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RESEARCH ARTICLE

Molecular Characterization, Risk Factor Analysis and Hematological Alterations Associated with *Anaplasma phagocytophilum* in Domestic Cats of Pakistan

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ABSTRACT

Anaplasma phagocytophilum (*A. phagocytophilum*) is a tick-borne rickettsial organism of zoonotic significance that has been alleged for frequent clinical infections in domestic animals including cats. The current study aimed to investigate the molecular prevalence of *A. phagocytophilum* in domestic cats. The study also focused on the assessment of possible risk factors and hematological alterations associated with this infection. For this purpose, 384 blood samples were first examined microscopically and then by PCR targeting the *16S rRNA* gene. The samples found positive on PCR were purified and sequenced later on. The data for the evaluation of risk factors was recorded and statistically analyzed. The present study found 3.39% and 7.03% of cat samples positive for *A. phagocytophilum* on microscopy and PCR, respectively. The sequences of *A. phagocytophilum* presented high similarity with isolates from Thailand with relatively less similarity with isolates from India and China. Chi-square analysis revealed previous tick history, use of ectoparasiticide, housing type and grooming practices as potential risk factors ($P<0.05$) for disease dynamics. Comparative hematological analysis revealed that the number of leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets was significantly decreased ($P<0.05$) in cats suffering from *A. phagocytophilum* compared to the healthy ones. To our knowledge, no study has been performed in the past to detect the presence of *A. phagocytophilum* in domestic cats of Pakistan. The current study may help in the adoption of adequate tick-control strategies for the control of *A. phagocytophilum* infection in domestic cats.

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INTRODUCTION

Domestication of cats is increasing day by day in developing countries due to their peculiar social behavior and fondness for human beings. The increasing population of domestic as well as feral cats around the human population has led to the increased risk of zoonosis e.g., rabies, toxoplasmosis, leptospirosis, ehrlichiosis and anaplasmosis that call for one health approach (Day, 2011). Tick-borne pathogens of domestic and feral cats, especially *Theileria sp., Babesia sp. and Anaplasma sp.* recently emerged but prevail in developing countries like Pakistan. Anaplasmosis is regarded as one of the most prevalent arthropod-borne diseases in sub-tropical and tropical regions around the globe (Torina *et al.*, 2012; Gomes *et al.*, 2013; Ghaffar *et al*., 2020; Ghauri *et al*., 2021; Atif *et al*., 2022; Ishaq *et al*., 2022) caused by gram-negative obligatory intracellular bacteria which infects not only cats but also dog, horse, cattle, buffalo, sheep, goat, donkey, cervid and human species. The species of *Anaplasma* cause feline granulocytic anaplasmosis which is a serious zoonotic disease (Baneth *et al.*, 2012). The primary pathogens infecting cats include *A. phagocytophilum* and *A. platys*.

A. phagocytophilum, an intracellular obligatory alphaproteobacterium of zoonotic importance, is also found to proliferate in platelets. This organism has primarily been documented in dogs, where it produces canine infectious cyclic thrombocytopenia which was discovered in a dog in Florida for the first time (Dumler *et al.*, 2001). Additionally, this infection has been discovered and confirmed in domestic cats using PCR and DNA

sequencing in various countries (Hegarty *et al.*, 2015; Lima *et al.*, 2010). In cats, it leads to granulocytic anaplasmosis that is associated with thrombocytopenia (Mylonakis and Theodorou, 2017). *A. phagocytophilum* is transmitted mainly by ixodes tick species (Stuen *et al*., 2013) whereas the tick species responsible for the transmission of *A. platys* is *Rhipicephalus sanguineus*. Other species like *A. platys* and *A. bovis* have also been reported in cats along with other livestock and wild animals (Atif, 2016). Various techniques including blood smear examination, complete blood count, ELISA and immuno-fluorescent assay (IFT) have been used for the detection of different *Anaplasma spp.* However, PCR is the most reliable of all the methods. Compared to dogs, cats may have less circulating neutrophilic granulocytes with *A. phagocytophilum*, which could result in false-negative PCR results (Schäfer and Kohn, 2020).

Anaplasmosis has been reported in various species including bovines, camels and equines in Pakistan (Azmat *et al*., 2018; Saleem *et al*., 2018), but studies on molecular characterization of *A. phagocytophilum* in domestic cats are limited. Therefore, it is need of hour to assess the infection load of this particular pathogen as this pathogen is of zoonotic nature. This study reports the molecular prevalence of *A. phagocytophilum*, along with the investigation of various risk factors and the several variations in the hematological profile due to this illness in domestic cats of Pakistan.

MATERIALS AND METHODS

Sample collection: An overall 384 whole blood samples, regardless of age, sex, and breed were collected by using convenient method from various government, private hospitals, veterinary laboratories, and clinics from 10 different towns of district Lahore, Pakistan (Fig. 1). The cats complained of being bitten by ticks or displayed any of the clinical symptoms like increased body temperature, anorexia, or anemia., weakness, listlessness, lameness, hemoglobinuria, swelling of lymph nodes, petechial hemorrhages on the conjunctiva, rough hair coat or icterus were targeted for sampling and selected as inclusion criteria. Prior to sampling, owners' consent was obtained and a data collection form was immediately filled out with details about the animals, owners, environment and management to elaborate on the associated risk factors.

After disinfecting the target area and proper clipping the hair, blood samples were collected by pricking the tip of ear by a lancet processed for blood smear examination. Furthermore, jugular venipuncture was used to collect 3 mL blood into EDTA-coated vacutainers aseptically and was sent to lab.

Microscopic examination: Thin blood smears made from all cat samples were stained with Giemsa stain. Initial screening was done by microscopy under a 100X oil immersion lens for the presence of inclusion bodies resembling *Anaplasma*.

DNA Extraction and PCR amplification: DNA extraction was done from the blood samples following the manufacturer's guidelines using GeneAll® Exgene™ Blood (SV mini 105-101) followed by the purity and concentration analysis using Nanodrop 260/280nm. After this DNA was stored at -20°C for further examination. The primers used for the amplification of a 345 bp of 16S *rRNA* gene of *Anaplasma spp.* consisting of forward primer 16SD: 5'-GGTACCYACAGAAGAAG TCC-3'and reverse primer 16SR: 5'-TAGCACTCATC GTTTACAGC–3' and PCR conditions and reaction mixture were the same as those used by Ahmed *et al.* (2020). The PCR reaction mixture was prepared in final volume of 20µl, consisting of 10µl of Master Mix, 2µl of template DNA, 2µl of each primer and 4µl DEPC-treated water. Initial denaturation was done (5 minutes at 95°C), followed by 35 cycles of denaturation (95°C for 30 sec), annealing (58°C for 30 sec) and extension (72°C for 30 sec). The final extension was done for 10 minutes at 72°C (Saleem *et al.,* 2018). The amplified PCR products were observed as positive bands (345bp) by running on 1.5% agarose gel using a 100bp ladder under UV light illuminators.

Sequencing and phylogenetic analysis: The positive samples were characterized at the species level by sequencing. The purification of the positive bands was done by using gel extraction kit (GeneAll® ExpinTM Gel SV 102-150) after cutting with a sterile blade under UV light. The protocol for gel extraction and purification was followed by the guidelines of (Ghaffar *et al*., 2020; Nawab *et al*., 2023). These pure DNA samples were sequenced and analyzed for phylogenetic analysis using suitable bioinformatics tools such as BioEdit and Mega X software. Multiple sequence alignment was performed by BioEdit software through *Clustal W* multiple alignment to compare the sequence of our isolates with the other highly similar published sequences from different selected countries. The phylogenetic tree was conferred by the maximum likelihood method using a phylogeny testing of 1000 bootstrap replications through MEGA XI software to compare and analyze the sequence homology of local isolates with each other and also with other respective gene sequences retrieved from NCBI.

Hematological analysis: Comparative hematological analysis was conducted by evaluating the alterations in hematological parameters of five *A. phagocytophilum* positive and five *A. phagocytophilum* negative, apparently healthy cats. Blood samples (3 mL) were obtained from jugular venipuncture and collected immediately into an EDTA vacutainer. Various hematological parameters under study included packed cell volume (PCV), total Leukocytes count (TLC), total erythrocytes count (TEC), platelets count, hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC) and Hemoglobin (Hb).

Statistical analysis: The risk factor analysis was done by non-probability testing using the chi-square method and those determinants which had ($P<0.2$) were selected for the final multivariable logistic regression technique. The *p*-value ($P<0.05$) and odds ratios ($OR>1$) were considered to be strongly correlated with disease dynamics. The results of the comparative hematological analysis were evaluated using student's *t*-test with a 95% confidence interval. All these statistical analyses were done using SPSS version 22.00.

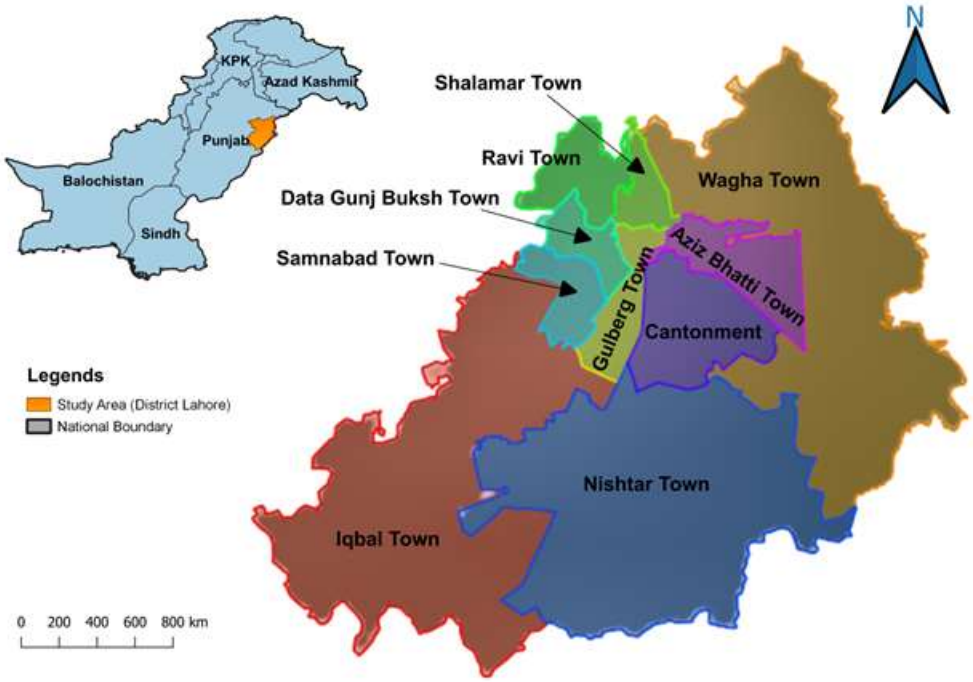

Fig 1: Study areas for collection of blood samples from domestic cats.

RESULTS

Microscopic and molecular detection of *A. phagocytophilum* in cats: An overall 384 blood samples from cats including Persian (n=103), domestic short hair (n=191) and Siamese (n=90) were examined for *A. phagocytophilum* in this study. Microscopic examination revealed 3.39% of cats positive for *Anaplasma*-like inclusion bodies while the molecular confirmation by PCR and sequencing results confirmed the presence of *A. phagocytophilum* in 7.03% of the samples. The prevalence of *A. phagocytophilum* infection was 1.94 and 7.77% in Persian cats, 3.66 and 6.28% in domestic short-hair cats and 4.44 and 7.78% in Siamese breed based on microscopy and PCR respectively. The detail of the findings are presented in Table 1.

Risk factor analysis associated with *A. phagocytophilum*: Risk factor analysis revealed that the anemia was found to be substantially linked with the incidence of *A. phagocytophilum* in cats (P=0.011). Moreover, housing status (P=0.001), ectoparasiticide use status (P=0.003), history of a previous tick-borne disease (P=0.016) and grooming practices (P=0.035) significantly correlated with the *A. phagocytophilum* prevalence in cats. On statistical analysis, risk factors such as age, gender, breed and vaccination status showed insignificant (p>0.05) association with *A. phagocytophilum* infection (Table 2).

Based on logistic regression, the main possible risk variables for disease dynamics were the status of ectoparasiticide use (OR = 3.289; CI = 1.437-7.529), previous tick-borne disease history (OR = 2.635; CI = 1.173-5.920), Grooming practices (OR = 2.442; CI = 1.042-5.724), house hygiene (OR = 4.129; CI = 1.702-10.017) and anemia (OR = 3.182; CI = 1.254-8.070). The cats in which ectoparasiticides were never used were at 3.29 times more risk of having infection compared to those cats in which these were used. In a similar manner, cats with poor housing conditions were 4.13 times more likely to contract *A. phagocytophilum* infection than cats with acceptable housing conditions. The probability of disease development was 2.64 times higher in cats with a history of tick-borne illness than in cats without such a history. The cats which were not properly groomed developed 2.44 times more *Anaplasma* infection than those cats in which grooming was practiced regularly (Table 3).

Molecular characterization of *A. phagocytophilum*: The sequences of *A. phagocytophilum* were submitted and accession numbers were attained as (ON796004), (ON796005), (ON796006), (ON796007) and (ON796003). The sequences were compared with already reported isolates on NCBI using BioEdit software and ClustalW tools. The current study's assessment of isolates revealed no substitution by (ON796004), (ON796005), (ON796007) and (ON796003) isolates at any position. While the isolate ON796006 showed substitutions at different positions i.e. 64, 65, 75, 114, 165, 171, 172, 220, 272, 302, 308 and 320, respectively. The current study isolates ON796004 and ON796007 found similar pattern with each other than that of isolates (ON796003), (ON796005) and (ON796006).

The comparative analysis of study isolates with that of already published sequences for *16S rRNA* on NCBI revealed more resemblance to *A. phagocytophilum* isolated from Goats from Thailand (Accession number: MZ687417) with less similarity with *A. phagocytophilum* isolated from ticks from India and China (Accession numbers: DQ648489 and OL855699). The current study isolates showed significant differences from the isolates of *Anaplasma* among various species in different regions of world. The isolates of a cat from Korea and a dog from Japan with GenBank accession numbers (KR021165) and (LC334014) respectively showed the least resemblance with our study isolates making an out-group (Fig. 2).

Table 1: Microscopic and molecular prevalence of *A. phagocytophilum* in domestic cats of district Lahore.

Group type	No. of animals tested	Microscopic examination		PCR	
		Positive samples	(%)	Positive samples	(%)
Persian	103	2	1.94	8	7.77
Domestic short hair	191	7	3.66	12	6.28
Siamese	90	4	4.44	7	7.78
Total	384	13	3.39	27	7.03

Table 2: Association of risk factors with *A. phagocytophilum* in domestic cats of district Lahore.

Risk factors	Variables	Study Population (n=384)	Positive No.	(%)	*p*-Value
Age	≤ 1 Year	166	10	6.02	0.501
	>1 Year	218	17	7.80	
Gender	Male	221	16	7.24	0.852
	Female	163	11	6.75	
Breed	Domestic short hair	139	12	8.63	0.390
	Persian	162	08	4.94	
	Siamese	83	07	8.43	
Housing	Indoor only	218	07	3.21	0.001*
	Open access to outdoor	166	20	12.05	
Grooming	Practiced	189	08	4.23	0.035*
	Not practiced	195	19	9.74	
Previous tick-borne disease history	Yes	157	17	10.83	0.016*
	No	227	10	4.41	
Vaccination status	Vaccinated	167	12	7.19	0.917
	Non vaccinated	217	15	6.91	
Ectoparasiticide use status	Used	231	09	3.90	0.003*
	Never used	153	18	11.76	
Anemia	Present	208	21	10.10	0.011*
	Absent	176	06	3.41	

* Indicates significant risk factors

Table 3: Assessment of potential risk factors by logistic regression model.

Risk Factors	Variables	Study Population (n=384)	Standard error	C.I 95%	*p*-Value
Housing	Open access to outdoor	4.129	0.452	1.702 – 10.017	0.002*
	Indoor only	1			
Grooming	Not Practiced	2.442	0.435	1.042 – 5.724	0.040*
	Practiced	1			
Previous tick-borne disease history	Yes	2.635	0.413	1.173 – 5.920	0.019*
	No	1			
Ectoparasiticide use status	Never Used	3.289	0.423	1.437 – 7.529	0.005*
	Used	1			
Anemia	Present	3.182	0.475	1.254 – 8.070	0.015*
	Absent	1			

*indicates potential risk factors

Table 4: Effect of *A. phagocytophilum* on hematology of domestic cats of Lahore.

Parameter (Normal range)	Non-infected cats	Infected cats	F-value	Mean Difference	CI (95%)	*p*-value
	Mean ± SD	Mean ± SD				
WBCs×10^3 /µL (4.5-14.5)	10.48±3.13	18.94±3.68	0.391	8.46	3.47 – 13.44	0.004*
RBCs ×10^6/µL (7 - 10.5)	9.18±0.83	5.52±0.84	2.062	3.66	0.85 – 6.46	0.017*
Platelets×10^3 /µL (180 – 500)	452.80±119.06	173.60±95.09	0.205	279.20	122.05-436.34	0.003*
Hb g/dL (10 - 16)	12.64±1.80	7.86±1.40	0.168	4.78	2.42 – 7.13	0.002*
PCV (%) (30 - 50)	45.66±9.76	23.50±3.74	13.001	22.16	11.37 – 32.94	0.001*

* indicates statistically significant difference

Hematological evaluation: Comparative hematological alteration of *A. phagocytophilum* affected and healthy cats were accessed (Table 4). A significant ($P<0.05$) decrease in mean±S.D of WBCs (18.94±3.68), RBCs (5.52±0.84), PCV (23.50±3.74), HgB (7.86±1.40) and platelets (173.60±95.09) was observed in *A. phagocytophilum* infected cats in comparison to healthy ones while other hematology parameters such as neutrophils, lymphocytes, eosinophils, MCV, MCHC and MCC showed non-significant association with diseased cats.

DISCUSSION

In both veterinary and human medicine, the introduction of novel tick-borne pathogens, as well as the recurrence of previously treated pathogens, is of utmost importance (Dantas-Torres *et al.,* 2012), especially in domestic cats which is one of the major concerns of researchers nowadays. Previously there is a report on molecular evidence of *Anaplama* spp. in domestic cats in Pakistan (Ahmed *et al.*, 2020), but there is a lack of species-specific information. In this study, 7.03% prevalence of *A. phagocytophilum* in domestic cats was found based on PCR. These findings were very similar to those of (André *et al*., 2014) in which it was found that free-roaming cats in Brazil were 8% positive to *Anaplasma* species on molecular basis that are closely related to *A. phagocytophilum*. Similarly, domestic and stray cats with anaplasmosis have been found to have a molecular prevalence of 5.4% in Southern Portugal (Maia *et al.,* 2014). In Pakistan, previously an overall 13% and 8% prevalence of *Anaplasma* spp. based on PCR and blood smear examination, respectively, has been reported (Ahmed *et al.,* 2020). The prevalence documented in the

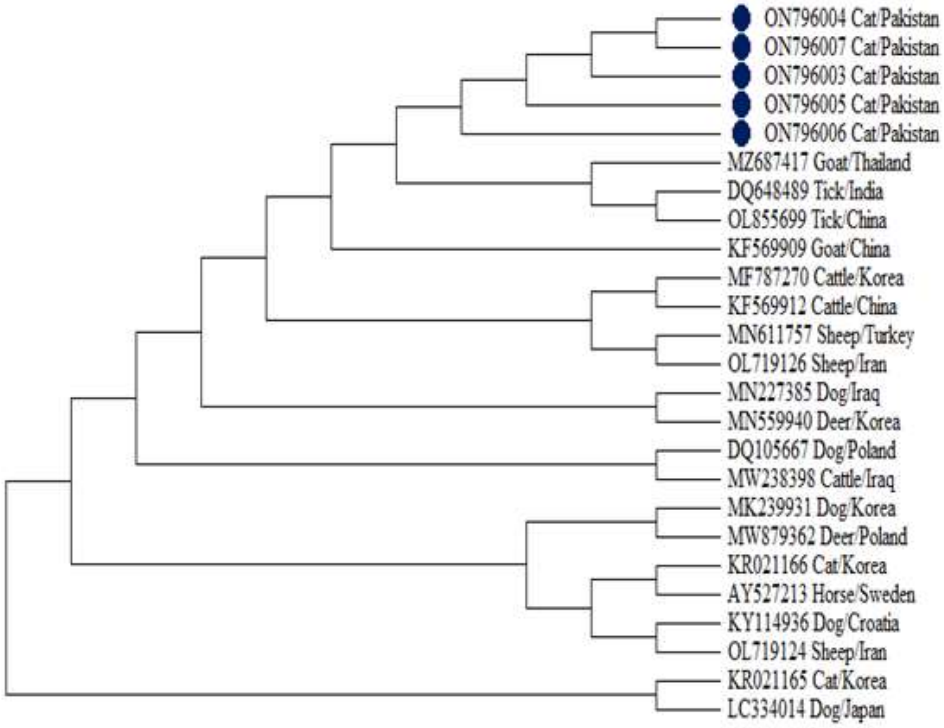

Fig 2: Phylogenetic tree of *A. phagocytophilum* using Maximum Likelihood method exhibiting the sequence homology with reported gene sequences.: The blue color is showing study isolates.

present research is contrary to the findings of previous studies that reported a molecular prevalence of 1.8% and 0.9% of *A. phagocytophilum* infection in cats from Spain and Korea respectively (Solano-Gallego *et al.*, 2006; Lee *et al.*, 2016). Additionally, a PCR and genotypic sequencing-based investigation carried out in Angol that showed that about 1% of domestic cats were found positive for *A. bovis* (Oliveira *et al.*, 2018). This increased prevalence of *A. phagocytophilum* in household cats as discussed in the present study which contradicts the already conducted research could be due to the hot and humid environment of Pakistan which favors the growth of ticks considerably (Batool *et al.*, 2019) leading to many tick-borne diseases including *A. phagocytophilum* in domestic cats. Moreover, another potential explanation for this greater incidence is that only cats with tick infestations and any clinical evidence of pyrexia, lethargy, or anemia were included in the study.

In the current research, male cats showed a higher prevalence (7.24%) as compared to female cats (6.75%). These results are in contrast to the findings of Ahmed *et al.* (2020) and Ebani and Bertelloni (2014) who stated a slightly increased prevalence in female cats as compared to male cats. The findings, however, correspond with those of Maia *et al*. (2014), who also noted a increased incidence in males (6.1%) cats as compared to females (4.8%). Moreover, the infection rate in adult cats of age more than one year (7.80%) was slightly higher as compared to younger cats of age less than one year (6.02%) was also published which was similar to the findings of Ebani and Bertelloni (2014) who also found that adult cats have higher prevalence (5.1%) of disease as compared to younger ones (0%). These results are also advocating the findings of previous studies (Vilhena *et al.*, 2013; Maia *et al.*, 2014; Ahmed *et al.*, 2020). Since mature cats are more likely to come into contact with ticks, the greater infection rate in adult cats may eventually be attributed to tick-borne illnesses. In the current research, the highest prevalence rate was reported in Mixed/non-descriptive breeds and domestic short-haired followed by Siamese and Persian. These results were supported by the previous studies who also noted a higher prevalence in domestic short-hair in comparison to other breeds (Vilhena *et al.*, 2013; Maia *et al.*, 2014; Ahmed *et al.*, 2020). The lower prevalence of infection in Siamese and Persians spoke of the fact that these breeds are usually housed indoors getting a minimum chance to be exposed to the outside environment. Additionally, grooming like brushing is regularly practiced in indoor cats which promptly removes any possible arthropod residing on the skin as it takes almost 24-48 hours for the ticks to transmit infection after attachment to the body of animals (Ebani and Bertelloni, 2014). Therefore, regular grooming practices help cats keep at low risk of tick infestation (Ahmed *et al.*, 2020). Housing type was another significant risk factor associated with the occurrence of *A. phagocytophilum* as cats with outdoor access revealed a higher prevalence which was in accordance with the outcomes of previous studies (Maia *et al.*, 2014; Ahmed *et al.*, 2020). These results were also in accordance with the study of Vilhena *et al.* (2013). These are because of the fact that cats frequently going outside have more chances of getting tick-borne diseases as they are more exposed to their vectors than those cats who are totally kept indoors. The present study also revealed that the cats exposed to tick infestation or presented with a history of tick infestation in the past were having higher prevalence rates as compared to cats with no such history. These results support the findings of previous studies (Ebani and Bertelloni, 2014; Ahmed *et*

al. 2020). Tick-borne diseases in domestic cats can be prevented by using ectoparasiticide. In this report, cats with no history of ectoparasiticide use manifested higher prevalence (11.76%) as compared to cats in which ectoparasiticide were used (3.90%). This was in agreement with previous studies (Maia *et al.*, 2014; Ahmed *et al.*, 2020). These risk factors were found to be strongly linked to the development of disease, indicating that ticks have a significant role in the spread of *A. phagocytophilum* infection to healthy ones. As a result, increased tick exposure or inadequate tick control methods will increase the likelihood of infection in cats.

The combinations of abnormal hematological parameters in *A. phagocytophilum* infection can vary depending on how long the infection persisted before sampling (Heikkilä *et al.*, 2010). In the present research, cats infected with *A. phagocytophilum* had significantly lower levels of hemoglobin, platelets, PCV, red blood cells and white blood cells. Thrombocytopenia, which is often mild to moderate in cats with *Anaplasma* infection, is the most prominent and frequent sign. A significant drop in platelets was seen in the current study, which was consistent with the findings of previous studies (Adaszek *et al.*, 2013; Ahmed *et al.*, 2020). This thrombocytopenia is attributed to the destruction of platelets (Carrade *et al.*, 2009) but the exact mechanisms of thrombocytopenia need more clarification. The potential causes could be enhanced phagocytosis of platelets by macrophages, decreased production of platelets due to immunological breakdown and enhanced phagocytosis of platelets cells by the macrophages and hypoplasia of bone marrow (Adaszek *et al.*, 2013). The true indices of anemia are a decrease in RBCs, HGB and PCV. The results of the current research were in harmony with (Adaszek *et al.*, 2013) who also reported a decrease in RBC count, HGB and hematocrit levels in cats with *A. phagocytophilum* infection. Similar findings to our results were also documented by previous studies (Gorna *et al.*, 2013; Ahmed *et al.*, 2020). Similar to thrombocytopenia, leukocytosis was too seen in the infected cats, which has been stated in many investigations (Kirtz *et al.*, 2005; Ahmed *et al.*, 2020).

Conclusions: This study depicts the molecular evidence of *A. phagocytophilum* in household cats of Lahore, Pakistan. The increasing incidence of infection in domestic cats was found to be significantly associated with potential risk factors, including prior ticks history and tick infestation, use of ectoparasiticides, housing style and grooming habits. Infected domestic cats revealed a significant decrease in platelets, hemoglobin, erythrocytes count, and PCV. These results may be useful in developing the disease's effective control strategies in the future. Due to the zoonotic impact of this malaise and in perspective of one health, endemic regions of the disease should be identified and possible control measures should be implemented in these regions to minimize the spread of the disease to non-endemic regions of the world.

Authors contribution: SNA and MA planned and designed the trial. RZA and MHS executed and collected the data. All the authors participated in the preparation of the manuscript. AKM revised the manuscript and all authors approved the final version of the manuscript.

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REFERENCES

Adaszek L, Górna M, Skrzypczak M, *et al.*, 2013. Three clinical cases of Anaplasma phagocytophilum infection in cats in Poland. J Feline Med Surg 15:333-7.

Ahmed A, Ijaz M, Ghauri HN, *et al.*, 2020. Molecular evidence of Anaplasma infection in naturally affected domestic cats of Pakistan. Comp Immunol Microbiol Infect Dis 72:101524.

André MR, Denardi NC, de Sousa KC, *et al.,* 2014. Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil. Ticks and tick-borne dis 5:545-51.

Atif FA, 2016. Alpha proteobacteria of genus Anaplasma (Rickettsiales: Anaplasmataceae): Epidemiology and characteristics of Anaplasma species related to veterinary and public health importance. Parasitology 143:659-85.

Atif FA, Abbas RZ, Mehnaz S, *et al.*, 2022. First report on molecular surveillance based on duplex detection of Anaplasma marginale and Theileria annulata in dairy cattle from Punjab, Pakistan. Trop Animal Health Prod 54:155. doi: 10.1007/s11250-022-03158-y.

Azmat M, Ijaz M, Farooqi SH, *et al.,* 2018. Molecular epidemiology, associated risk factors, and phylogenetic analysis of anaplasmosis in camel. Microb pathogen 123:377-84.

Baneth G, Bourdeau P, Bourdoiseau G, *et al.*, 2012. Vector-borne diseases-constant challenge for practicing veterinarians: recommendations from the CVBD World Forum. Parasit Vectors 5:1-3.

Batool M, Nasir S, Rafique A, *et al.*, 2019. Prevalence of tick infestation in farm animals from Punjab, Pakistan. Pak Vet J 39:406-10.

Carrade DD, Foley JE, Borjesson DL, *et al.*, 2009. Canine granulocytic anaplasmosis: a review. J Vet Intern Med 23:1129–1141.

Dantas-Torres F, Chomel BB and Otranto D, 2012. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol 28:437-46.

Day MJ, 2011. The immunopathology of canine vector-borne diseases. Parasit Vectors 4:1-13.

Dumler JS, Barbet AF, Bekker CPJ, *et al.*, 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: Unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combi. Int J Syst Evol Microbiol 51:2145-65.

Ebani VV and Bertelloni F, 2014. Serological evidence of exposure to Ehrlichia canis and Anaplasma phagocytophilum in Central Italian healthy domestic cats. Ticks Tick Borne Dis 5:668-71.

Gomes J, Soares R, Santos M, *et al.*, 2013. Detection of Theileria and Babesia infections amongst asymptomatic cattle in Portugal. Ticks Tick Borne Dis 4:148-51.

Gorna M, Adaszek L, Policht K, *et al.*, 2013. Detection of Anaplasma phagocytophilum in a cat. Vet Med (Praha) 58.

Ghaffar A, Ijaz M, Ali A, *et al.*, 2020. First report on molecular characterization of anaplasmosis in small ruminants in Pakistan. J Parasitol 106:360-8.

Ghauri HN, Ijaz M, Ahmed A, *et al.*, 2021. Molecular investigation and phylogenetic analysis of anaplasmosis in dogs. J Parasitol 107:295-303.

Hegarty BC, Qurollo BA, Thomas B, *et al.*, 2015. Serological and molecular analysis of feline vector-borne anaplasmosis and ehrlichiosis using species-specific peptides and PCR. Parasit Vectors 8:1-9.

Heikkilä HM, Bondarenko A, Mihalkov A, *et al.*, 2010. Anaplasma phagocytophilum infection in a domestic cat in Finland: case report. Acta Vet Scand 52:1-5.

Ishaq M, Ijaz M, Lateef M, *et al.*, 2022. Molecular characterization of Anaplasma capra infecting captive mouflon (Ovis gmelini) and domestic sheep (Ovis aries) of Pakistan. Small Rumin Res 216:106837.

Kirtz G, Meli M, Leidinger E, *et al.*, 2005. Anaplasma phagocytophilum infection in a dog: identifying the causative agent using PCR. J Small Anim Pract 46:300-3.

Lee S-H, VanBik D, Kim N-H, *et al.*, 2016. First molecular detection and genetic analysis of Anaplasma phagocytophilum in shelter cats in Seoul, Korea. Infect Genet Evol 46:71-3.

Lima MLF, Soares PT, Ramos CAN, *et al.*, 2010. Molecular detection of Anaplasma platys in a naturally-infected cat in Brazil. Brazilian J Microbiol 41:381-5.

Maia C, Ramos C, Coimbra M, *et al.*, 2014. Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. Parasit Vectors 7:1-8.

Mylonakis ME and Theodorou KN, 2017. Canine monocytic ehrlichiosis: an update on diagnosis and treatment. Acta Vet Brno 67:299-317.

Nawab Y, Muhammad II, Ayyub RM, *et al.*, 2023. Molecular Detection of Theileria annulata Infection: An Emerging Disease of Pet Dogs in Pakistan. Kafkas Univ Vet Fak Derg 29: 41-8.

Oliveira AC, Luz MF, Granada S, *et al.*, 2018. Molecular detection of Anaplasma bovis, Ehrlichia canis and Hepatozoon felis in cats from Luanda, Angola. Parasit Vectors 11:1–6.

Saleem S, Ijaz M, Farooqi SH, *et al.*, 2018. First molecular evidence of equine granulocytic anaplasmosis in Pakistan. Acta Trop 180:18–25.

Schäfer I and Kohn B, 2020. Anaplasma phagocytophilum infection in cats: A literature review to raise clinical awareness. J Feline Med Surg 22:428–41.

Solano-Gallego L, Hegarty B, Espada Y, *et al.*, 2006. Serological and molecular evidence of exposure to arthropod-borne organisms in cats from northeastern Spain. Vet Microbiol 118:274-7.

Stuen S, Granquist EG and Silaghi C, 2013. Anaplasma phagocytophilum—a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol 3:31.

Torina A, Agnone A, Blanda V, *et al.*, 2012. Development and validation of two PCR tests for the detection of and differentiation between Anaplasma ovis and Anaplasma marginale. Ticks Tick Borne Dis 3:283-7.

Vilhena H, Martinez-Díaz VL, Cardoso L, *et al.*, 2013. Feline vector-borne pathogens in the north and centre of Portugal. Parasit Vectors 6:1-6.