0.001
Pet clinic B	33.3 (22/66)	33.3 (21.96–44.71)	1	
Pet clinic A	22.6 (21/93)	22.6 (14.08–31.08)	0.583 (0.288–1.182)	0.133
Pet clinic C	6.7 (3/45)	6.7 (0–13.95)	0.143 (0.040–0.513)	0.001
Pet clinic D	4 (1/25)	4 (0–11.68)	0.083 (0.011–0.657)	0.004
Time of sampling [a]				
2017–2018	20.9 (27/129)	20.9 (13.91–27.95)	-	
2025	20.0 (20/100)	20.0 (12.16–27.84)	0.944 (0.494–1.806)	0.863
Breed [a]				
Purebred	27.5 (22/80)	27.5 (17.72–37.28)	-	
Cross bred	16.8 (25/149)	16.78 (10.78–22.78)	0.532 (0.277–1.020)	0.055
Age groups [b]				0.164
≤1year	22.2 (24/108)	22.22 (14.38–30.06)	0.549 (0.245–1.234)	0.144
1–5 year	34.2 (13/38)	34.21 (19.13–49.29)	1	
>5 year	15.6 (5/32)	15.63 (3.04–28.21)	0.356 (0.111–1.143)	0.076
Unknown	9.8 (5/51)	9.8 (1.64–17.97)		
Season [a]			0.212	
Spring (March–May)	25.0 (33/132)	25 (17.61–32.39)	1	
Summer (June–August)	15.22 (14/92)	15.22 (7.88–22.56)	0.538 (0.270–1.076)	0.077
Autumn (September–November)	0.00 (0/4)	0.00		
Winter (December–Feburary)	0.00 (0/1)	0.00		
Gender [b]				
Male	18.3 (23/126)	18.25 (11.51–25.00)	0.595 (0.310–1.142)	0.117
Female	27.3 (24/88)	27.27 (17.97–36.58)	1	
Unknown	0.00 (0/15)	0.00		
Influenza [c]				
Yes	53.8 (7/13)	53.85 (26.75–80.95)	1	
No	23.3 (34/146)	23.29 (16.43–30.14)	0.260 (0.082–0.827)	0.041
Unknown	8.6 (6/70)	8.57 (2.01–15.13)		
Gastroenteritis [c]				
Yes	71.4 (5/7)	71.43 (37.96–100)	1	
No	23.7 (36/152)	23.68 (16.93–30.44)	0.124 (0.023–0.667)	0.013
Unknown	8.6 (6/70)	8.57 (2.01–15.13)		
Vermifuge used or not [b]				
No	58.8 (20/34)	58.82 (42.28–75.37)	1	
Yes	23.4 (22/94)	23.4 (14.84–31.96)	0.214 (0.093–0.492)	<0.000
Unknown	5.0 (5/101)	4.95 (0.72–9.18)		
Total	20.5 (47/229)	20.52 (15.29–25.76)		

[a] Sampling record.; [b] Chief complaint; [c] Veterinarian diagnosis.

As shown in Table 2, co-infection with *A. ovis*, *A. platys*, and *A. capra* was not detected in any of the cats. Mixed infections were only found between *A. ovis* and *A. capra* (3.5%, 8/229) and between *A. ovis* and *A. platys* (1.7%, 4/229), resulting in an overall mixed PCR positivity of 5.2% (12/229).

3.2. Phylogenetic Analysis

Regarding *A. capra*, a total of 12 positive samples were identified based on the amplification of at least one target gene, including 5 positive for *gltA*, 11 for *msp4*, and 1 for *groEL*. Sequence analysis of *gltA* revealed two distinct sequence types: one (comprising three isolate sequences, MW520362) exhibited 100% identity with *A. capra* isolates from China, which have been previously detected in humans (KM206274), sheep (MG869277), dogs (MK838608), and ticks (KX987362) (Figures 1 and S1). The other type (represented by two isolate sequences, MW520361) displayed 99.7% identity with human-derived *A. capra* isolates (KM206274) (Figure S1). Phylogenetic analysis based on *gltA* placed both sequence types within a monophyletic clade of *A. capra*, supporting their close affinity to this species while distinguishing them from other established *Anaplasma* species (Figure 1). All *msp4* gene sequences of *A. capra* obtained in this study (MW520360) exhibited 100% identity. The results showed that these sequences shared 100% identity with those isolated from humans (KM206277), dogs (MK838607), goats (OP974547), sheep (KX417357), and ticks (KR261641), but only 79.4% similarity with the sequence isolated from takin (MH192358) (Figure S1). Correspondingly, in the phylogenetic tree, the variants from cats, dogs, humans, goats, sheep, and ticks clustered together on the same evolutionary branch, which was distinct from that of the takin isolate. All these sequences formed a clade corresponding to *A. capra*, clearly separate from other *Anaplasma* species (Figure 1). For the *groEL* gene, only one sequence was acquired in this study. It shared 100% identity with the sequence from a dog in China (MK862099), and 99.9% identity with sequences obtained from humans (KM206275), Persian onager (MZ222248), sheep (KX417341), and ticks (KX987393, PQ249220) (Figure S1). Phylogenetic analysis based on *groEL* sequences revealed that the isolate clustered within the *A. capra* clade but was distinct from other *Anaplasma* species (Figure 1).

Regarding the *msp4* gene of *A. ovis*, five distinct sequences (MW520355–MW520359) were obtained from cats in the present study. Among these, only one sequence (MW520355) showed 100% similarity with a human-derived isolate from Cyprus (FJ460443). The sequence MW520356 differed by a single SNP (C→T at position 366) compared to the human reference (FJ460443). Sequences MW520357 (C→A at position 286 and C→T at position 470) and MW520358 (C→T at positions 366 and 470) each contained two SNPs relative to FJ460443. Another sequence, MW520359, exhibited three SNPs (C→T at 366, C→T at 470, and C→T at 537). With the exception of MW520355, the other four sequences shared 96.9–99.8% similarity with reported sequences of *A. ovis* from humans (FJ460443), goats (MZ502498), camels (MN094835), cattle (MW535731), dogs (KX236056), and ticks (MF346331) (Figure S1). Phylogenetic analysis revealed that the *A. ovis* sequences obtained in this study formed a well-defined branch, distinct from other established *Anaplasma species* (Figure 1).

Based on 16S *rRNA* gene sequence analysis, all *A. platys* sequences obtained in this study were identical (PV834200) and shared 100% identity with cattle (MZ148474) and tick (MZ230571) isolates from China, clustering with them on the same phylogenetic branch (Figure 1).

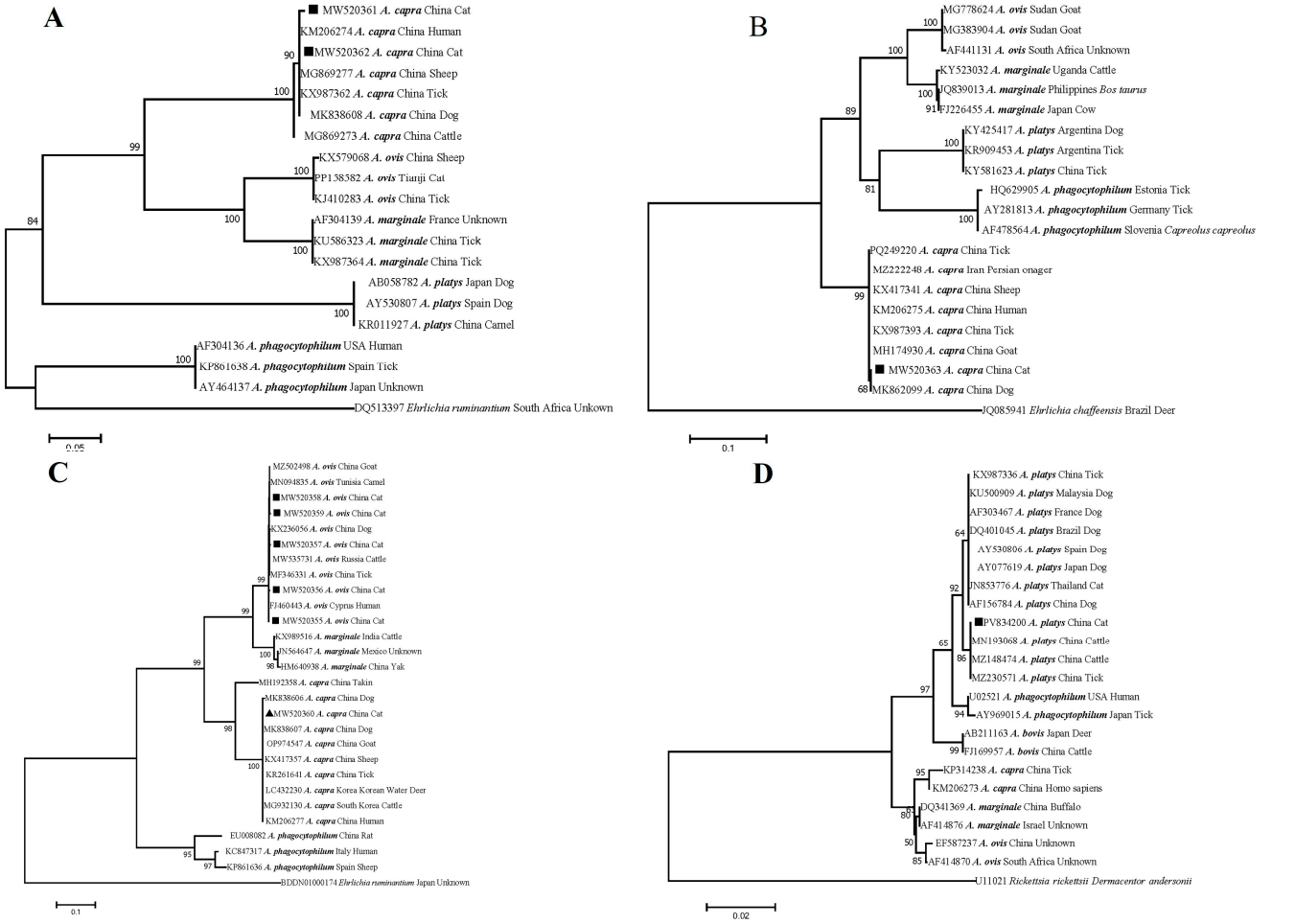


Figure 1. Phylogenetic analyses of *A. capra* based on *gltA* (**A**), *groEL* (**B**), and *msp4* (**C**); of *A. ovis* based on *msp4* (**C**); and of *A. platys* based on 16S *rRNA* (**D**). Phylogenetic trees were constructed using the neighbor-joining method with the Kimura 2-parameter model and 1000 bootstrap replicates. Numbers at branch nodes indicate the percentage of bootstrap replicates that reproduced the topology of each clade (only values > 50% are shown). Sequences generated in this study are indicated by black squares and triangles. Sample labels consist of GenBank accession numbers followed by the corresponding *Anaplasma* species names. *Ehrlichia ruminantium*, *Ehrlichia chaffeensis*, and *Rickettsia rickettsii* were used as outgroups.

4. Discussion

Currently, dogs and cats remain the most popular companion animals in China. According to 2024 statistics, 64.3% of first-time pet owners chose cats, while 57% opted for dogs (2025 China Pet Industry White Paperps, https://pdf.dfcfw.com/pdf/H3_AP202512111798269901_1.pdf) (accessed on 1 November 2026). Furthermore, from a "One Health" perspective, pet cats not only act as direct sentinels for human infections due to their close proximity but may also facilitate zoonotic transmission via intense contact and a shared living environment [4,28]. *Anaplasma*, a significant zoonotic pathogen, is transmitted through vectors. While there have been documented cases of this pathogen in dogs across China, reports of its occurrence in cats remain scarce [3,23,29–33]. To our knowledge, this study documents *Anaplasma* spp. infection in domestic pet cats in Henan and provides molecular detection frequency of *A. capra*, *A. ovis* and *A. platys* in cats from four veterinary hospitals in Zhengzhou City. The overall molecular detection frequency of *Anaplasma* spp. was 20.5% (47/229, 95% CI: 15.29–25.76), indicating a considerable exposure risk in this region. Notably, species-specific PCR positivity varied substantially: *A. ovis* was the most prevalent

(17.5%, 40/229), followed by *A. capra* (5.2%, 12/229) and *A. platys* (3.1%, 7/229), with statistically significant differences among them.

A. capra was first isolated from asymptomatic goats and subsequently found in patients with a history of tick bites in northern China [1,9,34]. Subsequently, *A. capra* has been detected worldwide across a broad spectrum of hosts, including domestic animals (e.g., goats, sheep, cattle, yaks, and dogs), wildlife (e.g., takins, muntjacs, water deer, musk deer, onagers, serows, and brown hares), and various tick species (e.g., *I. persulcatus*, *H. longicornis*, *H. qinghaiensis*, *D. abaensis*, *D. nuttalli*, and *R. microplus*) [32,34–41]. However, there have been no reports of *A. capra* infection in cats to date. In this study, *A. capra* was detected in 12 of 229 (5.2%) domestic cat samples, suggesting that cats are susceptible to *A. capra*; however, their epidemiological role remains unknown.

A. ovis is considered a tick-borne pathogen of small ruminants and causes ovine anaplasmosis [42]. The pathogen was first reported in sheep in 1912, and subsequently described in sheep, goats and some wild ruminants, cattle and dogs throughout the world [3,19,29,42–45]. Recently, an *A. ovis* variant was detected in a patient, indicating the zoonotic potential of this agent [8,46]. Furthermore, *A. ovis* had been detected in some vectors such as ticks (e.g., *D. nuttalli*, *D. silvarum*, *H. longicornis* and *H. qinghaiensis*), *Melophagus ovinus* and *Lipoptena cervi* [46–48]. *A. ovis* was first detected in a clinically healthy cat in Tianjin of China in 2021, with the PCR positivity 0.6%, which is markedly lower than PCR positivity observed in the present study (17.5%). Differences in the study population and sample size may be potential factors underlying this contrast.

A. platys was primarily considered a canine pathogen that causes a decrease in platelet count in infected dogs [49]. Nowadays, this pathogen has been identified in a variety of animals, such as cattle, goats, sheep, camels, foxes, wild boars, and red deer [50–53]. It was not until 2013 that the first DNA sequence-confirmed human case of *A. platys* infection was reported, involving a veterinarian [17]. Subsequently, the pathogen was detected in a doctor near Chicago, Illinois, as well as in the doctor's daughter and the family dog—all three of which shared completely identical genetic sequences [16]. Furthermore, *A. platys* was detected in 2014 in two women from Venezuela with a history of peridomestic tick exposure [15]. From then on, *A. platys* has been regarded as a potential zoonotic pathogen [50]. To date, sporadic cases of *A. platys* infection in cats have been documented in North America, Brazil, and Cyprus [54–57]. Subsequently, the identification of both *A. platys*-like and *A. platys* strains has been successively reported in feline populations across Italy, Chile, Turkey, and Saudi Arabia [21,58–60]. In this context, the present study provides, to our knowledge, the first evidence of *A. platys* infection in domestic cats in China, demonstrating an overall PCR positivity of 3.1% (7/229). This PCR positivity is comparatively higher than that reported in Saudi Arabia (2.27%) yet lower than the rates observed in free-roaming domestic cats from Chile (6.6%, 2/30) and household cats from Turkey (30.5%) [21,59,60]. These interregional discrepancies are likely attributable to variations in sampling time, geographic location, rearing practices, and sample size.

Since *Anaplasma* species are transmitted by arthropod vectors, which can carry multiple pathogens, mixed infections are expected to be frequent. However, in our study, no cat was found to be co-infected with all three agents (*A. ovis*, *A. platys*, and *A. capra*); rather, we observed 12 cases of dual infection, yielding an overall mixed-PCR positivity of 5.2% (12/229). This figure is marginally higher than the co-infection rate previously reported in stray dogs in Anhui, China [30]. These findings underscore that, in the context of feline anaplasmosis diagnosis and control, the clinical manifestations resulting from mixed infections deserve due attention, as they may inform targeted prevention strategies.

Sequence analysis of *gltA* and *msp4* sequences of *A. capra* from our feline samples (MW520362, MW520360) showed 100% identical to isolates from humans, sheep, dogs, goats,

and ticks, while the *A. ovis* msp4 sequence (MW520355) matched a human-derived isolate from Cyprus [1,8,32,35,61–63]. These partial-marker similarities raise public-health vigilance; nevertheless, complete genome comparisons are required to definitively evaluate whether these feline strains are genetically indistinguishable from human-origin zoonotic isolates.

5. Conclusions

In conclusion, this study provides the first molecular evidence documenting *A. capra* infection in domestic cats from China. Three *Anaplasma* species, namely *A. ovis*, *A. capra*, and *A. platys*, were detected in feline blood samples by nested PCR, with dual-species co-infections observed among sampled cats. Partial gene sequences of feline-origin *A. capra* and *A. ovis* shared high nucleotide identity with previously reported zoonotic isolates from humans, animals and ticks. Nevertheless, the epidemiological role of domestic cats, their reservoir competence for these *Anaplasma* pathogens, and the actual risk of pathogen transmission from cats to humans remain unknown. Further investigations including full-genome sequencing, experimental infection studies and field-based epidemiological surveillance are required to address these open questions. These findings highlight the necessity to conduct routine molecular surveillance of *Anaplasma* pathogens in companion domestic cats in urban China.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/vetsci13111229/s1, Figure S1. Homology analyses of *Anaplasma* species based on various genetic markers. (A–C) *A. capra* based on *gltA*, *msp4*, and *groEL* sequences, respectively; (D) *A. platys* based on 16S *rRNA* sequences; (E) *A. ovis* based on *msp4* sequences.

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Institutional Review Board Statement: Ethical approval for this study was granted by the Independent Ethics Committee of Henan Agricultural University on 26 March 2017 (Reference No. IEC-HENAU-20170326-428-08). Before sample collection, each cat owner was informed of the purpose of the study, the sampling procedure, and the intended use of the data. Verbal informed consent was obtained from each owner, and all animals were enrolled with the owner's prior approval.

Informed Consent Statement: Informed consent was obtained from all animal owners involved in the study.

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

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