

Seroprevalence of toxoplasmosis in humans and animals in Pakistan: a systematic review and metadata analysis

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Abstract

Objective: To review the seroprevalence of toxoplasmosis in Pakistan.

Method: The systematic review comprised search on Science Direct, Google Scholar, PubMed and Scopus databases for studies related to the seroprevalence of toxoplasmosis in Pakistan published between 2006 and 2020 which used serological diagnostic tests to detect *Toxoplasma gondii*. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were used throughout the review and statistical analysis was done using forest plot and random effect model.

Results: Of the 7093 human studies initially found, 20(0.28%) were reviewed. Of the 16,432 animal studies, 16(0.09%) were selected for detailed review. The pooled seroprevalence of toxoplasmosis in humans, calculated in this review was found as (76%) (95% confidence interval: 69-83%). Seroprevalence of human toxoplasmosis was higher in Khyber Pakhtunkhwa (31.7%) than Punjab (20.4%). Pooled seroprevalence in animals calculated in this review was found as (69%) (95% confidence interval: 64-74%). Seroprevalence in animals was higher in Khyber Pakhtunkhwa (44.7%) than Punjab (29.4%).

Conclusions: The seroprevalence of toxoplasmosis in both humans and animals should be studied in other parts of Pakistan as well.

Key Words: Livestock, Humans, Random-effect model, *Toxoplasma gondii*, Seroprevalence.

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Introduction

Toxoplasmosis is a zoonotic disease caused by parasite *Toxoplasma (T.) gondii*. The modes of transmission of the

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parasite include the consumption of the meat of sheep, cattle, goat, and pigs having bradyzoites of *T. gondii*, and the consumption of milk of goat, sheep and cattle, or contaminated chicken eggs.¹ More commonly, humans acquire infection through unintentional ingestion of oocysts excreted by infected felines.² The principal reservoir for *T. gondii* is wild and domesticated cats, and the oocysts are produced in cats through sexual stages of their lifecycle. During pregnancy, *T. gondii* can cause abortion in the pregnant host, an infection of the placenta and in the foetus, leading to miscarriage, foetal mummification and or death in all types of mammalian hosts and among animals, especially in sheep and cattle.³ *T. gondii* has a huge zoonotic potential and causes infection in humans across the globe. High seroprevalence in the population leads to higher ratio of infections.⁴ It can cause different neurological disorders in immunocompromised or human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients.⁵

Pooled seroprevalence of toxoplasmosis in Pakistan has been determined by compiling data.

It has been found that 30-50% of human population is infected with *T. gondii*, worldwide.⁶ Almost 20 million new cases of congenital and acquired toxoplasmosis were reported in 2010.⁷ On the basis of public and animal health, interaction of microorganisms and environment, horticulture, gardening, trade effects as well as socio-economic impacts, *T. gondii* is graded as one of the top 10 food-borne parasites.⁸ *T. gondii* is identified through direct methods, like microscopy and detection of deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR), while the segregation of *T. gondii* is done by various bioassays.⁹ *T. gondii* is also identified through indirect methods, like the detection of antibodies against *T. gondii* in the serum of humans and animals.¹⁰ Various serological techniques, such as Sabin-Feldman Dye test, enzyme-linked immunosorbent assay (ELISA), latex agglutination test (LAT), indirect haemagglutination test (IHAT) and rapid diagnostic test strip (RDTs), have been employed for the identification of *T. gondii* antibodies in humans and animals.¹¹ In Pakistan, various epidemics cause morbidity and death in the livestock sector. Due to these diseases, Pakistan faces an estimated loss of around PKR79 billion.¹² Toxoplasmosis is one of the cosmopolitan parasitic

diseases of the animals and humans, and it is ranked third in terms of the rate of global spread. Approximately 2.34 billion people are assumed to have been infected with *T. gondii* worldwide.^{13, 14} The current systematic review was planned to evaluate the seroprevalence of toxoplasmosis in humans and animals in Pakistan.

Materials and Methods

The systematic review comprised search on Science Direct, Google Scholar, PubMed and Scopus databases for studies related to the seroprevalence of toxoplasmosis in Pakistan published between 2006 and 2020 which used serological diagnostic tests to detect *T. gondii*. The Cochrane and the European Food Safety Authority (EFSA) instructions¹⁵ as well as Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed.¹⁶

The key words used included “Toxoplasmosis”, “Seroprevalence”, “Humans”, “Livestock”, “Sheep and Goat”, “Cattle and Buffalo”, “Poultry”, “Pakistani”, and “Pakistan”. Vivid studies, case reports, reviews, letters to editors, studies not representing the target population, duplicates, and studies not using immunological assays were excluded.

The studies found were screened and data was retrieved from the ones that were shortlisted for detailed review. Data was extracted separately if an article contained multiple studies, like with different animals.

Seroprevalence was approximated with 95% confidence interval (CI). Further, the random effect model of forest plot, I² and Cochrane Q statistics were used, as appropriate.

Results

Of the 7093 human studies initially found, 20(0.28%) were reviewed (Figure 1). Of them, 11(55%) were from Khyber Pakhtunkhwa (KP) and 9(45%) were from Punjab. A total

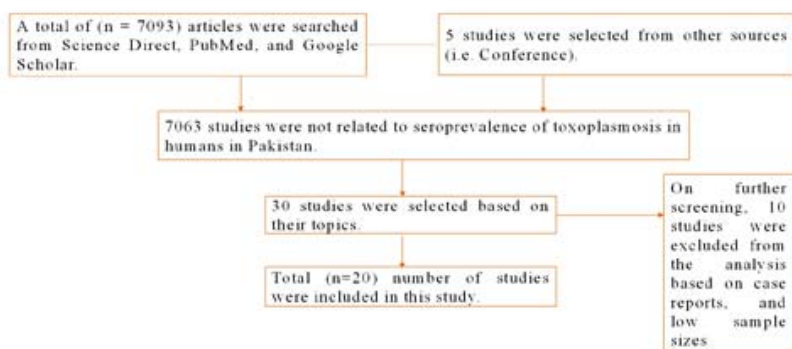


Figure-1: Study flowchart related to human-based literature

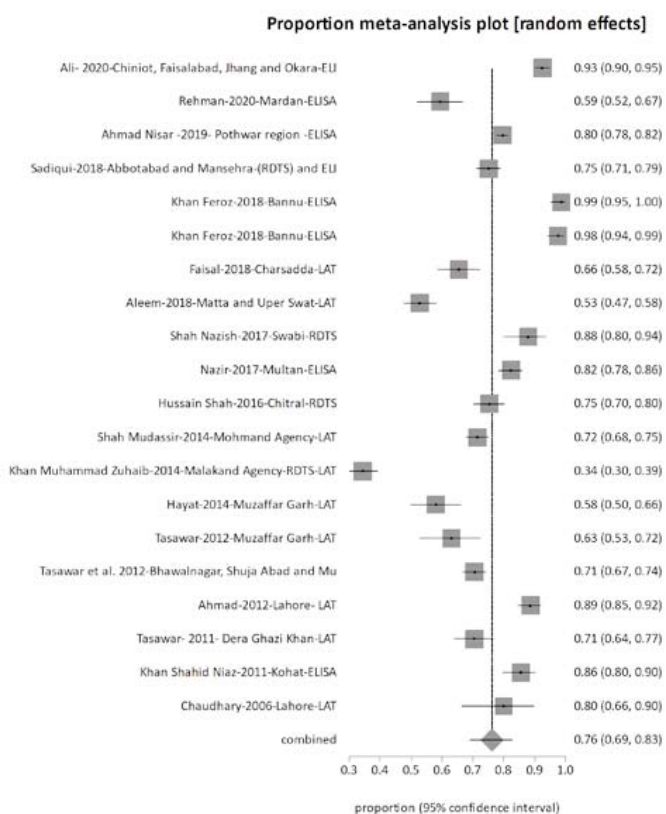


Figure-2: Forest plot of *Toxoplasma (T.) gondii* infection in humans in Pakistan using random effect model.

of 7145 individuals were part of these studies, and 1813(25.37%) were seropositive for toxoplasmosis. A broad difference was seen in the estimation of seroprevalence among various studies (Q=863.34; df=19; p<0.001; I²=97.8%; 95% CI: 97.5-98.1%) The pooled seroprevalence of toxoplasmosis was determined as (76%) (95% CI: 69-83%) (Figure 2). Seroprevalence was higher in KP (31.7%) compared to Punjab (20.42%) (Figure 3). The seroprevalence was detected using LAT in (10%) studies (Table 1).

Of the 16,432 animal studies, 16(0.09%) were selected for detailed review (Figure 4). Of them, 4(25%) were done in KP and 12(75%) in Punjab. A total of 8,519 animals were part of these studies, and 2,647(31%) of them were seropositive. A wide range of difference was seen in the seroprevalence estimation among the studies (Q=504.5; df=19; p<0.001; I²=95.4%; 95% CI: 94.5-96.1%). The pooled seroprevalence of toxoplasmosis in animals was determined as 69% (95% CI: 64-74%) (Figure 5). The

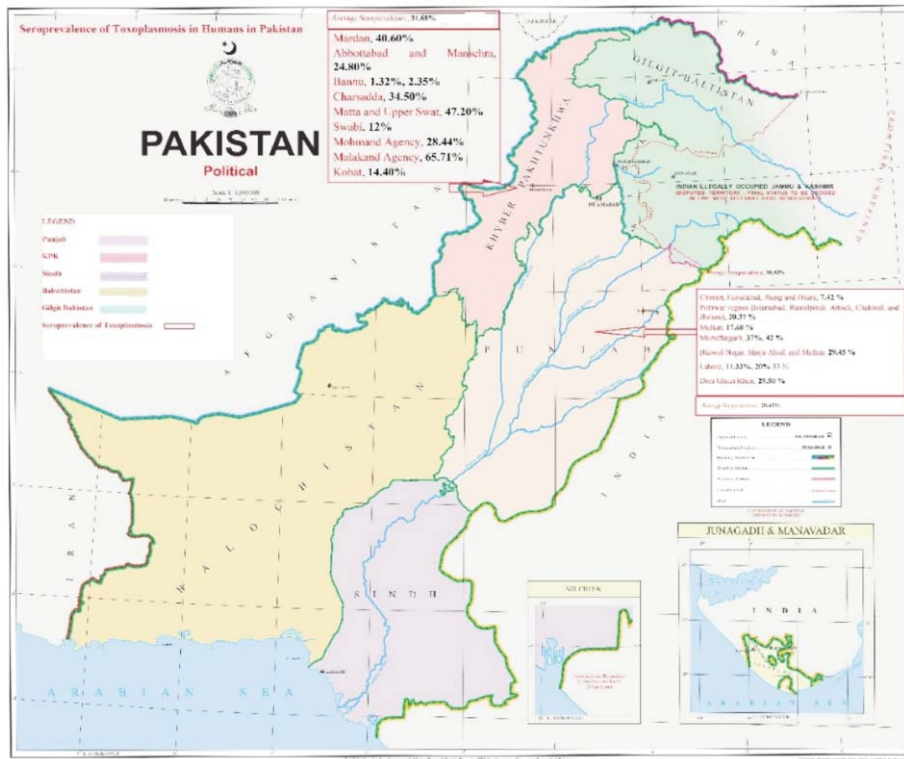


Figure-3: Topographical distribution of human toxoplasmosis in different regions.⁶⁶

Table-1: Summary of the reviewed.

Serial No.	Areas/ Regions	Provinces	Total individuals	Positive Cases	Prevalence (%)	Diagnostic Methods	References
1	Chiniot, Faisalabad, Jhang and Okara	Punjab	593	44	7.4%	ELISA	30
2	Mardan	Khyber Pakhtunkhwa	180	73	40.6%	ELISA	31
3	Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal, and Jhelum	Punjab	1659	338	20.4%	ELISA	32
4	Abbottabad, and Mansehra	Khyber Pakhtunkhwa	500	124	24.8%	RDTs, ELISA	33
5	Bannu	Khyber Pakhtunkhwa	150	2	1.3%	ELISA	34
6	Bannu	Khyber Pakhtunkhwa	170	4	2.3%	ELISA	35
7	Charsadda	Khyber Pakhtunkhwa	200	69	34.5%	LAT	36
8	Matta and Upper Swat	Khyber Pakhtunkhwa	360	170	47.2%	LAT	37
9	Swabi	Khyber Pakhtunkhwa	100	12	12%	RDTs	38
10	Multan	Punjab	403	71	17.6%	ELISA	39
11	Chitral	Khyber Pakhtunkhwa	300	74	24.7%	RDTs	40
12	Mohmand Agency	Khyber Pakhtunkhwa	580	165	28.4%	LAT	41
13	Malakand Agency	Khyber Pakhtunkhwa	420	276	65.7%	RDTs, LAT, and ELISA	42
14	Muzaffargarh	Punjab	150	63	42%	LAT	43
15	Muzaffargarh	Punjab	100	37	37%	LAT	44
16	Bhawal Nagar, Shuja Abad, and Multan	Punjab	550	162	29.5%	LAT	45
17	Lahore	Punjab	300	34	11.3%	LAT	46
18	Dera Ghazi Khan	Punjab	200	59	29.5%	LAT	47
19	Kohat	Khyber Pakhtunkhwa	180	26	14.4%	ELISA	48
20	Lahore	Punjab	50	10	20%	LAT	49

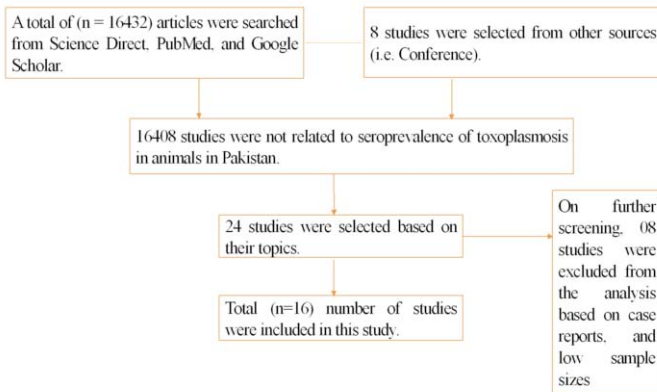


Figure-4: Study flowchart related to animal-based literature.

seroprevalence of toxoplasmosis in animals was higher in KP (44.7 (%)) compared to Punjab (29.4 (%)) (Figure 6).

LAT, ELISA and IHAT were used for the diagnosis of toxoplasmosis in animals (Table 2).



Figure-6: Geographical distribution of *Toxoplasma (T.) gondii* infections in animals in different regions of Pakistan. 66

Proportion meta-analysis plot [random effects]



Figure-5: Forest plot for random effect meta-analysis of *Toxoplasma (T.) gondii* infection in animal population of Pakistan..

Discussion

Besides causing a high disease load in humans, *T. gondii* also causes considerable economic loss to the livestock sector.¹⁷ The pooled seroprevalence of toxoplasmosis in humans and animals determined in the current systematic review was found as 76% and 69%, respectively. The findings are like those in Central America, Latin America, Europe and Asia (75-85%), while northern Europe and the United States have shown low seroprevalence.¹⁸

In Pakistan, the average seroprevalence of toxoplasmosis in humans was 20% between 2006 and 2020. No data were reported from the Sindh, Balochistan, Gilgit-Baltistan and Azad Jammu and Kashmir (AJK) in this regard. Thereafter, the seroprevalence of toxoplasmosis in humans has ranged between 1.3-65.7%.^{19 20}

Also, in Pakistan, the average seroprevalence of toxoplasmosis in animals was 31% between 2006 and 2020. In other studies, it has ranged from 10% to 55.4%.²¹

Seroprevalence of toxoplasmosis in the region has been reported in humans and animals to be 39.3% and 31%, respectively, in Iran, and 12.5% and 23.7%, respectively, in China.^{22, 23}

Toxoplasmosis is associated with numerous risk factors.²⁴ Abundance of cats is one of the vital sources of the spread of *T. gondii* infection in various regions of Pakistan. Cats can discharge millions of oocysts after eating even one tissue cyst containing bradyzoites.^{14, 25} In addition,

Table-2: Summary of various studies on Prevalence of *T. gondii* infection in the Animal-populations of Pakistan

Sr No.	Area/ City	Year of Study	Target Animal	Total Animals	Infected with Toxoplasmosis	Seroprevalence (%)	Diagnostic Method	References
1	Thal and Cholistan	2019	Camel	897	360	40.1%	Indirect ELISA	50
2	Multan	2018	Sheep and Goats	250	107	42.8%	LAT	51
3A	Charsadda	2018	Cows	139	77	55.4%	LAT	52
3B	Charsadda	2018	Goats	149	62	41.6%	LAT	52
4A	Multan and Khanewal Districts	2016	Sheep	500	168	33.6%	LAT	53
4B	Multan and Khanewal Districts	2016	Sheep	500	137	27.4%	ELISA	53
5A	Bhalwal, Kotmomin, Sahiwal, Shahpur, Silanwali and Sargodha Districts	2016	Sheep	470	123	26.2%	ELISA	54
5B	Bhalwal, Kotmomin, Sahiwal, Goahpur, Silanwali and Sargodha Districts	2016	Goats	530	227	42.8%	ELISA	54
6A	Cholistan Desert and Agricultural Areas of Rahim Yar Khan and Rajan Pur (Punjab) Pakistan	2016	Sheep	335	125	37.3%	LAT	55
6B	Cholistan Desert and Agricultural Areas of Rahim Yar Khan and Rajan Pur (Punjab) Pakistan	2016	Goats	865	252	29.1%	LAT	55
7	Faisalabad, Lahore and Gujranwala	2015	Horses (Equines)	272	91	33.5%	LAT	56
8A	Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)	2015	Sheep	413	75	18.2%	ELISA	57
8B	Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)	2015	Goats	419	60	14.3%	ELISA	57
9	Bahawalpur	2014	Camels	100	10	10%	LAT	58
10	Faisalabad	2014	Backyard Poultry	300	109	36.3%	LAT	59
11A	Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)	2014	Cattle	400	79	19.7%	ELISA	60
11B	Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)	2014	Buffaloes	422	64	15.2%	ELISA	60
12A	District Mardan	2013	Goats	350	148	42.3%	IHA	61
12B	District Mardan	2013	Sheep	290	128	44.1%	IHA	61
13	Multan	2011	Goats	200	104	55%	LAT	62
14	Dera Ghazi Khan, Multan and Khanewal	2010	Sheep	518	103	19.9%	LAT, ELISA	63
15A	Rahim Yar Khan	2009	Goats	110	28	25.4%	LAT	64
15B	Rahim Yar Khan	2009	Sheep	90	10	11.2%	LAT	64
16	Cholistan	2015	Sheep	335	125	37.3%	LAT	65

the sporulated oocysts can live for months and even for years in the soil. Moreover, earthworms, flies and beetles can escalate the oocysts and pass these on to the food chain.²⁶

In Pakistan, such high rate of seropositivity of *T. gondii* may be due to lack of awareness, lack of education among people, and improper hygienic conditions of the population. Lack of awareness about the infection, its sources of spread, its routes of transmission and ignorance of the outcomes of the diseases are important factors in the risk of infections.²⁷ The chances of infections can be reduced by increasing the level of education and

spreading awareness among the people.²⁸

Various serological methods are regularly used for the identification of *T. gondii* antibodies.²⁸ The current review noted that the most common methods used were LAT and ELISA. However, Toxoplasma immunoglobulin M (IgM) chemiluminescence ELISA (CLIA) is a robotic test that is performed in a very short time but is not a regular method used in either private or public laboratories in Pakistan.

Epidemiological investigations reckon important information for devising precautions for the control of toxoplasmosis. Awareness of seroprevalence of

Toxoplasma, disease problems and precautionary methods can lead to potent incidence curtailment.²⁹

The current systematic review has some limitations, like missing information about the seroprevalence of toxoplasmosis in different regions of Pakistan because of the absence of relevant studies, use of different methods for the identification of toxoplasmosis with variable levels of sensitivities and specificities, and diverse epidemiological outcomes. All these factors can have an influence on the overall seroprevalence rate mentioned in the review.

Conclusion

There is a need to create awareness among the masses to control toxoplasmosis in Pakistan. The relevant authorities should execute a screening programme in all provinces using standardised methods in order to estimate the actual prevalence and risk factors of toxoplasmosis in the country. Finally, there is a dire need to perform studies in Balochistan, Sindh, GB, and AJK regarding the seroprevalence of toxoplasmosis in human and animal populations

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