



# Tick-Borne Diseases-Possible Threat to Humans-Dog Interspecies Bond

SAID SAJJAD ALI SHAH<sup>1\*</sup>, MUHAMMAD ILYAS KHAN<sup>1</sup>, RAFIULLAH<sup>1</sup>, MIRZA ALI KHAN<sup>1</sup>, HAMIDULLAH KHAN<sup>1</sup>, ANWAR ALI<sup>1</sup>, MUHAMMAD IJAZ ALI<sup>1</sup>, RAFIULLAH JAN<sup>2</sup>

<sup>1</sup>Veterinary Research Institute, Peshawar, Pakistan; <sup>2</sup>The University of Agriculture, Peshawar, Pakistan.

**Abstract** | Dog (*Canine lupus familiaris*) is one of the most neglected species in the research institutes in Pakistan. Humans are at great risk of zoonotic diseases due to close association with dogs and humans-dog interspecies bond is at danger due to these diseases. The aim of the study was to determine the haemo-parasitic diseases of dogs, its effect on hematological profile and to correlate it with general health status of dogs. Total of 121 blood samples were collected from district Peshawar and Charsadda from public and private sectors including armed forces. Microscopic results showed 34 and 19% prevalence of anaplasmosis and canine babesiosis, respectively. Prevalence of haemo-parasitic diseases in dogs were studied with respect to different risk factors i.e. season, location, breed, owner, sex and age. Statistically significant ( $P < 0.05$ ) relation was recorded with respect to season, breed, ownership and age while no significant ( $P > 0.05$ ) relation was observed with respect to location and sex. Anemia was striking feature in hematological parameters with significant ( $P < 0.01$ ) decrease in red blood cells, hemoglobin and hematocrit values and can be classified as normocytic hypochromic with significant decrease ( $P < 0.01$ ) in Mean corpuscular hemoglobin concentration (MCHC). Ticks are important vector for the transmission of the haemo-parasites and ticks infestation is favored by hot and humid environment, the reason for higher prevalence of haemo-parasites in the summer season. Awareness among the community should be initiated through media talks, print and electronic media for periodical screening of their dogs for haemo-parasitic diseases and along with that control strategies should be designed.

**Keywords** | Haemo-parasitic diseases, Prevalence, Hematology, Anaplasmosis, Canine babesiosis, Anemia

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\***Correspondence** | Said Sajjad Ali Shah, Research Officer (CPP), Veterinary Research Institute, Peshawar, Pakistan; **Email:** sajjadsheikh0695@gmail.com

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## INTRODUCTION

Humans and dogs lived for years together and now it turns out to be one of the best human's friend and become valued pet. Dogs are closest to humans due to their splendid and remarkable social behaviors (Ugbomoiko et al., 2008). Due to higher influence on society and numerous roles, dog has given the sobriquet, "man's best friend". Dog evolved from gray wolves and is considered to be the

most abundant carnivore (Vila et al., 1999; Thalmann et al., 2013; Zhenxin et al., 2016). Dogs fulfill its duty for humans in the form of hunting, herding sheep and goats, protection, assisting security forces and more recently, dogs are used to identify thefts (Savolainen et al., 2002).

In Pakistan, dogs are mainly kept by security forces, as watch dog in houses and for herding of sheep etc by shepherds (Jafri and Rabbani, 1999). Despite of its definite

role in society, large number of dogs still live as feral and stray dogs which receive minimal public attention. Though a felony offence but in most parts of the country dogs are kept for fighting purposes. There is growing trend of keeping dog as pet animal in urban population of Pakistan and mostly kept by middle and upper-middle class households. Different breeds of dogs i.e. German shepherd, Pointer, Labrador and Spaniel are kept by corps which aids them in routine security operations as sniffer dogs which perform search duties with high sense of responsibility. Apart from corps, local population kept Bull Terrier (Local name Gull Terr, for security and fighting purpose), German shepherd (Popular name in locals, Alsatian dog) and Hounds (Tazi, for hunting).

Dog population is faced with different viral, bacterial, fungal and parasitic diseases. Tick borne diseases i.e. Canine babesiosis and anaplasmosis are most important parasitic diseases of dogs. Etiological agents of Canine babesiosis are *Babesia canis*, *Babesia gibsoni* and *Babesia vogeli* in which *Babesia canis* is of significant importance (Toboada, 1998; Birkenheuer et al., 1999). Canine babesiosis is transmitted by different tick vectors i.e. *Hyalomma*, *Rhipicephalus sanguineus* and *Dermacentor variabilis*. Anaplasmosis is caused by *Anaplasma phagocytophilum*, an intracellular parasite, transmitted by tick vector *Ixodes* (Dumler et al., 2001; Cohn, 2003). *Anaplasma phagocytophilum* has wide range of host including sheep and goats and also have zoonotic potential.

Currently, there is limited data available about dogs in Pakistan, and this is the most neglected specie in the research institutes of the country. Presently only few published reports are available on canine babesiosis but none about anaplasmosis. Due to huge population of stray dogs in urban areas of the country, it is of utmost importance to study various canine diseases. Aim of the present study was to find out the prevalence and hematological changes of haemo-parasites in dogs and to correlate it with its health status.

## MATERIALS AND METHODS

### STUDY AREA

This study was conducted in district Peshawar and Charsadda. Peshawar district lies at 34.04°N latitude and 71.5°E longitude with an altitude of 359m and has area of 1257 km<sup>2</sup> While district Charsadda is located at 34.8°N latitude and 71.43°E longitude with an altitude of 276m (908 feet) and lies 29 km from the provincial capital, Peshawar.

### SAMPLE COLLECTION

This study was conducted from January, 2015 to December, 2016. Blood samples (n=121) were collected and received

from different areas of Peshawar and Charsadda in the Center of Parasitology and Poultry (CPP) and Center of Microbiology and Biotechnology (CMB), Veterinary research institute, Peshawar. Whole blood samples collected in anti-coagulant added vacutainer tubes were transported to Veterinary Research Institute, Peshawar for onward processing.

### MICROSCOPIC EXAMINATION

Blood smears were prepared, fixed with methanol for 5 min and stained with 10% giemsa. Microscopic examination of the stained smears was performed at 100× objective for the presence or absence of parasites (Ahmad et al., 2007).

### HEMATOLOGY

For estimation of hematological alterations, anti-coagulant added blood was submitted to Pathology Lab, Veterinary Research Institute, Peshawar and were processed for complete blood count through Automatic Hematology analyzer (Urit Vet 2900) (Yasini et al., 2012).

### STATISTICAL ANALYSIS

Data thus collected was analyzed by Chi square test for prevalence of tick borne diseases with respect to season, location, breed, age and sex while student t-test was used for analysis of hematological data. Means of hematological parameters were compared by LSD at a probability level ≤ 0.05 using Statistical Package for Social Services (SPSS) version 16.0.

## RESULTS AND DISCUSSION

### PREVALENCE OF HAEMO-PARASITES

Results of the study showed that out of 121 blood samples 64 (52.8%) samples were positive for Haemo-parasites. Anaplasmosis was recorded as 34%, while Canine babesiosis as 19% (Figure 1).

### RISK FACTORS FOR HAEMO-PARASITES

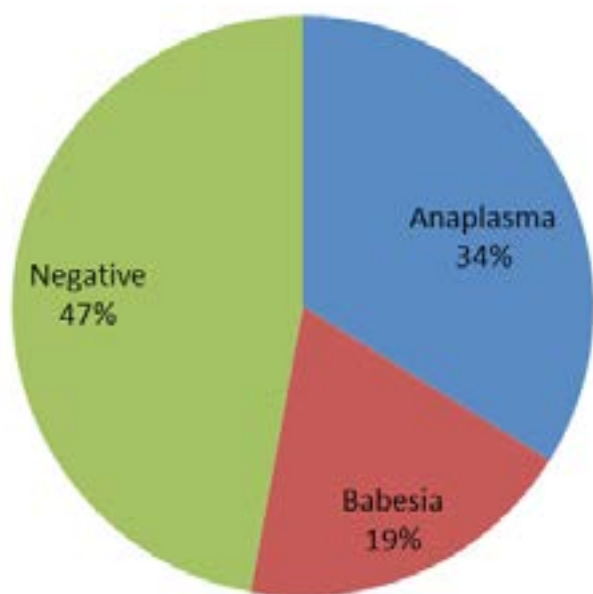
Different risk factors associated with occurrence of canine babesiosis and anaplasmosis were studied in detail. Results of the study showed that haemo-parasites were more in summer season (62%) as compare to winter (35.7%). Statistically significant difference (P<0.05) was recorded in the incidence of haemo-parasites with respect to season. This study was conducted in two districts of Khyber Pakhtunkhwa i.e. Peshawar and Charsadda. Slight difference in incidence was recorded in both districts but was statistically non-significant (P>0.05). Breed wise prevalence was recorded as 62.2, 30, 22.2, 14.2, 80.9 and 38% for German shepherd, Spaniel, Labrador, Pointer, Gull Terr and Tazi, respectively. Statistically significant difference (P<0.05) was recorded with respect to breed. Dog owners were also studied as risk factor in the present study and it was observed that occurrence of haemo-parasites were more in

**Table 1:** Prevalence of Haemo-parasite in dogs with respect to season, location, breed, owner, sex and age

| Variables | Category        | N   | Haemo-parasites prevalence (%) | Chi square value | P value |
|-----------|-----------------|-----|--------------------------------|------------------|---------|
| Season    | Winter          | 42  | 35.7                           | 8.18             | 0.01*   |
|           | Summer          | 79  | 62%                            |                  |         |
| Location  | Peshawar        | 101 | 52.4                           | 0.58             | 0.74    |
|           | Charsadda       | 20  | 55                             |                  |         |
| Breed     | German shepherd | 53  | 62.2                           | 21.24            | 0.01*   |
|           | Spaniel         | 10  | 30                             |                  |         |
|           | Labrador        | 9   | 22.2                           |                  |         |
|           | Pointer         | 7   | 14.2                           |                  |         |
|           | Gull Terr       | 21  | 80.9                           |                  |         |
| Owner     | Tazi            | 21  | 38                             | 11.38            | 0.00*   |
|           | Farmer          | 78  | 64.1                           |                  |         |
| Sex       | Govt            | 43  | 32.5                           | 0.182            | 0.91    |
|           | Female          | 4   | 50                             |                  |         |
| Age       | Male            | 117 | 52.9                           | 39.5             | 0.00*   |
|           | Adult           | 60  | 81.6                           |                  |         |
|           | Young           | 61  | 24.5                           |                  |         |

\* shows significant difference at level  $P < 0.01$ ; N: Total number of cases

**Prevalence of Haemo-parasites**



**Figure 1:** Overall prevalence of Haemo-parasites in dogs in district Peshawar and Charsadda

dogs kept by farmers individually as compare to dogs kept by security forces and difference was statistically significant ( $P < 0.05$ ). Occurrence was recorded more in males as compare to females but statistically non significant ( $P > 0.05$ ). In these study two age groups *i.e.* adults (age group more than 3 years) and young (age group less than 3 years) were studied. Haemo-parasites occurrence was found statistically higher ( $P < 0.05$ ) in adults *i.e.* 81.6% as compare to young dogs *i.e.* 24.5% (Table 1).

**Table 2:** Hematological parameters of infected and non-infected dogs

| Parameter                               | Negative                        | Infected                        | P-value |
|---|---------------------------------|---------------------------------|---------|
| TEC ( $\times 10^6/\mu\text{l}$ )       | 6.01 <sup>a</sup> $\pm$ 0.08    | 3.90 <sup>b</sup> $\pm$ 0.12    | 0.00*   |
| Hb (g/dl)                               | 16.02 <sup>a</sup> $\pm$ 0.37   | 9.65 <sup>b</sup> $\pm$ 0.32    | 0.00*   |
| HCT (%)                                 | 39.4 <sup>a</sup> $\pm$ 0.55    | 25.32 <sup>b</sup> $\pm$ 0.81   | 0.00*   |
| MCH (pg)                                | 26.58 <sup>a</sup> $\pm$ 0.49   | 25.04 <sup>b</sup> $\pm$ 0.46   | 0.02**  |
| MCV (fl)                                | 65.69 <sup>a</sup> $\pm$ 0.45   | 65.16 <sup>a</sup> $\pm$ 0.49   | 0.43    |
| MCHC (g/dl)                             | 40.47 <sup>a</sup> $\pm$ 0.61   | 38.38 <sup>b</sup> $\pm$ 0.51   | 0.00*   |
| TLC ( $\times 10^3/\mu\text{l}$ )       | 15.41 <sup>a</sup> $\pm$ 1.3    | 10.32 <sup>b</sup> $\pm$ 0.85   | 0.01*   |
| Granulocytes (%)                        | 51.12 <sup>a</sup> $\pm$ 3.29   | 44.38 <sup>a</sup> $\pm$ 3.07   | 0.13    |
| Lymphocytes (%)                         | 33.53 <sup>a</sup> $\pm$ 2.85   | 29.85 <sup>a</sup> $\pm$ 2.07   | 0.29    |
| Monocytes (%)                           | 22.03 <sup>a</sup> $\pm$ 0.90   | 18.86 <sup>a</sup> $\pm$ 1.22   | 0.04**  |
| Platelets ( $\times 10^3/\mu\text{l}$ ) | 217.65 <sup>a</sup> $\pm$ 13.07 | 122.77 <sup>b</sup> $\pm$ 10.43 | 0.00*   |

<sup>a, b</sup> means with different superscript with in row are significant at \* $P$ -value  $< 0.01$  and \*\* $P$ -value  $< 0.05$ ; **TEC:** Total erythrocytes count; **Hb:** Hemoglobin; **HCT:** Hematocrit; **MCV:** Mean corpuscular volume; **MCH:** Mean corpuscular hemoglobin; **MCHC:** Mean corpuscular hemoglobin concentration; **TLC:** Total leukocytic count

**HEMATOLOGICAL PROFILE**

Hematological results showed that there was significant decrease ( $P < 0.01$ ) in the total erythrocyte count in infected animals. Similarly, hemoglobin and hematocrit values of infected animals were lowered from that of normal animals and these were found highly significant statistically ( $P < 0.01$ ). Results of Erythrocytic indices showed that there was significant decrease in the value of Mean corpuscular hemoglobin (MCH) ( $P < 0.05$ ) and MCHC ( $P < 0.01$ ) while no significant changes were observed in the value

of Mean corpuscular volume (MCV) ( $P > 0.05$ ). Significant changes were observed in the value of Total leukocytes count (TLC) ( $P < 0.01$ ) and monocytes ( $P < 0.05$ ), whereas no significant changes were observed in granulocytes and lymphocytes ( $P > 0.05$ ). Platelets value of infected dogs were lowered from non-infected and was found significant statistically ( $P < 0.01$ ) (Table 2).

Due to close association and dependency on each other, dogs and humans lived for years together and dogs had performed various roles for human beings. Dogs were used in the past only for guarding, fighting, hunting and herding of sheep and goats, but over the years its role has changed and it can be kept as pet, as sniffing dog by security forces for narcotics, ammunition and also for identification of thefts and culprits. Due to its numerous roles and services for human, it is fully justified to call it “man’s best friend” (Vila et al., 1999; Savolainen et al., 2002; Thalmann et al., 2013). Despite of its definite role in society, huge population of dogs still present as feral and stray dogs in Pakistan and has nominal public attention. Due to this negligence, no proper statistics is presently available about dog’s population in the country and researchers are still reluctant to work in this neglected area.

Overall incidence of haemo-parasites in the current study was recorded as 19 and 34% for canine babesiosis and anaplasmosis, respectively. Prevalence of canine babesiosis was almost similar to results of (Ahmad et al., 2007) who recorded 14% prevalence of canine babesiosis in Lahore, Pakistan. These results are further supported by findings of (Mas, 1990) (20%) and (Cabannes et al., 2002) (14%). (Laha et al., 2014) reported higher prevalence (56%) of canine babesiosis which might be due to the reason that (Laha et al., 2014) studied molecular prevalence of canine babesiosis, while microscopy was used in the present study which is less sensitive comparatively. Prevalence of anaplasmosis in the present study was found similar with the findings of (Pennisi et al., 2012) who reported 38% prevalence of anaplasma. Findings of (Bhattacharjee and Sarmah, 2013) are not in agreement with current study and the possible reasons may be that (Bhattacharjee and Sarmah, 2013) reported only *Anaplasma platys* and did not reported *Anaplasma phagocytophilum*.

Findings of (Hamel et al., 2015) were not in agreement with the results of the present study who reported 24% prevalence of anaplasma species. Higher prevalence in the present study might be due to the fact that Peshawar and Charsadda districts offer favorable environment for tick infestation and tick borne diseases in other domestic animals.

Prevalence of anaplasmosis and canine babesiosis were studied in detail with respect to different risk factors *i.e.*

season, location, breed, owner, sex and age. Prevalence of haemo-parasites was higher in summer season as compare to winter and this was found statistically significant. In summer season, ticks infestation is high comparatively due to which the occurrence of tick borne diseases are higher. This finding is supported by many workers that season play key role in occurrence of parasitic diseases (Lorusso et al., 2010; Ahmad et al., 2011; Jalali et al., 2013). The present study was conducted in two districts of Khyber Pakhtunkhwa and to the authors knowledge no publish material is available currently about the prevalence of anaplasmosis and canine babesiosis in Khyber Pakhtunkhwa, Pakistan. Prevalence of haemo-parasites was more in district Charsadda as compare to Peshawar but the differences were not statistically significant. Increased humidity and rural environment in district Charsadda might be the possible reason for slightly higher occurrence of haemo-parasites. These findings are fully supported by the study conducted by (Jalali et al., 2013) that prevalence is higher in rural environment as compare to urban.

Samples collected were from two different sources *i.e.* public and private sectors including armed forces. Occurrence was higher in dogs kept at personal level as compare to dogs from government institutions and it was found statistically significant. Government agencies kept dogs for special purposes and they treat their dogs regularly whereas, lack of awareness in farmers may be the cause for higher prevalence. Secondly, government agencies kept dogs in kennels which are cleaned regularly whereas, farmers kept dogs in close vicinity of cattle, sheep, goats and are highly exposed to ticks infestation by ticks migration. Individual farmers mostly kept Gull Terr dog for the purpose of protection and fighting, German shepherd and Tazi dog for hunting, while government agencies have different breeds for different purposes *i.e.* German Shepherd, Spaniel, Labrador and Pointer. Breed wise occurrence was higher in Gull Terr and German shepherd and was found statistically significant. Similar findings were recorded by (Shrivastava et al., 2014) that prevalence was higher in German shepherd. Sex was also considered as an important indicator for the prevalence of haemo-parasites in dogs by many workers (Bashir et al., 2009), but in the present study no significant differences were observed because most of samples received or collected were from male dogs. Occurrence of haemo-parasites is age dependent and in young animals its prevalence is lower as compare to adults. In adult dogs, significantly higher incidence was recorded comparatively. These findings are in congruent with the study conducted by (Bashir et al., 2009; Jalali et al., 2013).

There was significant decrease in the value of TEC, Hb and PCV which are important indicators of anemia. Many workers in the past also considered anemia as an important outcome of haemo-parasitic diseases in dogs. Anemia

may be due to direct parasite damage to red blood cells or due to auto antibodies directed against erythrocytes cell membrane. Similar results of hematology were also reported by (Day, 1999; Pederson, 1999; Taboada and Lobetti, 2006; Salem and Farag, 2014). On the basis of erythrocytic indices, anemia can be classified as normocytic hypochromic because MCHC was lower and MCV was in normal range. Along with parasitic diseases normocytic hypochromic anemia can also be attributed to iron and folate deficiency (haematinics).

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## CONFLICT OF INTEREST

There is no conflict of interest.

## AUTHERS' CONTRIBUTION

SSAS and MAK conceived and designed the study. RJ helped in sample collection and processing. Rafiullah and AA provided the hematology data. SSAS, MAK, MJA, HK drafted the manuscript. All authors revised the manuscript and approved the final version.

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