

RESEARCH ARTICLE

Molecular Detection and Therapeutic Evaluation of *Anaplasma platys* Infection in Domestic and Stray Dogs in Jhang district, Pakistan

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ABSTRACT

Anaplasma platys, a tick-borne pathogen, infects canines, livestock, and occasionally humans worldwide. A total of 384 cephalic blood samples were obtained from domestic dogs (n=230) and stray dogs (n=154) for the detection of *A. platys* infection using PCR (16S rRNA) from district Jhang. The information regarding host and management was collected on a pre-tested questionnaire. The present study revealed an overall prevalence of *A. platys* 10.16% (39/384) comprising domestic dogs (7.42%) and stray dogs (15.62%). The logistic regression analysis revealed health status as the significant risk factor. For the therapeutic clinical trial, the domestic dogs were selected and randomly separated into five groups containing five dogs each. The animals of Group 1 (n=5) received doxycycline @10mg/kg, PO (21 days). The dogs of Group 2 (n=5) were given enrofloxacin at the rate of 2.5mg/kg, IM for 21 days. The dogs of Group 3 (n=5) were given doxycycline (10mg/kg) PO and prednisolone at the dose rate of 3 mg/kg body weight PO for 21 days. Group 4 (n=5): enrofloxacin 2.5mg/kg IM for 21 days IM and prednisolone 3mg/kg body weight PO for 21 days. A substantial increase of platelets and packed cell volume was noticed with the recovery rate of 60, 40, 100, and 80% for Groups I, II, III and IV; respectively. It can be concluded that canine anaplasmosis is endemic in dogs of Jhang, Pakistan. Doxycycline combined with prednisolone showed greater hematological improvement compared with the other treatment regimens evaluated in this study.

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INTRODUCTION

Dogs have historically been utilized for guarding, fighting, hunting, and herding sheep and goats. With time, their roles have diversified, and they are now widely kept as companion animals and used by security agencies as sniffer dogs for the detection of narcotics and stolen property. Because of their multifaceted roles and loyal services, dogs are commonly regarded as “man’s best friend.” (Justiniane *et al.*, 2025). Ticks and tick-borne diseases are a major concern for dogs. The stray dogs may be a source of the spread of tick-transmitted pathogens to domestic dogs and humans. Canine anaplasmosis caused by *A. platys* is considered an emerging disease of dogs. *A. platys* is an obligatory intracellular gram-negative tick-

borne pathogen that belongs to family *Anaplasmataceae* and order *Rickettsiales*. This pathogen was first identified in Florida (USA) and described as a Rickettsia-like, platelet specific organism in dogs, formerly known as *E. platys* (Dyachenko *et al.*, 2012). Replicating *A. platys* infection directly damages the platelets and develops thrombocytopenia followed by the appearance of the pathogen in platelets, which is a cyclic process. Later, parasitemia disappears, and subsequent thrombocytopenic episodes develop immune-mediated thrombocytopenia. The immune system loses tolerance to self-antigens and acts against its own platelets causing destruction and rapid decrease in circulating platelets (Atif *et al.*, 2021). Generally, clinical manifestations such as depression, anorexia, lymph adenopathy, pale mucous membrane, and

elevated temperature are encountered during infection (Dumler *et al.*, 2001). The microorganism is usually spread by the *Rhipicephalus (R.) sanguineus*, known as brown dog tick. The brown dog tick has the ability to adapt to the environment quickly. The *A. platys* infection has worldwide distribution, this has been reported from various countries/regions such as Taiwan (Nantou County) 11.43% (Yuasa *et al.*, 2017), India (Punjab) 57.3% (Kumar *et al.*, 2026), Thailand (Central and Northeast) 10.8% (Purisarn *et al.*, 2022); Pakistan 6.67% (Faisalabad, Islamabad, and Okara) (Alvi *et al.*, 2025); China (Hainan) 6% (Lin *et al.*, 2023), Egypt (Cairo) 6.5% (Selim *et al.*, 2021); Algeria (Algiers) 14.6% (Azzag *et al.*, 2015); Kenya (Northern part) 15.9% (Matei *et al.*, 2017); Italy 3.5% (De la Fuente *et al.*, 2006), Mediterranean Basin (Bouzouraa *et al.*, 2016); Mexico (Veracruz) 27% (Jeréz-Sulvarán *et al.*, 2024); Argentina (Eastern Salta) 10.91% (Rios *et al.*, 2026); Brazil (Rio Grande do Norte) 21.5% (Araújo *et al.*, 2025) and Australia 45.45% (Martin *et al.*, 2005). Area, host, targeted genes, and test method used; determine the occurrence of anaplasmosis (Ahmed *et al.*, 2020; Hassan *et al.*, 2024; Atif *et al.*, 2025a). Exposure to ticks and climatic conditions are the risk factors associated with *A. platys* infections (Pérez-Macchi *et al.*, 2019). Global warming makes the environment suitable for ticks' propagation (Ramzan *et al.*, 2021). Diagnosis is generally based upon the presence of basophilic inclusion (morulae) in platelets or buffy coat smears (Silaghi *et al.*, 2017). But blood smear has low sensitivity due to the cyclic nature of infection. Serological tests are preferred to blood smear but there are issues of false positive or negative results as well as they also do not differentiate the clinical or carrier infections (Atif *et al.*, 2021). Nonetheless, PCR has higher sensitivity and specificity. Chemotherapy is important to control infection and mortality. Most of the earlier studies used doxycycline and/or dexamethasone/prednisolone against tick-borne diseases associated with immune-mediated hemolytic complications (Jayatilaka *et al.*, 2018; Milošević *et al.*, 2024) but aim of our study is to use doxycycline with prednisolone. Dexamethasone is indicated for *A. platys* infection and co-infected with *Bartonella henselae* and *Candidatus Mycoplasma haematoparvum* (Gaunt *et al.*, 2010; Maggi *et al.*, 2013; Atif *et al.*, 2021). Dexamethasone is a powerful, long-acting glucocorticoid with a higher possibility of causing adverse effects. Its prolonged action and high glucocorticoid activity increase the risk of serious snags, particularly with long-term use and higher dose rate. Major adverse effects include adrenal suppression, severe psychiatric disturbances, osteonecrosis and immunosuppression that can increase susceptibility to infections. As far as we can ascertain, first integrated study that creates a baseline epidemiological and molecular data on *A. platys* in dogs from Jhang District, Punjab, Pakistan. Moreover, the present study compares infection prevalence and associated risk factors between owned and stray dogs. Molecular confirmation is usually required through PCR for more accurate detection of *A. platys*, especially in dogs with low or fluctuating bacteremia. Therapeutic evaluation provides evidence on the effectiveness of treatment in relation to clinical and hematological parameters for the assessment of *A. platys*

infection in dogs. Therefore, the study has been planned to assess the epidemiological status, risk factors, and therapeutic evaluation of *A. platys* in stray and domesticated dogs from Jhang, Pakistan.

MATERIALS AND METHODS

Study area: The present study was conducted in the Jhang district, Pakistan from March 2020 to March 2021. The study area is located in the central Punjab, having four tehsils viz., Jhang, Athara Hazari, Shorkot, and Ahmadpur Sial. The study district is situated on the left and right banks of river Chenab at latitude 30.98° (North) and longitude 72.25°. The meteorological conditions of district Jhang are comparable to desert which ranges from 5°C to 40°C temperature with 248mm of annual rainfall (Fig. 1).

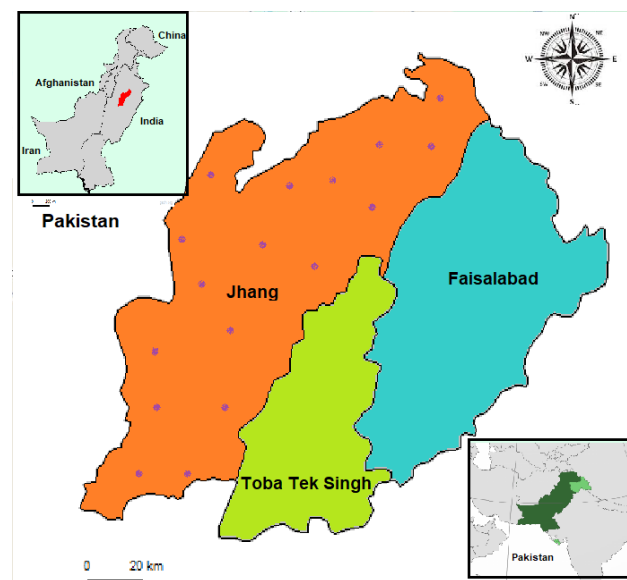


Fig. 1: Map of the Jhang district, Pakistan (orange colour), indicating sampling sites (purple color). The raw maps were retrieved from www.gadm.org.

Sample size and source of samples: A total of 384 blood samples were obtained from domestic dogs (n=230) and stray dogs (n=154) originating from different breeds, age and sex for the detection of *A. platys* infection as described by Thursfield (2007) assuming 50% prevalence at a confidence level of 95% with a desired precision of $\pm 5\%$.

The blood samples were aseptically collected in a 3mL disposable plastic syringe from cephalic vein and preserved in EDTA coated blood vacutainers. The samples were transferred in an icebox containing ice packs and brought to the Post-graduate Laboratory of Medicine, Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan for further processing. The samples were restored at 4°C for 4 to 7 days or at -20°C.

Detection:

DNA extraction: The DNA was extracted by using DNA extraction kit (Gene JET, Whole Blood Genomic DNA Purification Mini Kit of Thermo Scientific; Catalogue No. K0781), following the procedure as described by the manufacturer. A blood sample (200μL) was taken in a

1.5mL microcentrifuge tube with proteinase K and lysis solution. The mixture was incubated at 56°C for 10min to ensure cell lysis then ethanol 95% was added. The mixture was transferred into the genomic column and passed through washing solutions (wash buffer-1 and wash buffer-2) for removal of debris. Finally, the DNA was eluted in a micro centrifuge tube by centrifugation after the addition of 200µL elution buffer. The elution step was repeated to enhance the yield and quality of the extracted DNA. The DNA integrity and approximate concentration of the extracted DNA was assessed with the help of Gel electrophoresis. The DNA samples were stored at -20°C for further processing.

Polymerase chain reaction: For the detection of *A. platys*, *16S rRNA* was targeted through PCR by using specie specific-primers including the forward 5'-GATTTTGTCTAGCTTGCTATG-3' and the reverse primer 5'-TAGCACTCATCGTTTACAGC-3' as mentioned by Carvalho *et al.* (2017) and Jamil *et al.* (2024). The PCR was performed in a Thermal Cycler T100 (Bio-Rad, USA). A known positive control (obtained from a verified clinical case) and negative control (laboratory-grade nuclease-free water) of *A. platys* genomic DNA was mostly added in each run. The PCR mixture of 25µL consisted of a DNA sample (5µL), Primers (2µL) each, MgCl₂ (4µL), dNTPs (4µL), Taq-DNA polymerase (1µL), Taq-buffer (5µL) and nuclease free water (2µL). A total of 30 cycles were run with initial denaturation at 95°C for 5min. followed by denaturation at 94°C for 40sec (40 cycles), annealing at 56°C for 40sec and extension at 72°C for 30sec and final extension at 72°C for 5min. The amplified PCR product was either visualized or stored in a refrigerator at -20°C.

Gel electrophoresis: For visualization of the PCR product, the electrophoresis was performed with 1.5% agarose gel. The gel was loaded with 4µL of PCR product mixed with 2µL of 6X DNA loading dye and run in 1X TAE buffer for 30min at 120 volts. A ladder of 50bp was also run along with PCR products for comparison. Our gene of interest was amplified, and a PCR band length of 349bp was visualized on UV illuminator.

Estimation of risk factors: A pre-tested questionnaire including both open and close ended questions was used to collect information regarding area (Jhang, Athara Hazari, Shorkot, and Ahmadpur Sial), breed (Pakistani Shepard, German Shepherd, Labrador Retriever and Non-descriptive local), sex (male/female) and age (<1 years, 1-4 years, > 4 years), dog density (1-2, 3-5, >5), veterinary care (intensive, moderate, least), health status (excellent, good, fair and poor), tick infestation (yes/no), from domestic and stray dogs. A pre-tested questionnaire was used for the evaluation of factors and their levels of validity.

Chemotherapeutic clinical trial: For chemotherapeutic trials, male dogs of 1-2 years of age were selected and screened through PCR. After confirmation of their disease status, dogs were alienated into 5 groups, containing five animals each. The domestic male dogs who expressed clinical signs of fever lethargy pale mucus membrane

petechial hemorrhages, epistaxis, lymphadenopathy and PCR positive were included in the study. The dogs identified with any major infectious or non-infectious disease, other than anaplasmosis, were omitted from the study. The following drugs were administered to *A. platys*-infected dogs for the evaluation of therapeutic efficacy.

Group 1 (n=5): Doxycycline (Vibramycin, Pfizer) @10 mg/kg PO for 21 days.

Group 2 (n=5): Enrofloxacin (Encure 10%, Nawan Laboratories Pvt. Ltd) @ 2.5mg/kg IM (21 days).

Group 3 (n=5): Doxycycline @10mg/kg, PO (21 days) plus prednisolone (Deltasol, Progressive Laboratories Pakistan Pvt. Ltd.) PO at the dose rate of 3mg/kg for 21 days.

Group 4 (n=5): Enrofloxacin 2.5mg/kg IM (21 days) and Prednisolone 3mg/kg body weight PO for 21 days.

Group 5 (n=5): Healthy control.

The experiment was conducted at dog shelter house in the Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan for the period of 21 days.

Evaluation parameters: The complete blood counts and clotting time were monitored along with retrieval of clinical signs to determine the animal recovery (Wongtawan *et al.*, 2024).

Statistical analysis: The prevalence was statistically analyzed with Chi-square to associate risk factors. Logistic regression was performed to evaluate the risk factors. The two-way repeated-measures ANOVA and t-test was performed to evaluate therapeutic trial using statistical software SPSS-22. The *P*-value less than or equal to 0.05 was pondered significant.

RESULTS

The study was conducted to quantify the molecular epidemiology of *A. platys* in stray and domesticated dogs from four tehsils, viz., Jhang Sadar, Athara Hazari, Shorkot, and Ahmadpur Sial of district Jhang, Punjab, Pakistan. During the current study the overall prevalence of *A. platys* based on PCR was 10.16% (39/384). The amplification of *16S rRNA* gene of *A. platys* yielded a 349bp product (Fig. 2). Out of 384 samples, 169, 84, 68, and 63 samples were collected from Jhang, Athara Hazari, Shorkot and Ahmadpur Sial tehsils, respectively from dogs. Overall prevalence of *A. platys* in district Jhang was 10.16% (39/384). Tehsil-wise prevalence was highest in Athara Hazari 16.67% (14/84), followed by Jhang 10.06% (17/169), Ahmadpur Sial 7.94% (5/63); and Shorkot 4.41% (3/68). The Chi square analysis revealed a statistically not significant association among dogs of district Jhang ($X^2=6.70$, $df=3$, $P=0.082$).

With respect to dog type, the infection of *A. platys* in stray dogs 15.63% (20/128) and domesticated dogs 7.42%

(19/256) had a significant association ($X^2=6.29$, $df=1$, $P=0.012$). Breed-wise occurrence of canine anaplasmosis indicated that the infection was highest in German shepherd 17.78% (16/90), followed by Labrador retriever 12.50% (6/48); Pakistani shepherds 10.19% (11/108), and non-descriptive dog breeds 4.35% (6/138). The statistical analysis revealed that the breed variable had a significant effect ($X^2=11.12$, $df=3$, $P=0.011$). The host determinant of sex revealed that infection was highest in female (12.05%, 20/166) compared to male (8.72%, 19/218) with a non-significant association ($X^2=1.147$, $df=1$, $P=0.28$). The age-wise results of the present study elaborated that highest positivity was noticed in dogs of less than 1 year of age 13.89% (20/144) followed by 1-4 years 11.11% (12/108) and more than 4 years of age 5.30% (7/132). The statistical analysis revealed a non-significant associated with respect to age ($X^2=5.71$, $df=2$, $P=0.057$). Nonetheless, health status ($X^2=18.537$, $df=3$, $P=0.000$) had a significant association with the occurrence of *A. platys* infection in dogs.

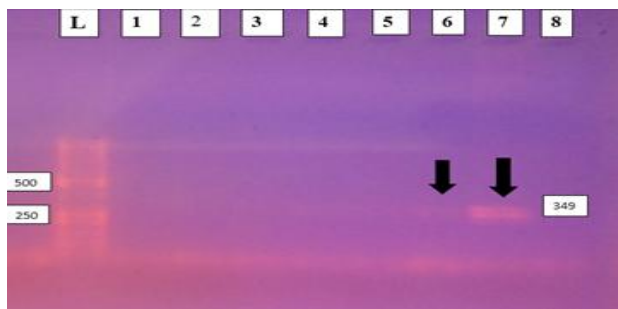


Fig. 2: Gel image of *Anaplasma platys* showing band on 349 base pair length in well 7. Whereas, "L" is ladder of 50 bp length.

The results of the current study regarding environmental determinants elaborated that the infectivity of *A. platys* was highest in dogs, where dog density was more than 5 (27.08%, 13/48), followed by 3-5 (10.61%, 14/132) and 1-2 per house/location (5.88%, 12/204). The

statistical analysis expounded that the frequency of anaplasmosis was significantly associated with the number of dogs. Similarly, veterinary services and tick infestation expressed a significant association with the existence of *A. platys* infection in dogs (Table 1).

To evaluate the multicollinearity and determinants of canine cyclic anaplasmosis, the linear and logistic regression were performed, respectively. The variables of area (Jhang, Athara Hazari, Shorkot, and Ahmadpur Sial), breed (Pakistani Shepard, German Shepherd, Labrador Retriever, and Non-descriptive local), sex (male and female), age (<1 year, 1-4 years and >4 years), and veterinary care (intensive, moderate, leas) showed collinearity among various variables.

Among the variables assessed based on univariate analysis, only health status was expressed as a significant risk factor for canine cyclic thrombocytopenia ($P=0.035$). The positive regression coefficient ($\beta=1.005$) and odds ratio (2.732) suggested that dogs with poor or compromised health were nearly 2.7 times more likely to develop disease than healthy dogs. This finding was further supported by the confidence interval @ 95% (1.072–6.964), confirming statistical significance. In comparison, dog density was not significantly related to disease occurrence ($P=0.799$). Although the odds ratio (1.151) was more than 1 with a broad confidence interval (0.390–3.397) along with a non-significant p-value, it indicated that dog density had no meaningful effect on the probability of infection within the study population. Likewise, dog type had no significant associated with cyclic thrombocytopenia ($P=0.141$). The negative regression coefficient ($\beta=-0.799$) and odds ratio below 1 [$\text{Exp}(\beta)=0.450$] imply a potential protective effect for dog category relative to the reference group; however, because the 95% confidence interval (0.155–1.304) included 1, the association was not statistically significant. (Table 2). In addition, none of the variable was recorded as significant during multivariate logistic regression analysis (Table 3).

Table 1: Prevalence of anaplasmosis in dogs of district Jhang, Pakistan.

Variables	Categories	Total No. of animals tested	No. of positive animals	Prevalence (%)	χ^2	P-value
Area	Jhang	169	17	10.06	6.70	0.082
	Athara Hazari	84	14	16.67		
	Shorkot	68	3	4.41		
	Ahmadpur Sial	63	5	7.94		
Breed	Pakistani	108	11	10.19	11.12	0.011
	German Shepard	90	16	17.78		
	Labrador Retriever	48	6	12.50		
	Non-descriptive	138	6	4.35		
Sex	Male	218	19	8.72	1.147	0.284
	Female	166	20	12.05		
Age	<1 year	144	20	13.89	5.71	0.057
	1-4 years	108	12	11.11		
	>4 years	132	7	5.30		
Dog density	1-2	204	12	5.88	19.18	0.000
	3-5	132	14	10.61		
	>5	48	13	27.08		
Veterinary services	Moderate	87	4	4.60	7.686	0.021
	High	119	9	7.56		
	Least	178	26	14.61		
Health status	Excellent	79	2	2.53	18.54	0.000
	Good	123	7	5.89		
	Fair	117	16	13.68		
	Poor	65	14	21.54		
Tick infestation	Yes	123	24	19.51	17.36	0.000
	No	261	15	5.75		
Type of dog	Domestic	256	19	7.42	6.29	0.012
	Stray	128	20	15.63		

Table 2: Univariate logistic regression analysis to evaluate the risk factors associated with canine cyclic thrombocytopenia in district Jhang, Pakistan.

Variable	β	S.E.	Wald	df	Sig.	Exp (β)	95% C.I. Lower	95% C.I. Upper
Dog density	0.141	0.522	0.65	1	0.799	1.151	0.390	3.397
Health status	1.005	0.477	4.434	1	0.035	2.732	1.072	6.964
Type of dog	-0.799	0.543	2.164	1	0.141	0.450	0.155	1.304
Constant	-4.052	0.649	38.962	1	0.000	0.017	—	—

C.I.= Confidence interval; S.E.= Standard error; β = Regression coefficient**Table 3:** Multivariate analysis to evaluate the risk factors associated with canine cyclic thrombocytopenia in district Jhang, Pakistan.

Variable	β	S.E.	Wald	df	Sig.	Exp (β)	95% C.I. Lower	95% C.I. Upper
Health status	—	—	14.025	3	0.003	—	—	—
Good	-3.128	0.954	10.738	1	0.001	0.044	0.007	0.285
Fair	-2.285	0.741	9.499	1	0.002	0.102	0.024	0.435
Poor	-0.959	0.525	3.338	1	0.068	0.383	0.137	1.072
Type of dog (Stray)	-0.770	0.554	1.930	1	0.165	2.159	0.729	6.395
Constant	-1.293	0.302	18.962	1	0.000	0.275	—	—

C.I.= Confidence interval; S.E.= Standard error; β = Regression coefficient**Table 4:** Evaluation of hematological parameters before and after treatment in *A. platys* infected and control dogs.

Drug	Parameters	0 day (Before treatment)	7 th day (Post-treatment)	14 th day (Post-treatment)	21 th day (Post-treatment)	Healthy (control)	non-treated
Doxycycline	PCV (%)	32.4±0.51	34.0±0.84*	36.8±0.66**	41.8±1.5**	44.6±0.51**	
	Platelets($\times 10^6$ /ul)	79.2±1.39	125.8±1.59*	174.8±2.35**	218.2±4.18**	255.8±4.44**	
Enrofloxacin	PCV (%)	33.2±0.58	35.2±0.73*	36.4±0.87**	41.2±0.86**	44.6±0.51**	
	Platelets($\times 10^6$ /ul)	83.2±1.24	114.8±6.55*	161.8±1.88**	209.6±2.11**	255.8±4.44**	
Doxycycline + PCV		31.2±0.66	35.4±0.68*	39.2±1.07**	44.2±0.80**	44.6±0.51**	
Prednisolone	Platelets($\times 10^6$ /ul)	71.2±1.52	186.4±2.33*	214.8±2.85**	253.6±3.78**	255.8±4.44**	
Enrofloxacin + PCV		32.2±1.30	35.2±1.30*	37.2±1.79**	42.2±0.8**	44.6±0.51**	
Prednisolone	Platelets ($\times 10^6$ /ul)	75.6±1.43	161.2±2.51*	197.8±1.06**	242.8±1.46**	255.8±4.44**	

*P<0.05; **P<0.001

Importantly, the drug $\times$ time interaction was highly significant, $F(9,48)=44.30$, $P<0.001$. This means that the pattern of platelet recovery over time differed significantly among the four treatment groups. The largest increase was observed with doxycycline+prednisolone, from 71.2 ± 3.4 at day 0 to $253.6\pm8.5\times10^6/\mu\text{L}$ at day 21. The Enrofloxacin+Prednisolone group also showed substantial recovery. The rate of recovery of dogs during the therapeutic trial was highest on day 21. The dogs with significant improvement in clinical signs and platelets counts were considered cured. Based on the results of the treatment trials revealed 60, 40, 100, and 80% recovery rate for Group I, Group II, Group III and Group IV, respectively (Table 4).

DISCUSSION

Anaplasma platys is the major etiological agent of canine cyclic thrombocytopenia in dogs. Limited studies are available on this important pathogen in Pakistan. The current study revealed a 10.16% occurrence of *A. platys* in dogs based on PCR targeting the *16S rRNA* gene, with the highest disease frequency in Athara Hazari. The higher existence of disease in Jhang is attributed to poor tick control practices, the incidence of *Rhipicephalus sanguineus* ticks, stray dog population, and higher humidity. Ghauri and colleagues depicted the disease positivity at 11.34% using *16S rRNA*-PCR (Ghauri *et al.*, 2021). Likewise, prevalence in the present study was in agreement with Faizal and colleagues, they reported 11.76% infection of *A. platys* using *groEL*-PCR from Indonesia (Faizal *et al.*, 2019). Whereas, lower PCR-based occurrence was reported from Peninsular-Malaysia 12% (Sipin *et al.*, 2020) and Thailand targeting *16S rDNA* gene with positivity rate of 10.8% (13/120) and with *gltA* gene 5.8% (7/120) (Purisarn *et al.*, 2022). Conversely, higher PCR-based infection of *A. platys* was recorded in

dogs from countries such as Vietnam 25.37% (34/134) targeting *16S rDNA* (Vo *et al.*, 2026), Argentina (Buenos Aires) 37.5% using *16S rRNA* gene (Cicutin *et al.*, 2013), Italy 52.9% (18/34) using *16S rDNA* gene based real-time PCR (Ramos *et al.*, 2014) and Saudi Arabia 57.1% (40/70) using *16S rRNA* gene based real-time PCR (Alanazi *et al.*, 2020). The tick infestation, geographic location, season, dog management, age, environmental conditions, use of acaricides, diagnostic methods influence the occurrence of disease in dog population.

Anaplasma platys chiefly infects platelets, and its typical intracellular inclusions (morulae) may be hard to detect consistently on peripheral blood smears. Besides, the fraction of infected platelets can fluctuate during the organism's cyclic bacteremia. Therefore, a negative microscopic examination does not exclude *A. platys* infection. Conversely, PCR detects *A. platys* DNA and may identify infection even when the organism is present at levels below the threshold required for reliable microscopic detection. Consequently, PCR generally provides greater sensitivity than peripheral blood smear examination for identification of *A. platys* infection. A study conducted by Rucksaken *et al.* (2019) on the comparison of blood smear and PCR from Thailand, documented 0% sensitivity of blood smear and 100% specificity.

Some earlier studies depicted the occurrence of *A. platys* along with other vector-borne diseases. Such as, Hussain *et al.* (2023) collected 224 blood samples from 159 dogs and 65 cats from Hong Kong and further investigated the serological, hematological, and molecular characterization of vector-borne diseases in the summer season. They reported the highest sero-positivity for *E. canis* or *E. ewingii* (10.7%), trailed by *Dirofilaria (D.) immitis* (5.7%) and *A. phagocytophilum* or *A. platys* (2.5%) infection using the SNAP 4Dx Plus test. Molecular analysis further revealed the presence of *Babesia gibsoni*

(3.7%), *E. canis* (3.1%), *D. immitis* (5.7%), and *A. phagocytophilum* (1.3%). Similarly, Aral and collaborators detected four tick-borne pathogens, including *Babesia spp.*, *Ehrlichia canis*, *Hepatozoon canis*, and *A. platys* (16S rRNA), with respective infectivity rates of 26.09, 5.87, 3.52, and 2.93% using PCR. These results were attained from 341 canine blood samples collected in the Kathmandu, Pokhara, and Chitwan regions of Nepal. The overall prevalence of tick-borne infections was 31.09% (Aryal *et al.*, 2025). These studies observed a lower frequency of canine anaplasmosis. The *A. platys* infection has wide host range and geographic distribution. According to the study of Costa-Júnior and co-researchers, the variable of area was a major risk factor in rural dogs from Minas Gerais State, Brazil (Costa-Júnior *et al.*, 2013). The diagnostic technique, area, existence of vectors, use of acaricides, immune status of the study animals and agro-climatic conditions may have credited to the inconstant frequency of disease.

The results of the present study based on chi-square for the determinants of breed, dog density, health services, tick infestation, and type of dog had significant associations. Based on the univariate results health status was the significant risk factor. While none of the variables were found significant based on multivariate regression analysis but this determinant was considered as protective based on negative regression coefficient. The variable of the type of dog is in line with the findings of Aryal *et al.* (2025); they identified stray dogs as one of the potential risk factors associated with the presence of canine tick-borne pathogens (Aryal *et al.*, 2025). The results of the present study based on chi-square are in line with the findings of Aryal *et al.* (2025); they identified dog type (stray or pet) as one of the potential risk factors associated with the existence of canine tick-associated infections (Aryal *et al.*, 2025). Nonetheless, Pérez-Macchi and colleagues mentioned higher dog density and anti-tick treatment as the major risk factor for canine cyclic thrombocytopenia associated with 16S rRNA/p44 gene-based PCR positivity from Asunción, Paraguay (Pérez-Macchi *et al.*, 2019).

Risk factors differ across regions (Atif 2025a,b; Xin *et al.*, 2025). Studies from South and Southeast Asia identified age, sex, tick infestation, lack of tick control methods, and climatic factors (high humidity during monsoon seasons) as the key determinants of tick-borne diseases (Perera *et al.*, 2025; Atif *et al.*, 2025b). Additionally, a Nepali study identified geographic location, dog type (stray or pet), and health status as potential risk factors linked with the occurrence of canine tick-borne pathogens utilizing multiple logistic regression analysis (Aryal *et al.*, 2025). The highly correlated independent variables lead to multicollinearity. This problem was successfully eliminated in the current study, which may lead to unstable coefficient estimates, inflated standard errors, reduced statistical power, sign reverse paradox, and misleading significance (Kim, 2019). The results of the present study based on the univariate regression test identified that the health status of dogs as the significant risk factors for *A. platys* infection. While variable of dog density was not a significant risk factor with a wider confidence interval. In addition, the variable

of type of dog was non-significant and negatively correlated with risk variable, indicating a protective effect compared to the reference category. Furthermore, the multivariate logistic regression revealed none of the variables as a significant risk factor. A study conducted in Lima (Peru) identified sex and weight as the significant risk factor among dogs based on multivariate regression analysis (Ruiz and Velarde, 2024).

The domestic male dogs who expressed clinical signs of fever, lethargy, pale mucus membrane, petechial hemorrhages, epistaxis, and lymphadenopathy were included in the study. It was revealed that dogs of Group-3 treated with doxycycline (Vibramycin) at 10mg/kg BW, PO for 21 days, and prednisolone (Deltasol) at the dose rate of 2mg/kg body weight showed the highest effect against *A. platys* infection in dogs. The hematology parameters have shown a normocytic normochromic anemia in infected dogs. A substantial increase of platelets, along with the increase of total leukocytes, and lymphocytes, was observed in recovered dogs from all treated groups (Group I to IV). The rate of recovery of dogs during the therapeutic trial was highest on day 2. Furthermore, Wongtawan and colleagues used doxycycline at the dose rate of 10mg/kg single in a day for 28 days for the effective clearance of *Babesia canis vogeli*, *Anaplasma platys* and *Ehrlichia canis* triple infections in dogs (Wongtawan *et al.*, 2024). Likewise, Situmorang and co-researchers successfully treated *Anaplasma* infection in dog using doxycycline at the above mentioned dose along with ivermectin and supportive treatment (Situmorang *et al.*, 2024). Prednisolone does not directly play a role in the treatment of *Anaplasma platys*. Instead, it is used as supportive therapy to control serious immune-related complications triggered by the infection, such as immune-mediated thrombocytopenia. In addition, prednisolone is not an anti-rickettsial drug. Improvement reflected is due to anti-inflammatory effect and suppression of immune-mediated thrombocytopenia rather than superior antimicrobial efficacy. However, the use of glucocorticoids must be used with caution, immunosuppressive effect may lead to the relapse of the subclinical infection (Sainz *et al.*, 2015).

The 16S rRNA gene has high level of common identity; it is difficult to separate distinct species and co-infection with genetically similar pathogens. The sequencing and phylogenetic analysis are required after PCR-based molecular detection for species and strain confirmation. The large sample size is preferred, as the small sample size limits the reliability of the therapeutic trial. These are major limitations of the study.

Conclusions: The overall occurrence of *A. platys* infection was 10.16%, indicating that the pathogen is endemic in the domestic and stray dogs of district Jhang. The highest prevalence was recorded in the Tehsil Athara Hazari. The univariate analysis determined that health status is the major risk factor of canine cyclic thrombocytopenia. Doxycycline combined with prednisolone produced the greatest hematological improvement among the regimens evaluated, although larger controlled studies with molecular follow-up are required before definitive therapeutic recommendations can be made. By combining epidemiological data with an

in vivo trial, this study provides a framework for selecting effective therapeutics and optimizing control strategies against canine anaplasmosis.

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Conflict of Interest: The authors have no competing interests.

Data Availability: The data was available upon reasonable request.

Ethics statement: The study was permitted by the Directorate of Advance Studies, University of Veterinary and Animal Sciences, Lahore, vide letter No. DAS/538-110, dated: June 18, 2020. This was a clinical and prevalence study, the animals were registered after gaining informed consent from their corresponding custodians. The research plan was conducted in full compliance with the ethical guidelines and regulations outlined in Pakistan's Prevention of Cruelty to Animals Act (1890) and the Punjab Wildlife Protection, Preservation, Conservation and Management Act (1974).

Authors Contribution: The FAA contributed to the conceptualization, original draft writing, methodology, review and editing, supervision, and resource provision. LK was involved in the data curation, methodology, formal analysis, investigation, and resource provision. MK and MFQ participated in review writing, editing, and supervision.

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