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Investigations of infected cases in Kirkuk city center through quantitative and qualitative methods of *Toxoplasma gondii*

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ABSTRACT: Blood samples were taken from 100 blood donations using private laboratories in Kirkuk city. The IHAT, LAT and ELISA tests were used on their blood plasma to see if they had become infected with the *Toxoplasma gondii* parasite. Researchers learned that, according to direct hemagglutination, the overall infection rate was 44% and by indirect hemagglutination, the rate reached 60%. According to ELISA, 71% of the samples were infected with the virus. After testing methods, it was observed that 44% of samples became infected with a parasite by direct hemagglutination, while 60% became infected by indirect hemagglutination. The infection was found with a 71% positive result by ELISA. The authors observed that the ELISA approach was the most precise method of all in their study, with indirect hemagglutination coming in second and direct hemagglutination ranking third in detecting antibodies. Similarly, *T. gondii* infection rates were mainly observed in people younger than 35. Our study revealed that the infection rate in people from cities was (75%), while the rate in rural areas was just (25%). The important factors supporting the spread of the parasite are contact with soil, the level of education and keeping cats. ELISA testing revealed that of the infected cases, 75% showed positivity for the IgG antibody compared to 25% for the IgM antibody.

KEYWORDS: Toxoplasma gondii, quantitative , qualitative , Kirkuk city

INTRODUCTION

The parasite *Toxoplasma gondii* is present in all parts of the world. The rate of infection with the parasite changes based on a community's culture, how wealthy it is, its location, local climate and the ways it can transmit the disease. As people age, infection becomes more common, especially when the air is hot and humid (Yan, *et al*., 2016).

Most people infected with parasites become ill without realizing it, but the disease can still lead to birth defects in children, including meningoencephalitis, due to the very early transfer of the parasite from the pregnant mother to her child (de Noya & Ruiz-Guevara,2022). From birth, an infant with infection is easy to identify based on a bulging skull, big head, mental disorders, feeding difficulties and unusual breathing rhythm(Bhowmik, 2023). The disease generally takes the life of a newborn within the first few months. Should the child live past ensuing mothers' love, it will be mentally retarded and learn slowly(Landsman, 2003).

When infection happens later, it causes delays in intellectual and motor development, an unusually large skull and in those whose immune system is compromised, results in a rash together with infected meninges, heart or lungs which can be fatal(Cordeiro, *et al*., 2015).

Raw meat, unboiled milk, raising cats, infection passed from a mother's placenta to the newborn and blood transfusion are the most significant factors leading to human infections(Givens & Marley , 2008). In France, undercooked meat is said to be responsible for the majority of infections with this parasite (Ducrocq , *et al*.,2021) .

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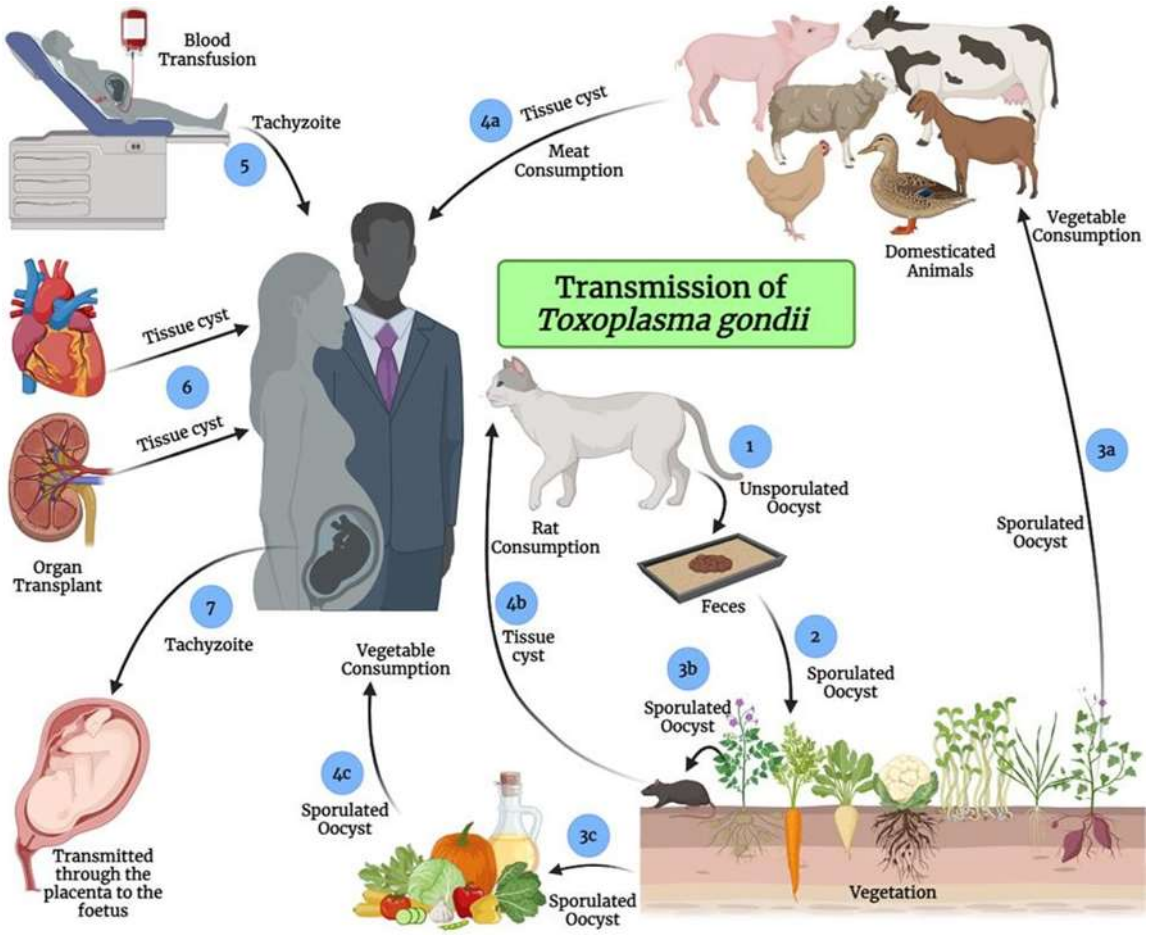

Fig. 1 : Zoonotic transmission of *Toxoplasma gondii* via different routes (Naeem *et al*.,2023)

About 50% of *Toxoplasma gondii* cases in the USA lead to 375 deaths each year among the 750 people infected and these deaths occur after eating meat that is not cooked thoroughly enough (Singh & Munawwar , 2010). According to the same study, the parasite infection rate in the US is 22.5% and in pregnant women, it is 15% – that's 30% higher in them(Taghipour,*et al*.,2021). Evidence revealed that older individuals are more affected by the infection, showing that raw or undercooked meat and not contact with soil, is the main route of transmission in the United States(Shang,*et al*.,2010). According to their observation, people with less education hold low-status jobs and thus spend much time on the ground which makes them more likely than others to be infected by the parasite(Mutebi ,*et al*.,2018).

The main objectives of conducting research

1- Studying *Toxoplasma gondii* both quantitatively and qualitatively and comparing its detection by direct, indirect methods and ELISA.

2- Find out the number of cases of the parasite in Kirkuk and examine the conditions that help the parasite to spread.

MATERIALS AND METHODS

Sample Collection

The research was performed on 100 women who were residents of Kirkuk city. A blood sample was collected from each individual using clean tubes and the blood was set aside and stored at -20°C for further research.

Experiments were done to explore and measure the level of antibodies against the *Toxoplasma gondii* parasite. I tested each sample in three ways: I did a direct hemagglutination assay (LATEX) on one, an indirect hemagglutination assay (IHA) on another and conducted an Enzyme Linked Immunosorbent Assay (ELISA) on the third.

Estimation of antibodies to *Toxoplasma gondii* using the direct agglutination (LAT) test.

Blood is drawn into a 50 ml micropipette and then put on the specific cards used for analysis. The special solutions are added to the serum and mixed with a piece of plastic. After that, they are put onto a vibrating machine. After finishing the protocol, the cards are taken out and the plasmid granules are studied. Infected samples display the granules, so their presence confirms that the sample

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is contaminated. Experts say that no change to the card's contents will take place if the selected microorganisms are absent (Chernecky & Berger, 2012).

Evaluating the number of antibodies against *Toxoplasma gondii* using the Indirect Hemagglutination Test (IHAT)

They carry out the test using special plates called ELISA plates. To dilute the serum in the sample, transfer 50 microliters of the sample serum and 75 microliters of the dilution solution form the test kit into a small outside test tube(Bhat ,*et al*.,2020) . We pick a certain column of the ELISA plate, as it has 8 wells set vertically.

1. Add 50 microliters of solution to the first well in the column you have chosen for the ELISA plate.
2. Add 50 microliters of the dilution solution to the well directly below Column 1, Upper-Right in the ELISA plate.
3. Pour 50 microliters from the first well into the following well and give the mixture a good shake. Moving to the next step, add 50 microliters of the fourth well stock to the eighth well to get dilutions of 1/32, 1/64, 1/128 and 1/256.
4. Transfer 50 microliters of the Toxoplasma gondii antigen from the test kit into wells 2 to 8. Give the mixture a gentle shake and then stir it. After that, leave it at room temperature for 3 hours.
5. Watch for the rings created in the wells. If the result is positive, the formed ring has a large diameter and this diameter decreases as the concentration becomes more negative. When the result is negative, there will be a dot in the center of the holes in the ELISA plate.

Enzyme-Linked Immunosorbent Assay is an ELISA test.

The method can identify IgG, IgM and IgA antibodies. How it works is by first adding soluble antigens and then adding the serum. The presence of antibodies for the parasite in the serum leads to their linking with the parasite. It can be detected by using antibodies attached to enzymes.

Blood was stored at 37°C for 15 minutes and then centrifuged using 2,000 rounds per minute for another 10 minutes. After centrifugation, we moved the sera to a microtube, froze them and placed them in storage for testing using ELISA(Cork ,*et al*.,2019). To carry out ELISA tests using the kits, I followed the instructions provided by the company. We use an ELISA reader at 450 nm to measure the optical absorbance of the standards and samples in the ELISA test. Now, test kits are designed to detect IgM and IgG antibodies for the parasite and they are very specific.

Data Collection

A survey was undertaken to understand what might lead to the transmission of the parasite. Among the questions on the survey were: age, having a job, level of education, owning a cat and contact with soil.

RESULTS AND DISCUSSION

This research involved collecting blood from 100 volunteers living in Kirkuk and analyzed it for *Toxoplasma Gondii* antibodies using direct damping, indirect bloody damping and ELISA.

The rate of parasite spread in the samples, as reported by the test:-

Using the IHA method

Based on this method, out of a total of 100 samples, only 60% are positive or were injured, because the remaining 40% are negative, meaning there is a moderate to slightly high proportion of injury in these samples.

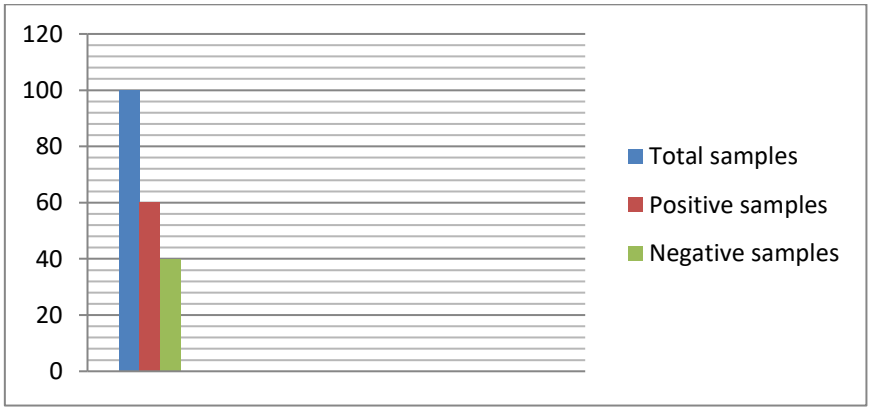

Figure 2, the IHA test sample is shown with the breakdown of positive and negative *Toxoplasma Gondii* cases

The LAT method showed that 44% of the samples tested positive and 56% of them were found to be negative. This means that by comparing the two rates, the average difference is 16%. Based on these results, the indirect hemoglobin agglutination method detects

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antibodies more accurately than Latex and the percentage is much like the levels found in (Hussien,*et al*.,2018) or (Al-Adlan, 2007). The variation in LAT results may reflect differences in economy, lifestyle and food customs. For instance, pregnant women in Asia prefer their food to be well cooked, but pregnant women in Europe are more likely to eat meat that has not been fully cooked (Ashraf ,*et al*.,2007). Moreover, keeping pets like cats and dogs and eating undercooked food increase the risk of reactivating the viral infection and you might also have experienced these risk factors when you were younger (Ahmed ,*et al*.,2021).

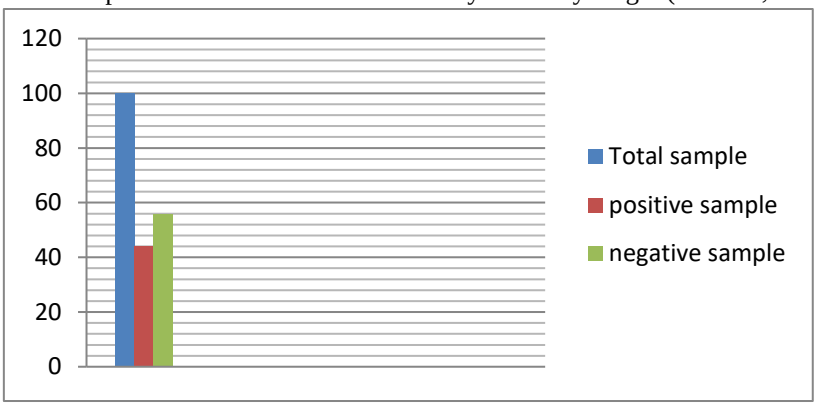

Figure 3 results of positive and negative cases of *Toxoplasma Gondii* using the Latex method

As the ELISA method indicates, out of 74 patients, 71 presented with the infection and the rest 29 were not infected. It is clear from Figure 5 that the difference between other rate methods was rather high. The outcome approximated the conclusions drawn by (Ibrahim,*et al*.,2009), (Noorbakhsh ,*et al*.,2002).

Figure 4 illustrates the percentage of positive and negative cases observed with the *Toxoplasma Gondii* using the Enzyme Linked Immunosorbent Assay (ELISA) method

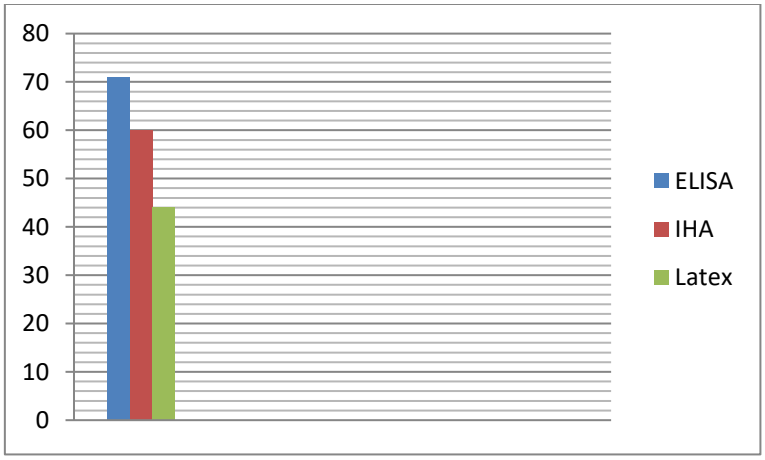

Figure 5 illustrates how many positive cases were found for *Toxoplasma gondii* using ELISA, Latex and IHA

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The rate at which individuals of various ages experience infection

The Latex method is used.

Figure 6 reveals that individuals within the 25-35 age group ended up with infection at the highest rate which is 21%. 13% of people aged between 15 and 25 and 10% of those from 36 to 45 were found to have infections.

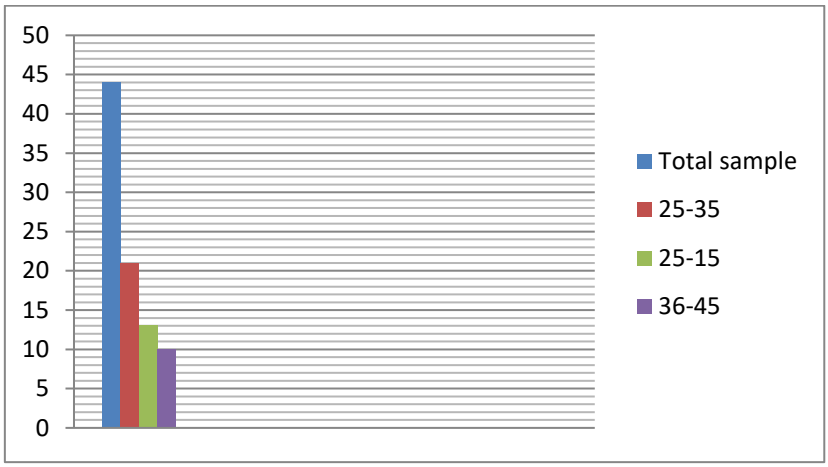

Figure (6) illustrates how many groups of people are getting infected, displayed with the Latex approach

According to this figure, 31% of infections were found in the age group (25-35) through the IHA method and the other groups (15-25) had an infection rate of 18%, while the (36-45) age group had a rate of 11%.

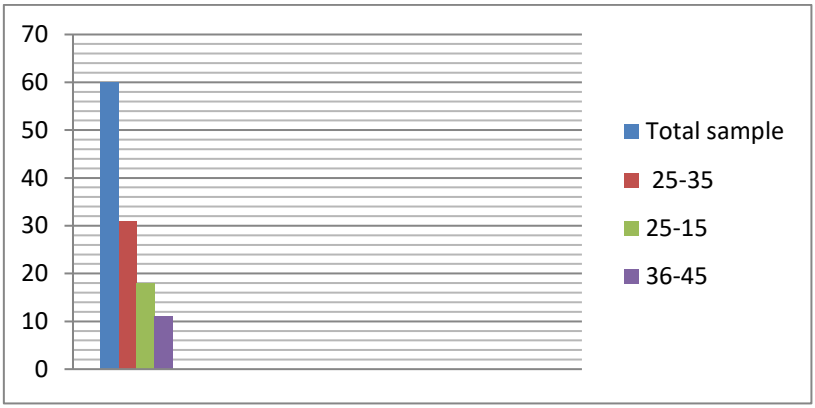

Figure (7) indicates the rate of infection in individuals divided by age groups using the IHA method

You can see from the graph that those in the (25-35) age group had the highest infection rate on ELISA which was 45% and the age groups (15-25) had 21% and (36-45) had 4%. The largest number of positive cases were found in people below 35 years. The reason for the surge in infection rates among women ages 25-35 may come from them being busier with household tasks, making them more likely to face risks and infections during this time(Mabaso,*et al*.,2018). It is often during this time that most women in eastern countries such as Iraq, decide to get married and have children. Experts have found that age itself is not a contributing cause of trichomoniasis, but as people get older, they become more vulnerable to infection(High, 2004). The results from this study suggest that older women are more cautious about getting parasites or that the chance to be infected decreases as women grow older(Felizardo,*et al*.,2018). As supported by general parasitology data, older people are less likely to have lymes infection compared to younger people (Izhar & Ben-Ami , 2015).

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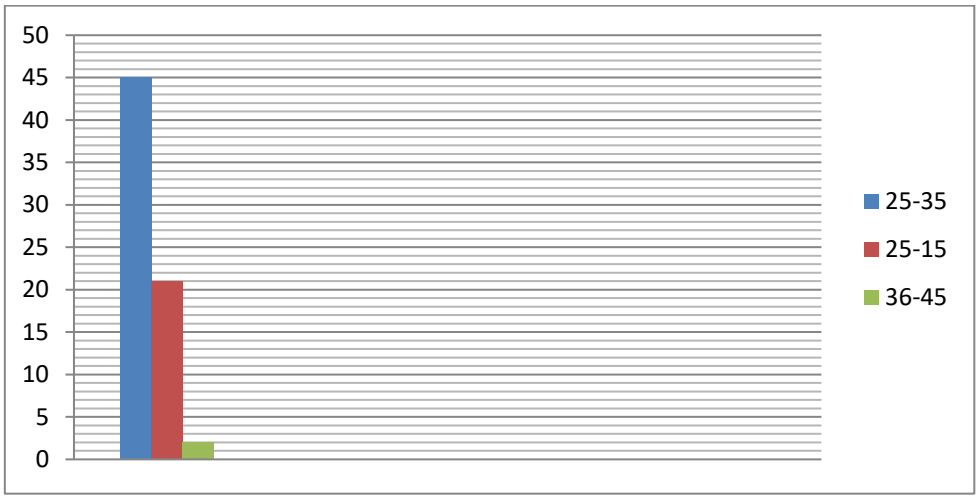

Figure (8) shows the infection rate in different age groups using ELISA testing

Percentage of infections caused by each type of infectious factors

Based on what facilitates infection, the study found that 30 of the female donors had jobs outside their homes. 15% and 30% were found to be the infection rates using the Latex Agglutination Test and Hemagglutination Indirect Test, respectively. 56% percent of students became infected. In women who were first found to be infected, the LAT found that education made a difference: 12% of those with no education, 7% with primary school education, 10% with secondary education and just 2% with university education were reported infected. Almost a quarter of the uneducated group had the infection, 21% of those with only primary education, 13% of those completing secondary education and 10% of those with university qualifications. Uneducated people, those with only primary schooling and 10% with secondary education were found to have an infection rate of 30%, 25% and 13%, respectively, by the Enzyme Linked Immunosorbent Assay. It is possible that higher educational levels lead to better behavior, resulting in a reduced risk of *Toxoplasma gondii* infection among pregnant women(Rasheed& Al-Sowielem, 2003). It could have a non-direct effect on the usual environmental and cultural reasons behind the parasite being found. If someone's education is low, there is a greater risk of them eating contaminated food(Darnton-Hill& Mkparu,2015) .

As for those who donate blood, only a small number of them enjoy raising cats; of the 60 participants who had no direct contact with cats, nearly one-fifth were found to be infected with the virus. Direct contact with cats involved 40 women and the frequency of the disease with the Test Agglutination Latex was ten percent. Using the Test Hemagglutination Indirect raised the risk to 30% and the infection rate for Enzyme Linked Immunosorbent Assay was 60%. The rare infection found in women who own cats could be simply because of the mild effect this has on seroprevalence of Toxoplasma gondii parasites. When cats' waste with egg sacs contaminates a person's skin or mouth, there is a risk of picking up this parasite(Overgaauw, 2020). When a cat stays indoors, avoids hunting and avoids eating raw meat, it is very unlikely to carry the Toxoplasma gondii parasite, so there is no significant risk for humans(Englar, 2019).

Besides, egg sacs do not appear in cat fur and they prefer to put their cautions in the ground, so it is not the cats that cause the issue(Jumaian, 2005). Out of 82 women who avoided contact with the soil, 5% were found to have infections by LAT, 22% had them from THI and 45% tested positive for ELISA, respectively. For the other 18 who came in contact, LAT yielded infection rates of 7%, THI yielded 20% and ELISA 61%. The more soil is handled, the more likely one is to develop the infection and this risk can impact pregnant women as well (Berger,*et al*.,2010). A high rate of infection from soil could be linked to the fact that reaching water or foods contaminated by soil is a risk factor for getting diseased(Dixit,*et al*.,2024). Only for two weeks every life, cats shed egg sacs (more than 10 million each day) when they become infected with the first parasite(Board,*et al*.,2017).

Two to five days after being shed, the egg sacs start infecting people and are passed through contact with water in rivers and lakes (Yan,*et al*.,2016). As a result, being in touch with soil or water and cats increases your chance of getting infected with Toxoplasma gondii. It is also a problem found across the world because it is hard to stop (Sandee, 2016). In some cases, up to 68% of infections happen because of parasites from the soil which occurs when large numbers of stray cats eat raw meat with tissue cysts and cats reproduce quickly and are easily infected again by the parasites in the soil(Tun, 2016). As a result, kitties carrying the parasite soil their home gardens which leads to a concentration of egg cysts in the soil(Zhang,*et al*.,2009).

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Compared cases involving infections for various types of residence

The diagram below demonstrates the prevalence of infection among residents of Kirkuk city, according to the results of (IHAT), (LAT) and ELISA. Of patients in the study, those from urban areas experienced a parasitic infection at a rate of 75%, compared to 25% for those living in rural areas. Of those living in the city as shown by (LAT) test, positive tests were recorded in 18% of cases, for (Test Hemagglutination Indirect Test) it was 28% and infection cases were 75%. People in rural areas had a 5% infection rate with the (LAT) test, but 17% had the infection with (Test Hemagglutination Indirect) and 46% carried it as tested by ELISA. It is not surprising that rural women are less affected by the parasite, as the most significant factor is cats depositing disease-laden feces; this occurs more often in the countryside where cats live in larger groups, yet city cats only use a few restricted areas for their defecation. For this reason, eggs from Toxoplasma gondii are highly concentrated which raises the risk of infection (Zhu,*et al*.,2023). Since rural cats spread out the egg lining in farm areas, the likelihood of getting the infection is reduced(Mahmood,*et al*.,2022).

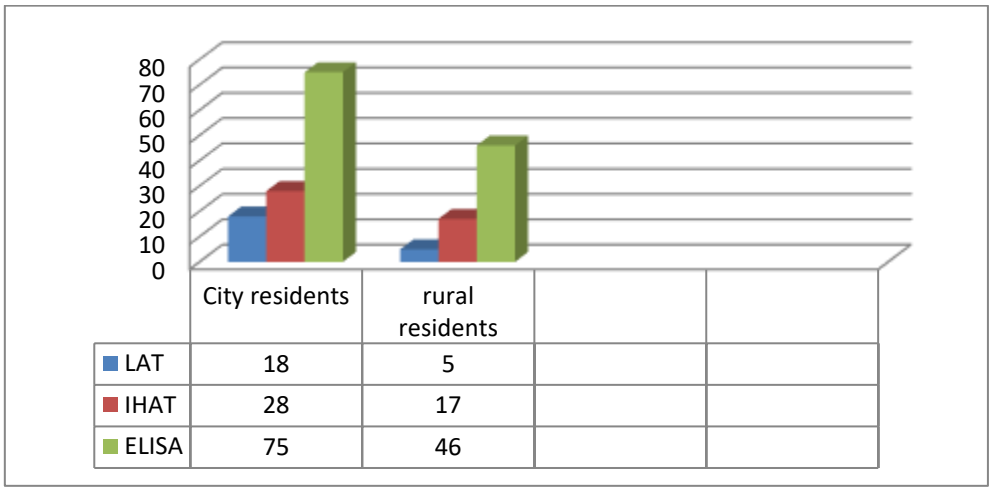

Figure (9) Infection prevalence by residential type

Detecting IgM and IgG antibodies in an infection using ELISA

In Figure 10, the distribution of study subjects who tested positive for infection and their antibodies using the ELISA method is shown. Out of the examined samples, the technique revealed 80 positive results. We have found that 75% of all positive cases had IgG antibodies and only 25% had IgM antibodies. The findings match those obtained by other researchers(Luo,*et al*.,2021). IgM antibodies quickly appear when someone gets infected, but IgG antibodies are present for a much longer period afterward(Dailey, 2017).

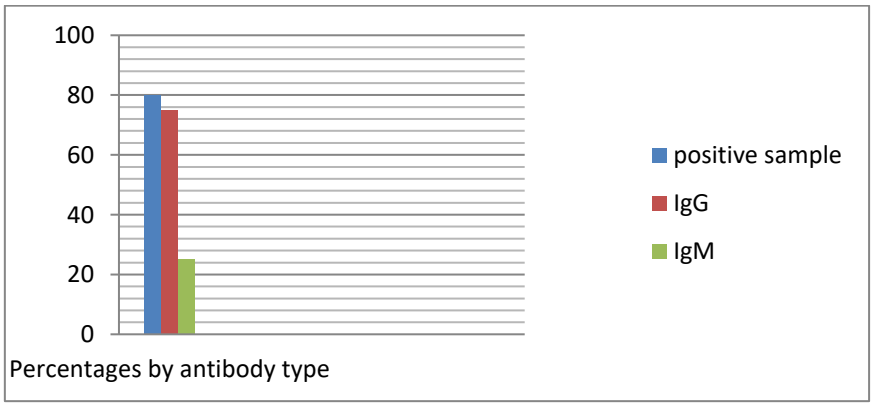

Figure (10) Infection by IgM and IgG Antibody Type Using ELISA

CONCLUSIONS AND RECOMMENDATIONS

Many people in Kirkuk city center seemed to be infected with *Toxoplasma gondii* and the illness was found at notably different rates among different age groups. A link was found between the infection and routes such as keeping cats, a person's age, their educational level and being exposed to soil.

Using ELISA and other quantitative methods was more effective for determining how severe an infection was, while serological tests helped with identifying the infection.

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It was found that those aged 25 to 35 years are most likely to get infected, as they are very active both socially and professionally. It was found that having a standard of living below the norm made a person more likely to get infected because of less awareness about health and negative environmental factors. The data pointed to a link between keeping cats at home and contracting the parasitic infection, making it clear that cats carry Toxoplasma.

Among suggestions, I recommend teaching people about safe food practices and caring for pets to avoid toxoplasmosis. Regularly examining women who can get pregnant and those who are expecting to catch the infection as early as possible and reduce the chance of it affecting their fetus. Encouraging others to cook their meat carefully, wash their hands and cleanse their skin after touching cats or soil. It is recommended for health institutions to use ELISA or PCR tests, as they lead to more accurate diagnoses and help assess the extent of the infection.

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