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Prevalence of Toxoplasmosis Among Women of Reproductive Age in Mukalla City, Yemen: A Comparative Study of Rapid Cassette Test and Cobas e411

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Abstract

Toxoplasma gondii is an obligate intracellular parasite that affects a wide range of mammals, including humans, which serve as intermediate hosts. This cross-sectional study enrolled 85 women during its initial stage. The second stage was the laboratory investigation that included the Cassette and Cobas e 411 tests. The overall seroprevalence of *T. gondii* in the study area was 58.82%. The seropositivity of anti-Toxoplasma IgG (58.82%) was higher than that of anti-toxoplasma IgM (1.18%) by the Cobas test, whereas IgG-positive and IgM-positive cases accounted for 55.29% and 4.71%, respectively, by the Cassette test. This suggests that *T. gondii* IgG antibodies in women are a reflection of chronic or past infection, while IgM reflects recent and acute *T. gondii* infection. The highest seropositive rate (70%) was recorded in the age group 33-38. The low sero-positive rate (54.55%) was recorded in the age group 21-26. The present study revealed that 50 % statistic in the abortion results. The results show that women with one previous abortion had a high (53.85%) seroprevalence of *T. gondii* antibodies. Higher sero-positive toxoplasmosis was recorded among women from Al-Deas zones (68.75%), revealing that the rate of infection among low-level women was high (75%). The ownership of animals in relation to the infection was studied. The highest seropositive rate was recorded among women who kept sheep and goats (71.43%), compared to 50% for cat owners, showing a statistically significant difference ($P < 0.001$). In conclusion, toxoplasmosis is a major public health issue among the studied population in Yemen. The Cobas e411 system offers superior sensitivity over the cassette method for antibody detection. Community-wide educational programs emphasizing hygienic animal handling are recommended, alongside the adoption of highly accurate testing protocols to improve diagnostic accuracy.

Keywords: Cobas e411; Risk Factors; Seroprevalence; *Toxoplasma gondii*, Yemen.

INTRODUCTION

Toxoplasma gondii is an animal coccidian parasite that causes toxoplasmosis (Alkhali, 2015). *Toxoplasma gondii* is an obligate intracellular parasite affecting a wide range of mammals and birds, including humans. Though human toxoplasma infection is very common, affecting nearly one-third of the world's population, clinical manifestations are relatively rare, mostly restricted to opportunistic infections in immunocompromised persons and congenital infection in the fetus (Sastry and Bhat, 2014). Toxoplasma parasites were first discovered in 1908 by Charles Nicolle and Louis Monceaux at the Pasteur Institute, in the North African rodent called the gundi, hence the species name gondii. At the same time, Alfonso Splendore described the organism from laboratory rabbits at the Portuguese Hospital in São Paulo, Brazil. However, the complete life cycle was not determined until 1970 (Garcia, 2007). Its importance as a human pathogen was recognized only much later, when Janku in 1923 observed the cyst in the retina of a child with hydrocephalus and microphthalmia, Wolf and Cowen in 1937 identified the first congenital brain infection, and Pinkerton and Weinman recorded postnatal infection in 1940. With the discovery in 1948 of the Sabin-Feldman dye tests, the first serological assay for Toxoplasma antibody, the scope and extent of the infection became open to study (Petersen, 2007). Animals of the family Felidae, only domestic and wild cats, are known as definitive hosts of toxoplasmosis. Oocysts are shed with the feces of the definitive host and are non-infective in freshly passed feces. Sporulation, the process by which Oocysts become infective, requires oxygen and occurs outside the host body in 1–5 days depending on temperature, humidity, and aeration (Asbakk and Prestrud, 2013). A human may become

infected by accidentally ingesting oocysts passed in cat feces through contaminated soil or handling of cat litter and ingesting tissue cysts within raw or undercooked meat (lamb, pork, and beef), drinking unpasteurized milk, contaminated water, or unwashed fruits or vegetables (Satoskar et al., 2009). Transmission can also occur by transfusion and during organ transplantation (Duan, 2012). Mothers who develop acute toxoplasmosis in the first trimester have a much lower fetal transmission rate than in the third trimester, but fetuses exposed early are at much higher risk for severe symptoms or death and spontaneous abortion (Satoskar et al., 2009). Patients can minimize their risk factor of developing toxoplasmosis by avoiding the consumption of undercooked meat and careful hand washing after contact with soil (Al-Nahari, 2010). The life cycle of the parasite consists of 3 stages as follows: (a) Tachyzoites, the rapidly multiplying trophozoites which invade and multiply within cells, (b) Bradyzoites, the slowly multiplying forms inside tissue cysts, seen during latent and chronic infection, and (c) Sporozoites inside oocysts, which are shed in cat feces and remain in the environment. In cats, which are the definitive hosts (Assafa, 2004). Both schizogony and gametogony take place in the epithelial cells of the small intestine. This is known as the enteric cycle. Cats shed Oocysts for 1 to 2 weeks, and large numbers may be shed, often more than 100,000 per gram of feces. Oocysts survive in the environment for several months to more than 1 year and are resistant to disinfectants, freezing, and drying. However, they are killed by heating to 70° C for 10 minutes. (Tille, 2014). The oocysts are 9 to 11 µm wide by 11 to 14 µm long and contain two sporocysts, each containing four sporozoites (Borges, 2004). They are produced through gametogonium and subsequently shed in the feces of definitive hosts (cats). Following excretion, these unsporulated oocysts sporulate in the environment, becoming infective within soil or water. If ingested by felines, the parasite completes its enteroepithelial cycle. Alternatively, upon ingestion by non-feline avian or mammalian intermediate hosts, the sporulated oocysts release sporozoites that subsequently invade intestinal epithelial cells. Within these cells, the parasites replicate via endodyogeny, differentiating into rapidly dividing tachyzoites (Diaz-Suarez, 2009). The subsequent rupture of host cells releases these tachyzoites, which disseminate hematogenously and lymphatically to infect any nucleated cell within various target tissues and organs. This is known as the exocentric cycle. Primary infection with the parasite may be asymptomatic, acute, or chronic (Parlak, 2015). In chronic infections, tissue cysts are produced within muscles and other tissues. When other intermediate hosts ingest these tissue cysts, the asexual cycle is repeated. When cats ingest the tissue cysts, they become infected, and in them both asexual and sexual cycles are repeated (Jones, 2009). Although the tissue cysts may develop in visceral organs such as the lungs, liver, and kidneys, they are more prevalent in neural and muscular tissues, including the brain, eyes, and skeletal and cardiac muscle. Intact tissue cysts can persist for the life of the host and do not cause an inflammatory response (Iqbal, 2007). Toxoplasmosis is one of the most common parasitic zoonotic infections affecting a wide range of mammals and birds. Its prevalence in humans varies from 5–75% and depends on various risk factors, such as the geographical area (Montoya, 2008). It commonly affects older age and fetuses, as well as exposure to cats and cat feces, Food habits, ingestion of uncooked cat and other animal meat, at higher risk (Malarvizhi, 2012). Immune status patients with HIV, malignancies, and other immunocompromised conditions are at high risk, and Patients undergoing blood transfusions or organ transplantations are at higher risk (Khurana, 2010). Congenital toxoplasmosis results when infection is transmitted transplacental from mother to fetus. This infection occurs when the mother gets primary toxoplasma infection, whether clinical or asymptomatic, during pregnancy (Khalil, 2016). The risk of fetal infection rises with the progress of gestation, from 25 % when the mother acquires primary infection in the first trimester, to 65 % in the third trimester (Kamal, 2015). Conversely, the severity of fetal damage is highest when infection is transmitted in early pregnancy. Most infected newborns are asymptomatic at birth and may remain so throughout. Some cases develop clinical manifestations of toxoplasmosis weeks, months, or even years after birth (Jones, 2001). Although a congenital infection can result in abortion, stillbirth, or neonatal disease with encephalitis, chorioretinitis, and hepatosplenomegaly, Fever, jaundice, and intracranial calcifications are also seen (Jeannel, 2020). Infection acquired postnatally is mostly asymptomatic. Clinical toxoplasmosis may present in different forms. The most common manifestation of acute acquired toxoplasmosis is lymphadenopathy, the cervical lymph nodes being most frequently affected (Janvier, 2013). Fever, headache, and splenomegaly are often present. Another type of toxoplasmosis is ocular. Primarily, toxoplasmosis involving the central nervous system is usually fatal and is often found in AIDS patients. Toxoplasmosis is particularly severe in immunodeficient patients. (Garly, 2021). Other manifestations of Toxoplasma infections are meningoencephalitis, hepatitis, pneumonitis, and myocarditis. (Diza, 2005). While acute infection with *T. gondii* can produce psychotic symptoms similar to those displayed by persons with schizophrenia (Bouhamdan, 2010). Most *T. gondii* infections are not diagnosed because individuals who are exposed never feel ill or become symptomatic, or through a mistaken belief that a febrile disease, such as a cold or flu, is an innocuous viral infection. (Pishak, 2006). Serological testing for IgM antibodies against T. gondii is currently the most commonly employed diagnostic test for acute infections (Ridley, 2012). Convalescent IgG antibodies will occur

late in the infection after the acute phase has passed. Finding *T. gondii* antibody can aid in diagnosis (Roberts, 2009). Numerous serological assays are available to detect humoral antibodies against the parasite. These include the Sabin–Feldman dye test (DT), indirect hemagglutination assay (IHA), indirect fluorescent antibody test (IFAT), modified agglutination test (MAT), latex agglutination test (LAT), enzyme-linked immunosorbent assay (ELISA), and immunosorbent agglutination assay (ISAGA). Furthermore, molecular techniques are increasingly employed, particularly in the preparation and standardization of antigenic reagents (Saif, 2014). In recent years, attempts have been made to develop diagnostic techniques that rely on nucleic acid probes to detect small amounts of parasite DNA. These probes are made using PCR (polymerase chain reaction) amplification of parasite ribosomal DNA. Such techniques allow for the detection of single organisms in tissue samples (Samue, 2001). The most common drug combination used to treat congenital toxoplasmosis consists of pyrimethamine and a sulfonamide, plus folinic acid in the form of leucovorin calcium to protect the bone marrow from the toxic effects of pyrimethamine (Satti, 2011). Spiramycin is recommended for pregnant women with acute toxoplasmosis when fetal infection has not been confirmed in an attempt to prevent transmission of *T. gondii* from the mother to the fetus (Tekkesin, 2012). Antibiotics may be used in pregnant women, immunocompromised patients with organ involvement, congenitally infected infants or individuals with ocular disease. Treatment of acute infections during pregnancy reduces the risk of infection in the fetus by approximately 50%. Healthy non-pregnant individuals may not be treated, as the infection is self-limiting and typically mild (Tlamcani, 2017). Prevention is accomplished through proper personal hygiene and avoiding the feces of cats and certain other animals, such as pigs and sheep. Meat should be properly cooked before eating to ensure that the organisms are destroyed. It might be added that a great deal of exposure occurs in daily life, as up to one-third of the world's population shows antibodies against the organism (Zaki 2016).

This study aims to determine the prevalence of Toxoplasmosis among women of reproductive age in Mukalla City, Yemen, and to evaluate and compare the diagnostic performance of the Rapid Cassette Test and the Cobas e 411 in detecting *Toxoplasma gondii* infection. Additionally, the study seeks to identify potential factors associated with Toxoplasmosis infection in this population and to provide recommendations for effective screening and diagnostic strategies for reproductive-aged women in Yemen.

METHODS

In this study, two diagnostic methods were used, namely Electrochemiluminescence immunoassay (ECLIA), which is intended for use on (Cobas e 411) immunoassay analyzers, and One Step Toxoplasma – IGM /IGG Tests, Rapid Immunochromatographic Assay (cassette) in diagnosing toxoplasmosis. A total of 85 (83 pregnant and 2 non-pregnant) women attending the above health centers were investigated for toxoplasmosis. Blood samples were collected from each woman in plain tubes, allowed to clot, then centrifuged, sera were separated from the clot, and then tested to determine IgM & IgG anti-TOXO antigen on the same day by using two different techniques in laboratories. TOXO- IgG and TOXO- IgM Cassette test. The Advanced Quality One Step TOXO-IgM Test is a colloidal gold-enhanced immunoassay for the determination of IgM anti-TOXO antigen (anti-TOXO-IgM) in human whole blood, serum, or plasma. The nitrocellulose membrane was immobilized with the TOXO antigen in the test region. During the assay, the specimen is allowed to react with the colored conjugate mixture and then migrates chromatographically on the membrane by capillary action. For a positive result, a colored band with the specific immuno-complex will form on the membrane. Absence of this colored band in the test region suggests a negative result. A colored band always appears in the control region, serving as procedural control regardless of the test result. The Advanced Quality One Step TOXO-IgM, IgG Test is a colloidal gold-enhanced immunoassay for the determination of IgG anti-TOXO antigen (anti-TOXO-IgM, IgG) in human whole blood, serum, or plasma. The nitrocellulose membrane was immobilized with the TOXO antigen in the test region. During the assay, the specimen is allowed to react with the colored conjugate mixture and then migrates chromatographically on the membrane by capillary action. For a positive result, a colored band with the specific immuno-complex will form on the membrane. Absence of this colored band in the test region suggests a negative result. A colored band always appears in the control region, serving as procedural control regardless of the test result.

TOXO - IgM Cobas e 411 testing. The principle of Toxo-IgM. U-Capture test principle. Total duration of assay: 18 minutes. 1st incubation: 10 µL of the sample is automatically prediluted 1:20 with Diluent Universal. T. gondii-specific recombinant antigen labeled with a ruthenium complex) is added. Anti-Toxo IgM antibodies present in the sample react with the ruthenium-labeled T. gondii-specific recombinant antigen. The 2nd incubation: Biotinylated monoclonal h IgM specific antibodies and Streptavidin-coated microparticles are added. The complex becomes bound to the solid phase via the interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Pro Cell /Pro Cell M. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier. Results are

determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration. The analyzer automatically calculates the cutoff based on the measurement of TOXIGM Cal1 and TOXIGM Cal2. The result of a sample is given either as reactive or non-reactive, as well as in the form of a cutoff index (signal sample/cutoff). Interpretation of the results: Results obtained with the cobas e 411 Toxo IgM assay can be interpreted as follows: Non-reactive: < 0.8 COI, Indeterminate: ≥ 0.8 - < 1.0 COI, Reactive: ≥ 1.0 COI. Samples with a cutoff index < 0.8 are non-reactive in the Toxo IgM assay. Samples with a cutoff index between ≥ 0.8 and < 1.0 are considered indeterminate.

TOXO - IgG Cobas e 411 testing: The principle of Toxo- IgG

Sandwich principle. Total duration of assay: 18 minutes.1st incubation: 10 µL of sample, a biotinylated recombinant *T. gondii* specific antigen, and a *T. gondii*-specific recombinant antigen labeled with a ruthenium complexa form a sandwich complex.2nd incubation: After the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier. The analyzer automatically calculates the analyte concentration of each sample in IU/mL. Interpretation of the results: Results obtained with the cobas e 411 Toxo IgG assay should be interpreted as follows, taking into consideration the respective algorithm which is used for the screening of Toxoplasma in pregnant women according to national or regional guidelines or recommendations. Toxo IgG testing is used as a first-line screening assay. Non-reactive: < 1 IU/mL, Indeterminate: ≥ 1 - < 3 IU/mL, Reactive: ≥ 3 IU/mL. Samples with concentrations < 1 IU/mL are considered non-reactive in the Elecsys Toxo IgG assay. Samples with concentrations ≥ 3 IU/mL are considered positive for IgG antibodies to *T. gondii* and indicate either acute or latent infection.

RESULTS

Table 1. Sero-incidence of toxoplasmosis among Mukalla city women according to home address.

No.	Address Home	Total cases examined	Positive	%	Negative	%
1	Mukalla	14	6	42.86	8	57.14
2	Deas	16	11	68.75	5	31.25
3	Sharg	19	11	57.89	8	42.11
4	Fowah	24	15	62.50	9	37.50
5	Bwaesh	8	5	62.50	3	37.50
6	Broom	4	2	50.00	2	50.00
Total		85	50	58.82	35	41.18

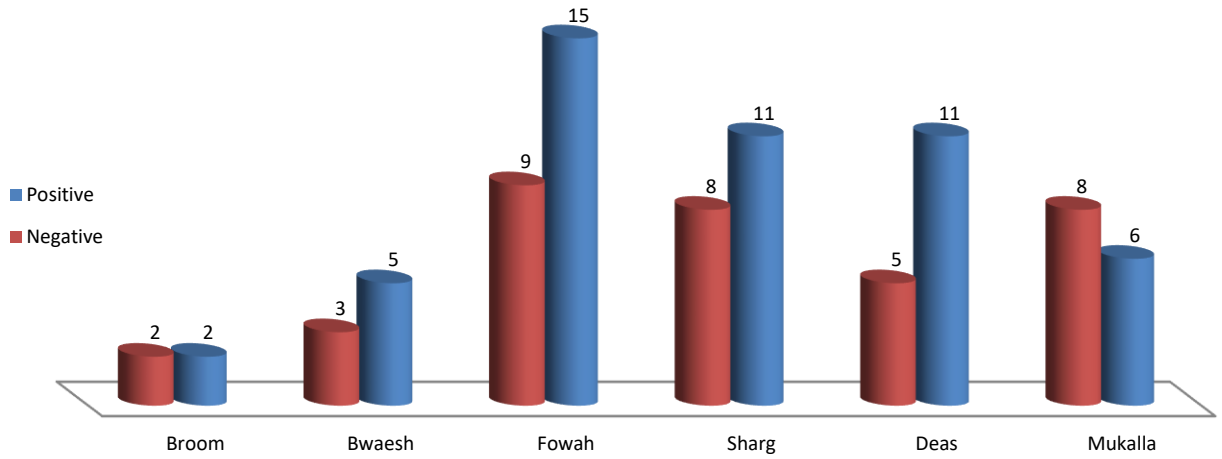

Figure 1. Sero-incidence of toxoplasmosis among Mukalla city women according to home address.

The highest seroprevalence of toxoplasmosis among Mukalla city women was recorded in the homes of 50 (58.82 %), and the highest seropositive rate (68.75 %) was recorded in Al-Deas zones in comparison with other areas of Al-Mukalla city, as shown in Table 1. There are statistically significant differences in the degree of prevalence based on home (P=0.017).

Table 2. Seropositive anti-toxoplasmosis IgG & IgM according to different diagnostic techniques.

Diagnostic technique	Sero-positive rate of immunoglobulin				
	No. of test	IgG	%	IgM	%
cassette	85	47	55.29	4	4.71
cobas e 411	85	50	58.82	1	1.18

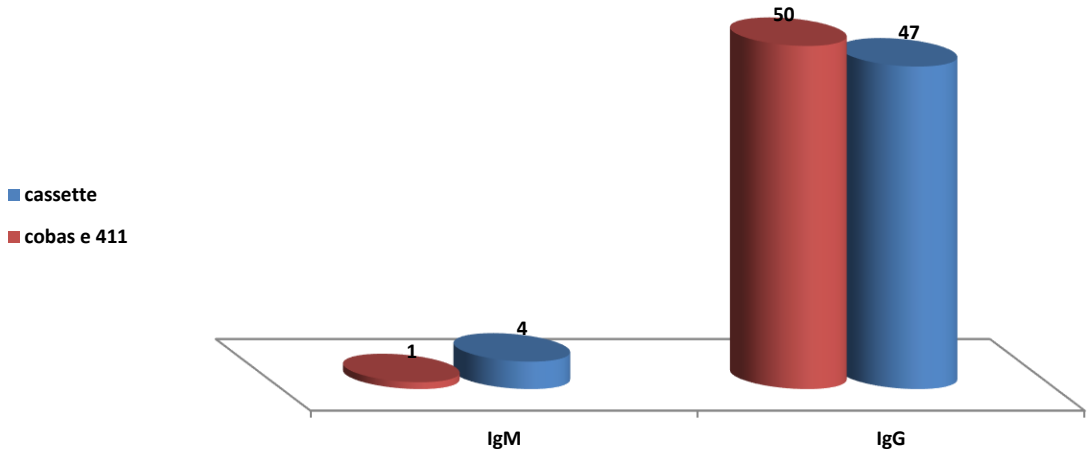

Figure 2. Seropositive anti-toxoplasmosis IgG & IgM according to different diagnostic techniques.

Seropositive anti-toxoplasmosis IgG & IgM according to different diagnostic techniques (cassette and Cobas e 411). The overall seropositive rate of IgG was higher than the seropositive rate of IgM, as shown in Table 2. Cassette test revealed the highest seropositivity of IgG antibodies, 47 (55.29%), whereas IgM antibodies were 4 (4.71 %). The seropositive rate of IgG and IgM antibodies detected by the cobas e 411 test was 50 (58.82%) and 1 (1.18 %), respectively. Not significant (P=0.169), there is no relationship between diagnostic technique and seropositive anti-toxoplasmosis.

Table 3. Comparison between different results of anti-toxoplasma (IgG & IgM) by cassette and Cobas e 411 tests.

Anti-toxoplasmosis	Different cases result	First sample results				Second sample results			
		Positive	%	Negative	%	Positive	%	Negative	%
IgG result by Cassette	4	1	25	3	75	3	75	1	25
IgG result by Cobas e 411	4	3	75	1	25	3	75	1	25
IgM result by Cassette	3	3	100	0	0	0	0	3	100
IgM result by Cobas e 411	3	0	0	3	100	0	0	3	100

The current study shows different results in seven cases between the cassette and the Cobas test. The highest seropositive rate of IgG in 4 cases (75%) was recorded in the first sample by the Cobas test, whereas 25% was recorded by the cassette test. In the second sample, after 2 weeks, a seropositive rate of IgG (75%) was recorded by the Cobas test, whereas 75% was recorded by the cassette test, as shown in Table 3. From these results, we conclude that Cobas e 411 is highly sensitive to detect Toxoplasma antibodies from the cassette.

A seropositive rate of IgM in 3 cases (0%) was recorded in the first sample by the Cobas test, whereas 100% was recorded as weakly positive by the cassette test. In the second sample, after 2 weeks, a seropositive rate of IgG (0 %) was recorded by the Cobas test, whereas 0% was recorded by the cassette test, as shown in Table 3. From these results, we conclude that all weak positive IgM results were false positives, as shown in Fig. 7, due to IgM antibodies disappearing in the second sample by two techniques.

Table 4. Distribution of toxoplasmosis according to age groups.

Age groups	Total cases examined	Positive	%	Negative	%
15 - 20	14	9	64.29	5	35.71
21 - 26	22	12	54.55	10	45.45
27 - 32	34	19	55.88	15	44.12
33 - 38	10	7	70.00	3	30.00
> 38	5	3	60.00	2	40.00
Total	85	50	58.82	35	41.18

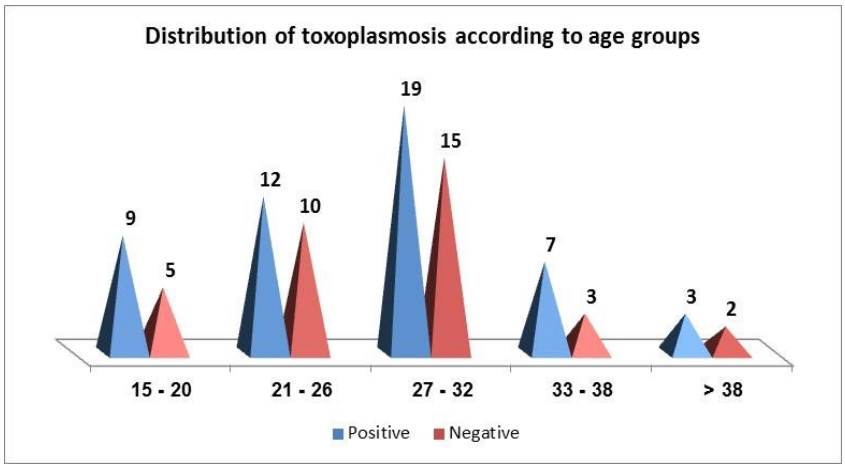

Figure 3. Distribution of toxoplasmosis according to age groups.

The distribution of toxoplasmosis according to age groups. The highest seropositive rate (70 %) was recorded in the age group 33-38 years in comparison with other age groups, as shown in Table 4. Only 64.29 % of females were under the age of 21 years (15-20 years). Also, 60% of the female samples tested were over 38 years of age. Toxoplasmosis in relation to age groups was also studied. There are statistically significant differences between the degree of prevalence based on age groups ($P = 0.006$).

Table 5. Number of abortions in relation to toxoplasmosis among women.

No. of abortions	No. of women examined	No. of women infected	Positive rate (%)
Once	13	7	53.85
Twice	4	2	50.00
Three times	5	2	40.00
Total	22	11	50.00

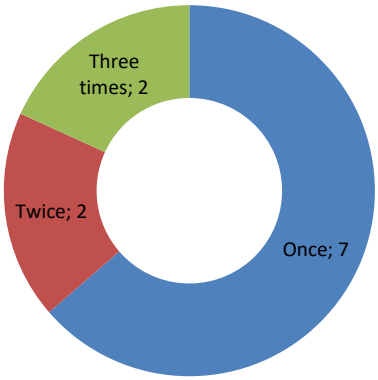

Figure 4. Number of abortions in relation to toxoplasmosis among women.

The study examined the relationship between the number of abortions and Toxoplasmosis among women. The results indicated that women with a history of a single previous abortion exhibited the highest seropositive rate for toxoplasmosis at 53.85%, compared to women with fewer prior abortions, who had a seropositive rate of 40%, as shown in Table 5. However, there was no statistically significant difference (P = 0.338) in the positive rates based on the number of abortions.

Table 6. Socio-Demographic Characteristics of Participating Women-Socioeconomic Level.

Socioeconomic level	Cases examined	Positive	%	Negative	%
Poor	4	3	75.00	1	25.00
Good	76	44	57.89	32	42.11
Very good	5	3	60.00	2	40.00
Total	85	50	58.82	35	41.18

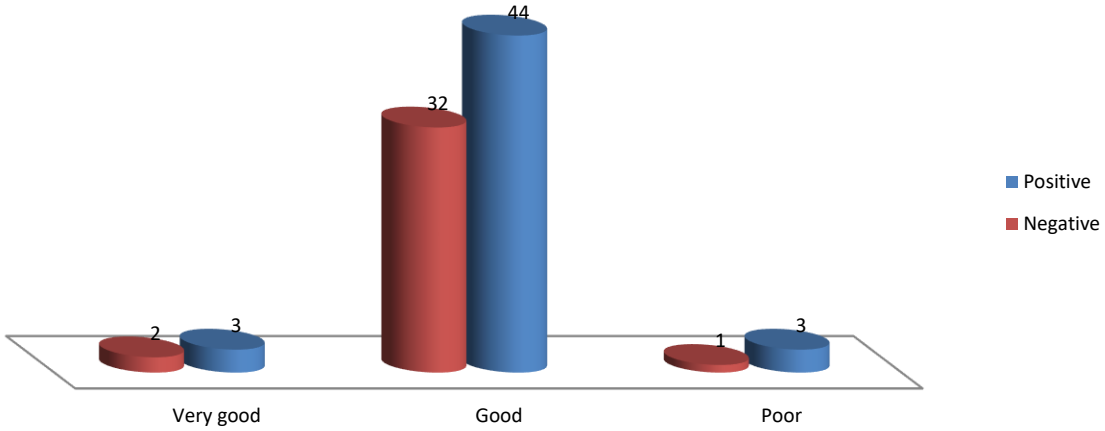

Figure 5. Socio-demographic characteristics of Socioeconomic levels.

The analysis of the questionnaire, as detailed in Table 6, based on the demographic characteristics of the participating women, showed that women living in poverty had the highest seropositive rate at 70%, compared to women from good and very good socioeconomic levels, who had rates of 57% and 60%, respectively. A statistically significant difference was observed in the prevalence rates across different socioeconomic levels ($P = 0.000$).

Table 7. Socio-demographic Characteristics of Participating Women-Ownership of Animals.

Ownership of animals	Cases examined	Positive	%	Negative	%
Sheep & goat	21	15	71.43	6	28.57
Cats	2	1	50.00	1	50.00
Total	23	16	69.57	7	30.43

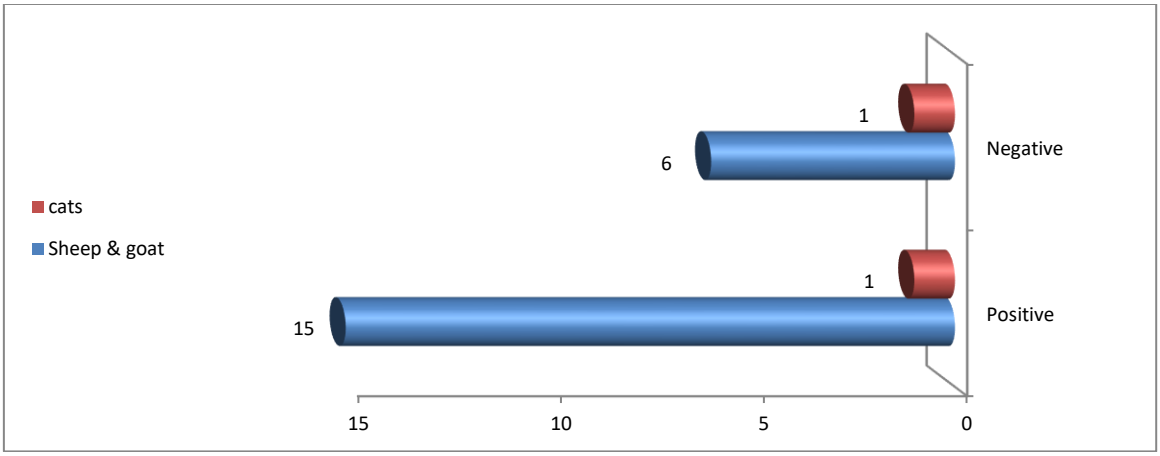

Figure 6. Socio-demographic characteristics of ownership of animals.

In the present study, the ownership of animals in relation to the infection was studied. The highest seropositive (71.43%) was recorded among women who had sheep and goats at home, followed by cats (50%). Results show a statistically significant correlation between toxoplasmosis and ownership of cats and other animals. There are statistically significant differences between the degree of prevalence based on the Ownership of animals ($P = 0.000$). In addition to that, the highest seropositive rate (66.67%) was recorded among women drinking raw milk.

Table 6. Socio-demographic Characteristics of Participating Women-Consumption of Raw Milk.

Drink raw milk	Cases examined	Positive	%	Negative	%
	3	2	66.67	1	33.33

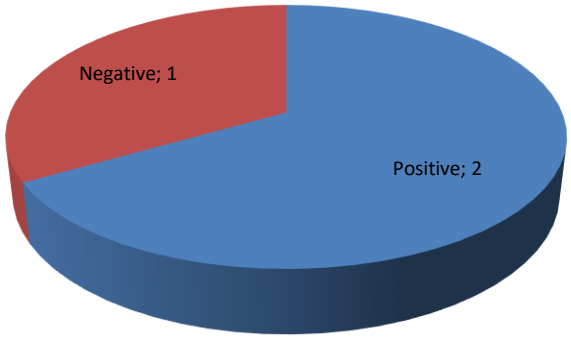

Figure 7. Socio-demographic characteristics of drinking raw milk.

CONCLUSIONS

Based on the findings of the study, it can be concluded that there is a high seroprevalence of *Toxoplasma gondii* among women in Mukalla City, which appears to be a significant factor contributing to pregnancy-related abortions. Accurate serological diagnosis, utilizing combined IgG and IgM testing, is essential for reliable detection of infection status. Furthermore, implementing effective *T. gondii* infection management strategies, including health education for women of reproductive age, is critical for preventing primary infections during pregnancy and reducing associated adverse outcomes. These measures are imperative for improving maternal and fetal health outcomes in the region.

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Competing Interests: The authors declare that there are no competing interests.

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