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SHORT COMMUNICATION

Coccidiosis: Prevalence, Epizootiological Risk Factors, Hematological and Serum Biochemical Profile in Clinically Infected Pet Dogs

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ABSTRACT

The present study was designed to investigate the prevalence, epizootological profile, and haematological and serum biochemistry changes in coccidiosis infected pet dogs of Rawalpindi-Islamabad, Pakistan. For this purpose, 150 blood and faecal samples were collected from dogs of various breeds. Data regarding the epizootolgical risk factors were collected through a questionnaire at the time of sample collection. The faecal examination illustrated a 16.67% prevalence of coccidiosis in dogs. Data analysis of associated risk factors revealed that locality (P = 0.001), domestication (P = 0.000), and sanitary conditions (P = 0.000) have a significant impact. Whereas there was a non-significant association among age, gender, breed and faecal consistency in coccidiosis infected animals. Various hematological parameters were compared and revealed the non-significant difference in coccidiosis positive samples compared to the control group. The biochemical parameters showed significant differences in serum total protein (P = 0.001) and serum albumin (P = 0.003) among infected and control animal samples. This study findings provide baseline data and valuable information to the owners, public health authorities, researchers and the veterinary community on the risk factors, complications and probable transmission of canine coccidiosis.

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INTRODUCTION

Dogs are companion animals, globally adopted for their possessor's social, physical and sentimental well-being (Wells *et al.,* 2022). They have a close association with humans and their habitats, which are the major source for transmitting various intestinal parasites. The most common intestinal parasites are *Coccidia, Giardia*, *Entamoeba, Balantidium coli* and *Trichomonas*. The intestinal parasites frequently induce growth retardation, reduced performance, and increased susceptibility to other infectious disorders (Raza *et al.*, 2018).

Among these, coccidia is the most common intestinal protozoan parasite of Livestock, humans, cats and dogs (Imran and Alsayeqh, 2022). The infection is transmitted via the faecal–oral route or by ingestion of the tissues of the infected host (e.g., mice, rats, hamsters and other vertebrates). The infection is more common in young animals, and has two phases: subclinical (no signs and symptoms) and clinical (Papazahariadou *et al.,* 2007). The second phase is characterized by oocyst formation, anorexia, fever, watery diarrhoea, vomiting, dehydration, gastrointestinal bleeding, and abdominal discomfort (Lappin, 2010). In severe cases, respiratory signs, dysentery, neurological disorders, and mortality can occur. After recovery, animals can develop varying

degrees of immunity. In canine population, survival of their oocysts against disinfection may contribute to disease occurrence (Liberato *et al.,* 2018).

Despite their significant health impact, little interest is attributed to dogs' intestinal parasites, and limited research was conducted in Pakistan. Previously, two exclusive studies on coccidiosis have reported a prevalence of 18% and 16.33% in Lahore (Nisar *et al.,* 2009, Younas *et al.,* 2014). Currently, there is no information regarding the epidemiology of coccidiosis in Rawalpindi-Islamabad, Pakistan. Therefore, the present study was designed to investigate the prevalence, associated risk factors, hematological and biochemical profile in coccidiosis infected dogs.

MATERIALS AND METHODS

Sample and data collection: The sample size was calculated at a 95% confidence level using statistical formula (Thrusfield, 2018). A total of 150 faecal and blood samples were collected from dogs in two cities, Rawalpindi and Islamabad, Pakistan. All the dogs were companion animals, and informed consent was obtained from pet owners before enrolling them in the present study. Samples were collected from apparently healthy and diseased dogs visiting pet clinics with clinical symptoms of fever, vomiting and diarrhoea. The faecal samples were collected from rectum in polythene bags, while blood samples were collected directly from the cephalic/saphenous vein through collection needles (Becton Dickinson and company, Franklin Lakes, U.S.A) in EDTA-coated vacutainers. A predesigned questionnaire was filled at sampling site in order to acquire disease related information (age, gender, breed, body temperature, faecal consistency, hydration status, and dysentery).

Faecal examination: The faecal samples were processed using direct smear, and floatation techniques to detect coccidia oocysts. The direct smears were made by putting a drop of water on a clean slide and the faecal sample was mixed. Finally, the cover slips were placed, and the slides were examined under the light microscope. Faecal samples found negative using direct smear, were subjected to the floatation method with a ratio of 1:3 with saturated zinc sulphate solution (1.180 specific gravity). After centrifugation, the small amount of supernatant was examined under the microscope. The oocysts were identified according to the standard morphological criteria (Zajac *et al.,* 2021).

Hematological analysis: Complete blood count (CBC) of infected and non-infected dogs was carried out to determine various hematological parameters such as red blood cells count, total leukocyte count, packed cell volume, hemoglobin, erythrocyte sedimentation rate, and mean corpuscular volume using haematology analyzer (Abbott Cell-Dyn 3700), Illinois USA).

Serum biochemistry: Various biochemical parameters, including serum total protein, albumin, alanine aminotransferase (ALT), and serum globulins, were determined on Metertek SP-8SO spectrophotometer (Korea), using AMP diagnostic kit (Austria) according to manufacturer instructions.

Statistical analysis: All the data were analyzed with Statistical package Minitab (version 16, USA) using One Way ANOVA and Chi-Square test. The results are shown as Mean ± Standard deviation. The P value < 0.05 was considered as significant.

RESULTS

The microscopic examination of faecal samples revealed that 25 (16.67%) dogs were positive for coccidiosis (Table 1). The prevalence of coccidiosis varied significantly ($P = 0.001$) among the localities such as rural (33.3%) and urban (15.6%) areas (Table 1). The pups (22.9%) presented the highest frequency of infection compared to sub-adult (16%) and adults (7.6%); similarly, male dogs (19.1%) were more infected than females (13.1%) (Table 2). A significant difference ($P = 0.000$) was observed among dogs of poor (16% dogs), moderate (84% dogs) and good (0% dogs) sanitary conditions (Table 3). When analyzed for the purpose of domestication (hunting, search, shepherd, amusement, and security), the coccidiosis was highly significant ($P = 0.000$) in the hunting dogs (25%) (Table 4). Further, the associated risk factor faecal consistency was not associated ($p > 0.05$) with the incidence of disease (Table 2). The prevalence of coccidiosis among assorted breeds of dogs was significant ($P = 0.04$), with the highest incidence in Spaniel (33.33%), Stray (33.33%) and Gultair (33.33%), while lowest was observed in German shepherd (4%) followed by 0% in Accession, Pit-bull, Pug, Floopy, Shih Tzu, Husky and Dalmation breeds of dog (Table 3). The hematological profile of *coccidian* positive (n=25) and negative blood samples (n=125) revealed that most of the studied parameters, such as (red blood cell count, total leukocyte count, packed cell volume, hemoglobin, erythrocyte sedimentation rate, and mean corpuscular volume) were non-significant ($p > 0.05$) (Table 4). Analyses of the biochemical profile of the serum samples showed a significant reduction in serum total protein ($P = 0.001$) and serum albumin ($P = 0.003$) in coccidiosis positive samples as compared to negative samples (Table 5).

DISCUSSION

Companion animals, owing to their close proximity to humans, are a potential source of intestinal parasites (Akram *et al.,* 2019). As they are a global health problem, understanding the epidemiology of intestinal parasitic infections in dogs helps minimize the risk to humans. The overall prevalence of coccidiosis is similar to previous studies conducted in Pakistan, which reported 18% and 16.33 % infection rates, respectively (Nisar *et al.,* 2009, Younas *et al.,* 2014). However, a higher incidence was observed among the dogs in rural areas, which could be attributed to the poor sanitary and hygienic conditions compared to urban areas. The data regarding age and gender of the animals indicated that young pups and male dogs were found more susceptible to infection, as compared to the sub-adult, adults and female dogs, respectively. The findings are similar with previous studies that reported young dogs were more prone to coccidiosis (Buehl *et al.,* 2006, López *et al.,* 2006) due to

Table 1: Prevalence of coccidiosis in dogs

Prevalence	Total samples	Coccidiosis Positive	Percentage%	Chi-sq	P-value
Overall	150	25	16.67%	2.192	0.139
Urban	141	22	15.6%		
Rural	9	3	33.3%	10.287	0.001*

*P<0.001 = highly significant

Table 2: Association of Gender, age and risk factor with prevalence of coccidiosis

Parameters		Total Sample	*Coccidiosis* Positive	Chi-sq	P-Value
Sex	Male	89	17 (19.1%)		0.139
	Female	61	8 (13.1%)	2.192	
Age	Pup	61	14 (22.9%)		0.063
	Sub Adult	50	8 (16%)	5.528	
	Adult	39	3 (7.6%)		
Sanitary condition	Good	09	0 (0%)		
	Moderate	96	21 (84%)	20.740	0.000*
	Poor	45	4 (16%)		
Faecal consistency	Watery		5 (20%)		
	Semisolid		15 (60%)	5.556	0.062
	Solid		5 (20%)		
	All	150	25 (16.67)		

*P<0.001 = highly significant.

Table 3: Prevalence of coccidiosis in domestication and various breeds of dogs

Risk factor		Total Sample	*Coccidiosis* Positive	Chi-sq	P-Value
Purpose of keeping dogs	Hunting	4	1 (25%)		0.000*
	Search	6	0 (0%)		
	Shepherd	4	0 (0%)	45.486	
	Amusement	113	22 (19.4%)		
	Security	23	2 (8.6%)		
Breed with at least 1 positive sample	Labrador	30	6 (20%)		0.044
	Cross	17	5 (29.4%)		
	Bull terroir	10	3 (30%)		
	German Sheperd	49	2 (4%)		
	Rottweiler	7	2 (28.5%)		
	Symonds	5	1 (20%)		
	Pug	5	1 (20%)		
	Stray	3	1 (33.3%)	25.486	
	Boxer	4	1 (25%)		
	Spaniel	3	1 (33.3%)		
	Pointer	5	1 (20%)		
	Gultair	03	1 (33.3%)		
	All	150	25 (16.67)		

*P < 0.001 = highly significant.

low specific immunity (Ramírez-Barrios *et al.,* 2004). The analysis of associated risk factors revealed that the prevalence of coccidiosis varied among purposes of domestication and breeds. The significantly higher incidence was observed in hunting and stray dogs, as they roam the open areas, exposing them to risk factors of disease transmission (Sukupayo and Tamang, 2023).

Regarding hematological changes, packed cell volume, hemoglobin, and total erythrocyte count were non-significantly low in infected dogs than non-infected dogs. This fluctuation in the parameters could be due to dysentery and hemorrhages on intestinal mucosa caused by coccidiosis (Mahmoud *et al.,* 2001). The influence of disease on various biochemical parameters in infected dogs revealed significant decrease in serum total protein and serum albumin, these findings were in agreement with that of (Stockham and Scott 2013; Hashemnia *et al.,* 2014).

There is an increased risk of infection if owners are unaware of the possibility of transmission and associated risk factors. Consequently, diagnostic research, along with the development of communication strategies focused on educating owners of these animals, represent an essential step in preventing intestinal parasites (Raičević *et al.,* 2021).

Table 4: Comparative hematological values in coccidiosis positive and negative dogs.

	Coccidiosis		
Parameters	Positive Mean ± SD	Positive Mean ± SD	P-value
PCV (%)	29.62±10.39	32.73±10.23	0.168
HGB (g/dl)	11.240± 4.215	12.098± 4.049	0.338
ESR (mm/h)	1.2400±0.4359	1.2240±0.4186	0.863
TEC (10^{12}/l)	4.850±1.697	5.528±1.582	0.055
TLC (10^9/l)	15.227± 8.802	15.079±7.788	0.933
Neutrophils %	68.76±11.00	72.00±12.11	0.217
Basophils%	0.1600± 0.3742	0.0800± 0.2724	0.212
Eosinophils%	2.480± 1.447	2.680±1.440	0.527
Lymphocyte%	20.800± 9.220	17.184±9.379	0.080
Monocyte%	7.680±3.288	7.936±3.574	0.741
MCV (fl)	61.064±7.928	60.334±6.924	0.639
MCH (pg)	22.820+3.523	22.598+3.409	0.768
MCHC (g/dl)	37.464+4.513	37.150+3.651	0.707

PCV = Packed cell volume, HGB = Hemoglobin, ESR = Erythrocytes sedimentation rate, TEC = Total erythrocytes count, TLC = Total leukocytes count, MCV = Mean volume of Erythrocytes, MCH = Mean Content of Hemoglobin, MCHC = Mean Concentration of Hemoglobin in Erythrocytes.

Table 5: Comparative biochemical values in coccidiosis positive and negative samples.

Biochemical parameters	Coccidiosis		
	Positive Mean ± SD	Negative Mean ± SD	P-value
Total protein g/dl	4.793±0.859	6.680±1.678	0.001*
Albumin g/dl	2.700±1.106	4.000±1.125	0.003*
Globulin g/dl	2.093±0.891	2.680±1.77	0.263
ALT u/l	70.02±25.76	67.47±21.67	0.772

*P<0.001 = highly significant.

Conclusions: The present study has provided baseline data and valuable information regarding prevalence, epizootiology risk factor and haemato-biochemical profile of coccidiosis to the owners, public health authorities, researchers and the veterinary community. Therefore, the better understanding of canine coccidiosis will lead to the development of more effective disease control strategies.

Authors contribution: MQ, IAK designed this study. MR, MN and ME participated in its coordination. QUA and SA participated in sample collection and performed the experiments. MAH and MNA conducted data analysis. MIM drafted the manuscript. The TUR, and GHD critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interest: The authors declares that they have no conflict of interest.

Ethical statement: All the procedures for animal handling and lab protocols were approved by the ethical committee of Department of Zoology, Pir Mehr Ali Shah (PMAS) Arid Agriculture University Rawalpindi, Pakistan.

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