



## **TOXOPLASMOSIS IN ANIMALS AND HUMANS FROM PAKISTAN: PAST AND PRESENT**

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**Running Title:** Toxoplasmosis in animals and humans from Pakistan

**NOVELTY OF MANUSCRIPT:** The current study is novel in its kind because no history of toxoplasmosis in Pakistan has been reported so far.

## **TOXOPLASMOSIS IN ANIMALS AND HUMANS FROM PAKISTAN: PAS AND PRESENT**

### **ABSTRACT**

Toxoplasmosis being a cosmopolitan parasitic infection, has been reported from all around the world. However, majority of the farmers doesn't not know what is toxoplasmosis? The current study was carried out to compile and review the published data available at various electronic databases such as Google Scholars, PubMed, Science Direct, Scopus and Research Gate. The studies containing information related with toxoplasmosis in food animals, commensals and humans of Pakistan from 1990 to 2017 were included in the present review. The overall *T. gondii* infection rates ranged between 0% and 53.8% animals. The results indicated that toxoplasmosis was found 2.5% to 45% in sheep, 19% to 57.1% in goats, 19% to 22% in cattle, 15% to 22% in buffaloes, 10% camels, 26.43 to 63% in cats, 28% to 50% in dogs, 36% to 58% in mice; and in humans 27.7% to 63%. Furthermore, the results from different studies showed the variation in the occurrence of *Toxoplasma gondii* infections with time and space. The present study suggested that toxoplasmosis is overall increasing in Pakistan with the passage of time. The current report would provide an insight for the future research on toxoplasmosis in Pakistan.

**KEY WORDS:** Epidemiology; cattle; small ruminants; human toxoplasmosis; *Toxoplasma gondii* infection

## INTRODUCTION

Pakistan is basically an agriculture based economy (Begum and Yasmeen, 2011) including food and staple crops as well as livestock animals. About 11% of the GDP of Pakistan is shared by the livestock animals (GOP, 2009) including sheep, goats, cattle, buffaloes, camels and poultry. These livestock animals are not only the source of earning the cash but also are vital for meeting the milk and meat requirements. The infected food animals subsequently become the potential source of *Toxoplasma* transmission to humans through unpasteurized milk (Dehkordi et al., 2013; Al-Jebouri et al., 2013), meat (Montoya and Liesenfeld, 2004) and eggs (Al-Jebouri et al., 2013).

Among livestock animals, the buffaloes and cows are more important contributing approximately 66% and 32% respectively of the total milk produced in Pakistan (Ahmad et al., 2012). Ranking second highest milk producers in the world (FAOSTAT, 2010) and considered as the black gold of Pakistan, the buffaloes are the main producers of milk in the country. Perhaps due to these animals Pakistan now is the third major producer of milk in the world (Ahmad et al., 2012). On the other, meagre studies have been carried out for the assessment of toxoplasmosis in these important Bovids in Pakistan. Sheep and goats are not only reared for their skin, wool or/and hair in the fabrics and chees industries (Ashraf et al., 2013) and production of 2% of the total yield of milk collectively in Pakistan (Ahmad et al., 2012) but also for mutton production particularly at the sacrificial occasions (Qureshi et al., 2010). These animals have been established transmitting toxoplasmosis through zoonosis (Gebremedhin et al., 2013). Likewise, camels are important source of flesh and milk, hair and hide (FAO, 2002) and also vital means of transport particularly in the Cholistan and Thal and Thar Desert of Pakistan. These food animals can transmit infection of *T. gondii* to humans through milk and meat (Muskin et al., 2011). In Pakistan, the livestock animals suffer from a variety of diseases inflicted by different species of parasites. One of the

major diseases toxoplasmosis, caused by a heteroxenous apicomplexan *T. gondii* (Tenter et al., 2000).

### **Epidemiology of *T. gondii***

*Toxoplasma*, being polyxenous parasite has many routes of transmission to a variety of species of hosts (Dubey, 2009<sup>a</sup>) completing its life cycle in two phases, sexual and asexual (Dubey, 2009<sup>a</sup>). The parasite completes the sexual phase of life cycle strictly in the felines (Boothroyd, 2009) while the asexual phase may be completed either in primary hosts, the felines (Boothroyd, 2009) or the secondary host from class mammals or birds (Hill et al., 2005; Boothroyd, 2009).

*T. gondii* has many advantages which enable it to survive successfully in water, air, soil and a variety of host species but the most important is its capability to change its shape and structure as per environment. The routes of transmission may either be vertical, via placenta or horizontal, from species to species. After the completion of life cycle in cats, *Toxoplasma* is shed as the oocysts which bear the sporozoites formed within it (Petersen and Schmidt, 2003). The ingestion of the oocysts contaminated food by the hosts facilitates *T. gondii* to get into the gut of secondary host that can be any of the warm blooded species including the food animals such as sheep, goats, cows, buffaloes, camels, pigs and many birds' in which the asexual phase of the life cycle is completed (Urquhart et al., 2003). The food animal species may, on the other hand, be infected through water contaminated with tachyzoites (de Moura et al., 2006) that are the active proliferative forms of *T. gondii*. The water bodies contaminated with oocysts can also be a potential source of transmission of *T. gondii* from the environment susceptible animal species because some studies have suggested that the oocysts can sustain in water up to several months at a moderate temperature from 20° to 37°C (Dumetre and Darde, 2003). The infected food animals subsequently become the potential source of *Toxoplasma* transmission to humans through unpasteurized milk

(Dehkordi et al., 2013; Al-Jebouri et al., 2013), meat (Montoya and Liesenfeld, 2004) and eggs (Al-Jebouri et al., 2013).

The occurrence of toxoplasmosis varies in humans from population to population ranging between 9% and 78% (Flegr, 2013), from region to region (Ahmad and Tasawar, 2015<sup>a</sup>) and different age groups and genders of animals (Ahmad and Tasawar, 2016<sup>a</sup>; Ahmad and Tasawar, 2016<sup>b</sup>). In humans *T. gondii* has been reported causing various health problems such as abortion (Tenter et al., 2000), blindness (Holland, 2003) that may lead to permanent blindness, encephalitis (Dubey, 2009<sup>a</sup>) and mental disorders resulting in behavioral changes (Flegr, 2013) such as schizophrenia (Yolken et al., 2009) causing road accidents by drivers suffering toxoplasmic schizophrenia (Flegr, 2013). Some studies have also established *T. gondii* facilitating HIV infection to the humans (Dubey and Jones, 2008). The transmission of *T. gondii* in the horizontal and vertical routes has been established by several studies (Asgari et al., 2011; Lopes et al., 2013) in animals and humans (Dubey, 2009<sup>b</sup>). Although toxoplasmosis has been studied throughout the world yet it is the most neglected disease in Pakistan like the other developing countries. As the slaughter animals are widely accepted the potential source of toxoplasmosis (Gebremedhin et al., 2013), we carried out this study to highlight toll of this disease in Pakistan.

## DISCUSSION

### **Toxoplasmosis in sheep and goats**

Seroprevalence of *T. gondii* has been found in varying rates from 0% to 100 % reported by different studies (Tenter et al., 2000) owing to the ecological factors (Ahmad and Tasawar, 2015) and the other determinants such as age of host animal and management techniques practiced by livestock farmers (Tasawar et al., 2012). In Pakistan also, the occurrence of toxoplasmosis in

sheep has been reported at differential rates. For example, Zaki (1995) reported 2.5% infection of *T. gondii* from South-West of Pakistan in a very close agreement with 4.4% revealed by (Yang et al., 2013) in China. The later studies on sheep toxoplasmosis demonstrated the higher *T. gondii* infection rates. For instance, 11.2% from Rahim Yar Khan (Ramzan et al., 2009), 19.8% from the sheep populated in districts Khanewal, Multan and Dera Ghazi Khan (Lashari and Tasawar, 2010), 36.0% from Northern mountainous regions (Shah et al., 2013) and 37.3% from Cholistan desert and two districts Rajan Pur and Rahim Yar Khan, in the Indus plains of Pakistan (Ahmad and Tasawar, 2015) coinciding with 37.5% obtained in Iran by Asghari et al. 2011. The findings of Ahmad and Tasawar (2015) were also very close to 34.5% reported in Somalia (Kadle, 2014) and 33.6% from Multan and Khanewal (Hanif and Tasawar, 2016). These disparities in the toxoplasmosis rates might be linked with the disease differential resistance in ovines inhabiting various climatic regions and/or perseverance of *T. gondii* oocysts in varied regions (Meerburg and Kijlstra, 2009). At gender level, the findings of different authors varied, evaluating higher toxoplasmosis in female (24%) than male (19%) (Ramzan et al., 2009) in agreement with higher in females (38%) and lower in male (36%) animals (Shah et al., 2013). On the other hand, Ahmad and Tasawar (2015) evaluated *T. gondii* infections, lower in ewes than rams, perhaps due to immigration and emigration of ovines herds at different intervals of sampling period. At breed level in sheep, Ahmad and Tasawar (2015) reported differential occurrence of anti-*T. gondii* antibodies in Kajli 45.0%, Thalli 44.1%, Cholistani 39.68% and Lohi breeds 28.6%. Earlier, results obtained by Lashari and Tasawar (2010) for Kacchi (22.7%) and Lohi breeds 15.7% respectively were, however, significantly (P-value 0.05).

Different studies reported varying results of toxoplasmosis in goats in various regions of Pakistan. Ramzan et al. (2009) conducted a survey in Rahim Yar Khan reporting 19% infection

rates. The prevalence of infection of *T. gondii* was found elevated (29.1%) in subsequent years (Ahmad and Tasawar, 2016<sup>a</sup>) (Table 1). At breeds level infections of *T. gondii* in Beetal and Teddy goats Tasawar et al. (2011) assessed significantly ( $P < 5$ ) higher rates of toxoplasmosis in Beetal (52%) and lower in Teddy animals (46%). Similar differential trends in infection rates, although nonsignificant ( $P$ -value= 0.52), were obtained by (Ahmad and Tasawar, 2016<sup>a</sup>) while investigating toxoplasmosis in four goat breeds namely Beetal (33.3%), Nachi (30.9%), Dera Din Panah (21.0%) and Teddy breed (29.0%) populated in Cholistan desert and two districts in South Punjab. Almost similar results 26.7%, have been reported by Kadle (2014) in Somali goats perhaps experiencing analogous environmental conditions. In Pakistan Tasawar et al. (2011) established the higher toxoplasmosis rates (57.1%) in females as compared with (25%) in the male animals varying with (Ahmad and Tasawar, 2016<sup>b</sup>) who have reported the non-significantly ( $P$ -value 0.89) lower infection rates in males as compared with female goats. The variation in findings can be attributed to the management techniques of farmers in different study regions (Cenci-Goga et al., 2013). The differences in the study techniques can also be accounted for the different rates of toxoplasmosis.

### **Toxoplasmosis in buffaloes, cattle and camels**

Chaudhary et al. (2006) investigated the comparison of prevalence of anti-*T. gondii* antibodies in buffaloes and found a positive correlation between LAT and PCR results showing toxoplasmosis at the rate of 22% (Table 1). Shah et al. (2013) reported 16% occurrence of toxoplasmosis in these large ruminants in some federally administered tribal areas (FATA) of Pakistan coinciding with 15.5% from North Punjab (Ahmad and Qayyum, 2014). Ecologically the (FATA) are at the high altitude and a temperature lower than Northern Punjab and Lahore region. The results brought to the suggestion that higher altitude facilitates the survival of *T. gondii* particularly in the large ruminants.

Zaki (1995) evaluated *T. gondii* infection in cattle from Pakistan. The infection rates increased to 87% (Tasawar et al., 2013) higher than 15.9% from Iran (Nematollahi and Moghddam, 2008), 5.7% from China (Zhou et al., 2012), 2.6% also from China reported by Qiu et al. (2012). At gender level, Tasawar et al. (2013) reported seroprevalence of toxoplasmosis higher (47%) in cows than bulls (39%) from Khanewal, Pakistan in agreement with (Qiu et al., 2012) who established same trend of higher prevalence of toxoplasmosis in female cattle (3.4%) than in male (2.5%) in China. *T. gondii* infections in camels occurring between 3.1% and 90.9% has been reported by Dehkordi et al. (2013) in Iran and Utuk et al. (2012) in Turkey respectively. In Pakistan, toxoplasmosis was first reported by Chaudhary et al. (2014) establishing the overall 10% *Toxoplasma* infections with higher rates (11.1%) in females than (8.6%) in male camels. The overall infections were lower than 22% for Abu Dhabi (Youssef et al., 2005) but higher than 6.3% for Somalia (Kadle, 2014). The camels have been reported as potential source of toxoplasmosis in the pastoral people (Muskin et al., 2011). Additionally, in the present time, the camel milk is winning its likelihood by the populace of urban areas due to its medical importance. Thus it is hypothesized that a large population of nomads relying upon the camel milk and meat in Pakistan might be getting infested from toxoplasmosis via camels.

### **Toxoplasmosis in chickens**

Toxoplasmosis in domestic chickens inhabiting Pakistan was first studied by Zaki (1995) evaluating zero percent rates of disease. In the later years it was found 18.8% (Mahmood *et al.*, 2014) for chicken from Khyber Pakhtunkhwa with a higher prevalence 20.5% in free living fowls as compared with 5.9% in caged broilers suggesting higher chances of the environmental transmission of parasite. On the other hand, in Faisalabad, Akhter et al. (2014) established 36.3% occurrence of toxoplasmosis with an increasing trend of infection along the age of chickens.



## **Toxoplasmosis in rodents, cats and dogs**

The first report on toxoplasmosis in two species of rats from different localities in Lahore, Pakistan was presented by Ahmad et al. (2012) evaluating 58.5% in *Rattus rattus* and 36.6% (Table 2) in *Mus musculus* via latex agglutination technique. Perhaps the higher prevalence in *Rattus* than *Mus* might be due to the difference in sample size of subjects. The genus *Rattus* has been reported dominating *Mus*, leading to the hypothesis that the former might be existing in a larger population as compared with the latter. The same study reported the coexistence of *Toxoplasma* infection in humans ranging up to 28%. The rodents are the successful commensals of humans and the incidence of toxoplasmosis. The coexistence of *Toxoplasma* infection in the humans up to 28% and rodents ranging between 36.6% and 58.5% warrants the further proliferation of toxoplasmosis.

Cats are the natural reservoir of *T. gondii* thus being the primary hosts are the major contributors of resistant eggs to the environment. Many studies have been carried out around the world providing the evidence of the cats' role in proliferation of toxoplasmosis. In Pakistan, Ahmad et al. (2001) evaluated toxoplasmosis in cats at the rates of 60%. The report also established the higher incidence of infection in the stray cats (66.6%) as compared with the indoor animals (57.1%). In a later study from Multan, Tasawar et al. (2013) reported 63.4% incidence of *Toxoplasma* infection in the cats by using the Fecal floatation technique. The findings of Tasawar et al. (2013) showed the prevalence of infection higher than (Ahmad et al., 2001) Faisalabad. The results at gender level (67.0% in male and 59.2% females) by (Tasawar et al., 2013) also disagreed with (Ahmad et al., 2001) who evaluated toxoplasmosis 70% in females and 40% in males. The differential findings might be attributed to the use of different techniques and different ecological regions (Ahmad and Tasawar, 2015).

The first survey on toxoplasmosis in dogs was carried out by Ahmad et al. (2001) who established the occurrence of anti-*Toxoplasma* antibodies at the rate of 50% through LAT technique. The also revealed the elevation of infection along the age of animals that might be due to the vertical transmission. The congenital transmission in dogs is also justified by the fact that the stray dogs have the free chance of mating without any screening of disease with exception of pet dogs. This notion was also proved by the data showing higher infection rates 57.1% in the local breeds as compared with 46.1% the exotic canines reared as pets. Ahmad et al. (2001) also reported the positive correlation between the cats and dogs toxoplasmosis. Dogs and cats are found in close contact with herds of animals facilitating the parasite to proliferate horizontally. The felines and canines share the water bodies with the flocks of food animals. Therefore, these commensals are not only the source of transmission of toxoplasmosis to humans rather to the domestic food animals as well.

### **Toxoplasmosis in humans**

In human, *T. gondii* infection was first reported by Bari and Khan (1990) from selected areas of the than North West Frontier Province of Pakistan, now named as Khyber Pakhtunkhwa, some parts of Punjab and Azad Kashmir. The study established 46% and 27.7% seroprevalence of IgG and IgM respectively in the human subjects. Furthermore, the study also demonstrated the highest prevalence (63%) from Punjab (Table 3), lower (48%) from Kashmir (Table 3) and the lowest (38%) occurrence of infection was found in Khyber Pakhtunkhwa. However, a later study conducted by Faisal et al. (2013) in Khyber Pakhtunkhwa, ascertained the occurrence of toxoplasmosis at lower rates (19.2%) in the pregnant women coming from Swabi area. Perhaps the infection rates lower than previous reported infections rates (38%) from KPK (Bari and Khan, 1990) might be due the small study area district Swabi and short period of six months. The overall

toll of disease 46% from Northern Punjab, KPK and Kashmir (Bari and Khan, 1990) was very close to 42% human toxoplasmosis from Muzaffargarh, Pakistan in the recent past (Hayat et al., 2014). The latter study further reported the increasing trend of infection along the increasing age of subjects except for some deviations in some age groups. However, the former reports of *T. gondii* infection were higher than 29.45% reported by Tasawar et al. (2012) in humans from Southern Punjab. Significant disparities in the occurrence of infection in male (35.89%) and female subjects (25.9%) were also observed in the results computed from Southern Punjab (Tasawar et al., 2012).

## CONCLUSION

Although toxoplasmosis has been studied throughout the world yet it is the most neglected disease in Pakistan. Tracing back from the first report in animals in Pakistan (Zaki, 1990) to the latest report by Ahmad and Tasawar (2016) the *T. gondii* infection rates ranged between 0% in goats and chicken (Zaki, 1995) and 53.8% in goats evaluated by Shah et al. (2013) brought to the conclusion that toxoplasmosis presents an ever increasing health issue in Pakistan, perhaps due to the least attention evinced by the authorities. The occurrence of toxoplasmosis almost at equal rates in humans and commensal species of animals such as rodents suggested the transmission of *T. gondii* from mice to humans as well. It is therefore recommended that animals and humans simultaneously to evaluate the zoonotic impact of *T. gondii* in all the provinces of Pakistan.

## AUTHORS' CONTRIBUTIONS

Saghir Ahmad conceived the idea and supervised the research work to evaluate the history of toxoplasmosis in animals from Pakistan and the research was carried out by Warda Hanif (MSc Zoology research scholar) and Sana Younis (BS Zoology, research scholar).

## COMPETING INTEREST

The authors declare that there is no competing interest.

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**Table 1: Toxoplasmosis in food animals from Pakistan**

Category	Location	Number positive	Prevalence	Test used	Reference
Sheep	South-West Pakistan	1/40	2.5%	LAT	Zaki, M (1995)
	Dera Ghazi Khan, Multan, Khanewal	103/518	19.8%	ELISA & LAT	Lasahari and Tasawar (2010)
	Mohmand Agency	36/100	36.0%	LAT	Shah et al. (2013)
	Cholistan, RYK, RPR	125/335	37.3%	LAT	Ahmad and Tasawar (2015)
	KHW & MTN	137/500	27.4%	ELISA	Hanif and Tasawar (2016)
Goats	South-West Pakistan	0/58	0%	LAT	Zaki, M (1995)
	Rahim Yar Khan	**	19%	LAT	Ramzan et al. (2009)
	Multan	104/200	52.0%	ELISA	Tasawar et al. (2011)
	Mohmand agency	56/104	53.8%	LAT	Shah et al. (2013)
	Cholistan, Rahim Yar Khan, Rajan Pur	252/865	29.1%	LAT	Ahmad and Tasawar (2016)
Cattle	South-West Pakistan	25/100	25.0%	LAT	Zaki, M (1995)
	Khanewal	87/200	43.5%	LAT	Tasawar et al. (2013)
	Northern Punjab	79/400	19.7%	ELISA	Ahmad and Qayyum (2014)
Buffaloes	Lahore Region	11/50	22.0%	LAT & PCR	Chaudhary et al. (2006)
	Mohmand agency	16/100	16%	LAT	Shah et al. (2013)
	Northern Punjab	64/422	15.1%	ELISA	Ahmad and Qayyum (2014)
Camels	Bahawalpur	10/100	10.0%	LAT	Chaudhary et al. (2014)
Chickens	South-West Pakistan	0/64	0%	LAT	Zaki M (1995)
	KPK	101/536	18.8%	IHA	Mahmood et al. (2014)

Table 2: Toxoplasmosis in cats, dogs and mice from Pakistan

Category	Location	Number positive	Prevalence%	Technique	Reference
Cats	Faisalabad	06/10	60.0%	LAT	Ahmad et al. (2001)
	Multan	104/164	63.4%	Fecal floatation	Tasawar et al. (2013)
	Northern Tropical Region	111/420	26.4%	ELISA	Ahmad et al. (2014)
Dogs	Faisalabad	20/40	50%	LAT	Ahmad et al. (2001)
	Northern Tropical Region	116/408	28.4%	ELISA	Ahmad et al. (2014)
Rodents	Lahore		36.6%		Ahmad et al. (2012)

Table 3: Toxoplasmosis in humans from Pakistan

Location	No. positive	Prevalence	Test used	Reference
Muzaffergarh	63/150	42.0%	LAT	Hayat et al. (2014)
Swabi	155/805	19.2%	LAT	Faisal et al. (2014)
Southern Punjab	162/550	29.4%	LAT	Tasawar et al. (2012)

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