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Seroprevalence of *Toxoplasma gondii* Infection in *Gallus domesticus* of District Mardan, Khyber Pakhtunkhwa, Pakistan

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Abstract.- The aim of present study was to determine the rate of prevalence of *Toxoplasma gondii* in chickens and to apprise the community about the negative effects of the *T. gondii* in the study area. A total of 536 blood samples of caged and free range chicken were tested, of which 18.85% were found infected with *T. gondii*. In 64 blood samples from caged 5.90% were found sero-positive. While out of 468 free range chickens 20.70% were found with anti-toxoplasma antibody. The seroprevalence rate was 22.20% detected in female birds of free range chickens, while 17.80% were found sero-positive in male free range chickens. A greater number of samples were found positive in free range chickens, while little number of caged chickens was with negative result. The present research study shows that infection of toxoplasmosis is high in chickens in Mardan, Pakistan, which is a public health threat in the study area.

Key Words: Toxoplasmosis, indirect hemagglutination antibody (IHA), *Toxoplasma gondii*, Chickens, Mardan

INTRODUCTION

Toxoplasma gondii is an obligate, intracellular protozoan parasite, cosmopolitan in distribution and a causative agent of toxoplasmosis (Blader and Saeij, 2009; Aldebret *et al*., 2011; Shah *et al.,* 2014). Its infection is generally caused by ingesting the oocyst stage, reaching to body tissues via water and food contacted by cat's faeces (Schlundt *et al*., 2004; Webster, 2007; Robert-Gangneuxa and Darde, 2012). The condition is also passed via placenta from mother to offspring while feeding the child during pregnancy (Sibley *et al*., 2009; Hajsoleimani *et al*., 2012). The other possible cause of toxoplasmosis transmission occurs through blood or leucocytes from infected blood donors to patient (Zhou *et al*., 2011; Bodaghi *et al.,* 2012).

Prevalence rate of *T. gondii* varies in different countries (Shah *et al.,* 2014). Toxoplasmosis is reported 30% in Europe and generally 10% observed across USA (Hill and Dubey, 2002). A highest rate of toxoplasmosis is recorded across Serbia and Poland with 58% and 60% (Hasan, 2011). Estimatidly, about 33% of the human population may harbour this parasite, however its prevalence rate varies with climate, nutritional factors, geographic factors, sociocultural habits and transmission route (Shah *et al*., 2013b). Improved animal husbandry practices and increased awareness can well counter the possible risks of toxoplasmosis across the globe (Weiss and Dubey, 2009; Shah *et al.,* 2013a).

The previous studies recorded 42.28% and 44.13% toxoplasmosis infection in goats and sheep, respectively in district Mardan. However, overall infection recorded in district Mardan was 43.12 % (Shah *et al.,* 2013a). Similarly toxoplasmosis was recoded in federally adminisrted tribal area, in Mohamand agency with 32.29% in farm animals (Shah *et al.,* 2013b). Recently, the seroprevalence of *T. gondii* recorded in human population of the nearby district Mardan with 28.44% infection (Shah *et al.,* 2014).

Initially toxoplasmosis infection asymptomatic or specified with only generally symptomatic with mild flue (Goz *et al.,* 2007; Selseleh *et al.,* 2012). Medically it leads to encephalitis (Hill and Dubey, 2002; Blader and Saeij, 2009; Jones and Roberts, 2013), abortions (Mori *et al*., 2011) and other congenital defects in humans (Chaudhary *et al*., 2006). Other conditions

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caused are lymphadenopathy, retino-choroiditis in new borns which readily leads to blindness (Blader and Saeij, 2009; Weiss and Dubey, 2009). *Toxoplasma gondii* may also become the cause of diseases like pericarditis, pneumonia, and other neurologic disorders (Cabanas *et al*., 2012; Lee *et al*., 2012), central nervous system damage, mental retardation, chorioretinitis, blindness, meningoencephalitis, hydrocephalus, intracranial calcifications and epilepsy with fetal results or spontaneous abortion in about 10% of cases (Khan *et al*., 2011; Mori *et al*., 2011; Cabanas *et al*., 2012). It also leads to ocular disease (Jones and Roberts, 2013). The congenital infection can form lesions in the retina of the eye which can result in eye pain and might loss the vision power of the eye (Weiss and Dubey, 2009).

Toxoplasmosis occurs in goats, sheep (Stormoen *et al*., 2012) and cats (Holzworth, 1987), dogs (Aiello and Mays, 1998) and horses (Nicolle and Manceaux, 1908). It also causes mortality in birds of different species including domesticated and commercially available chickens (Devada *et al*., 1998; Murao *et al*., 2008; Wu *et al*., 2011). Other birds are pigeons (Biancifiori *et al*., 1986), sparrows (Pinowski *et al*., 1999). As the birds are the intermediate hosts of *T. gondii* so chickens are also victimized by *T. gondii* infection worldwide and can create serious problems for humans (Devada *et al*., 1998). Meat from *T. gondii* infected poultry (chickens) is consumed widely in many countries and is known to be the primary source of infection for humans (Dubey *et al*., 2007)

The present study aims to record the incidence of toxoplasmosis in chickens of district Mardan Khyber Pakhtunkhwa province in Pakistan. This investigation will further open new gates for the molecular level findings of *T. gondii* infection in the region and will help in general awareness of the locals using commercial chicken as a preferable source of protein source.

MATERIAL AND METHODS

Serum collection

The present study was conducted to determine the sero-prevalence of *T. gondii* in chickens including commercially available as well as domesticated chickens in Mardan District of Khyber Pakhtunkhwa province in Pakistan. Samples were randomly selected across the study area with a 1632 km^2 area. A total of 536 samples were collected from 03 geographically different areas (tehsil Mardan, tehsil Katlong and tehsil Takht Bhai). About 02 mL of blood was collected from wings by puncturing through disposable needle in vein. Collected blood was immediately transferred to collecting tubes containing anticoagulant. The samples were centrifuged at 3500 rpm for 5 min at 25-30°C or room temperature for the extraction of serum. The serum collected was stored in eppendorff tubes at -20°C until further analysis to be carried out (Chumpolbanchorn *et al*., 2009).

Serological examination

The commercial Indirect Hemagglutination Antibody (IHA) test kits were used according to manufacturer protocol (SERFIB, France) for detection of antibodies of *T. gondii*. A serial two fold dilution was prepared starting from 1:40, up to 6th dilution. A drop of sensitized red blood cells was poured in the each vile containing diluted serum. While un-sensitized and sensitized blood was used as positive and reagent control. The samples were properly mixed and the plate was allowed to stand for 02 hours before recording the reading. All sera reactivated at $\geq$1:80 were considered as positive (Shah *et al*., 2014).

Statistical analysis

The results were expressed in percentages. The values between different sex groups were analyzed by using Chi Square test for Windows (Release 16.0 standard version). The P value < 0.05 was considered as statistically significant.

RESULTS

The present study was conducted regarding "seroprevalence of *Toxoplasma gondii* infection in domesticated and caged chickens in District Mardan". The study area was divided in three geographically different zones *i.e.* Tehsil Mardan, Tehsil Takht Bhai and Tehsil Katlang with a total area of 1632 km^2 area. A total of 536 blood samples were collected in Mardan district. There were 101

positive samples (18.85%) and 435 (81.15) were with no anti-toxoplasma antibody detection. Over all a total of 18.85 % toxoplasma infection was recorded across the study area (Table I).

Table I.- Toxoplasmosis in caged (briolers) and free or domesticated chickens.

Chickens	N	Positive (%)	Negative (%)
Caged (Broiler)	68	4 (5.90)	64 (64.10)
Free range (Domestic)	468	97 (20.70)	371 (79.30)
Total chickens	536	101 (18.85)	435 (81.5)

Table II.- Toxoplasmosis in caged (briolers) and free or domesticated chickens according to sex.

Chickens	N	Positive (%)	Negative (%)
Caged/Broiler			
Male	10	-	10 (100.00)
Female	58	4 (6.90)	54 (93.10)
Total	68	4 (5.90)	64 (94.10)
Uncaged/Domestic			
Male uncaged	152	27 (17.80)	125 (82.20)
Female uncaged	316	70 (22.20)	246 (77.80)
Total	468	97 (20.70)	371 (79.30)
Grand total	536	101 (18.85)	435 (81.15)

The data collected shows that 04 (5.90%) out of 68 samples were found positive for toxoplasma infection in caged chickens (broilers). 94. 10 % sample was recorded negative for toxoplasmosis antibody in their blood (Table II). However the rate of infection was quite similar in both sexes of caged broilers. Table II, shows 468 blood samples were collected from free range (domestic) chickens for the detection of anti-toxoplasma antibody in district Mardan. 97 (20.70%) out of total 468 samples were found positive (20.70%), while 371 (79.30%) samples were recorded negative by testing for *T. gondii* infection. Regarding sex, toxoplasmosis was recorded high in female birds (22.20%) as compared to male uncaged or domesticated chickens, as seen in Table II. The data showed that female have little high rate of prevalence (22.20%) as compare to male (17.80 %) domestic chicken.

DISCUSSION

The birds are the important hosts of *T. gondii* and also considered as epidemiologically important for toxoplasmosis infection. Usually toxoplasma infection is passed to ground-foraging birds when coming in contact with soil contaminated with toxoplasma oocysts. Cats are the commonly known for frequent infection of *T gondii*. Previous studies showed that *T. gondii* causes major mortalities in wild birds of different species (Murao *et al*., 2008; Wu *et al.,* 2011). Among birds, chickens also serve as the intermediate hosts of *T. gondii*, so chickens are readily victimized across the globe and even causing problems for human population as well (Devada *et al.,* 1998). Regarding prevalence, *Toxoplasma* infection is very much low in caged birds and chickens. However, the seroprevalence of toxoplasmosis is significantly high in free-range or backyards chickens. Due to their habit of feeding close to the ground, free-range chickens are usually counted as a good indicator of environmental contamination by *Toxoplasma* oocysts (Dubey, 2010; Shah *et al.,* 2013a).

Out of 536 sera samples, obtained from Mardan district, Pakistan, 101 (18.85%) were found seropositive for *T. gondii*. This figure for seroprevalence is quite lower than 64% toxoplasma infection recorded in chickens from Ghana. About 24.4% toxoplasmosis was reported in Indonesia, 30% in Poland, and 24.2% in Vietnam (Dubey *et al*., 2008). However seroprevalence recoded in USA (12.5%) is quite lower than our figures (18.85%) (Dubey *et al*., 2008). A study conducted in adjacent province, in Punjab (Pakistan) shows that there is no anti-toxoplasma antibody in chickens (Zaki, 1995). The difference in rate of prevalence may be due to the geography, climate socioeconomic conditions, traditions, and customs and due to life style of the people living at there.

The infection rate in the present study in caged and uncaged (free-range) chickens is 20.70% and 5.90% respectively, which is nearly similar to a

study conducted in Beni Suef, Egypt, showing seroprevalence of 20% and 9.6% in free-range and farmed chickens, respectively (Aboelhadid *et al*., 2013). Our investigations are in full commitment with the findings of Xu *et al*. (2012) Liaoning Province, China showing 18.8% and 5.6% prevalence in free-range and caged respectively with a significantly higher prevalence in free-range chickens. The prevalence rate in present study is higher than 11.4% (of 361) in free-range and 4.1% (of 244) in caged chickens reported from Southern China (Yan *et al*., 2009). Another study suggest that prevalence in caged and free-range chickens is 6.23% and 10.19% respectively, the study was conducted in North-West China (Cong *et al*., 2012). A study from China recorded a total of seropositive rate of 30.36% (of 1173) in free-range chickens reported from 13 provinces/municipalities of China (Zhao *et al*., 2012). A study conducted in Addis Ababa, Ethiopia revealed that seroprevalence of T. gondii antibodies in free-range chickens was high as 38.4% (Tilahun *et al*., 2013).

A study from Egypt reveals high rate (38.1%) of toxoplasmosis in Kafr El-Sheikh, Capital of Kafr El-Sheikh Governorate, Egypt (Harfoush and Tahoon, 2010). There occurs a variation between rate of prevalence in caged and free ranged chikens. The high prevalence in free ranged chickens as compared to caged chickens, is because of environmental contamination with oocysts. The free ranged chickens become mainly infected by feeding from ground or soil as readily contaminated with oocysts of *T. gondii* (Edelhofer and Prossinger, 2010; Tilahun *et al*., 2013).

In the present study anti-toxoplasma antibodies were found to be more common in females (22.20) as compared to males (17.80%) domestic free range chickens. A significant difference was observed in male and female infection in the present study. However, there are limited studies worked out gender based studies of toxoplasmosis infection in chickens.

This was the ever first scientific investigation conducted across the study area regarding toxoplasma infection in chickens. In fact, it will pave a way for future line of action and will create a better awareness regarding the problem in locals of the study area.

CONCLUSIONS

This is the first ever study regarding seroprevalence of *T. gondii* infection in chickens of Mardan, Pakistan. The results of the present study showed that chickens (caged and uncaged) are commonly victimized by *T. gondii* infection. The overall infection rate was 18.85 % in chickens of study area. The infection rate was higher in uncaged/free range (20.70%) as compared to caged chickens (5.90 %). The infection rate was higher in females as compared to males. Susceptibility to toxoplasmosis is more in females as compared to male chickens. The high prevalence in free ranged chickens as compare to caged indicates the environmental contamination with oocysts because free ranged chickens become mainly infected by feeding from ground or soil and the ground is contaminated with oocysts of *T. gondii*. It was concluded that the main reason of the prevalence of the toxoplasmosis was the transmission may be due to cats presence in homes or unhygienic condition of the society. It is suggested that more research work should be carried out to find out other possible routes of transmission other than cats and chickens.

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