Research Article

Hematological Changes in Free-Range Chicken (*Gallus domesticus*) Naturally Infected with *Toxoplasma Gondii*

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Abstract | The aim of present study was to determine the seroprevalence and hematological changes in free-range chicken (*Gallus domesticus*) naturally infected with *Toxoplasma gondii* (*T. gondii*). Blood samples of chicken (n=71), were collected from Cholistan areas of Bahawalpur (Punjab), Pakistan. Serum samples were tested for toxoplasmosis with a commercial latex agglutination kit (Antec Diagnostic products, UK). The overall prevalence was 26.76%. The significant (P<0.05) higher seroprevalence was observed in males (34.28%) than in females (19.44%). For analysis of hematological parameters blood samples were collected. The mean values for hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells Count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells count (WBC), Heterophils (%), Eosinophils (%), Basophils(%), Monocytes(%), and Lymphocytes(%) values of negative chickens were 9.43±0.49 g/dl,,25.81±1.34%,1.84±0.13 x10^6/µL,151.7±11.0 fl, 3.85±0.22 pg, 39.34±2.07%, 3.98±0.13 x10^3/µL, 45.71±1.26%, 2.08±0.29%, 1.54±0.17%, 2.58±0.29% and 48.10±1.36% and for positive chickens were 9.35±0.89 g/dl, 24.63±2.21%, 1.68±0.17 x10^6/µL, 168.9±17.5 fl, 5.92±1.95 pg, 59.3±19.5%, 3.98±0.23 x10^3/µL, 45.74±2.76%, 3.05±0.45%, 1.58±0.23%, 0.95±0.19% and 48.74±3.15, respectively. MCH, eosinophils and monocytes showed significant difference. Hb, PCV, RBC, MCV MCHC, heterophils, basophils and lymphocytes values showed non-significant difference. Furthermore, some of the hematological parameters are altered by *T. gondii* but not all. In literature a little information is present regarding the hematological changes due to *T.gondii* in chickens of Cholistan, Bahwalpur, Pakistan. This is pilot study one of a kind and need for further studies with larger populations and samples across the country.

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Introduction

Protozoan parasite *Toxoplasma gondii* (*T. gondii*) is found worldwide that potentially infects all warm-blooded vertebrates including mammals, birds and humans (Wang et al., 2015). It is one of the most widespread zoonotic pathogens in the world. Recent studies have focused on this agent and shown that it may be responsible for the emergence of several different health problems. It causes a cosmopolitan zoonotic disease Toxoplasmosis (Dubey, 2010a). This parasite also transmits in human beings by accidental ingestion of contaminated food or water with oocytes shed by cat or by ingestion of infected raw or undercooked meat (Tenter et al., 2000). As the birds are the intermediate hosts of *T. gondii* so chickens are

also victimized by its worldwide and can create serious problems for humans (Vieira et al., 2018). Meat from infected poultry (chickens) is consumed widely in many countries and is known to be the primary source of infection in humans (Dubey et al., 2007).

Worldwide seroprevalence of *T. gondii* in chickens, ducks and geese are summarized by Dubey (2010b). Across the world the prevalence of toxoplasmosis is variable, in different countries ranging from zero to hundred percent depending upon their civilizations, societies, inhabitant's life styles, animal's age and climate conditions (Smith, 1991; Tenter et al., 2000). In Pakistan, seroprevalence studies have shown 36.33% prevalence in backyard poultry in district Faisalabad (Akhtar et al., 2014) and 18.85% in domesticated and caged chickens in district Mardan, Khyber Pakhtunkhwa, Pakistan (Mahmood et al., 2014).

Previous study has been carried out about the prevalence of *T. gondii* in world and as well as in Pakistan. However, such studies allied with hematological parameters of infected chicken from Cholistan desert of Pakistan have not yet been reported. Therefore, the main objective of this study was to determine the *T. gondii* seroprevalance and effects on hematological parameters in chickens from Cholistan desert, Pakistan.

Materials and Methods

Study period and area

The present study was conducted in the month of April- September, 2017 in Cholistan desert. The study was conducted in Cholistan (27º42´and 29º45 N 69º52´and 75º24 E), 112m above the sea level.in Bahawalpur, Pakistan. Area has characteristics of arid, hot subtropical climate with mean rainfall 180mm annually. The average annual temperature of the area is 28.33˚C but in June the temperature exceeds 45˚C (Farooq et al., 2010).

The study was approved by the Directorate of Research, Innovation and Commercialization of the Islamia University of Bahawalpur, Pakistan through the Department of Life Sciences and University College of Veterinary and Animal Sciences (UCV and AS).

Study birds

A total of n=71 (males=35 and females=36) chickens (*Gallus domesticus*) was included in this study. Chickens are arranged into three age groups (6-12 months), (13-18 months) and (≥ 19 months).

Sampling and data collection

Blood samples (3mL) were collected from wing vein of chickens (n=71) as per recommended protocol. Blood samples were stored as two aliquots: clotted for harvesting serum and un-clotted (0.5 M EDTA) for hematological analysis. The needed data about age, gender from each chicken was collected at the time of sampling.

Serological diagnosis

The samples sera were screened for anti-*T. gondii* antibodies by Latex agglutination test (LAT) using commercially available kit (Antec Diagnostic products, UK). The assay was performed according to the producer's instructions.

Hematological analysis

The blood samples were examined for hematological parameters, using Sahli's haemoglobinometer method for hemoglobin (Hb), microhematocrit was used for Packed Cell Volume (PCV) and Neubauer's haemocytometer was used for counting Red Blood Cells (RBCs) and White Blood Cells (WBCs). The erythrocytic indices *viz.* Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using the formula given by Jain (1998). For differential leukocyte counts (DLC), a drop of whole blood was used for making a thin smear which was air dried, fixed in ethyl alcohol and stained using Field's Stain (SDL, Pakistan) (Chunge et al., 1989).

Statistical analysis

The results of prevalence were expressed in percentages whereas those for hematology in Means and SE. The values between different gender, age groups analyzed by Chi square and hematological parameters analyzed by using t-test through Minitab version 2013. The $P \leq 0.05$ was considered as statistically significant.

Results and Discussion

The overall prevalence

Samples sera (n=71) obtain from free ranged *Gallus domesticus* in Bahawalpur n=19 (26.76%) were found seropositive for *T. gondii* (Table 1). The same

prevalence rate has been reported 26.6% for Indonesia (Dubey et al., 2008). The prevalence rate recorded in present study is lower than those reported in some regions including 66% in Amazon, Brazil (Dubey et al., 2006); 53% in Argentina (More et al., 2012); 47.2% in Giza, Egypt, (El-Massry et al., 2000); 36% in China (Zhao et al., 2012); 36.33% in Pakistan (Akhtar et al., 2014).

Table 1: *Overall prevalence of T. gondii in male and female chickens of Cholistan areas, Pakistan.*

Parameters	Male chicken	Female chicken	Total
No. of chicken examined	35	36	71
No. of chicken infected	12	7	19
Prevalence (%) age	34.28[a]	19.44[b]	26.76

Different superscripts in rows shows significant difference (P<0.05).

Moreover, the prevalence recorded in present study is higher than those reported in some regions including Pakistan (18.85%) (Mahmood et al., 2014); Israel (18%) (Dubey et al., 2004); India (17.9%) (Sreekumar et al., 2003); Egypt (13.95%) (Aboelhadid et al., 2013); northwest China (7.26%) (Cong et al., 2012). The difference in rate of prevalence may be due to the geography, climate, feeding and living styles, and number of cats may contribute to the differences in *T. gondii* seropositivity in chickens.

Gender wise seroprevalence

In present study, higher prevalence (P<0.05) was observed in males (34.28%) as compared to that in females (19.44%) (Table 1). Similar results have been reported in different areas of Bangladesh by Nath et al. (2014). In contrast to the present study, higher prevalence was recorded in females as compared to male chicken by Gicik and Arslan (2001) in Turkey and Naqvi et al. (2017) in Pakistan. Males are less susceptible to protozoan's parasites then females. There are various factors *e.g.*, sex associated hormones, age, environmental factors and nutrition (Alexander and Stimson, 1988; Roberts et al., 2001).

Age wise seroprevalence

In this study, a significant difference (P<0.05) was detected in seropositive older chickens as compared to younger chickens (Table 2). The logic behind this might be that the older animals had more possibilities to get infected than the younger ones. (Zhao et al., 2012). In previous studies it is reported that the cats play an important role in transmission of disease to other animals including chickens (Yan et al., 2009; Zhao et al., 2012).

Table 2: *Age wise prevalence of T. gondii in chickens of Cholistan areas, Pakistan.*

Age group (Months)	Chicken examined	No. of positive	Positive (%) age
6-12	54	11	20.37
13-18	14	7	50
≥ 19	3	1	33.3

Hematological parameters

Present study showed significant hematological changes with statistically high level of eosinophils, monocytes and MCH (Table 3). Present study indicates the high level of eosinophils similar to other studies. According to Irizaary-Rovira (2004) in peoples with parasitic infections large numbers of eosinophils are produced and eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration). Eosinophils are typically associated with allergic reaction and parasitic infections and rarely seen in high number except in raptor species (Samour, 2004). Samilar results are reported by Advincula et al. (2010) that seropositive cats lacked or had very few monocytes. By Smart et al. (1973), it has been reported that the toxins released into the blood by certain microorganisms can cause absence or low level of monocytes and the leucopenia is often related with toxoplasmosis.

Table 3: *Mean±SEM values for hematological parameters of T. gondii positive and negative chickens.*

Parameters	Negative chicken n=52	Positive chicken n=19
Haemoglobin (g/dL)	9.43±0.49[a]	9.35±0.89[a]
Packed cell volume (%)	25.81±1.34[a]	24.63±2.21[a]
Red blood cells count (x10^6/µL)	1.84±0.13[a]	1.68±0.17[a]
Mean corpuscular volume (fl)	151.7±11.0[a]	168.9±17.5[a]
Mean corpuscular hemoglobin (pg)	3.85±0.22[a]	5.92±1.95[b]
Mean corpuscular hemoglobin concentration (%)	39.34±2.07[a]	59.3±19.5[a]
White blood cells count (x10^3/µL)	3.98±0.13[a]	3.98±0.23[a]
Heterophils (%)	45.71±1.26[a]	45.74±2.76[a]
Eosinophils (%)	2.08±0.29[a]	3.05±0.45[b]
Basophils (%)	1.54±0.17[a]	1.58±0.23[a]
Monocytes (%)	2.58±0.29[a]	0.95±0.19[b]
Lymphocytes(%)	48.10±1.36[a]	48.74±3.15[a]

Different superscripts in rows shows significant difference (P<0.05).

The present study indicates the low hemoglobin, PCV, RBC count, MCV values and higher MCHC, heterophils, basophils, lymphocytes percentages in infected chickens however statistically non-significant. There is no change occur in WBCs of infected chicken. Similar results were found by Irizaary- Rovira (2004) and Wakenell (2010) as reported in coccidiosis caused by *E. tenella* and *E. brunette*. The reduction in the RBC is due to the loss of blood into the gastrointestinal tract (external blood loss) and infectious disease (Irizaary-Rovira, 2004). Similar results recorded by Atmaca et al. (2015) that higher percentage of neutrophils was observed in *T. gondii*-infected group as compared to control group. Heterophils also contain a variety of granules that contribute to the first line host defense against protozoa, bacteria, fungi and some viruses (Wakenell, 2010). It is also reported that the neutrophils are important for controlling toxoplasmosis in humans and mice (Bliss et al., 1999; Del Rio et al., 2001). Bosophils are one of the first leukocytes to inter tissue as part of the early inflammatory response in birds (Koutsos et al., 2007). Irizaary-Rovira (2004) reported that the increased lymphocytes count may be associate with the effect of caeca and intestinal inflammation. Chronic antigenic incentive may result in increased lymphocytes count because its primary function is formation of humoral antibodies for cell mediated immunity. By Tonin et al. (2013) is reported that the higher percentage of lymphocytes was present in infected rats. WBC counts indicates the state of immunity, high values may indicate infection, whereas low values indicate immunosuppression. (Campbell, 1995). The results of this study were contrast to those reported by Rose et al. (1979) that number of leukocytes increased in chicken blood infected with *E. maxima* and *E. acervulina*. Based on this study, it is concluded that overall prevalence of *T gondii* in chicken (*Gallus domesticus*) is 26.76% in Cholistan, Desert Pakistan. The seroprevalence is higher in males and adults chicken. Furthermore, some of the hematological parameters are altered by *T. gondii* but not all. In literature a little information published regarding the hematological changes due to *T.gondii* in chicken Cholistan, Pakistan. This is a preliminary study one of a kind that need for further studies with larger populations across the country.

Author's Contribution

Mushtaq Hussain Lashari: Design the study and wrote the manuscript.
Fozia Afzal: Lab. work and data acquisition.
Umer Farooq: Performed proof reading of the draft and corrected references.

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