

Toxoplasmosis in Naturally and Experimentally Infected Goats

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Abstract: One hundred slaughtered goats (2-3 years old) were used for diagnosis of toxoplasmosis in naturally infected goats, and 12 healthy pregnant and nonpregnant goats were used to study the pattern of toxoplasmosis as experimental study. Prevalence of toxoplasmosis in 100 slaughtered goats revealed that 29 (29%) and 27 (27%) were seropositive by LAT and IHA tests, respectively. There were agreement between LAT and IHA 97.3% in seronegative and 93.1% in seropositive sera in goats. There were complete concordance between LAT and bioassay in cats and mice. While the agreement between IHA result and bioassay in cat and mice was 93.1% in goats. Clinical examination of experimentally infected goats revealed that all goats had slight rise of body temperature; depression, anorexia, cough, muscular hyperthesia and diarrhea by day 5 and returned to normal by day 11. The age of fetus at the time of *T. gondii* infection is one of the known causes for the variability in clinical response. As infection of goats in early stage of pregnancy result in fetal reabsorption, while infection in mid pregnancy lead to abortion in one goat at 28 days post-infection and the other was aborted at 40 days post-infection. Moreover infections in late pregnancy resulted in delivery of viable kids. On the other hand controls goats were clinically normal and pregnant does were birth viable kids. LAT showed rapid response after 14 days post-infection, while IHA detected antibodies after 3 weeks post-infection. The antibody titers of both tests remained high until the end of experiment (48 weeks), while the titers were decreased around abortion or parturition and increased again after one week. Both LAT and IHA tests were insensitive in the pre-suckling kids from infected goats, whereas PCR gave positive results. In conclusion, PCR considered the most reliable tool for diagnosis of prenatal infection of toxoplasmosis, while LAT and IHA were considered unreliable tools for diagnosis of toxoplasmosis if they applied one week before or after kidding. [Journal of American Science. 2010;6(11):122-129]. (ISSN: 1545-1003).

Keywords: Prevalence, Toxoplasmosis, Goats, Abortion, Parturition.

1. Introduction

Improvement of goats breeding can be done through elimination of destructive factors affecting their productive potential. Toxoplasmosis is one of the most common zoonotic protozoal diseases caused by an obligatory intracellular apico-complexan protozoan. The disease is widely distributed affecting people worldwide. *Toxoplasma gondii* was firstly discovered by Nicolle & Manceaux (1908) in North Africa. *T. gondii* may be transmitted vertically by Tachyzoites to the fetus via placenta or horizontally through ingesting sporulated oocytes or tissue cysts of infected animals (Tenter et al., 2001). The infection of goats by *T. gondii* occurred through contaminated food and water by sporulated oocytes from infected cat feces (Dubey & Beattie, 1988). Therefore, toxoplasmosis in these animals was significantly associated with the presence of cats roaming in the farms. If the parasite is encountered during pregnancy, fetal infection, abortion and neonatal loss can occur, thus toxoplasma infection in goats has a major economic impact upon their farming (Dubey & Towle, 1986). The latent infected goats constitute potential source of human toxoplasmosis whose acquired infection mainly through ingesting of tissue cysts in undercooked meat

(Dubey & Beattie, 1988; El-On & Peiser, 2003). Several techniques employed for diagnosis of toxoplasmosis including coprological (feces), histological (tissues), bioassay (inoculation of cat and mice) and serological tests including dye test, IHA, LAT, ELISA and PCR (Hurtado et al., 2001; Pereira-Bueno et al., 2002; Pierglili-Fioretta, 2004). In view of the above argument, this work was planed to investigate the seroprevalence and pattern of toxoplasmosis in naturally infected and experimentally challenged goats with *T. gondii*.

2. Material and Methods

2.1. Animals

2.1.1. Goats: One hundred goats (2-3 years old) that slaughtered at Cairo and Zagazig abattoirs were used for diagnosis of toxoplasmosis in naturally infected goats. Moreover, 12 healthy pregnant and nonpregnant goats (2-3 years old) were used to study the pattern of toxoplasmosis as experimental study. The experimental animals were proved to be free from parasitic infestation after clinical and parasitological examination (Radostits, 2007).

2.1.2. Cats: seventy four apparent normal cats 2-3 months age were used in this study at different

intervals. After parasitological examination, they proved free from; Nematoda, Cestodes, Isospora, Eimeria and *T. gondii* oocysts and serologically negative against *T. gondii* infection. They fed *ad libitum* diet according to Charles (1979). Cats were used for tissues bioassay.

2.1.3. Mice: one hundred and twenty three white Swiss laboratory mice, about 25 gm body weights were obtained from Unit of Laboratory Animals, Faculty of Veterinary Medicine, Zagazig University. They proved to be free from toxoplasmosis. They were used for blood bioassay.

2.2. Samples

2.2.1. Blood Samples: Ten ml of blood were collected in clean sterile dry screw capped bottle from examined goats and cats. The collected blood were left to clot at room temperature for one hour and centrifuged at 3000 rpm for 15 minutes. Sera were aspirated by Pasteur pipette in other clean dry crocked bottle which labeled in a serial number and stored at -20°C until used. Moreover, 10 ml of blood collected from goats one week post infection were put in a tube containing EDTA (ethylene diamine tetra acetic acid) and subsequently inoculated subcutaneous (S/C) into mice and also used for detection of *T. gondii* antigen by PCR.

2.2.2. Faecal Samples: The entire faeces from each cat were examined for oocysts as described by Dubey (2001). Moreover fecal samples from goats that used for experimental studies were examined before experiment according to Soulsby (1986).

2.2.3. Tissue Samples: Tissue specimens were obtained from skeletal muscles (diaphragm) of slaughtered goats; in addition to placenta and tissues of aborted fetuses. The tissue specimens were used for bioassay in mice and cats according to Dubey (2001).

2.3. Seroprevalence of toxoplasmosis in slaughtered and experimental goats

Serum samples were collected from slaughtered goats as well as from experimentally infected and control goats weekly interval. The serum samples were tested serologically by LAT and IHA according to (Jacob, 1973) and Camargo & Leser (1976), respectively. Blood samples on EDTA were taken one week post infection and tissue specimens from each aborted fetus and placenta examined by PCR according to Esteban-Redondo et al. (1999) and bioassay in mice according to Dubey et al. (1997).

2.4. Polymerase chain reaction (PCR)¹ according to Esteban-Redondo et al., (1999): Primer 1 corresponds to B1 gene nucleotides 694 to 714 (5' - GGAAGTGCATCCGTTTCATGAG) and primer 2 is of the opposite sense and corresponds to nucleotides 887 to 868 (5' -TCTTTAAAGCGTTCGTGGTC) on the antisense strand.

2.5. Diagnosis of toxoplasmosis in serologically positive slaughtered goats through bioassay:

Fifty gm of fresh meat (from diaphragm) were collected from serologically positive slaughtered goats (29) against toxoplasmosis. Twenty-five grams from each animal were taken and used for determination of infection by bioassay in mice through digestion technique (3 mice for each sample). Another 25gm were used for determination of infection by bioassay in cats (2 cats for each sample).

2.5.1. Determination of infection by digestion technique

Twenty five grams from each carcass were minced and digested in 250 ml of Pepsin hydrochloric acid; after one hour at 37°C , the suspension was filtrated via gauze and the filtrate was centrifuged for 15 min. at 2000 rpm. The supernatant was removed and the sediment was washed with sterile saline then centrifugation was repeated. To the final precipitate, 10ml of saline containing 500 units of penicillin and 0.5 mg of Streptomycin were added. Three ml of suspension were inoculated I/P into 3 mice. The mice were killed two weeks post-inoculation and their peritoneal exudates were examined microscopically for *T. gondii* tachyzoites (Dubey et al., 1997).

2.5.2. Determination of infection by bioassay in kittens

Twenty-five grams of diaphragms from each animal were minced and fed to 2 kittens. Entire feces of each cat were collected daily post infection and examined microscopically for the presence of *T. gondii* oocysts according to Dubey (2001). The sporulation and testing the infectivity of isolated oocysts were done according to Dubey & Beatlie (1988). These sporulated oocysts were used for experimental study. Before inoculation of infective sporulated oocysts in goats, H_2SO_4 was neutralized by 3.3% NaOH.

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2.6. Ultrasonic pregnancy detection of goats

A real-time ultrasound scanner equipped with transrectal and transabdominal transducers (Pie-medical, Genus 240, Japan) was used for this study, and a well lubricated 6 MHz transducer with Carboxymethyl-cellulose conducted gel was introduced as described by Haibel (1990); Kaehn (1994) and Hesselink & Taverne (1994). Two methods of ultrasonographical examination (trans-rectal and trans-abdominal) were used to check pregnant goats

2.6. Disease pattern in experimental goats

Twelve goats were divided into 5 groups. Each animal (in group 1-4) was inoculated with 60,000 sporulated oocysts:

- Group-1 included 2 goats at early gestation (1-2 month).
- Group-2 included 2 goats at mid gestation (3 months).
- Group-3 included 2 goats at late gestation (≥ 4 months).
- Group-4 included 2 goats (nonpregnant).
- Group-5 (control) included 4 goats (2 pregnant and 2 nonpregnant).

Daily observation of goats for any clinical manifestation was applied according to Radostits et al. (2007). Serum samples were collected from each animal in the experimental and control groups weekly (up to 48 weeks) for estimation of infection by toxoplasmosis by LAT and IHA, and another blood samples were collected on EDTA (Ethylene Diamine Tetra-acetic Acid) to detect infection by PCR and bioassay in mice.

2.7. Statistical analysis

The obtained data were analysed for significance using T-test (Selvin, 1996), and the variabilities were done by variant NOVA (SAS, 1996).

3. Results

3.1. Seroprevalence results

Out of 100 goats sera samples that collected from Cairo and Zagazig abattoir 29(29%) and 27 (27%) of goats were seropositive against toxoplasmosis by LAT and IHA tests. The seroprevalence was higher at Zagazig abattoir (28%) than that at Cairo abattoir (24.2%) (Table 1). The titer of LAT were ranged from 1/16 to 1/512, with the most frequency 1/32 to 1/256, while the titer of IHA were ranged from 1/80 to 1/1280 with the most frequently 1/160 (Table 2).

3.2. Disease pattern in goats

Daily investigation of all infected goats revealed slight rise of body temperature. The temperature started to rise at day 5, reached a peak of

41°C at day 6 and 7 and return to normal at day 11. All goats showed depression, anorexia, cough, muscular hyperthesia, and diarrhea. Experimentally infections of goats in early stage of gestation (Group I) result in fetal reabsorption and were negative by ultrasonographical examination one-month post-infection (Image1a,b). While infection of 2 mid pregnant does (group II) result in one of these does was aborted at 28 days post-infection, whereas the other was aborted at 40 days post-infection. Moreover infection of 2 late pregnant does (Group III) were birth 4 normal viable kids their weight were 1.9 - 2 kg. The main gross lesions in the placenta were multiple focal areas of necrosis and calcification which were grayish white in color and firm in consistency (Image 2). On the other hand control does (Group V) were clinically normal and pregnant does were birth normal viable kids their weight ranged from 2 - 2.2 kg.

Table 1: Seroprevalence of toxoplasmosis in slaughtered goats.

Locality	Goats				
	Total No.	LAT		IHAT	
		+ve	%	+ve	%
Zagazig Abattoir	67	20	29.9	19	28.4
Cairo Abattoir	33	9	27.3	8	24.2
Total	100	29	29	27	27

LAT: Latex Agglutination Test; IHAT: Indirect Haemagglutination Test.

Table 2: Titers of LAT and IHAT during seroprevalence of toxoplasmosis in slaughtered goats.

LAT	Goats							
	Titre	No.	LAT				IHAT	
			-ve	1/80	1/160	1/320	1/640	1/1280
-ve	71	71	-	-	-	-	-	
1/16	5	2	1	2	-	-	-	
1/32	8	-	1	6	1	-	-	
1/64	7	-	-	4	2	1	-	
1/128	3	-	-	-	2	1	-	
1/256	4	-	-	-	1	2	1	
1/512	2	-	-	-	-	1	1	
Total	100	73	2	12	6	5	2	

LAT: Latex Agglutination Test; IHAT: Indirect Haemagglutination Test.

Tachyzoites of *T. gondii* were recovered from the blood of 5 out of 8 experimentally infected goats one week P.I. through blood bioassay in mice. Only 5 out of 24 mice injected with blood became infected (Image 3). *T. gondii* were isolated from all placenta and aborted fetuses of infected does using tissue bioassays in mice. There were 6 out of 12 mice

and 5 out of 6 mice injected with tissue of placenta and aborted fetuses became infected. Tissue bioassay in cats revealed *T. gondii* from all placenta and aborted fetuses of experimentally infected does. There were 7 out of 8 and 4 out of 4 cats became

infected and shed oocysts after feeding tissues of placenta and aborted fetuses of experimentally infected does (Image 3 and Image 4). Blood and tissues bioassay of non-infected control goats revealed no tachyzoites or tissue cysts (Table 3).

Table 3. Bioassay results in goats infected with sporulated oocysts.

Infected stage	Groups	No. of goats	Stages of gestation	Does for Each group	Bioassay in mice			Bioassay in cats	
					By blood	By placenta	By fetus	By placenta	By fetus
					No. of mice +ve/no. of inoculated	No. of mice +ve/no. of inoculated	No. of mice +ve/no. of inoculated	No. of cats +ve/no. of inoculated	No. of cats +ve/no. of inoculated
Sporulated oocysts	1 st group	1	Early	60,000 sporulated oocytes	0/3	-	-	-	-
		2	Early		1/3	-	-	-	-
	2 nd group	3	Mid		1/3	1/3	2/3	2/2	2/2
		4	Mid		1/3	2/3	3/3	2/2	2/2
	3 rd group	5	Late		0/3	1/3	-	1/2	-
		6	Late		1/3	2/3	-	2/2	-
	4 th group	7	Non		0/3	-	-	-	-
		8	Non		1/3	-	-	-	-
	Control	9	Mid		0/3	0/3	-	0/2	-
		10	Mid		0/3	0/3	-	0/2	-
		11	Non		0/3	-	-	-	-
		12	Non		0/3	-	-	-	-
Total		12			5/24	6/12	5/6	7/8	4/4

Serum samples collected from infected goats as well as control revealed that all goats were seronegative at the time of inoculation. LAT showed rapid response as antibody titer first appear after 10 days in one goat, while all infected does developed antibody titer of 1/32 or more after 12 days P.I. *T. gondii* serum antibodies peaked usually to a plateau level at 8 to 22 weeks after inoculation of the does and showing slight fluctuations until the end of experiment (48 weeks). Abortion or kidding coincided with or preceded these peak levels and antibodies titers of aborted does ranged from 1/64 – 1/128 (Table 4). Whereas IHA detected antibodies against *T. gondii* in some goats (2 does) on day 14 P.I. and all infected goats were seropositive after 3 weeks P.I. *T. gondii* serum antibodies peaked usually

to a plateau level at 9 to 26 weeks after inoculation of the does and showing slight fluctuations until the end of the experiment (48 weeks). Abortion or kidding preceded these peak levels and antibodies titers of aborted does ranged from 1/80-1/160 (Table 4). The results also revealed that antibody titer of either LAT or IHA were decreased around abortion or parturition and increase again after one week (Table 4). Both LAT and IHA were negative and insensitive in detection of congenital infection in pre-suckling kids. Whereas PCR gave positive results in the blood of all infected does one week P.I., but gave negative results in control group. Moreover, PCR gave positive results in the placenta and tissues of all aborted kids from infected does as well as in the blood of life congenitally infected kids (Table 4 and Image 5).

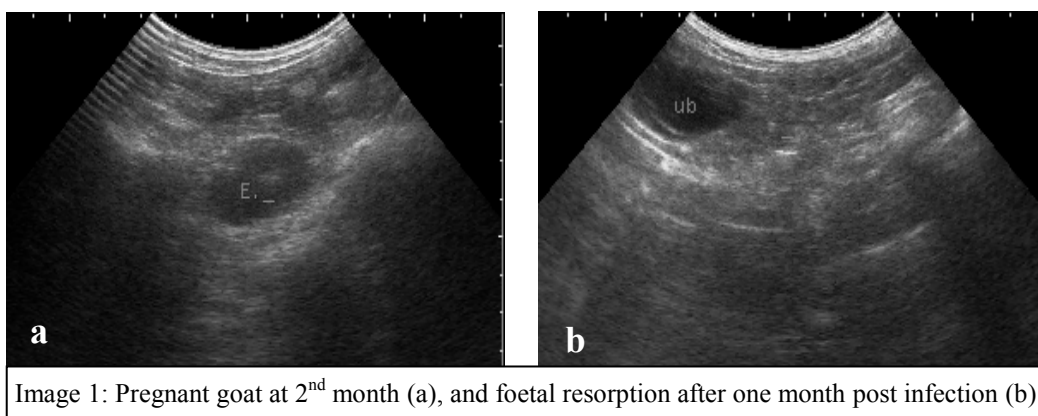


Image 1: Pregnant goat at 2nd month (a), and foetal resorption after one month post infection (b)

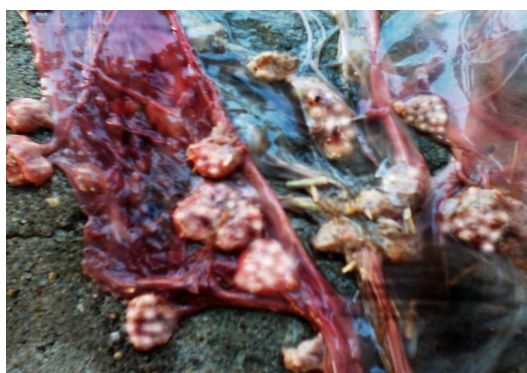


Image 2: A gross lesions in the placenta, where multiple focal areas of necrosis and calcification which were grayish white in

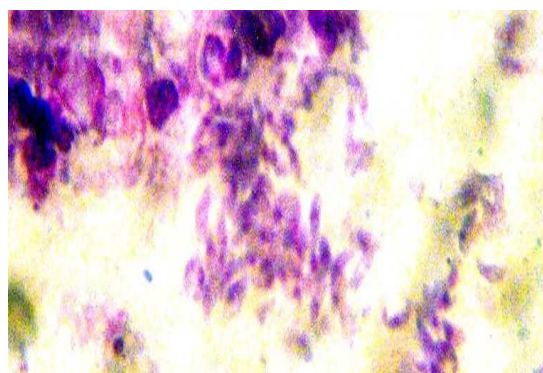


Image 3: Tachyzoites of toxoplasma gondii from peritoneal fluid of mice experimentally infected with blood of infected goats.



Image 4: Oocyst of *T. gondii* from cat faeces.

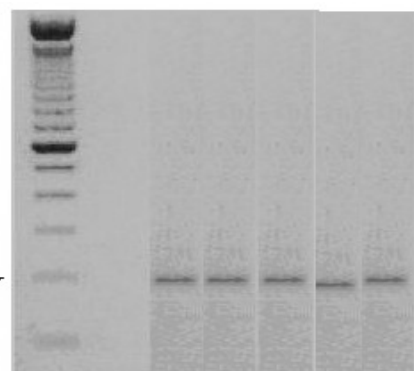


Image 5: Positive PCR results in the placenta and tissues of aborted kids from infected does as well as in the blood of life congenitally infected kid.

Table 4. Outcomes of pregnancy in experimentally infected goats with *T. gondii* oocysts

Animals		Early pregnant		Mid pregnant		Late pregnant		Non-pregnant		
Does	No	1	2	3	4	5	6	7	8	
	Serological results	LAT	Initial	<16	<16	<16	<16	<16	<16	<16
Peak/plateau			32768 (70)	16384 (63)	65536 (70)	32768 (70)	16384 (56)	16384 (63)	16384 (84)	2048 (63)
At abortion Or kidding			-	-	128	128	64	64	-	-
IHA		Initial	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		Peak/plateau	1/10240 (84)	1/10240 (70)	1/20480 (70)	1/10240 (84)	1/20480 (63)	1/10240 (70)	1/5120 (84)	1/10240 (84)
		At abortion Or kidding	-	-	1/160	1/160	1/80	1/160	-	-
PCR on blood		+	+	+	+	+	+	+	+	
At abortion or kidding days after inoculation		Fetal resorption	Fetal resorption	28	50	25	30	-	-	
Kids	Condition of kid		-	-	Fresh	Fresh	Viable	Viable	-	-
	Pre-suckling Antibody titer	LAT	-	-	<16	<16	<16	<16	-	-
		IHA			<1/80	<1/80	<1/80	<1/80		
		PCR			+ve	+ve	+ve	+ve		
	T.gondii isolation	Mice			+	+	+	+	-	-
Cat				+	+	+	+	-	-	

LAT: Latex Agglutination Test; IHA: Indirect Haemagglutination Test, PCR: Polymerase Chain Reaction.

4. Discussion

The sole source of infection of herbivore especially goats by *T. gondii* is sporulated oocysts in contaminated food and water from infected cat feces. Toxoplasmosis has a potential risk to human, in addition to its economic losses in goats. If the parasite is encountered during pregnancy, fetal infection; abortion and neonatal loss can occur (Buxton 1998; Bisson et al., 2000). Clinical symptoms of toxoplasmosis in goats are not specific especially in the early stage of infection. Therefore, the detection of specific toxoplasma antibodies appears to be an important tool for diagnosis of toxoplasmosis in ovine species.

Evaluation of serological tests becomes important in order to use sensitive and specific tests in serological surveys (Moreno et al., 1991). In the present study, out of 100 goats' sera slaughtered at Cairo and Zagazig abattoir, 29 (29%) and 27 (27%) of goats were seropositive against toxoplasmosis by LAT and IHA tests respectively. This percentage of infection among goats seems to be nearly similar to that reported by Pita-Gondim et al. (1999); Sharma et al. (2003) and Jittapalpong et al. (2005) using the same tests. Our result was different than those reported by Matsuo & Husin, (1996); Da Silva & Langoni (2001) and Mainardi et al. (2003). In the present study it was noticed that the percentage of infection varied from region to region. This variation may be attributed to the difference of the environmental and ecological condition, which affect the biology of the parasite or the system of breeding and hygienic measures inside farms.

Regarding the quantitative agreement in antibody titers obtained by IHA and LAT in 100 caprine serum samples examined for toxoplasmosis, it showed that out of 5 LAT positive sera at titers 1/16, 2 sera were IHA negative and all LAT result >1/32 was positive by IHA. This was in agreement with Chhabra et al. (1981), Dubey et al. (1987) and Tress et al. (1988) who found 100% correlation between LAT, IHA and dye test. They concluded that LAT is sensitive, reliable, rapidly responsive serological test and is efficient for screening purposes. Regarding to the comparison between serological results and the results of bioassay in mice and cats, the results revealed that there were complete concordance between LAT and bioassay in cats and mice, as all LAT positive sera samples of goats were positive by bioassay in cats and mice. While the agreement between IHA and bioassay in cat and mice were 93.1% in goats. These results reflect that LAT is more sensitive than IHA. This result agreed with that reported by Dubey et al. (1987); Tress et al. (1988); Figueiredo et al. (2001) and Lhafi et al. (2004) and Érica et al. (2010)

The results of clinical examination of experimentally infected goats revealed that all infected goats had slight rise of body temperature (41°C), depression, anorexia, cough, muscular hyperthesia, and diarrhea by day 5 and returned to normal by day 11. These results were in agreement with Dubey et al. (1980) and Nishi et al. (2001). Goats in group (I), which infected at early stage of gestation revealed fetal reabsorption and negative ultrasonographical examination, one month post-infection. This result was in agreement with that recorded by Dubey (1981). Infection of 2 mid pregnant does (group II), result in abortion of one at 28 days post-infection, whereas the other was aborted at 40 days post-infection. This result was concordant with that of Buxton (1998). Moreover infections of 2 late pregnant does (Group III) were birth normal viable kids their weight were 1.9-2 kg. This result coincided with that reported by Dubey & Beattie (1988). The previous results indicate that age of fetus at the time of *T. gondii* infection in the goats is one of the known causes for this variability in clinical response. This concordant with that reported by Blewett and Watson (1983)

Blood bioassay in mice revealed *T. gondii* tachyzoites from the blood of 4 out of 8 experimentally infected goats one week P.I. only 4 out of 24 mice injected with blood became infected. These results were in agreement with that recorded by Dubey et al. (1980) and Freyre et al. (2008) who detected parasitaemia in 7 out of 7 goats that lasted 3 to 10 days, and Nishi et al. (2001) who detected parasitaemia in 50% of infected goats by mice bioassay from 7 to 14 days P.I. and isolated viable *T. gondii* from all infected goats which killed after 8 weeks P.I. Tissue bioassays in mice and cats revealed *T. gondii* from all placenta and aborted fetuses of infected does. This result was in agreement with that recorded by Dubey (1981) who isolated *T. gondii* from the fetal placenta of 6 out of 7 goats as early as 10 and as late as 15 days after inoculation. Regarding PCR results, *T. gondii* was detected from blood of life fetuses, all placenta and aborted fetuses of infected does. This result was coincided with that reported by Sreekumar et al. (2004) who found that lung, muscles and mesenteric lymph node aspirates of the doe and lung tissue of the aborted fetus were PCR positive.

With regard to the results of serological tests on experimentally infected goats LAT showed rapid response as all infected does developed antibody titer of 1/32 or more after 14 days P.I., while IHA detected antibodies after 3 weeks P.I. These results were in agreement with that reported by Vitor et al. (1999) who found that antibodies against *T. gondii* in

the sera of experimentally infected goats were ranged from 1:256 and 1:32000.

Our results revealed that both LAT and IHA tests were insensitive in detection of *T. gondii* antibodies in the post-suckling lambs from infected does. These results coincided with that reported by Dubey et al. (1987). The antibodies of LAT and IHA remained high until the end of experiment (at 48 weeks P.I.), this clarified that the high antibody titers are not necessarily diagnostic of recent infection. This result consensus with that recorded by Dubey (1985) who reported that the concentration of antibody may remain high, even into the next breeding season.

It could be concluded that, clinical symptoms of toxoplasmosis in goats are not specific; therefore serological tests appeared to be an important tool for diagnosis. Moreover, PCR considered the most reliable tool for diagnosis of prenatal infection of toxoplasmosis, while LAT and IHA were considered unreliable tools for diagnosis of toxoplasmosis if they applied one week before or after kidding.

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