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SEROPREVALENCE OF *Toxoplasma gondii* AND ITS EFFECT OF HEMATOLOGICAL PICTURE IN COMMENSAL RODENTS IN FAISALABAD PAKISTAN

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People and different creatures, for example, wild rats (*Rattus rattus*) have been accounted for to be infected by *Toxoplasma gondii* (*T. gondii*). *T. gondii* infects rats through feed and water and these might transmit *T. gondii* contamination to people. The prevalence in the serum of rodents having antibodies (Abs) against *T. gondii* was assessed by utilizing Latex Agglutination Test. Sera tests of commensal rats, *R. rattus* (n = 71) and mice, *Mus musculus* (n = 41) were caught at various structures i.e., sequester settlements, government residences and luxurious habitations in Faisalabad city for *T. gondii* Abs and its hematological investigation. High rate of seropositive was recorded in *R. rattus* (45%) than *M. musculus* (36%). *T. gondii* was more common (72%) in female rats, and female mice (73%) when contrasted with male rats (28%) and mice (27%) with factual importance ($P<0.05$). The results in regards to the relationship between young and adult and *T. gondii* uncovered that prevalence of Toxoplasmosis was higher 78 and 73% in full grown rats and mice, individually with measurable hugeness ($P<0.05$). When compared the prevalence of *T. gondii* in commensal rats and mice caught from different areas, the parasite had most elevated prevalence (55 and 48%) in sequester settlements. Sequester settlements indicated more commonness of *T. gondii* in rats and mice than different structures with factual importance ($P<0.05$). Prevalence of *T. gondii* was recognized from the rats and mice of neighborhood. Blood examination demonstrated that seropositive rats and mice had essentially ($P<0.05$) higher variations from the norm on hemoglobin level, erythrocyte number, neutrophil and monocyte numbers contrasted with rats and mice, which were seronegative. While there was not noteworthy contrast for percent packed cell volume, leukocyte count, eosinophil and lymphocyte numbers ($P>0.05$) among sero-positive and sero-negative rats and mice. This demonstrates *T. gondii* disease might be a conceivable reason for hematological variations from the norm. Further studies on the angles to give more facility examination data on other creature wellbeing is in progress.

Keywords: Toxoplasmosis, *Rattus rattus, Mus musculus sequester settlement, latex agglutination test, heamotological analysis*

INTRODUCTION

Toxoplasmosis is a standout amongst the most widely recognized parasitic diseases worldwide, likely affecting around 33% of the human population (Tenter *et al*., 2000). The first human victim of Toxoplasmosis in China was accounted for in 1964 in Jiangxi Province. Toxoplasmosis is a zoonotic disease that is influenced by sanitary system (Jacobs *et al*., 1960).

Intermediate hosts (rodents, fowls, sheep, pigs, dairy cattle and so forth.) might get contaminated because of ingestion of sporulated oocysts. *T. gondii* contamination has turned into a noteworthy worldwide general well-being of the human immunodeficiency infection that has caused immunocompromised deficiency syndrome pandemic (Hooshyar*et al*., 2011; Wei *et al*., 2015).

Each warm-blooded living being, the intermediate hosts, transform into contaminated, for the most part by eating contaminated nourishment or beverage by oocysts emptied from felids and tissue cysts from other moderate hosts (Montoya and Liesenfeld, 2004; Dubey and Frenkel, 1998), reported the *T. gondii* in rats, which makes the survival difficult in its presence. Many scientists portrayed 5-10% recurrence of antibodies to *T. gondii* in rats and also effectively discovered the challenges by contrasting an assortment of serological screenings directed with a few techniques (Carleton and Musser, 2005).

The ingestion of water contaminated with oocysts is considered to be the primary source of infection *T. gondii* to the rodents, which might assume a vital part in the transmission of contamination. *T. gondii* causes a stand out amongst the most commonly known parasitic diseases in human and livestock populations (Dubey, 1994). Individuals from the Felidae family (essentially local felines) serve as complete hosts. It might prompt a transient disease described by lymphadenopathy, fever, exhaustion, arthralgia,

dermatosis, disquietude and cerebral pain (Webster, 1994).

Expanded rat populace in a territory can be specifically identified with the expanded zoonotic sicknesses in human population (Bradshaw, 1999). Rodents are bearers of ailments transmitted to people (rat borne zoonosis), are likewise incharge of extensive financial misfortunes regarding diminished workability, profitability and medicinal services costs. The most imperative negative effect of rat borne diseases is the loss of human well-being and lives can be relegated no sticker price. Urban rats do convey parasites that can bring about sickness and should represent a danger to the strength of groups where benchmarks of ecological and individual cleanliness are not looked after (Battersby, 2002).

In spite of the fact that many diagnostic tests have all the earmarks of being scientifically acceptable however, economic assessment should be considered. Latex agglutination test (LAT) is a fast showing reasonable sensitivity being ideal for field conditions aimed to conduct seroprevalence studies (Huang *et al.,* 2004). It has been reported that LAT has 98 and 100% specificity and sensitivity, respectively (Dubey and Jones, 2008).

In Pakistan, transmission of the toxoplasmosis is not established to an appropriate level while only a few reports have been published till to-date, which highlight the importance of wild rats (Rafique *et al*., 2009).

T. gondii disease might add to blood variations from the norm i.e., on haemoglobin level, red platelets number, neutrophil and monocytes checks, level of accessible potassium, then again packed cell volume, leukocyte count, eosinophil and lymphocytes stay within the normal limit (Huang *et al*., 2004). In Pakistan, most studies on toxoplasmosis are serologic in nature and studies on the impact of Toxoplasmosis on hematologic parameters of rats and mice are not yet reported. Blood pictures might be useful to comprehend therapeutic infirmities of the sufferers and might serve as gauge data to comprehend the impacts of Toxoplasmosis on rats and mice on account of their utilization as experimental animals.

Keeping the significance of commensal rats and mice, which are important players to transmit the disease to human beings, the present study was planned to explore seroprevalence and any blood irregularties on *T. gondii* infected commensal rats and mice through hematological examination.

MATERIALS AND METHODS

Faisalabad locale of Pakistan has a range of 5,856 km^2 and a population of about 5,340,771 heads. In Faisalabad district, the urban population is 2,280,360. The range itself developed in significance as the grain belt of the Punjab. Prosperous towns and rural areas have sprung up in the wake of colonization. The most extreme temperature in summer reaches upto 50°C or 122°F. In winter, it might fall beneath the freezing point. The winter begins from November and proceeds till March. December, January and February are the coldest months. The midyear begins from April and nonstop till October. May, June and July are the most blazing months. The live catching of commensal rats (*R. rattus*) and mice (*M. musculus*) was completed from November 2013 to April 2014 at various structures i.e., sequester settlements, government states and luxurious living arrangements in Faisalabad city, the region of Punjab. The rats and mice were caught and conveyed alive to the Department of Zoology, GC University, Faisalabad. The caught rats and mice were characterized into various gatherings in view of age, mature and immature, sex, and zone of capture. The blood was collected from the heart, one half of the blood was gathered in EDTA (Ethylene Diamine Tetra Acetic Acid) covered test tube for CBC (Complete Blood Count) and half blood was gathered in gel tube without anticoagulant to discrete the serum for serological studies for the commonness of *T. gondii*. Sera was acquired from blood tests in sanitized eppendorf tubes and centrifuged @ 3000 rpm for 10 min. to get clear supernatant. All information was put away in an electronic database.

***Latex agglutination test*:** *T. gondii* antibodies coated LAT kits was procured Global Toxoplasmosis Kit, having Latex reagents to be read as +ve and – ve along with controls. A 50 µl volume of diluted serum (1:16) was set on an expendable slide and blended with a drop of reagent and mixed with a disinfected stirrer. The test was completed with both positive and negative control Huang *et al.* (2004). The latex particles permit the antigen-antibody reaction and this reaction was observed following 5 minutes, if there was agglutination, the test was certain and show the antibodies of *T. gondii*. On the off chance that there was no agglutination, then test was negative and had no antibodies of *T. gondii*.

***Hematologic procedure*:** All rats and mice were subjected to Complete Blood Count (CBCs) investigation. The CBCs test was performed at Alrehmat Clinical Laboratory Faisalabad, Pakistan.

Consequences of hematology were broke down after the standard hematological qualities for rats and mice. For CBCs of rats, expected estimations of Hb ran from 11.5 to16.1g/dL, RBCs tally ran from 6.76 to 9.75 x $10^6/mm^3$, WBCs check went from 6.6 to 12.6 x 10^3/mm3, Platelets tally extended from $1.5x10^5$ to 4.0 x $10^5/mm^3$ and packed cell volume (PVC) with 37.6 to 50.6% typical reach. For CBCs of mice, expected estimations of Hb extended from 9.9 to 10.5g/dL, RBCs check ran from $7x10^6$ to 13x $10^6/mm^3$, WBC tally went from $5x10^3$ to 12x $10^3/mm^3$, Platelets tally ran from $3x10^5$ to 10x 10^5/mm3 and packed cell volume (PVC) with 39 to 49% ordinary extent (Huang *et al*., 2004).

***Measurable Analysis*:** Chi-square test was utilized for measurable investigation of antibodies of *T. gondii* and hematological results from seropositive and seronegative rats and mice. Contrasts were viewed as huge when $P<0.05$.

RESULTS AND DISCUSSION

***Serological data*:**In the present study, chosen structures were examined from November 2013 to April 2014. An aggregate of 112 examples of commensal rats and mice sera were tested for against *T. gondii* antibodies. Forty seven (47) were discovered infected with *T. gondii*, demonstrating a general prevalence was 42% (45% in rats and 36% in mice). There were obvious contrasts in percent predominance recorded among three territories in present study both in rats and mice, at houses in sequester settlements (55%), houses in departmental settlements (33%) and luxurious homes (25%) and mice (48, 37 and 14%), separately (Table 1). In the present study the prevalence of *T. gondii* antibodies in sera tests of commensal rats and mice was 42 percent (rats 45% and mice 36%) distinguished by LAT. Present serological information demonstrated a much higher rate of contamination in rodents conversely with the greater part of the referred to writing on prevalence of *T. gondii* (1-30%) in different nations (Dubey and Frenkel, 1998; Franti*et al*., 1976; Tizard *et al*., 1978). In UK, mean prevalence was recorded by (Webster, 1994) was 35%, which is three times higher rate in correlation with the past reports. Frenkel *et al.* (1995) reported 23.3% prevalence of *T. gondii* in rats of Panama though just 3% was accounted for by (Tizard *et al.*, 1978). El-Shazly *et al*. (1991) described 11.6% seropositive responses for Toxoplasmosis while dealing with four commensal rodents in Egypt. Just 2 (0.8%) of 238 rats were discovered positive in Grenada. Our outcomes are contrasted with (Salibay and Claveria, 2005) in Philippines prevalence (55.5% in rodents) and Ahmad *et al*. (2012), reported the 58.7% prevalence in *R. rattus* and 36.66% in *M. musculus*. The predominance of *T. gondii* was high in *M. musculus* (51.9%, 120/230) and lower in *R. norvegicus* (10.3%, 4/39) in Netherland and the United Kingdom. In UK, mean prevalence seen by (Webster, 1994) was 35%, which is three times higher rate in correlation with the past reports. It demonstrates that percent prevalence of *T. gondii* contamination is distinctive all through the world and shifts from one land territory to other (Salibay and Claveria, 2005). This variety might be because of distinction in hygienic condition, population, vicinity of transitional host and sustaining propensities for individuals of various nations of the world. Importance contrast in percent prevalence was found among three areas in present study both in rats and mice. The lesser rate of *T. gondii* disease at rich living arrangements was reported than other two territories i.e. at houses in sequester settlements (55%), houses in departmental settlements (33%) and opulent habitations (25%) and mice (48, 37 and 14%), separately might be because of good natural condition, instructive status, less number of stray felines.

In present rodents study, out of 32 tests of contaminated rats (*R. rattus*) 23 females were discovered positive appearing (72%) predominance of *T. gondii* while the male appeared (28%) predominance of *T. gondii*. The female indicated fundamentally ($P<0.05$) higher prevalence 72% when contrasted with male 28% (Table 2). Out of 15 tests of contaminated mice (*M. musculus*) 11 (73%) females were distinguished seropositive while male 4 (27%) indicated seropositive prevalence of *T. gondii*. The female indicated essentially ($P<0.05$) higher prevalence 73% when contrasted with male 27%. Yin *et al*. (2010) watched a higher seroprevalence in female than in male *R. rattus*, the distinction was not noteworthy ($P > 0.05$). The comparable study directed by (Yin *et al*., 2010) in China, the majority of the infected rats (3.2%; 7 out of 217) were female. No *T. gondii* antibodies were distinguished in male rats. Interestingly, in a study by (Salibay and Claveria, 2005) in the

Table 1. Number of rats and mice serologically positive to *T. gondii* infection in urban area of Faisalabad prevalence of *T. gondii* in sera of commensal rats and mice trapped from different localities

Species	Collection sites	Number examined	Seropositive	% Seropositive	Total (% Seropositive)
R. rattus	Houses in Sequester settlements	40	22	55[a]	
	Houses in departmental colonies	27	9	33[b]	71(45)
	Posh residences	4	1	25[bb]	
M. musculus	Houses in Sequester settlements	21	10	48[a]	
	Houses in departmental colonies	13	4	37[b]	41(36)
	Posh residences	7	1	14[bb]	
Total		112	47	42	112(42)

Values with different letters per parameter were statistically significant

Table 2. Prevalence of *T. gondii* among rats (*R. rattus*) and mice (*M. musculus*) in relation to sex and maturity

Species	Gender				Age				Total
	Male	% of male	Female	% female	Mature	% Mature	Immature	% Immature	
R. rattus	9	28[a]	23	72[b]	25	78[a]	7	22[b]	32
M. musculus	4	27[a]	11	73[b]	11	73[a]	4	27[b]	15

Values with different letters per parameter were statistically significant

Philippines, the *T. gondii* contamination rate was higher in male rats than in female rats. The prevalence of toxoplasmosis changes among mice and nations, the present investigation of toxoplasma contamination rate in mice was (36%), higher than that reported by (Morsy *et al*., 1994) 12.5%, (Defeo *et al*., 2002) 0.8%, and (Williams *et al*., 2005) 1% but it was lower than those reported by (Salibay and Claveria, 2005) 65%. In the vulnerability to parasitic contamination, hormonal contrasts in the middle of guys and females assume an essential part. It is broadly acknowledged that numerous hormones including the sex-related hormones straightforwardly impact on the immune system. Safety in females can be separated because of numerous variables e.g., age, sustenance, pregnancy and ecological elements.

The present study demonstrated 78% of adult and 22% of juvenile rats though 73% of mature and 27% of youthful mice contaminated with *T. gondii* (Table 2). The grown-ups indicated altogether ($P<0.05$) higher prevalence than immature both in commensal rats and mice. The prevalence of adult rodents (4.7%) was about double the prevalence of young rodents (2.6%) reported by (Williams *et al*., 2005). Our outcomes for seroprevalence in rats and mice were like those of past studies led by (Webster, 1994). Commensal rodents can be an imperative hotspot for the transmission of zoonotic parasites, for example, *T. gondii* to felines. The high predominance of *T. gondii* reported in these species shows the suggestion in a transmission cycle near people.

We ought to teaching feline proprietors about the significance of gathering of feline dung, decreasing the quantities of non-domesticated felines, diminishing the quantity of commensals rats and mice is likewise a critical for control of toxoplasmosis. This study will be the premise for further studies in the study of disease transmission of *T. gondii*. More studies are required to decide the seroprevalence rates among population of commensal rodents and different animals in various regions.

***Hematological results*:** It was found from the present work that hematological pictures were essentially adjusted with toxoplasmosis in both rats and mice. Beforehand, it has been watched that seropositive felines display a scope of irregularities connected with blood, which either be morphological qualities of platelets or their check. The development of anemia in both sero-positive rats and mice might be ascribed to diminished packed cell volume and Hb concentrations and RBCs consider contrasted with the ordinary scope of the above parameters.

In the present study both rats and mice, PCV did not demonstrate altogether distinctive values but rather the RBCs tally and Hb concentration were beneath the reference range (Table 3, 4). Low RBCs tally and Hb, among other joined variables, could bring about frailty which has been recorded in toxoplasmic felines and even man (Huang *et al*., 2004). The consequences of the present study were suggestive of *T. gondii* disease impelled impacts on rats and mice. The presence of leukocytes might indicate safety in living beings. Any adjustment in the WBCs might indicate genuine variations from the norm on wellbeing. In the present study, the differential counts on neutrophils and monocytes demonstrated obvious results. Imperfections in neutrophils capacities can be because of decrease of neutrophils number at noteworthy level cause less resistance or abnormal state might show a dynamic contamination.

Table 3. Number of seropositive and seronegative rats showing haematological abnormalities in complete blood count analysis

Blood count	Abnormal blood count Seropositive Rats (% based on N=32)	Seronegative Rats (% based on N=39)
Haemoglobin	24^{a}(75)	6^{b}(15.3)
Red blood cells(RBC)	32^{a}(100)	4^{b}(10.2)
Packed volume cell(PVC)	14^{a}(43.7)	11^{a}(28.2)
White blood cells(WBC)	21^{a}(65.6)	10^{a}(25.6)
Eosinophil	7^{a}(21.8)	4^{a}(10.2)
Lymphocytes	19^{a}(59.3)	16^{a}(41)
Monocytes	25^{a}(78.1)	5^{b}(12.8)
Neutrophils	22^{a}(68.7)	8^{b}(20.5)

Values with different letters per parameter were statistically significant

Table 4. Number of seropositive and seronegative mice showing haematological abnormalities in complete blood count analysis

Blood count	Abnormal blood count Seropositive Mice (% based on N=15)	Seronegative Mice (% based on N=26)
Haemoglobin	12^{a}(80)	3^{b}(11.5)
Red blood cells(RBCs)	15^{a}(100)	4^{b}(15.3)
Packed volume cell(PVC)	10^{a}(66.6)	13^{a}(50)
White blood cells(WBCs)	9^{a}(60)	12^{a}(46.1)
Eosinophil	5^{a}(33.3)	3^{a}(11.5)
Lymphocytes	9^{a}(60)	11^{b}(42.3)
Monocytes	12^{a}(80)	2^{b}(7.6)
Neutrophils	11^{a}(73.3)	5^{b}(19.2)

Values with different letters per parameter were statistically significant

Both conditions might be found in *T. gondii* disease relying on the hidden well-being state of host. In the present report, neutrophils number was altogether higher while the monocytes were not very high in both seropositive rats and mice.

The less monocytes level in the blood might happen in the light of the arrival of poisons into the blood by specific microorganisms, particularly microscopic organisms. Since for the most part rats and mice are utilized as trial animals, these are utilized as models, such sorts of results might be found among people and other warm blooded life forms of *T. gondii* contamination. The presence of toxoplasmosis might deliver numerous signs and side effects coming about because of the parasite's assault of extensive variety of tissues.

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