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Short Communication

STUDY ON THE PREVALENCE AND HEMATOLOGICAL ALTERATIONS IN *TOXOPLASMA GONDII* INFECTED CAPTIVE PHEASANT SPECIES OF BAHAWALPUR ZOO, PAKISTAN

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ABSTRACT

The purpose of this study was to identify the seroprevalence of *Toxoplasma gondii* (*T. gondii*) and hematological alterations in captive pheasant species of Bahawalpur Zoo, Bahawalpur, Pakistan. The blood samples of 100 birds belonging to three different species *viz*. ring-necked pheasant (*Phasianus colchicus*) (n=46), green pheasant (*Phasianus versicolor*) (n=40), and silver pheasant (*Lophura nycthemera*) (n=14) were analyzed through Latex Agglutination Test (LAT) and Enzyme Linked Immunosorbent Assay (ELISA). Seroprevalance through LAT in ring-necked, silver and green pheasants was 10.86%, 7.14% and 7.5%, and through ELISA was 32.60%, 22.5% and 14.28%, respectively. Adults and males had higher prevalence as compared to their counterparts, though, statistically nn-significant ($P \geq 0.05$). In all the studied species, hematological results revealed that mean values of Hb and TEC were higher ($P \leq 0.05$) in non-infected birds as compared to infected ones. This is a preliminary study of a kind and needs further research with large number of sample and population across the country.

Keywords: *Toxoplasma gondii*, ELISA, LAT, Seroprevalance, Oocytes, Hematology parameters.
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INTRODUCTION

Toxoplasma gondii (T. gondii) is an apicomplexan parasite which causes toxoplasmosis. *T. gondii* infection is more prevalent in birds and warm-blooded animals including human (Dubey *et al*., 2010). Domestic cats and other felines (dog, lions, tiger *etc*.) are definitive hosts of *T. gondii* in which sexual phase of parasite occurs and asexual phase occurs in intermediate hosts such as birds and warm-blooded mammals (Dubey, 2010a). Definitive host of *T. gondii* plays a significant role in its transmission through resistant oocytes into the environment which may provide a chance for intermediate host to get infected. Definitive hosts become infected with this parasite by consuming infected tissues from intermediate host such as rodents or birds (Dubey, 2010 a,b). There are various ways for the transmission of this parasite to its intermediate host. In humans, it is mostly transferred through consumption of raw/uncooked meat of infected livestock or birds (Dubey, 2010b). Birds become infected by taking oocysts from undercooked food, eating or drinking food and water contaminated with these oocysts. Birds are clinically resistant to *T. gondii* and are main source of infection in humans. (Dubey, 2010a).

Toxoplasmosis in birds and other animals (mammals including human) is often subclinical. In some cases, parasite causes symptoms in birds such as weight loss, lack of appetite, depression, diarrhea and decline in health. Seroprevalence of toxoplasmosis in wild and domestic birds such as pheasants, peafowls, partridges and owls has earlier been determined through enzyme-linked immunosorbent assay (ELISA), Latex Agglutination Test (LAT), Modified Agglutination Test (MAT) and Indirect Hemaggultination Test (IHA). Birds feed directly from soil, when they feed from contaminated soil with *T. gondii* oocytes they get infection. So, the prevalence study of *T. gondii* in birds is also used as an indicator of soil contamination (Zhang *et al.,* 2014). Studies on the prevalence of antibodies to *T. gondii* in animals can be useful tools in the determination of exposure within a population. There are a variety of serological methods to examine animal exposure to *T. gondii*. The MAT is often carried out as a test for the detection of infected birds as it shows high sensitivity and specificity, and it is can be used in multiple species (Dubey, 2010b; Jakubek *et al*., 2012).

The common pheasant (*Phasianus colchicus*) is a gallinaceous bird belonging to family Phasianidae and originates from central and eastern Asia. They have been

introduced worldwide including New Zealand, Europe, Australia, North, and South America. Pheasants are considered as best source of meat in many parts of the world (dos Santos *et al.,* 2007). There is scanty information regarding *T. gondii* seroprevalance in pheasants. In previous records, 2.4% prevalence of *T. gondii* in pheasants from Czech Republic has been reported by Literak *et al*. (1992). Pheasants feed consists of insects, grains and seeds. Different plumaged pheasants are present in Punjab and Sindh Province of Pakistan. A large population of pheasants is located in district Mansehra, Khyber Pakhtunkhwa. Pheasants are also being kept under captivity in Pakistan.

The main objective of this study was to assess seroprevalance of *T. gondii* in captive pheasants and alterations in hematological attributes of infected and non-infected pheasants.

MATERIALS AND METHODS

Blood samples from 100 pheasants including ring-necked (n=46), green (n=40) and silver (n=14) pheasants from Bahawalpur zoo, Bahawalpur were collected from June 2018 to February 2019. Blood was drawn from *vena ulnaris cutanea* in disposable syringe, using safety clothes and gloves to avoid any contamination. Total 3 mL of blood was collected in vacutainers. About 1.5 mL of the blood was immediately transferred in aliquot coated with EDTA to prevent blood clotting for hematology analysis. Remaining blood was taken in aliquot without anticoagulant for serum analysis. Serum was harvested and stored for further analyses.

Serological diagnosis

Latex Agglutination Test: The commercial agglutination kit (TOXO-100T, Antec Diagnostic Products, UK, CAT 151009) was used to detect Toxoplasma-specific antibodies in serum of hosts. Samples and reagents were brought to room temperature. The assay was performed according to the instructions manual.

Enzyme Linked Immunosorbent Assay: The test procedure was carried out according to the method described by Lind *et al.,* (1997) using CALBOTECH *Toxoplasma* IgG ELISA kit (Cenix Diagnostic GmbH, Germany, TOXOG 37305A) as per manufacturer's instructions.

Hematological analysis: For hematological analysis, Vitalab Flexor E Automatic Analyzer (Netherland) was used. The analyzer was meant for human blood samples and hence, was off-hand validated through blood samples of various birds. The values of different hematological attributes such as hemoglobin (Hb), Total Erythrocytic Count (TEC), Total Leukocytic Count (TLC), hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined.

Statistical analysis: The data was arranged as per age (young and adult), gender (male and female), and species (ring-necked, green and silver pheasants). The prevalence was compared by implying Chi-square through Minitab Version 13 and the hematological attributes between infected and non-infected birds were compared by using independence t-test through SPSS (SPSS for Windows Version12, SPSS Inc., Chicago, IL, USA).

RESULTS

The overall prevalence found in present study was 9 and 26% using LAT and ELISA, respectively (Table 1). Age-wise, higher prevalence was recorded in adult hosts as compared to young ones. Similarly, gender-wise results revealed that higher prevalence was recorded in males as compared to females. Species-wise results showed slightly higher seroprevalence in ring-necked pheasants both through LAT and ELISA (Table 1). All these results were, though, statistically non-significant ($P \geq 0.05$).

In all the studied species, hematological results revealed that mean values of Hb and TEC were higher ($P \leq 0.05$) in non-infected birds as compared to infected ones (Table 2).

Table 1. The seroprevalence of *Toxoplasma gondii* on the basis of species, gender and age in captive pheasants by using LAT and ELISA.

Parameters	No. of hosts observed	No. of Infected Host					
		Prevalence By Using Latex Agglutination	Chi-Square	P-Value	Prevalence By using ELISA	Chi-Square	P-Value
Overall Prevalence	100	9(9%)			26(26%)		
		Age					
Young	36	2(5.55%)	0.815	0.36	7(19.44%)	0.815	0.36
Adult	64	7(10.93%)			19(29.68%)		
		Gender					
Male	49	6(12.24%)	1.23	0.26	13(26.53%)	1.23	0.26

Female	51	3(5.88%)			13(25.49%)		
		Species wise prevalence					
Ring necked Pheasants	46	5(10.86%)			15(32.60%)		
Green necked Pheasants	40	3(7.5%)	2.27	0.36	9(22.5%)	0.36	0.833
Silver Pheasants	14	1(7.14%)			2(14.28%)		

Table 2. Mean±SEM values of hematology in infected and non-infected pheasants.

Parameters'	**Infected hosts Mean± S.EM**			**Non- infected Hosts Mean± S.EM**		
Hematology	**Ring necked pheasants**	**Green pheasants**	**Silver Pheasants**	**Ring necked pheasants**	**Green pheasants**	**Silver pheasants**
Hemoglobin g/dl	17.7±0.8[a]	17.4±0.5[a]	17.4±1.8[a]	18.4±0.2[b]	18.8±0.4[b]	18.1±0.8[b]
Total RBC/mm^3	2.5±0.08[a]	2.5±0.1[a]	2.7±0.1[a]	2.6±0.05[b]	2.6±0.09[b]	3.0±0.06[b]
HCT %	39.2±1.7[a]	42.4±1.7[a]	40.0±5.8[a]	40.7±0.91[a]	39.5±1.1[a]	40.5±2.3[a]
MCV f/l	144.7±2.8[a]	144.3±3.6[a]	146.8±12.7[a]	145.8±1.0[a]	147.2±1.2[a]	144.5±7.2[a]
MCH pg	57.7±2.2[a]	52.3±2.0[a]	60.7±8.8[a]	59.0±1.3[a]	56.4±1.4[a]	60.9±3.0[a]
MCHC G/dl	42.2±0.3[a]	42.2±0.3[a]	42.6±0.4[a]	41.9±0.2[a]	42.6±0.2[a]	41.8±0.5[b]
Platelets/mm^3	2143±340[a]	2533±322[a]	3333±667[a]	2563±195[a]	1968±176[a]	2245±493[a]
TLC/mm^3	148357±650[a]	101067±32479[a]	40700±3816[a]	137017±30549[a]	146238±29364[a]	51036±7783[a]
Neutrophils	4.4±0.9[a]	5.4±0.6[a]	2.0±0.01[a]	5.0±0.3[a]	5.1±0.3[a]	4.2±0.7[a]
Lymphocytes %	87.2±3.2[a]	88.5±1.5[a]	88.3±4.6[a]	87.1±1.1[a]	87.1±1.2[a]	86.3±2.0[a]
Monocytes %	2.8±0.9[a]	3.8±0.6[a]	1.6±0.3[a]	4.3±0.5[a]	4.8±0.5[a]	4.6±0.9[a]
Eosinophils %	3.1±0.8[a]	3.0±0.5[a]	2.0±0.5[a]	2.9±0.3[a]	2.2±0.2[a]	2.7±0.6[a]

The different superscripts showed significantly difference between infected and non-infected hosts within columns

DISCUSSION

The present study is the first of its kind being reported with an aim of presenting seropravelence of *Toxoplasma gondii* in various species of pheasants kept under captivity in Bahawalpur zoo, Bahawalpur. It also caters the objective of analyzing hematological attributes in Toxoplasma infected and non-infected pheasants.

According to this study, the overall seroprevalance through LAT was 9% and through ELISA was 26%. A lower prevalence in pheasants has been reported by Literak *et al.,* (1992) from Czech Republic being 2.4%. Various studies have reported different prevalence rates worldwide in various birds such as in wild birds (89.6%), chukars (67 %), rufous bellied thrush (63.1%), partridges (55%) turkeys (40%), guinea fowls (22%), and pheasants (0%) (Karakavuk *et al*., 2018), Sedlák *et al.,* 2000, Gennari *et al.,* 2014, Zhang *et al.,* 2000). In Iran, a survey conducted by Amouei *et al*., (2018) in migrating and domestic birds determined a seroprevalance of 52% and 51.3%, respectively. Similarly, a study performed by Uterák *et al*., (1992) in wild birds of Czech Republic found that the occurrence of *T. gondii* was 0.5% in house sparrows, 2% in pheasants, 8% in common buzzards, 12% in mallards, and 18% in rooks. In Turkey, 0.95% and 0.90% seroprevalance has been reported in domestic and wild pigeons, respectively by Karatepe *et al*., (2011). Most of the birds including pheasants are ground feeders, if soil is contaminated with parasite they have greater chance to get infected (Dubey *et al*., 2010). Seroprevalence of *T. gondii* in zoo-captive birds may be due to various management issues including mechanical transportation of oocytes shed by feral cats passing through zoo keeper dress, shoes or other cleaning tools.

In the present study, higher prevalence was observed in males than in females (Table 1). Similar results have been reported by Nath *et al*., (2014), Zhang *et al*., (2014), and Lashari *et al*., 2018 while contrasting results have been reported by Gicik and Arslan (2001) and Naqvi *et al*., (2017). Males are much less vulnerable than females to protozoan parasites. There may be different factors such as hormones related to gender, age, environmental factors and nutrition (Roberts *et al.,* 2001) for these variations.

Age-wise higher prevalence was observed in adults as compare to young ones in our study (Table 1). The reasoning behind this may be that there would be more chances for adult animals to get infected than younger ones as suggested previously (Zhao *et al*., 2012). It has been noted in previous research that cats play a significant role in disease transmission to certain livestock, including birds (Yan *et al*., 2009; Zhao *et al*.,

2012). Adults have wide range of feeding than young ones so, they might be having more chance to get infected.

In all the studied species, hematological results revealed that mean values of Hb and TEC were higher ($P \leq 0.05$) in non-infected birds as compared to infected ones (Table 2). Low level of RBCs and HCT may indicate the loss of blood in gastrointestinal tract due to infection similar results are reported by Irizaary- Rovira (2004) and Wakenell (2010). Level of neutrophils increase in infection but its level is decreased in current study which is in contrast to previous results. Neutrophils have granules (lysosomal bodies) which help these cells to fight against various infections causing agents (Wakenell, 2010).

Basophils cell play significant role in birds in response to any infection or inflammation (Koutsos *et al*., 2007). High level of lymphocytes is reported in current study which play significant role to maintain immunity. The TLC helps to indicate the level of infection in birds and in other animals. Tonin *et al*., (2013) reported that higher percentage of lymphocytes was present in infected rats. TLC count indicates the state of immunity, high values may indicate infection, whereas low values indicate immunosuppression (Campbell, 1995).

Conclusion: On the basis of present study, it is concluded that overall prevalence of *T gondii* in pheasants kept captive at Bahawalpur zoo, Bahawalpur is 9% by LAT and 26% by ELISA. The prevalence was higher in males and adult pheasants. Moreover, some of the hematological parameters were altered by *T. gondii* but not all, which helps us to assess the level of infection in hosts due to this parasite. In literature, scanty information is present regarding the prevalence and hematological alterations due to *T. gondii* in captive pheasants across the world. This is a preliminary research one of a kind that needs for further studies with larger populations across the world.

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