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Serum Toxoplasmosis Assessment of Libyan Sheep, Goats, and Chickens

[1] Sumaya Mustafa Allamoushi, [2] Mohamed Kalefa Mansur, [3] Najat Mohammed Mahmoud, [4] Maher Mohamed Abed El Aziz

[1, 2, 3] Department of Biology, Faculty of Education Qaser Bin Ghashir, University of Tripoli, Tripoli, Libya
[4] Department of Chemistry, Faculty of Education Qaser Bin Ghashir, University of Tripoli, Tripoli, Libya

Corresponding Author: **Maher Mohamed Abed El Aziz**

Abstract
One of the most widespread parasite zoonosis in the world, toxoplasmosis affects most warm-blooded animal species, including humans. *Toxoplasma gondii*, intracellular parasite with a single cell, is the cause of infection. Most animals and people serve as intermediate hosts, leaving cats as the only ultimate hosts. This disease has current medical and veterinary significance since it can lead to congenital disorders, abortion and neonatal death in both individuals and wild animals. Toxoplasmosis infection put the public's health at risk from food-borne epidemics and results in significant financial loss. Using the Latex Agglutination Test (LAT) protocol, the current study aimed to determine the extent of toxoplasmosis distribution in the blood of Libyan sheep, goats, and chickens. One hundred blood samples were collected randomly from sheep (40 samples), goats (20 samples), and chickens (40 samples). Blood samples were collected from six different areas including Tripoli city. Results show that the overall infection takes the order: chicken (85 %) > sheep (75 %) > goats (50 %). The high rate of positivity toxoplasmosis was observed among the older age group for the three investigated animal species. The data was treated statistically by applying four statistical parameters, namely, squared deviations, variance, standard deviation and abnormality analysis. High estimation of the prevalence of toxoplasmosis (90 %) in Libyan sheep was released after the anomaly value is excluded.

Keywords: Toxoplasmosis, Libyan Sheep, Goats, Chickens, Latex Agglutination Test

Introduction

Toxoplasmosis, a worldwide protozoan zoonosis, is caused by the obligatory intracellular protozoan parasite *Toxoplasma gondii*, which infects a wide range of hosts and causes significant morbidity and death [1, 2]. This parasite is thought to infect up to one-third of the world's human population [3]. All warm-blooded animals, including most cattle and people, are most likely intermediate hosts. Members of the Felidae family, such as household cats, are definitive hosts. *Toxoplasma gondii's* life cycle consists of asexual proliferation in various tissues of intermediate hosts and sexual reproduction in the intestines of definitive hosts [4]. Tachyzoites, bradyzoites contained in tissue cysts, and sporozoites contained in sporulated oocysts are the three infectious stages of Toxoplasma gondii's life cycle. For both intermediate and final hosts, all three phases are contagious. By transmitting tissue cysts between intermediate hosts (even in the absence of definitive hosts) and also by transmitting oocysts between definitive hosts, its life cycle may continue indefinitely [5]. According to Zewdu, E. G. *et al*, 2013 [6], *Toxoplasma gondii* infection during pregnancy can cause chorioretinitis, hydrocephalus, anomalies of the central nervous system, or even fetal or neonatal mortality. Sheep who have had toxoplasmic abortions often do not have repeated toxoplasmic abortions, making them suitable for breeding purposes in the future. To avoid infecting felids and other agricultural animals, fetal membranes and deceased fetuses should not be handled with bare hands and should instead be buried or burned. Cats shouldn't be permitted near lambs and goats that are pregnant. To avoid *Toxoplasma gondii* cyst infection, grain should be kept covered. It is important to inform the public, in particular cat handlers, women, and butchers, about the risks associated with consuming raw or undercooked meat as well as the sources of infection, means of disease transmission, and nature of the illness [1, 2]. The first study on *Toxoplasma gondii* in sheep in Libya is from Tripoli [7], which used the Latex agglutination test to report seroprevalence rates of 40.71% in adult sheep in Tripoli, Libya. A comprehensive survey on *toxoplasma gondii* infection and toxoplasmosis in North Africa was published in 2019 [8]. Compared to some African countries, a few research and review articles were published on toxoplasmosis, especially for Libyan cattle and farm animals [9]. Our present work aimed to evaluate the prevalence of toxoplasmosis disease in Libyan sheep, goats, and chickens using the Latex Agglutination Test (LAT) protocol.

Material and Methods

The current study was conducted on three types of Libyan farm animals, namely: sheep, goats, and chickens of different age groups and of both genders. Samples were collected within the end of March 2020 to the beginning of May 2020.

Blood Samples

One hundred blood samples were collected randomly from sheep (40 samples), goats (20 samples), and chickens (40 samples). Sheep blood samples were collected from Tripoli city, South of Tripoli, Tarhuna city, and Zletten city while goats and chicken blood samples were collected only from the south of Tripoli region including Qasr Bin Ghashir, Al-Sawani, Al-Azizia, and Airport Road. Chicken samples were collected from slaughterhouses of different villages in the south of Tripoli region. Investigated blood samples were drawn from the jugular vein of the animal using sterile blood drawing needles with 2-5 ml of each sample. All blood samples were placed in test tubes that did not contain anticoagulant like EDTA and left to coagulate for half an hour. After that, the samples were kept in the refrigerator for two hours, and then transferred to the laboratory for the purpose of separation and obtaining blood serum, using a fast centrifuge (3000 cycles/min for 5 minutes), and after the separation process, the sera were withdrawn with a sterile micropipette for each sample, and the sample number was recorded on it, and then kept at (-20 ºC) until the tests were performed. The serum samples were investigated using an examination of Latex Agglutination Test for toxoplasmosis for detection of anti-toxoplasma antibodies.

Toxoplasmosis Latex Test Kit

Latex Agglutination Test (LAT) kit contains three different reagents:

1. Reagent suspension, which is an alkaline solution of PH=8 of polyester latex particles covered with toxoplasma gonadotropin antigen.
2. Positive control solution, which is a human serum with IgG antibodies prepared from rabbits added to it.
3. Negative control solution, which is a diluted human serum without the addition of antibodies.

The kit also contains transparent plastic chopsticks for the purpose of mixing the ingredients well, as well as light plastic strips with a black background, as shown in Fig 1.

Fig 1: Latex Agglutination Test (LAT) kit

Latex Agglutination Test (LAT) Procedure

The refrigerated test kit and frozen serums were taken out and left until the laboratory temperature (25 °C) was reached. Drops of the positive control solution were placed on one of the circles of the plastic test slide as a standard solution indicating the presence of the parasite (shows stickiness or clotting). Also, drops of the negative control solution were placed on another circle of the plastic test slide as a standard solution indicating the absence of parasite infection (no stickiness or clotting). Accurate 50 µL of serum were placed by means of a micropipette on the circles of the plastic test slide for the test, and one drop of the reagent was placed on it, after shaking it well in order to homogenize its components. The two drops were mixed well with the plastic sticks of the kit, and they were moved in a slowly circular motion with the plastic sticks using the hand, and placed in a vibrator for 5 minutes, and the results were read after the end of the period, and so on with all samples, and the results were recorded, as indicated by the samples that showed clear adhesion to the eye with the latex granules indicating that it is positive for the test, while the samples that did not have adhesion indicated that it is negative for the test. Infected blood samples were expressed as percentage (%) where,

$$\%\ Infected\ samples = \frac{number\ of\ positive\ samples}{total\ number\ of\ samples} \times 100 \quad (1)$$

$$(P, \%) = \frac{n}{N} \times 100 \quad (2)$$

Results and Discussion

The level of spreading of toxoplasmosis in Libyan sheep, goats, and chickens was evaluated according to the gender of the animal, age, and the homeland of the examined animals.

Prevalence of Toxoplasmosis in Libyan Animals

Percentage of infection of toxoplasmosis in Libyan sheep, goat, and chicken was calculated and represented in Fig 2, from which it was found that the total infection of animals can be arranged in the following order:

Chicken (85 %) > Sheep (75 %) > Goats (50 %)

The above results prove that chicken are more susceptible for the infection of toxoplasmosis while goats are less susceptible for the same disease, this may be attributed to the fact that the natural immune system of goats are more developed than that of chicken [10]. It was noticed that female of goats are less infected animals by 41.7 %, and the high percentage of infection by toxoplasmosis was recorded for female chicken by about ~ 89 %. Percentage ratio of female toxoplasmosis infection for chicken to sheep to goats is (2.13: 1.95: 1) respectively. As shown in Fig 2, male of sheep and chicken are less infected than female, but male of goat is more infected than female. Increasing of toxoplasmosis infection in female may be due to the weakness of female during pregnancy and breeding period [11] and hence high immune deficiency. Unfortunately, all investigated Libyan animals show a positive agglutination

test towards toxoplasmosis with different extent starting from the lower value of 41.7 % for female goat to higher value of 88.9 % female chickens.

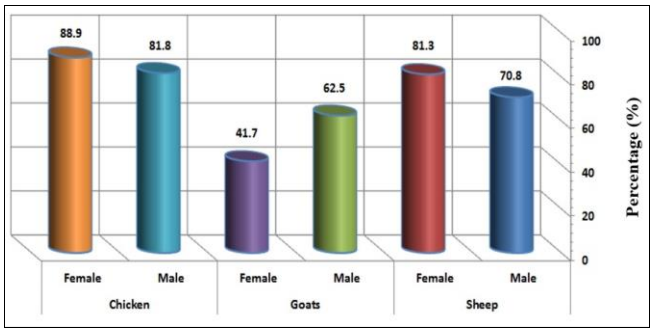

Fig 2: Prevalence of toxoplasmosis in Libyan sheep, goat, and chicken according to gender

According to EL-Gomatiet *et al*., 2008 [7], in Libya there is only one study was done concerning with the prevalence of toxoplasmosis in sheep, these study was done in Tripoli region and the results show that the percentage of infection was 40.7%. Another published work in 2013 [12] showed that the overall seroprevalence of antibody of Toxoplasma is 71%. The above two results prove that the toxoplasmosis disease was replicated by 1.74 times along five years. The present study shows that the overall prevalence of toxoplasmosis is 70 % in the Libyan animals. This result may reflect a type of balance or stability of toxoplasmosis disease along the last ten years, and this may be attributed to the increase of general awareness of parasites' disease.

Effect of Age of Animal on Prevalence of Toxoplasmosis

The effect of age of the animal on prevalence of toxoplasmosis was studied from below 6 months to above 3 years for both sheep and goats and from 30 to 60 days for chickens as shown in Fig 3, from which it was noticed that 100 % infection by toxoplasmosis was obtained with the age of animal (sheep and goats) above 3 years. This severe infection may be reflects the weakness of veterinary serveries in Libya especially with this range of age. In contrast, zero percent of infection was recorded with goats of age below 6 months. Results emphasizes that the high rate of positivity toxoplasmosis was observed among the older age group for the three investigated animals. This result is in agreement with the fact reported in previous literature for human [13]. In general, still goat under one year is less susceptible for the infection of toxoplasmosis.

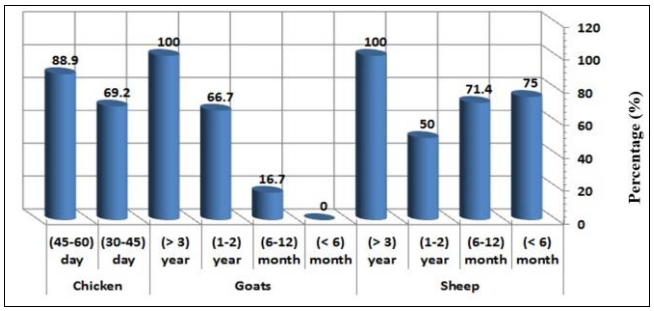

Fig 3: Prevalence of toxoplasmosis in Libyan sheep, goat, and chicken within different groups of ages

Effect of Homeland of the Animal

The effect of homeland of the animal on the prevalence of toxoplasmosis disease was studies for different four local regions. The obtained results were represented in Table (1), from which it was found that the percentage of infected sheep increased according to the following order:

South of Tripoli (60 %) < Tarhuna (83 %) ≈ Zletten (83 %) < Tripoli city (100 %)

The above-mentioned order is expected due to the fact that south of Tripoli region including Qasr Bin Ghashir, Al-Sawani, Al-Azizia, and Airport Road are considered of pure rural region with high availability of veterinary services centers, and therefore it was expected to save domestic animals from toxoplasmosis regularly. On contrast, Tripoli city are modern non-rural region with few of veterinary services centers, so domestic animals like sheep, goats, or chickens can easily infected by toxoplasmosis disease. Despite of the number of investigated samples from south of Tripoli region are greater than that of Tripoli city by 9 times, it was concluded that the same trend was observed for chickens, where the percentage of infected animals originated from south of Tripoli region are less that that originated from Tripoli city itself. Goats show a moderated percentage of prevalence of toxoplasmosis disease with 50 % infection.

Table 1: Prevalence of toxoplasmosis in different regions

Region	Sheep			Goats			Chicken		
	Samples	Infected	%	Samples	Infected	%	Samples	Infected	%
Tripoli city	8	8	100	Nil	Nil	Nil	4	4	100
South of Tripoli	20	12	60.0	20	10	50	36	30	83.3
Tarhuna	6	5	83.3	Nil	Nil	Nil	Nil	Nil	Nil
Zletten	6	5	83.3	Nil	Nil	Nil	Nil	Nil	Nil
Total	40	30	75.0	20	10	50	40	34	85

Statistical Analysis

Using statistical analysis aims to investigate several data behaviors such as trends, patterns, and relationships by well-operated quantitative data analysis. After collecting the data from the samples, it must be organized and summarized in many tables and figures using what we call descriptive statistics. After that, inferential statistics can be used to examine the hypotheses theoretically or mathematically to evaluate the investigated case. Therefore, using statistical analysis can lead to an interpretation and generalization of research findings that can easily draw a whole well-invented picture [14, 15]. The standard deviation is derived from variance and tells us, on average, how far each value lies from the mean. Standard deviation is the square root of variance. Standard deviation are mostly having so many benefits, among of them is the estimation of the quality of measurements, the tendency towards reality, and estimation of the undesired experimental errors. Mathematically,

variance and standard deviation are calculated using the following formulas [16, 17]:

$$Variance = \sigma^2 = \frac{\sum_{n=1}^{n=\infty}(X-\mu)^2}{N-1} \quad (3)$$

$$Standard\ Deviation = SD = \sqrt{\sigma^2} = \sqrt{\frac{\sum_{n=1}^{n=\infty}(X-\mu)^2}{N-1}} \quad (4)$$

Where:

X: Is the value of the data distribution.

μ: Is the average value of population sample.

N: The total number of observations or the size of samples.

Statistical analysis of the prevalence of toxoplasmosis disease in Libyan sheep was carried out applying the four statistical parameters, namely, squared deviations, variance, standard deviation and abnormality data analysis. Variance and standard deviation were calculated using sample estimation model forms whereas the abnormality data analysis was carried out using the plot diagram between squared deviations with the homeland of the investigated animal. Both standard deviation and standard error are treated as a single parameter of the same significance. Squared deviations (X-μ)^2 and variance have the same units of square power (X2) while standard deviation and standard error have the same units of unity power (X1) as in variables. Table (2) shows the final results of mathematical calculations of squared deviations, variance, and standard deviation/error for the prevalence of toxoplasmosis in Libyan sheep. Fig 4 represent the abnormality behavior of the same results. From Table (2), it was found that the overall values of variance and standard deviation are 0.284 and 0.533 respectively. Numerically, it was noticed that the studied statistical parameters (SD and σ^2) are very low values (closed to zero) for all variables (Xn: X1, X2, X3……); especially the value of Tripoli city with 100 % infection ⟦(σ⟧^2= 0.009, SD = 0.09) and this behavior is due to the fact that no errors or deviation with full maximum infection. Addition of standard deviation values (SD) to each variable (X) gives the validity of measured ranges as (X±SD), from which the minimum and the maximum value of each variable can be predicted as (Xmin & Xmax). Results show that the broadened rang was obtained with the animals originated from south of Tripoli region. So, it was concluded that these results seems to be significant only with very narrow range and all values out of this narrow range are considered insignificant. The calculated values of (Xmin & Xmax.) for south of Tripoli region are equal to (59.16 & 60.84 %) and the ratio of Xmin to Xmax equal to ~ (1.0: 1.03), so the acceptable prevalence values of toxoplasmosis in this studied area should be within this range. The prediction of the abnormality of the obtained results can be estimated using the squared deviation values. The relationship between the sample originality and the squared deviations was tested to show the abnormality of the obtained data and hence it gives us a relative measure of the total variation, especially abnormality values for specific sample. Fig 4 shows that the results of south of Tripoli region is considered as abnormal value, so it can be easily excluded to avoid the abnormal deviation in the final recorded results. Recalculation of prevalence of toxoplasma in Libyan sheep after exclusion of abnormality value leads to a high estimated prevalence (90 %).

Table 2: Squared deviations, variance and standard deviation values for the prevalence of toxoplasmosis in Libyan sheep

Region	Libyan Sheep						
	Prevalence			Statistical Parameters			
	Samples	Infected	%	$(X-\mu)^2$	σ^2	$SD = \sqrt{\sigma^2}$	[(X)±SD]
Tripoli city	8	8	100	0.25	0.009	0.09	100±0.09
South of Tripoli	20	12	60.0	20.25	0.698	0.84	60±0.84
Tarhuna	6	5	83.3	6.25	0.216	0.46	83.3±0.46
Zletten	6	5	83.3	6.25	0.216	0.46	83.3±0.46
Total	40	30	75.0	33	0.284	0.533	75±0.53

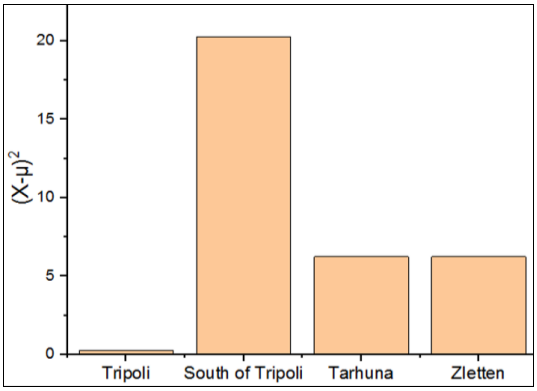

Fig 4: The abnormality behavior of South of Tripoli region

Conclusion

Toxoplasmosis infection put the public's health at risk from food-borne epidemics and results in significant financial tolerance and therefore more attention should be paid to this widespread disease. In Libya, there is noticed shortage of published research articles on toxoplasmosis infection, especially on farm animals. Our present study was done evaluating the prevalence of toxoplasmosis in one hundred samples of sheep, goats, and chickens. Results emphasized that the total infection is 85, 75, and 50 % for chickens, sheep, and goats respectively. Old animals are found to be more susceptible to the infection than the young animals. Statistical analysis shows that theoretical estimation of toxoplasmosis infection is more than the recorded values by 15 %.

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