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Seroprevalence of toxoplasmosis in human (*Homo sapien*) population of District Bannu Khyber Pakhtunkhwa (KP), Pakistan

Feroz Khan^{*1}, Muhammad Rooman², Hameed Ur Rehman³, Abdur Rab⁴, Faiz Ur Rehman⁴, Abid Ur Rehman⁴, Abdullah Khan⁵, Asad Ullah⁵, Maqsood Ali⁶, Afzal Ahmad⁷

¹Department of Zoology and Biology, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi, Pakistan

²Department of Zoology, Hazara University Garden Campus Mansehra, Khyber Pakhtunkhwa, Pakistan

³Department of Chemistry, Kohat University of Science & Technology, KUST, Khyber Pakhtunkhwa, Pakistan

⁴Department of Zoology, Kohat University of Science & Technology, KUST, Khyber Pakhtunkhwa, Pakistan

⁵Arrazi Medical Complex Jeddah Street near Tamimi Camp. Al Arifi area, Al-Jubail, Kingdom of Saudia Arabia

⁶Department of Biomedical Sciences, CHA University, South Korea

⁷Department of Microbiology, Abbotabad University of Science and Technology, Abbottabad, Pakistan

Key words: Seroprevalence, Toxoplasmosis, Human (*Homo sapien*), Population, District Bannu

<http://dx.doi.org/10.12692/ijb/12.5.255-264>

Article published on May 30, 2018

Abstract

Toxoplasmosis is a zoonotic disease, caused by the *Toxoplasma gondii*. It is a common parasite which is responsible of high morbidity and mortality in all warm-blooded organisms including human beings. The current study was conducted during April to December 2015 in District Headquarters Teaching Hospital (DHQTH) in district Bannu for the assessment of *T. gondii* in Humans population. Total of 170 blood samples were collected from humans population, of which 166 blood samples were negative and 4 were positive with *T. gondii* of IgM and not of IgG antibodies. The 90 blood samples were recorded in females and 80 were in males. Among the positive three were recorded in females, while one was in male respectively. The overall percentile prevalence of toxoplasmosis was recorded with range of (97.64%) negative blood samples and 2.36% positive blood samples. Among the positive blood samples these females were more infected than males with range of 1.77% and 0.59% respectively. The percentile range of negative blood samples were 47.05% and 52.95% in males and females respectively. The age groups 36-45, 46-55 and >66 were more susceptible to the toxoplasmosis. The rural area was more susceptible to the toxoplasmosis than the urban area. Similarly the illiterate humans populations were also more infected as compared to the educated categories.

* Corresponding Author: Feroz Khan ✉ 03449002451h@gmail.com

Introduction

Toxoplasma gondii is a common protozoan parasite, belonging to the phylum Apicomplexa, subclass Coccidiasina and family Sarcocystidae (Hill *et al.*, 2007; Pereira *et al.*, 2010). It is cosmopolitan infection, because 6 billion peoples have been suffered with *Toxoplasma gondii* in all over the world (Furtado *et al.*, 2011). Other study reveals that one third of the world human's population is documented to transfer the *Toxoplasma gondii* parasite (Ryan and Ray 2004). It has been also noticed that warm and humid areas have high prevalence of toxoplasmosis (Coelho *et al.*, 2003). *Toxoplasma gondii* is a common parasite which is responsible of high morbidity and mortality in all warm-blooded organisms including human beings. It is one of the most worldwide zoonotic parasite, causes infection with non-symptomatic in healthy individuals, but it may cause serious problems in immune compromised individuals including AIDS (Hill and Dubey, 2002). It also causes abortions and neonatal complications problems in pregnant women and deeply adverse effects on livestock production (Dubey and Jones, 2008). The estimation of the human seroprevalence is greatly different in different countries including cultural habits and among the vary groups living in the same area (Dubey and Jones, 2008). Approximately 29.5% of human Pakistani population in different districts like Dera Ghazi Khan and Punjab has serological infection of toxoplasmosis (Tasawar *et al.*, 2011). The aim of the current study was to find out the seroprevalence of toxoplasmosis in human (*Homo sapien*) population of District Bannu Khyber Pakhtunkhwa (KP), Pakistan.

Materials and methods

Study site description

District Bannu is located in between the 31.28° North latitude and 73.25° East longitudes. It is present in the southern area of districts Lakki Marwat, Karak boundaries and the North South Waziristan Agencies. According to the 1998 census the estimated population of the district Bannu is 677350 and growth rate is 2.8% annually. The total region of district Bannu is 1,227 square kilometers and 74196 Hectors area is under cultivated.

The ambient climate of the district Bannu is 48°C and 6°C in summer and winter season. Total of 45% area is irrigated through canal systems, while the remaining area is depend upon the rain fall (Fig. 1).

Blood samples collection

The current study was conducted during April to December 2015 in District Headquarters Teaching Hospital (DHQTH) in district Bannu for the assessment of *Toxoplasma gondii* in Humans population. For this study those individuals were selected who visited to the hospital with the complaints of symptoms of *Toxoplasma gondii* like fever, muscles pain, bones pain and visceral organs complications. 2mL of venous blood was taken in a sterile syringe from each patient and brought to the laboratory with in hospital for further process.

Toxoplasma gondii detection

The collected blood was centrifuged at the speed of 3500 rpm for 5 minutes for serum extraction. After that the serum was separated from tube through dropper and kept 3 drops on rapid diagnostic device for detection of IgG and IgM value. The device contains three values namely 'C' negative value (The serum does not contain antibodies), 'T1' indicates IgM value and 'T2' indicates IgG value respectively. The spot 'S' is used for serum falling, if the serum was stopped on 'C' value it indicates that sample was negative, if stopped on 'T1' and 'T2' values it indicates that samples were positive with IgM and IgG antibodies (Fig. 2).

History of *Toxoplasma gondii*

First the Nicolle and Manteaux (1908) has identified a protozoan parasite in the tissues of small burrowing rodent. The parasites namely *Toxoplasma gondii*, *Ctenodactylus gondii*, was used for Leishmaniasis in the Charles Nicolle research laboratory at the Pasteur Institute in Tunis. Initially Nicolle assumed that protozoan parasite called as a piroplasm (Jitender and Dubey, 2008). From the Leishmaniasis, as soon as the Nicolle and Manteaux had discovered a new pathogen called as *Toxoplasma gondii* on the basis of their morphological shape and host.

Finally their complete name is *Toxoplasma gondii* (Dubey, 2002). In survey the accurate name for the protozoan parasite should have been *Toxoplasma gondii*, while Nicolle and Manteaux (1908) had inaccurately recognized the host as *Ctenodactylus gondii* (Dubey, 2007). Similarly Splendor (1908)

identified the same parasite in a mammal like rabbit in Brazil, also incorrectly recognized it as Leishmaniasis, but he did not give a name to it. For the next 30 years *Toxoplasma gondii* like pathogens were also identified in several other hosts like birds species (Dubey, 2002).

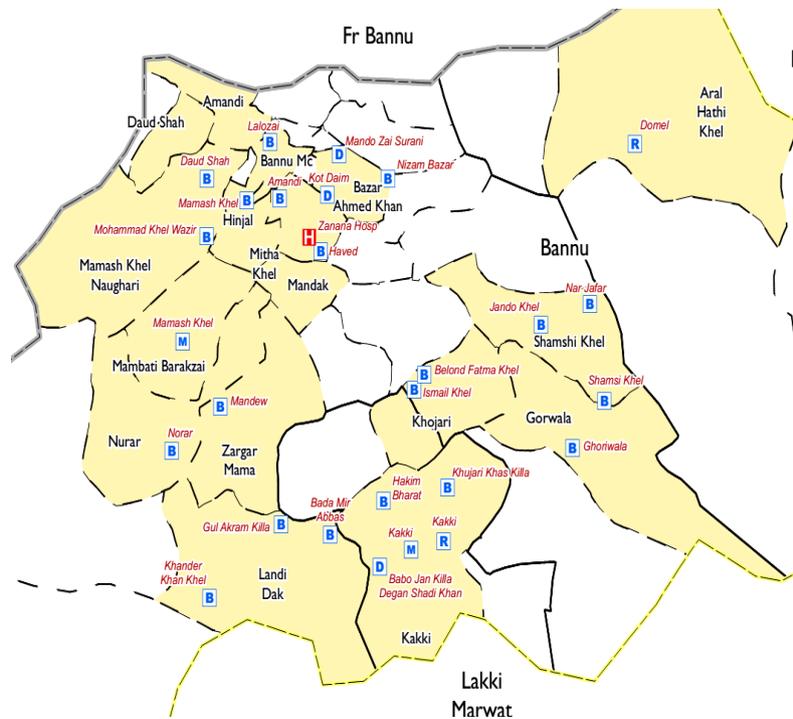


Fig. 1. Map of district Bannu Online (2015).

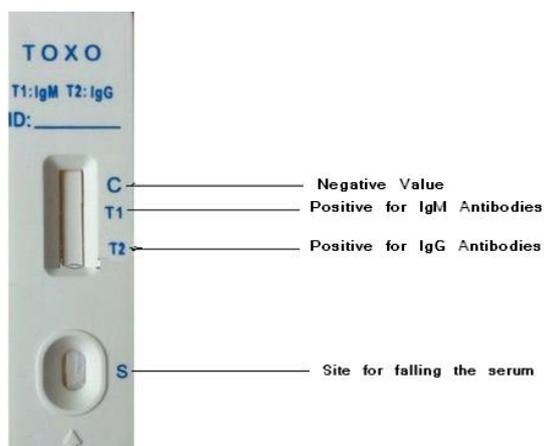


Fig. 2. Rapid diagnostic kit with visible positive, negative values and serum site.

Symptoms

The prevention of *Toxoplasma gondii* infection in pregnant women is very significant because it produce harmful complications in fetus. If the pregnant women is infected with toxoplasmosis

parasite, so it can causes poor growth and abortion. If the baby is born with toxoplasmosis infection can result of many complications like mental disabilities, hydrocephalus (water on the brain) and eye problems. Besides these it also affects many other organs like reproductive tract, muscles, bones, nervous system and visceral organs. It is a zoonotic disease also infect the birds and other domestic animals and produce many other different symptoms like weight loss, blindness and neck twisting etc. (Dolores and Dubey, 2014).

Hosts

There are following two types of hosts.

a. Definitive Host

Domesticated and wild cats are the individuals of felidae family, considered the definitive host of *Toxoplasma gondii*, in which their sexual stage takes place in their mucosa of intestine. During severe infection the cats release non-infectious oocysts in their fecal materials.

The main reason of infection in cats are improperly cooked meat and infectious prey. Kittens are more vulnerable to the parasite than adult cats. Those cats who hunt the wild animals are also susceptible to the infection. Domestic cats have less chance of getting the infection because they mostly used the pure and properly cooked meat (Jones and Wilson, 2003).

b. Intermediate host

Humans and other warm-blooded animals are considered the intermediate host. In healthy peoples, the infection of the parasite is more serious. In this stage the parasite attacks on cells and formed a specialized vacuoles called as parasitophorous containing bradyzoites, which propagated slowly form a parasite (Dubey, 2007).

These vacuoles synthesize the tissue cysts in muscles and brain, but the host immune system does not detect these cysts. These cysts show great resistance against different antibiotic and eliminate from the tissue very difficult. Within vacuoles the *Toxoplasma gondii* divided and finally released the tachyzoites, these tachyzoites are the motile and reproduce a parasite by asexually.

The infected tissue is eaten by cat, live within stomach of cat and infect soft layers of small intestine where they propagate sexually and produce oocysts release with feces. Animals and humans ingest these oocysts by eating the improperly cooked meat and unwashed vegetables become infected. The parasite enters the soft layers of small intestine and distributed via blood stream throughout the body (Nasreen, 2009).

Mode of Transmission

The *Neospora caninum* can be transmitted through placenta to the fetus called as transplacental transmission (Trees and Williams, 2005), or by the eating of infected meat with tachyzoites or cysts. It is also transmitted by the ingestion of infected food and drinking water contaminated with tachyzoites. When the tachyzoites are transferred from contaminated dam to her fetus during pregnancy (Dubey, 2007). Similarly the new born baby also becomes infected after drinking the milk contaminated with tachyzoites (Davison, *et al.* 2001), because the milk contain the

DNA of *Neospora caninum* including colostrum has been documented (Moskwa, *et al.* 2007).

Causes

The main cause of toxoplasmosis is the unpasteurized dairy products which contain the protozoan parasite, similarly the contaminated water with *Toxoplasma gondii* is not common in United State. Mostly the meat of Lamb and pork are infected with *T. gondii* parasite. Although kitchen instruments like knife, utensils and other materials are infected by the come in contact with meat containing cysts. All the used utensils should be washed with hot and soapy water. Similarly fruites and vegetables may also contaminate with cysts of *Toxoplasma gondii*, and they should be washed with pure water before eating. In other way the toxoplasmosis can also be transferred through blood transfusion and organ transplant, but it is very rare case (Dolores and Dubey, 2014).

Prevention

Human toxoplasmosis infection is prevented by washing of hands and other used materials like cutting board, knife and sink board with soap carefully after handling the meat containing cyst. The meat containing parasite can also be killed by exposure to extreme heat (67°C) for 4 minutes (Dubey *et al.*, 1990), and cool (-13°C) for 3 days (Dubey and Thayer, 1994). Cats and other domestic animals are key rule in the transmission of *Toxoplasma gondii* parasite and humans should be for away from their contact (Dolores and Dubey, 2014).

Treatment

The prescribed drugs for toxoplasmosis infection are Pyrimethamine (Daraprim) and Sulfadiazine respectively. These drugs have a useful affect during the stage, where the parasite is rapidly propagating. These drugs will not eliminate infection completely, but have little effect on infections. Few other drugs like clindamycin, spiramycin, diamino diphenylsulfone and atovaquone are also prescribed in very severe conditions (Dolores and Dubey, 2014). The purpose of current study was to investigate the seroprevalence of toxoplasmosis in human's population of District Bannu.

Results

The current study was conducted during April to December 2015 in District Headquarters Teaching Hospital (DHQTH) in district Bannu. Total of 170 blood samples were collected from humans population, of which 166 blood samples were negative and 4 were positive with *Toxoplasma gondii* of IgM and not of IgG antibodies. The 90 blood samples were recorded in females and 80 were in males. Among the positive three were recorded in females, while one

was in male respectively. For the prevalence of toxoplasmosis the age of the patients were divided in to six groups with ten year gape. The age >66 have more patients than the other age groups. From the below table it is cleared that the age of 36-45, 46-55 and >66 years were more susceptible to toxoplasmosis infection. Similarly the females were more susceptible to the *Toxoplasma gondii* than males (Table 1).

Table 1. Age wise prevalence of toxoplasmosis in district Bannu during April to December 2015.

Age (Years)	Male			Female		
	Negative	Positive	Total	Negative	Positive	Total
15-25	08	00	08	10	00	10
26-35	10	00	10	12	00	12
36-45	13	00	13	15	01	16
46-55	11	00	11	17	01	18
56-65	16	00	16	13	00	13
>66	21	01	22	20	01	21

The percentile range of negative blood samples were 97.64% (166), while positive were 2.36% (04) respectively (Fig. 3).

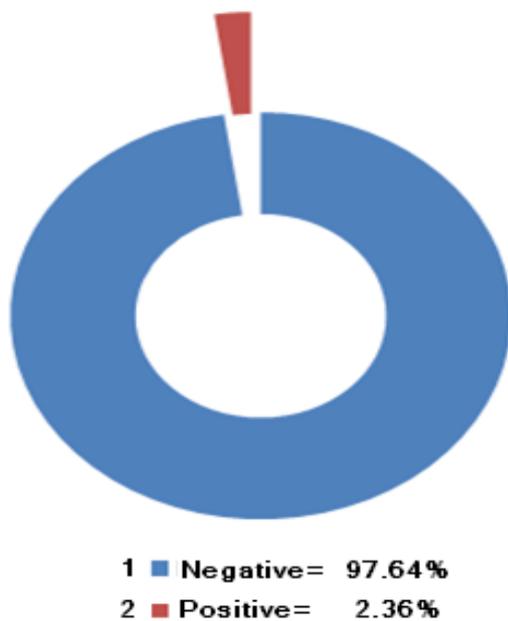


Fig. 3. Percentage wise prevalence of toxoplasmosis in humans population.

The percentile range of males negative blood samples were 47.05% (80), while females negative were 52.95% (90) respectively (Fig. 4).

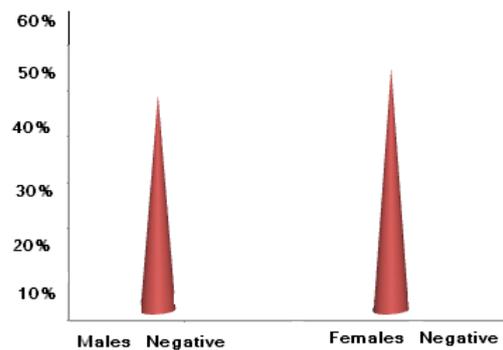


Fig. 4. Percentage wise prevalence of males and females negative blood samples of toxoplasmosis.

The percentile ranges of females positive were 1.77% (03), while male positive was 0.59% (01) respectively (Fig. 5).

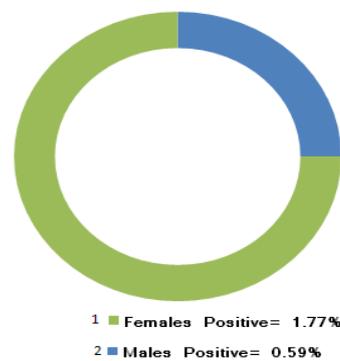


Fig. 5. Percentage wise prevalence of males and females positive blood samples of toxoplasmosis.

Mostly the females patients were belonged to the rural area, among the infected patients only one was located to the urban area, while the remaining three patients were located to the rural area. In male the urban patients were greater than the rural area patients (Table 2).

Table 2. Prevalence of toxoplasmosis on the basis of locality of the patients.

Locality	Negative		Positive	
	Male	Female	Male	Female
Urban	45	36	00	01
Rural	34	51	01	02

Illiterate humans populations were more susceptible to the toxoplasmosis rather than educated humans population. Similarly one female patient was recorded in educated categories, while the remaining two females and one male were recorded in illiterate categories (Table 3).

Table 3. Prevalence of toxoplasmosis in educated and illiterate humans population.

Education	Negative		Positive	
	Male	Female	Male	Female
Literate	23	37	00	01
Illiterate	56	50	01	02

Discussion

Toxoplasmosis is a zoonotic disease, caused by the *Toxoplasma gondii*. It is a common parasite which is responsible of high morbidity and mortality in all warm-blooded organisms including human beings. In the present study total of 170 blood samples were collected from humans population, among these 166 (97.64%) blood samples were negative while 04 (2.36%) blood samples were positive with *Toxoplasma gondii* infection. Among the positive blood samples 03 (1.77%) were recorded in females, while 01 (0.59%) was in male respectively. But all the positive cases were infected with IgM and not of IgG antibodies. The males negative blood samples were 80 (47.05%), while females negative were 90 (52.95%) respectively. The age groups 36-45, 46-55 and >66 years were more susceptible to toxoplasmosis infection. The rural area was more susceptible to the toxoplasmosis than the urban area.

Similarly the illiterate Humans populations were also more infected as compared to the educated categories.

A study was conducted by the Ahmad *et al.*, 2012, total of 300 humans blood samples were screened from different regions of Lahore Pakistan for *Toxoplasma gondii* infection. The overall prevalence of toxoplasmosis was recorded as 11.33% respectively. But the prevalence was recorded in different regions namely Walled City (15%), Railway Station (13%) and Allama Iqbal Town (6%) respectively. People of aged between 51-60 years have highest prevalence of infection than those of aged in between 41-50 years. Another study was put forwarded by Tasawar *et al.*, 2012 in Southern Punjab, Pakistan, total of 550 humans blood samples were screened. The overall prevalence of *T. gondii* infection was recorded as 29.45% respectively. Similarly the males have highest prevalence of toxoplasmosis as compared to the females with percentile range of 35.89% and 25.9% respectively. For prevalence the age groups of 1-10 years were more susceptible as compared to the age group of 61-70 years. Another study was conducted by the Sadaruddin *et al.*, 1991 in Islamabad Pakistan, the overall seroprevalence of toxoplasmosis was recorded as 17.4% from the humans population. The present study was less as compared to the above reports because the present findings have small sample size.

Many other reports were also documented from different parts of the world about the seroprevalence of toxoplasmosis. For example in Iran, 7.1% of seroprevalence of humans toxoplasmosis was recorded by Rostami *et al.*, 2006, 30.9% reported in Bombay, India by Meisheri *et al.*, 1997, 24.2% in Slovakia by Studenicovaa *et al.*, 2006, 49.8% by Chacin-Bonilla *et al.*, 2003 in Western Venezuela, Avelino *et al.*, 2004 reported 65.8% of prevalence in Brazil, Singh and Pandit, 2004 reported 45% in India and Sharif *et al.*, 2007 documented 77.4% in Iran respectively. The present findings were also small as compared to the mentioned reports because the blood samples which were collected have minimum age was 15 years. Less than 15 years of ages have low immunity, poor hygienic conditions, used unwashed fruits and careless.

Gender wise prevalence of toxoplasmosis was also reported from different regions of the world. In Italy, Moschen *et al.*, 1991 showed 18.2%, 17.5% of seroprevalence of toxoplasmosis in males and females respectively. Yaneza and Kumari 1994 reported 35.6% in males, while 1.9% in females in Saudi Arabia. Meisheri *et al.*, 1997 documented 34% in men and 26.2% in women in Bombay, India respectively. Lee *et al.*, 2000 showed 7.2% in females and 6.0% in males from the rural area of Korea. Sharif *et al.*, 2007 recorded 80% of seroprevalence in males and 77.6% in females in Iran. From the above findings the seroprevalence of toxoplasmosis was higher in males as compared to the females because of less immunity against the *Toxoplasma gondii* in males (Al-Qureshi, 2004) Similarly the females show great resistance against parasite infection as compared to the males because in males the testosterone hormone has immunosuppressive properties (Morales-Montor *et al.*, 2004). In our findings the females have high prevalence of toxoplasmosis rather than males. It is totally opposite to the above mentioned reports. Because in present results mostly rural area individuals were studied, these individuals are domesticated the animals in their houses and the females have closed contact in cleaning of their places, keeps the grass and extraction milk from their mammary gland. The literature indicates that it is a zoonotic disease.

Another study was conducted by the Gyang *et al.*, 2015 in Lagos City of Nigeria, total of 382 primary school children (PSC) were screened for *Toxoplasma gondii* infection. Among these 195 were females and 187 were males with an average age of 10.8 years. The overall seroprevalence was recorded as 24% and 83.5% respectively. The present findings were also small as compared to the study conducted by the Gyang *et al.*, 2015, because the mention study contained the primary school childrens (PSC) who have low immunity, body weakness and consumption of raw meat. The average age was recorded as 10.8 years, while in our study the average age was 15 years respectively. A study was put forwarded by the Mwambe *et al.*, 2013 in Tanzania, total of 350 pregnant women were screened.

Among these 108 (30.9%) were positive and urban area was more susceptible than those of rural area with range of 41.5% and 22.0% respectively. Hashemi and M. Saraei 2010 reported total of 400 women belonged to Qazvi community of Iran. The overall prevalence of toxoplasmosis was recorded as 34% respectively. The unemployed women have high prevalence as compared to the students with percentile of 38.3% and 22.6% respectively. Saki *et al.*, 2015 reported total of 130 women were screened for *T. gondii* infection in Iran. Among these 28 women were positive with percentage of 21.5% respectively. Olariu *et al.*, 2008 tested total of 104 women from urban and 80 from rural areas. Among these 70% prevalence was recorded in women belonging to rural area, while 48.1% from the urban area.

In rural area the age groups 30-39 (80%) and 40-45 (92.8%) years were more susceptible to the infection. Similarly the urban area of age groups 30-39 (48.7%) and 40-45 (57.1%) years have high prevalence as compared to the age <20 (14.3%) years respectively. In short the present findings were less as compared to the mentioned citation, because our results have short duration and small samples size.

Conclusion

Toxoplasmosis is a zoonotic disease, caused by the *Toxoplasma gondii*. It is a common parasite which is responsible of high morbidity and mortality in all warm-blooded organisms including human beings. The current study concluded that the 36-45, 46-55 and >66 years of age groups were more susceptible to the toxoplasmosis. The overall percentile prevalence of toxoplasmosis was recorded with range of (97.64%) negative blood samples and 2.36% positive blood samples. Among the positive blood samples the females were more infected than males with range of 1.77% and 0.59% respectively. But all the positive cases were infected with IgM and not of IgG antibodies. The percentile range of negative blood samples were 47.05% and 52.95% in males and females respectively. The rural area was more susceptible to the toxoplasmosis than the urban area. Similarly the illiterate Humans populations were also more infected rather than educated categories.

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