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Communication

[*Comunicação*]

Study on prevalence and liver function test enzymes of differently plumaged peafowls (Pavo cristatus) infected with *Toxoplasma gondii* in captivity

[*Estudo sobre enzimas de prevalência e teste da função hepatica de pavões de plumage diferente (Pavo cristatus) infectados com Toxoplasma gondii em cativeiro*]

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The birds in captivity in Pakistan and elsewhere face many infectious disease problems such as toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*). The *T. gondii* is an obligate intracellular parasite which causes toxoplasmosis in birds and mammalia (Tenter *et al.*, 2000). Felids or cats are its definitive hosts while birds and mammals are intermediate hosts (Dubey *et al.*, 2010). Cats shed oocysts in their stool that contaminate the environment and remain infective for more than one year, which permits the transmission of infection to intermediate hosts like birds, mammals, sheep, goats and humans.

In birds, seroprevalence of *T. gondii* is best indicator to access the soil contamination with sporulated oocytes. In birds, it causes loss of appetite, yellowing of mucous, maceration, diarrhea and abnormal functioning of the central nervous system. Like other birds, peafowl is also an intermediate host of *Toxoplasma gondii*. The infection rate of *Toxoplasma gondii* varies by country.

Studies on toxoplasmosis in peafowl are limited in Pakistan. Similarly, hematological and biochemical parameters show the health and metabolic status of the body, but no information is available about biochemical parameters altered due to the infestation of *T. gondii* in captive peafowls. Considering the role of birds in infection of *Toxoplasma gondii* to humans and also according to our knowledge, there is no documented report about the prevalence of toxoplasmosis in captive birds in Pakistan. Therefore, the present survey was conducted to examine the effect of toxoplasmosis in peafowl in captivity and its effects on serum activity of liver enzymes of both seropositive and seronegative peafowls.

The present study was conducted in in Bahawalpur Zoo, Bahawalpur, Pakistan from January to August 2018. The zoo was established in 1942 and is the fourth biggest zoo of Pakistan. Its total area is 25 Acres donated by Sir Nawab Sadiq Muhammad Khan Abbasi, the former 'Amir of Bahawalpur'. It houses about 870 animals including a number of primates, big cats,

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deer, antelope, ratites, reptiles and birds. It lies from 29.402764°N to 71.681601E° and 112m above the sea level where the average rainfall is 20 to 25 cm annually. The Animal Ethics Committee of the Islamia University of Bahawalpur (IUB), Pakistan approved this study.

Blood samples were collected from the peafowls (n=100) (45 male and 55 females) from differently plumaged peafowls including blue (n=28), white (n=10), pied (n=10) and black shoulder (n=52). Information about age, sex and physical condition of birds was gathered during blood sampling. Age-wise peafowls were divided into two groups *viz*. adults (24-36 months) and young (7-9 months). The blood samples were allowed to clot for nearly one hour and then subjected to centrifuge for 15min. Serum was harvested and kept at -20ºC until further analyzed for serum chemistry attributes at the Parasitology Research Laboratory of Department of Zoology, IUB.

The commercially available Toxo Latex Kit (Atlas Medical, Blankenfelde, Germany) was used to detect specific antibodies in serum of birds that consisted of a positive control, a negative control and a Latex Reagent. The reagent contained suspension of polystyrene particles coated with antigen of *T. gondii*. Positive control shows agglutination when added to serum and negative control does not show agglutination. Antigen- antibody reaction could take place when serums which contain antibodies against *T. gondii* were tested and this reaction can be easily visualized because of agglutination. Both the reagents and serum were brought at the room temperature prior to use. A serum sample of bird was serially diluted twofold in phosphate buffered saline from 1:2 to 1:8.

A sample found positive at 1:2 to 1:8 serial dilutions was tested at higher, doubling dilutions 1:16, 1:32, 1:64. One drop of diluted serum sample was placed onto the black area of the slide. The latex reagent was mixed well, and one drop was added to each serum drop. Both drops were mixed with the help of a stirrer and the slide was tilted. A clear positive reaction indicated the presence of *T. gondii* antibodies, which reflected either a past infection or an evolving infection. A negative reaction indicated the absence of *T. gondii* antibodies.

The *Toxoplasma* IgM ELISA kit (Calbiotech Inc. CA, USA) was used to examine *T. gondii* antibodies in birds as per manufacturer's instructions. Serum samples, positive and negative controls were added to the microwells. *T. gondii* antibodies if present in serum samples showed agglutination by forming an antibody-antigen complex. All unbound antibodies were washed away, and enzyme conjugate was added which bind to the antibody-antigen complex. Excess enzyme conjugate was washed away and the substrate (TMB) was added. The plate was allowed to incubate, and the color was developed. A blue solution appeared in the presence of antibodies, which turned yellow after the addition of the stop solution. There was no coloration in the absence of antibodies. The development of color was stopped by adding stop solution. The optical density (OD) of the microplate was read at 450nm with microplate absorbance reader 800 TS, BIOTK, UK. Results were written as the % age of the mean absorbance values of the sample (S) to the mean absorbance value of positive (P) control given with the diagnostic kit. According to manufacturer's reference, sera with S/P $\leq$ 40% = Negative, 40% > 50% Doubtful, 50%$\leq$S/P < 200% = Positive, S/P $\geq$ 200% = Strong positive.

Liver function test enzymes such as Albumin, bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were analyzed using commercial assay kits (Spinreact, Spain) and a chemistry analyzer. The data were subjected to Chi-square statistical analysis using the minITAB version 16.0 software. Results were tested against P$\leq$0.05 for significance. According to LAT, 37% (37/100) of peafowls were found to be positive. The overall prevalence in the pied, blue, black shoulder and white plumage peafowl was (4/10) 40%, (11/28) 39.28%, (19/52) 36.58% and (3/10) 30%, respectively. Gender-wise non-significantly higher prevalence was recorded in males (17/45) 37.77% as compared to females (20/55) 36.36%. Similarly, non-significantly higher prevalence (30/79) 37.97% was recorded in adults as compared to young ones (7/21) 33.33% (Table 1).

Table 1. The prevalence of *Toxoplasma gondii* in peafowl by LAT and ELISA

Groups	Category	By LAT Peafowls examined (n=100)	Infected Peafowls	Prevalence %	By ELISA Peafowls examined (n=100)	Infected Peafowls	Prevalence %
Plumage	Pied	10	4	40[a]	10	2	20[a]
	Blue	28	11	39.28[b]	28	8	28.57[a]
	Black shoulder	52	19	36.58[b]	52	17	32.69[b]
	White	10	3	30[b]	10	3	30[b]
Gender	Male	45	17	37.77[a]	45	16	35.55[a]
	Female	55	20	36.36[a]	55	14	25.45[b]
Age	Young	21	7	33.33[a]	21	5	23.80[a]
	Adult	79	30	37.97[a]	79	25	31.64[b]

In columns different superscripts show significant difference (P<0.05).

According to ELISA, the overall prevalence was 30% (30/100) and in different plumages such as pied, blue, black shoulder and white plumage peafowls, it was (2/10) 20%, (8/28) 28.57%, (17/52) 32.69% and (3/10)30%, respectively. Gender-wise, significantly higher prevalence was observed in males (16/45) 35.55% as compared to females (14/55) 25.45%. Age-wise, significantly higher (25/79) 31.64% infestation was recorded in adults as compared to young birds (5/21) 23.80% (Table 1). Level of bilirubin was non-significant, while the level of Albumin and ALP were significantly elevated in infected than in non-infected hosts. The ALT and AST decreased in the infected hosts than in non-infected peafowls (Table 2).

Table 2. Mean±SEM values of liver function test enzymes in infected and non- infected hosts

Parameters	infected hosts				Non-infected hosts			
Liver function tests	Black shoulder plumage	White plumage	Blue plumage	Pied plumage	Black shoulder plumage	White plumage	Blue plumage	Pied plumage
Bilirubin	1.7± 0.10[a]	1.56± 0.41[a]	1.92± 0.20[a]	1.45± 0.25[a]	1.71± 0.06[a]	1.47± 0.08[a]	1.72± 0.40[a]	1.46± 0.51[a]
Albumin	3.28± 0.18[a]	3.65± 0.46[a]	3.22± 0.33[a]	2.81± 0.51[a]	3.49± 0.15	2.84± 0.19[b]	3.22± 0.76[a]	3.09± 0.29[b]
ALT	58.53± 3.17[a]	41.66± 0.33[a]	61.63± 4.23[a]	64.5± 1.50[a]	62.71± 1.67	62.86± 2.44[b]	58.40± 11.27[b]	54.13± 3.38[b]
AST	68.53± 3.16[a]	73.33± 8.35[a]	68.50± 5.99[a]	76.5± 10.51[a]	75.37± 2.04	66.57± 7.12[b]	75.70± 11.35[b]	68.8± 8.40[b]
ALP	520.3± 66.41[a]	283.3± 21.90[a]	584.1± 91.81[a]	893± 0.50[a]	463.6± 38.11[b]	476.0± 114.[b]	478.2± 239.2[b]	298± 31.32[b]

In rows different superscripts show significant difference (P<0.05).

In Pakistan, no data is available on seroprevalence of *T. gondii* in peafowls. This is the first such study on seroprevalence of peafowls kept at Bahawalpur Zoo, Pakistan. Among birds, peafowls also serve as intermediate hosts which cause significant health problems to the public (Tian *et al.,* 2012). The results attained through LAT were re-tested by ELISA in order to enhance diagnostic accuracy in the present study. Previous researches have reported a higher seroprevalence of *T. gondii* in birds in different regions of the world including Iran (32.3%), China (31.8%), and

Iraq (31%, 67% and 56%) in geese, peafowls, ducks and chicken, respectively (Tian *et al.,* 2012, Lashari *et al.,* 2018). Lower prevalence rate has been recorded in Colorado (3.9%), Portugal (4.6%), and China (8.36%) by Dubey *et al.,* 2010, Waap *et al.,* 2008 and Zhang *et al.,* 2014 for pigeons, parrots and pet birds, respectively. The difference in prevalence could be attributed to difference in breed and species of birds.

In the present study, according to LAT and ELISA, a higher prevalence rate was recorded in males as compared to females. Zhang *et al.* (2104) in China reported higher prevalence of *T. gondii* in male (10.43%) parrots as compare to females (6.08%). Same results have been reported by Lashari *et al.* (2018). The relation of gender with susceptibility of host to toxoplasmosis might be due to genetic predisposition and difference in hormones. Testosterone is known for its immunosuppressive activity (Seli and Arici, 2002). The females can be immune because of different factors *e.g*. diet, age and surrounding conditions. Males are less susceptible to protozoan parasites than females. Causes of higher prevalence of *T. gondii* antibodies in males in present study may be due to steroid hormonal difference.

In the present study, a significant difference ($P<0.05$) was detected in seropositive adult hosts as compared to young hosts. Similar results have been reported by Zhang *et al.* (2104). Present and previous studies showed that adults have more chance to get an infection by ingestion of oocysts with contaminated food because they have a wide range of feeding area than young ones.

The present study showed significant biochemical changes with statistically high level of bilirubin, Albumin and ALP in the infected host as compared to non-infected hosts. ALT increased in infected blue and pied plumaged peafowls whereas level of AST elevated in infected white and pied plumaged peafowls. In general, the analysis revealed that there was a significant effect of toxoplasmosis on liver function test enzymes of hosts. Similar results were reported by Amany *et al*. (2010) from Egypt, Dawood and Mahmood (2012). In contrast to the current study, a decreased level of albumin and elevated level of ALT and AST have also been recorded in *T. gondii* infected hosts (Amany *et al*., 2010). In serum, biochemical profile level of AST and ALT indicate the working condition of liver cells.

These enzymes play a significant role in metabolic transmission of amino acid mostly present in the liver and some other tissues. In case of any cellular damage, AST and ALT spread into the surrounding tissues and its level of activity increased. In case of any hepatic injury activity level of AST and ALT increases (Adeyemi and Akanji, 2011). Toxoplasmosis causes infiltration of liver cells and in portal area, damage endothelial cells and necrosis in hepatocytes. Moreover, the elevated level of AST and ALT also provide information about infection and inflammation induced by *T. gondii*. Toxoplasmosis causes significant and permanent destruction to the liver, noteworthy developments of organisms arising in alterations in the metabolism of the liver (Amany *et al.,* 2010).

In a nutshell, toxoplasmosis is widespread in peafowls in Bahawalpur Zoo, Pakistan which indicates an environmental contamination with oocysts of *T. gondii.* According to LAT and ELISA highest prevalence was recorded in males as compared to females. Age-wise higher prevalence was recorded in adult peafowls. Toxoplasmosis also affects the liver function test enzymes of the hosts. The present study may provide preliminary data for the control of *T. gondii* in other zoo birds to minimize the load of *T. gondii*. We recommend the zookeepers, veterinary staff and allied stake holders to devise a directional strategy towards control and eradication of *T. gondii*.

RESUMO

O presente estudo foi realizado para determinar a prevalência geral de toxoplasmose em pavões de plumagem diferente e seu efeito nas enzimas de teste da função hepática dos hospedeiros. Um total de cem pavões de plumas diferenciais, como ombro preto (n = 52), azul (n = 28), branco (n = 10) e arlequim (n = 10) foram estudados no zoológico de Bahawalpur, no Paquistão, usando o Latex Agglutination Test (LAT) e ensaio imunossorvente ligado a enzima (ELISA). A prevalência geral por LAT e ELISA foi de 37% e 30%, respectivamente. Por LAT, observou-se uma prevalência não significativamente maior (P≥0,05) em gênero

(37,77%) nos machos do que nas fêmeas (36,36%), enquanto os adultos apresentaram uma prevalência maior (37,97%) em relação aos jovens (33,33%). De acordo com o ELISA, uma prevalência significativamente (P <0,05) maior (35,55%) foi observada nos machos do que nas fêmeas (25,45%) e significativamente (P <0,05) maior prevalência (31,64%) foi registrada nos adultos do que nos jovens (23,80%). A análise do perfil bioquímico sérico mostrou que o nível de bilirrubina não teve elevação significativa nos hospedeiros infectados, em comparação aos não infectados, enquanto a concentração de albumina, alanina aminotransferase (ALT), aspartato aminotransferase (AST), fosfatase alcalina (ALP) foi significativamente (P <0,05) diferente nos hospedeiros infectados. Conclui-se que a toxoplasmose afeta as enzimas do teste da função hepática. Essa é uma pesquisa preliminar e requer mais pesquisas em todo o país, com populações e amostras maiores.

Palavras-chave: Toxoplasmose, pavões, LAT, ELISA, enzimas de teste da função hepática

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