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Seroprevalence and risk factors of *Toxoplasma gondii* in sheep and goats in Benghazi city-East of Libya.

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Highlights

- **The study investigates the infection of *Toxoplasma gondii* in both goats and sheep around the city of Benghazi during the period from March to November 2019.**
- **Based on the *Toxoplasma gondii* antibodies (IgG) kites, 260 samples were found positive for this parasite.**

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ABSTRACT

In this study, serum samples from four hundred and seventy (470), comprising 234 local sheep (Ovis aries) and 236 goats (Capra aegagrus hircus) of different ages and gender were collected and analysed for *Toxoplasma gondii* antibodies (IgG) using the latex agglutination test (LAT) kits according to manufacturer's instructions. The result showed that of the 470 serum samples of tested animals (sheep and goats) analysed, 260 were positive for *Toxoplasma gondii* antibodies (IgG) given an overall prevalence of 55.3%. A significant difference in seroprevalence of *T. gondii* antibodies was observed between the two species of tested animals with a higher prevalence in sheep (P=0.02). No significant differences in the overall seroprevalence of *T. gondii* antibodies between males (61.5%) and females (45.5%) of both species (P=0.339). The overall seroprevalence of *T. gondii* was higher in tested animals of less than 2 years of age, though the difference was not statistically significant (p=0.225). According to the breeding site, Almagzha region showed significant (P=0.00) high seroprevalence (77.4%) compared with other sites. The highest *T. gondii* seroprevalence was recorded in the spring season. This study showed that local sheep and goats from Benghazi and the near by areas may represent a significant source of *T. gondii* infection in the human population through consumption of undercooked meat.

1. Introduction

Toxoplasma gondii is a zoonotic unicellular protozoon infecting a large range of warm-blooded animals (mammals and birds). It can cause spontaneous abortion and neonatal mortality in humans and animals. The only definitive hosts are members of the cats' family Felidae, including domestic cats (Levine, 1985; Dubey, 2016) and almost all domestic mammals including sheep, goats, cattle, humans, and birds serve as intermediate hosts. The main source of infection is primarily through the ingestion of meat containing tissue cysts (pradazoites) or milk and water contaminated with sporulated oocysts (Tenter, Heckeroth and Weiss, 2000) In Libya and most Arab and African countries, sheep, goats, cattle, chickens and camels represent the most consumed animal species (Tonouhewa *et al*., 2017), therefore these livestock animals play an important role in the epidemiology of the disease caused by *T.gondii* or Toxoplasmosis (Dubey, 2010; Hill and Dubey, 2013; Rouatbi *et al*., 2019). Toxoplasmosis is responsible for significant economic losses to the global sheep, goat, and cattle industry through abortions or debilitated offspring (Dubey and Beattie, 1988; Buxton *et al*., 1991; Kamani *et al*., 2010). The severity of the disease in livestock animals is associated with the stage of pregnancy. Infection during the early stage of gestation can result in fatal death, mummification, abortion, and stillbirth, while infection in the later stage of gestation may have no clinical effect, and lambs are usually born normal but infected and immune (Dubey and Beattie, 1988; Dubey, 2016; Buxton *et al*., 2017). Many studies have assessed the seroprevalence of *T.gondii* in sheep and goats in different parts of the world using different serological techniques (Dubey, 2009; Tonouhewa *et al*., 2017). In Libya, only a few documented studies on seroprevalence of *T. gondii* in animals (Azwai, El-Gammoudi and Gameel, 1993; EL-Gomati *et al*., 2008; Al-mabruk *et al*., 2013). From three locations in Tripoli, namely (El-Njala, El-Hani and El-Khadra). Azwai, El-Gammoudi and Gameel, 1993 reported seroprevalence rates of 50% in goats, 26.17% in sheep, 11.15% in cattle and 4.79% in horses. On the other hand, EL-Gomati *et al*., (2008) reported seroprevalence rates of 40.71 % in adult sheep using the Latex agglutination test, while Al-mabruk *et al*., (2013) studied the seroprevalence of *T. gondii* antibodies in sheep by using the same test (LAT) in different geographical areas in Libya (western, central, eastern, and southern area), where they showed the overall of seroprevalence of *T. gondii* antibodies which was 71%. The present study was carried out to determine the seroprevalence of *T. gondii* in sheep and goat which raised in the city of Benghazi and its around areas.

2. Materials and Methods

2.1 Study Area

This study was conducted in Benghazi and near by areas, which include small cities and villages. The city of Benghazi is situated in the Northeast of Libya, (32°10N & 20°06E). It is the second most populous in the country and the largest city in Cyrenaica with an estimated population of 631,555 in 2017, with an area of about 314 km^2. Benghazi has a Mediterranean climate of moderate humid

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winters and dry summer. The temperature occasionally comes down to less than 5°C at night during winter (November to February) and rises to 40°C during the daytime in summer (May to August). The wettest month is January with the highest rainfall 66 mm and the driest month is July with the lowest rainfall zero mm. The samples were collected from four locations (villages) in and around the city of Benghazi, namely, Salug 45 Km Jardinah 33Km, Alnawagia 20 Km, Almakzaha 30 Km, Boatnni 6Km and Tokrah 75 Km from the city of Benghazi, during the period from March to November 2019.

2.2 Experimental animals

A total of 470 (234 sheep & 236 goats) local sheep (Ovis aries) and goats (Capra aegagrus hircus) of different ages and gender were randomly selected from six locations.

2.3 Blood Samples Collection

Free-roaming sheep and goats were bled with the owner's consent. Approximately 5-10ml of blood was drawn from the jugular vein using plain vacutainer tubes and kept overnight at room temperature to clot for serum preparation. Aliquots of sera were obtained by centrifugation at 3000 revolutions per minute for 10min and the sera were transported to the Zoology Laboratory of the Faculty of Science in the icebox and stored at −20°C until they were tested. The owners of the sheep and goats whose herds and flocks were involved in the study were orally accepted and interviewed using a questionnaire. The questionnaire included the following information, site, date of collection, sex, age, species of the animal, physiological status, and husbandry.

2.4 Serology

All collected animal serum samples were tested for IgG antibodies against *T. gondii* using a commercial latex agglutination test, Toxoplasmosis Latex Test Kit (Toxo Latex Kit from Atlas Medical, William James House, Cowley Rd, Cambridge, CB4 0WX, UK). Atlas Toxo latex kit is an agglutination test to detect specific antibodies in serum. The testing protocol and interpretation of results were performed according to the manufacturer's instructions.

2.5 Data analysis

The generated data were stored in Microsoft Excel 2013 Spreadsheet and were subjected to descriptive statistics using statistical package for social sciences (SPSS) version 23.

3. Results

Four hundred and seventy (470) serum samples comprising 234 sheep and 236 goats were collected and analysed for *Toxoplasma gondii* antibodies (IgG) using the latex agglutination test (LAT) kits according to the manufacturer's instructions. The result showed that of the 470 samples analysed 260 were positive for *Toxoplasma gondii* antibodies (IgG) given an overall prevalence of 55.3% (Table 1). A significant difference in seroprevalence of *T. gondii* between the two species of tested animals was observed, with a higher prevalence (60.7%) in sheep (P=0.02). The result as in Table 2 showed that although the overall prevalence of *T. gondii* in males (61.5%) was higher than those in females (54.5%), but statistically was not significant (P=0.339). The same results of no significant differences in the prevalence of *T. gondii* between genders of both sheep and goats when compared separately, (P=0.344) and (P=0.678) respectively.

According to the classified groups of ages, the results revealed as shown in (Table 3) that, the overall prevalence of *T. gondii* was higher in tested animals' group of fewer than 2 years of age (59%) and lower in the age group over than five years though the difference was not statistically significant between the three groups (p=0.225).

The overall seroprevalence of *T. gondii* in tested animals according to rearing (breeding) areas is shown in Table 4. Significant differences were found between the five locations, in which the highest infection rates were found in Almagzha (77.41%) followed by AbouAtni (66.66%), Jerdina (63.1%).

The prevalence of *T. gondii* antibodies in tested animals were varied with the seasons, the highest prevalence was found in spring (67.8%) followed by autumn (53.6%), and then summer (Table 5). Statistically, there is a significant relationship between the prevalence and three seasons as the probability value is equal to (0.00) which is less than 0.05.

Table 1

The overall prevalence of *T. gondii* antibodies in sheep and goats.

Animal species	No. of examined	No. of infected	Prevalence %	P-value
Sheep	234	142	60.7	
Goats	236	118	50.0	p_value = 0.02
Total	470	260	55.3	
			$Chi_Square = 5.426,$	$d.f = 1$

Table 2

The overall prevalence of *T. gondii* in tested animals based on sex.

Gender	No. of examined	No. of infected	Prevalence %	P-value
Male	52	32	61.5	
Female	418	228	54.5	$p_value = 0.339$
Total	470	260	$Chi_Square = 0.915$	$d.f = 1$

Table 3

The overall prevalence of *T. gondii* antibodies in tested animals based on age groups.

Age	No. of examined	No. of infected	Prevalence %	
<2	232	137	59.1	
2-5	213	111	52.1	
>5	25	12	48.0	$p_value = 0.255$
total	470	260	55.3	

$Chi_Square = 2.736, \quad d.f = 2$

Table 4

Prevalence of *T. gondii* antibodies in tested animals based on location

Location	No. of examined	No. of infected	Prevalence %
Abou Atni	30	20	66.7
Almagzha	62	48	77.4
Salough	137	66	48.2
Tokrah	100	37	37.0
Jerdina	141	89	63.1
Total	470	260	55.3

$Chi_Square = 33.69, \quad d.f = 4, \quad p_value = 0.00$

Table 5

Prevalence of *T. gondii* antibodies in tested animals based on seasons.

Season		Toxoplasma	
	No. of examined	No. of infected	Prevalence %
The spring	174	118	67.8
The summer	100	37	37.0
The autumn	196	105	53.6
Total	470	260	55.3
	$\boldsymbol{Chi_{Square} = 24.814, \quad d.f = 2, \quad p_value = 0.00}$		

4. Discussion

The result of this study showed that 470 serum samples from sheep and goats were analysed by latex agglutination test (LAT), and 260 were positive for *T. gondii* antibodies given an overall prevalence of 55.3%. This result showed clearly the existence of *T. gondii* infection in sheep and goats in Benghazi and the nearby areas and they are more at risk to *T. gondii* infection hence the meat from such animals could be a potential risk to public health if consumed raw or undercooked. The latex agglutination test (LAT) is easy to perform, sensitive and has been used for the measurement of antibodies to *T. gondii* in serum of sheep, goats, pigs, camels, donkeys, humans, and cattle (Al-Ramahi, Hamza and Abdulla, 2010; Younis *et al*., 2015; Fereig *et al*., 2016; Atail *et al*., 2017; Ishaku *et al*., 2018; Etter, Neves and Tagwireyi, 2019; Julie, Paul and Antoine, 2019). The high overall seroprevalence (55.3%) recorded in this study is consistent with results reported by many authors from different parts of the world (Hove, Mukaratirwa and Lind, 2005; Atail *et al*., 2017; Etter, Neves and Tagwireyi, 2019). These authors recorded an overall seroprevalence of 67.0%, 52.0%, and 83.3% in small ruminants from Zimbabwe, Soudan, and South Africa respectively. In the meantime, lower prevalence rates than the result of this study were recorded from many countries including, 17.7% from Ethiopia (Gebremedhin *et al*., 2014), 26.7% from Egypt (Fereig *et al*., 2016), 35.1% from Jordan, 31.0% from Turkey (Oncel and Vural, 2006), 37.0% from Tunisia (Lahmar et al., 2015), 28.3 % from Gabon (Julie, Paul and Antoine, 2019) and 35.0% from Nigeria (Ishaku et al., 2018). The differences of seroprevalence in these studies could be related to the risk factors which include age, sex, breed, climatic variation, diagnosis test (sensitivity and specificity), geographical location, and samples size of population studies (Skjerve *et al*., 1998; Tenter, Heckeroth and Weiss, 2000; Gebremedhin *et al*., 2014; Lahmar *et al*., 2015; Dubey, 2016; Tonouhewa *et al*., 2017; Tilahun *et al*., 2018; Alghanaei and Abdulsalam, 2019). The statistically significant difference in *T. gondii* seroprevalence based on animal species (goats and sheep) with higher prevalence in sheep (P=0.02) observed in this study is in agreement with many results previously reported (Azwai, El-Gammoudi and Gameel, 1993; Negash *et al*., 2004; Gebremedhin *et al*., 2014; Lahmar *et al*., 2015; Fereig *et al*., 2016; Tonouhewa *et al*., 2017; Tilahun *et al*., 2018; Abdallah *et al*., 2019; Etter, Neves and Tagwireyi, 2019; Julie, Paul and Antoine, 2019). Meanwhile, other researchers reported seroprevalence of *T. gondii* in goats higher than in sheep (Al-Ramahi, Hamza and Abdulla, 2010; Al-Kappany *et al*., 2018; Tonouhewa *et al*., 2019). The difference between seroprevalences of *T. godii* in sheep and goats could be attributed to the susceptibility of species, feeding behavior, and samples size. The overall prevalence of *T. gondii* antibodies in males of tested animals (61.5%) was higher than those in females (54.5%), but statistically was not significant (P=0.02). A similar result was reported by Yin *et al*. (2015) in China, Tonouhewa *et al*. (2019) in Benin, Gebremedhin *et al*. (2014) in Ethiopia, Atail *et al*. (2017) in Sudan, Subedi *et al*. (2018) in Napal, Younis *et al*. (2015), Ibrahim *et al*. (2017), in Egypt and Oncel and Vural (2006) in Turkey. This observation could be related to the fact that both males and females shared the same breeding place, food, water and have the same opportunity of contact with oocysts shed by cats in the environment (Lahmar *et al*., 2015; Tonouhewa *et al*., 2017). Ishaku *et al*. (2018), reported overall *T. gondii* seroprevalence in males (sheep and goats) has a higher prevalence than in females. These authors have referred to the reason behind the difference to the fact that female animals usually are kept confined for breeding purposes so they have little contact with oocysts. On the other hand, other workers recorded significant higher seroprevalence in females than in males (Lahmar *et al*., 2015; Tilahun *et al*., 2018), the females being subjected to more stress (pregnancy & lactation) might make them less resistant to toxoplasmosis (Atail *et al*., 2017).

According to age groups, the results revealed that the overall seroprevalence of *T. gondii* was higher in tested animals of less than 2 years of age when compared with other groups though the difference was not statistically significant. The same conclusion of no significant difference in age groups was reported by many authors (Yin *et al*., 2015; Atail *et al*., 2017; Subedi *et al*., 2018; Tonouhewa *et al*., 2019). Other researchers found a positive relationship between age and prevalence of *T. gondii* antibodies (Chikweto *et al*., 2011; Gebremedhin *et al*., 2014; Al-Bajalan, Al-Nasiri and Mahmood, 2015; Younis *et al*., 2015; Ibrahim *et al*.,

2017; AL-Hatami, AL-Kardhi and May 2018). The variation in results could be due to the difference in selected age groups used in tested animals and the sample size of each study. The statistically significant relationship between the location and incidence of the *T. gondii* seroprevalence was observed in this study. The highest prevalence was seen in Almagzha (77.4%) followed by Abou Atni (66.7%). This variation could be related to geographical and environmental factors along with possible differences in breeding systems and the density of stray cats. A higher seroprevalence is associated with humid areas which are favorable for sporulation of oocysts that voided in cat feces. Moreover, oocysts are very resistant to environmental conditions and can remain infectious in moist or sand soil for up to 18 months (Tenter, Heckeroth and Weiss, 2000). The present study revealed a higher seroprevalence of *T. gondii* in the spring season than in both autumn and summer seasons. This could be related to transmission rates between seasons, in that more infectious take place in the wet months where the climate is more suitable for the survival of oocyst (Gebremedhin *et al*., 2014), and then the seroprevalence will be high in the dry season. Oocysts are distributed in the environment through wind, rain, and surface water or harvested feeds, hay, straw, and grain that had been contaminated with cat feces (Tenter, Heckeroth and Weiss, 2000). These factors could control the variation in *T. gondii* seroprevalences in different locations and seasons. The result showed a high seroprevalence of *T. gondii* antibodies in sheep, this result is consistent with results recorded by (Azwai, El-Gammoudi and Gameel, 1993) and (Al-mabruk *et al*., 2013) in Libya, (Julie, Paul and Antoine, 2019) in Gabon, and (Etter, Neves and Tagwireyi, 2019) in South Africa. Moreover, lower seroprevalences of *T.gondii* in sheep than in the present result were also recorded in different parts of the world which include, 40.7% in Libya (EL-Gomati *et al*., 2008), 33.7% in Ethiopia (Tilahun *et al*., 2018), 35.9 % in Ghana (Bentum *et al*., 2019), 31.0% in Turkey (Oncel and Vural, 2006) 51.8%, in Egypt (Ibrahim *et al*., 2017), 45.7% in Sudan, Atail *et al*. (2017), 36.2% in Iraq (Al-Ramahi, Hamza and Abdulla, 2010), 28.0% in Nigeria *(Ishaku et al*., 2018), 25.6% in Algeria (Abdallah *et al*., 2019), 1.4% in Benin (Tonouhewa *et al*., 2019) and 40.2% in Tunisia (Lahmar *et al*., 2015). The difference in seroprevalences could be related to many factors such as sample size, test techniques, geographical and environmental factors, management system, location, age, sex, and breed (Tonouhewa *et al*., 2019).

According to gender, the result showed that males have higher *T. gondii* seroprevalence antibodies than female's sheep but statistically not significant, this result in agreement with many reports (Gebremedhin *et al*., 2014; Yin *et al*., 2015; Atail *et al*., 2017; Ibrahim *et al*., 2017; Subedi *et al*., 2018; Abdallah *et al*., 2019; Bentum *et al*., 2019; Tonouhewa *et al*., 2019). As mentioned earlier the reason behind the higher prevalence in males than females are because female are usually kept confined for breeding purposes or because the females were subjected to more stress during pregnancy and lactation period (Atail *et al*., 2017). On other hand, Tilahun *et al.* (2018) and Younis *et al*., (2015) found a significantly higher seroprevalence of *T. gondii* antibodies in females' sheep when compared with males. Consideration of seroprevalence *T. gondii* antibodies in sheep with different ages, the result showed that young sheep of fewer than one year have a higher prevalence than the other two age groups, although there were no significant differences. This result is compatible with results recorded by previous studies (Yin *et al*., 2015; Subedi *et al*., 2018; Tonouhewa *et al*., 2019). However, the present result is inconsistent with reports from many other authors (Dumètre *et al*., 2006; Oncel and Vural, 2006; Chikweto *et al*., 2011; Gebremedhin *et al*., 2014; Lahmar *et al*., 2015; Tilahun *et al*., 2018; Abdallah *et al*., 2019; Etter, Neves and Tagwireyi, 2019) as these authors found that old sheep have a higher prevalence of *T. gondii* antibodies than young ones as they have a longer chance of exposure to the agent of infection and probably because they have less immunity. The result of this study showed that fifty percent of the total goats examined were found to be seropositive for *T. gondii* antibodies. Lower seroprevalence of *T. gondii* has been recorded by many authors from different parts of the world (Negash *et al*., 2004; Al-Ramahi, Hamza and Abdulla, 2010; Chikweto *et al*., 2011; Abu-Dalbouh *et al*., 2012; Gebremedhin *et al*., 2014; Lahmar *et al*., 2015; Fereig *et al*., 2016; Tonouhewa *et al*., 2017; Atail *et al*., 2017; Tilahun *et al*., 2018; Ishaku *et al*., 2018; Abdallah *et al*., 2019; Bentum *et al*., 2019; Julie, Paul and Antoine, 2019). On other hand, higher seroprevalences than the present results were also recorded (Younis *et al*., 2015; Al-Kappany *et al*., 2018; Etter, Neves and Tagwireyi, 2019; Tonouhewa *et al*., 2019). According to the gender of goats, the result revealed that males showed a higher prevalence than females but no significant differences were found (P=0.678). This result is consistent with results reported by many researchers (Gebremedhin *et al*., 2014; Fereig *et al*., 2016; Atail *et al*., 2017; Tonouhewa *et al*., 2019). The result revealed no differences found between the goats of different ages, the same results were recorded by Gebremedhin et al. (2014) in Ethiopia and (Atail *et al*., 2017) in Sudan. Meanwhile, many authors reported that old or aged goats have a higher seroprevalence to *T. gondii* antibodies than younger ones (Chikweto *et al*., 2011; Younis *et al*., 2015; Bentum *et al*., 2019).

5. Conclusion

This study showed a high seroprevalence of *T. gondii* antibodies IgG in sheep and goats from the city of Benghazi and its around areas which confirm the previous studies in Libya (Azwai, El-Gammoudi and Gameel, 1993; EL-Gomati *et al*., 2008; Al-mabruk *et al*., 2013). This suggests that the consumption of undercooked meat of these animals represents a potential risk of infection to humans and well alert the government, health a weariness and society to Toxoplasma infection as these animals are the main source of meat in Benghazi. *Toxoplasma gondii* is widely prevalent in Benghazi city (Kassem and Morsy, 1991; Bader, 2002; AL-Ghariane, 2006; Younis and Elamami, 2018). Although the source of infection remains unclear one cannot exclude the sheep and goat's meat as a source of infection, therefore, awareness of people on ways of transmission and prevention of *T. gondii* infection should be raised through education and further study should be conducted to explore the impact of the disease on food animal production.

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