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MOLECULAR DETECTION OF *TOXOPLASMA GONDII* DNA IN RAW GOAT AND SHEEP MILK WITH DISCUSSION OF ITS PUBLIC HEALTH IMPORTANCE IN ASSIUT GOVERNORATE

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ABSTRACT

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This study was carried out to investigate the incidence of *Toxoplasma gondii* in 105 sheep and goat raw milk samples in Assiut, Egypt. Milk samples were tested by microscopic examination, Latex agglutination test and 50 samples were tested with PCR assay. The incidence of *Toxoplasma gondii* in sheep and goat milk samples was 10.48% (11/105) by microscopic examination, 39% (41/105) by Latex agglutination test and 16% (8/50) by PCR assay. High significant differences were recorded in incidence of *Toxoplasma* in raw sheep milk in 8.62% (5/58) by micropscopic examination, in 39.66% (23/58)) by latex agglutination test and detection of B1 gene of *Toxoplasma gondii* in 10.71% (3/28) by PCR assay. While, significance differences were found in incidence of *Toxoplasma* in raw goat milk in 12.77% (6/47)) by microscopical examination, in 38.30% (18/47) by Latex agglutination test in addition to detection of B1 gene of *Toxoplasma gondii* in 22.73% (5/22) by PCR assay. The results showed that sheep and goats were excreting *Toxoplasma gondii* DNA in their milk. The results of this study revealed that Latex agglutination test and PCR assay could become useful tools to diagnose the incidence of *Toxoplasma gondii* in sheep and goat milk samples. This study is the first report of direct detection of *Toxoplasma gondii* in sheep and goat milk samples in Assiut Governorate, Egypt. Control measures and public heath importance were also discussed in this study.

Key words: *Toxoplasma gondii DNA antibodies caprine ovine milk Assiut Governorate.*

INTRODUCTION

Milk is raised as a complete food, especially for children and seniors as it has a high value for proteins, minerals, fats and vitamins. Milk from goats is more easily digested than cow's milk by children as ingestion of raw goat milk remains a common practice in the rural areas in Egypt, particularly among poor children and those that are allergic to cows' milk. Therefore, the hygienic quality of milk has a high importance from the public health point of view but sometimes *Toxoplasma* infection may occur.

Toxoplasmosis has priority as one of five parasitic diseases with public health action [Center for Disease Control and Prevention (CDC), 2013]. *Toxoplasma gondii* is an obligate intracellular protozoan that infects humans and a wide range of warm-blooded animals (Smith and Reduck, 2000). All mammals including man act as intermediate hosts who infected by ingestion of sporulated oocysts, cyst-contaminated meat, milk contaminated by tachyzoites or transplacentarily, except feline species which acts as a definitive host (Innes, 1997; Pepin *et al.*, 1997 and Jenum and Stray-Pedersen, 1998).

Toxoplasma gondii is one of the important zoonotic parasites of world-wide, based on geographic location; 15–85% of the human population is asymptomatically infected with *Toxoplasma gondii.* Up to one third of the human population in the world was chronically infected with toxoplasmosis. Sheep and goats were well known sources of human infection with toxoplasmosis among food animals. Drinking of unpasteurized goat milk was considered source of human infections (Aspinall *et al.*, 2002; Acha and Szyfres, 2003; Reischl *et al.*, 2003; Lehmann *et al.*, 2006; Havelaar *et al.*, 2007; Pappas *et al.*, 2009; Dubey, 2010; Al-Khatib, 2011; Dubey *et al.*, 2011 and Mancianti *et al.*, 2013).

Sheep and goat are more widely and most seriously affected by *Toxoplasma gondii* among livestock animals and show high seroprevalences in many areas of the world, up to 92 and 75%, respectively. Adult

goats were reported to have died from acute toxoplasmosis and this disease is a common cause of abortion, early embryonic death with resorption or mummification, stillbirth and neonatal death in sheep and goats. Polymerase chain reaction method was used for detection of *Toxoplasma gondii* DNA in the milk based on its B1 gene (Buxton, 1998; Dunn *et al.,* 1999; Tenter *et al.,* 2000; Petersen *et al.,* 2001; Dubey, 2004; Buxton *et al.,* 2007; Dubey, 2009a; Tenter, 2009; Ragozo *et al.,* 2010; Asgari *et al.,* 2011 and Tavassoli *et al.,* 2013).

Toxoplasmosis causes significant reproductive and economic losses in animals as well as public health concerns as consumption of contaminated milk damage the human health and can facilitate zoonotic transmission. The presence of a recent or active infection of animals could be an important source of transmission of the infection to men. Animals shed *Toxoplasma gondii* tachyzoites in all body fluids, including milk in the acute stage of the disease. Moreover, *Toxoplasma gondii* being reported to be more prevalent in goat milk than in their meat (Jittapalapong *et al.,* 2005 and Prelezov *et al.,* 2008; Camossi *et al.,* 2011 and Luptakova *et al.,* 2015).

Toxoplasmosis has a major public health importance in food safety issue as its tachyzoite has been found in unpasteurized milk of sheep and goats. Consumption of unpasteurized goat's milk has been associated with human toxoplasmosis, therefore, it is advisable that milk should be boiled or pasteurized before human consumption as these procedures will inevitably kill any potentially present tachyzoites (Tenter *et al.,* 2000; Hiramoto *et al.,* 2001; Ertug *et al.,* 2005; Jones *et al.,* 2009; Inpankaew *et al.,* 2010; Bezerra *et al.,* 2013; Dehkordi *et al.,* 2013; Flatt and Shetty, 2013; Mancianti *et al.,* 2013; Dubey and Jones 2014; Dubey *et al.,* 2014 and Da Silva *et al.,* 2015). Clinical toxoplasmosis in human has been attributed to drinking of unpasteurized goat's milk due to release of *Toxoplasma* tachyzoites in naturally infected goats milk (Sacks *et al.,* 1982; Chiari and Neves, 1984 and Skinner *et al.,* 1990) and was linked to toxoplasmosis in an infant (Riemann *et al.,* 1975 and Dubey and Lappin, 2006).

Abdel-Rahman *et al.* (2012) identified *Toxoplasma* specific antibodies in both serum and milk of naturally infected goats using IHAT. Moreover, Azab *et al.* (1992) and Haridy *et al.* (2010) detected *Toxoplasma gondii* specific antibodies in milk using IFAT and ELISA, respectively.

Ghoneim *et al.* (2009) recorded that drinking raw sheep or goat milk is one of the strongest risk factors of acquiring *Toxoplasma gondii* infection by high risk women at El-Fayoum, Egypt. Serological diagnosis represents the first and the most widely used approach to define the stage of toxoplasmosis. Diagnosis of infection in pregnancy can be improved by determination of *Toxoplasma* DNA. Also, (Balea *et al.,* 2012) highlighted the potential risk of human infection with *Toxoplasma gondii* in Cluj Country by consumption of raw milk from small ruminants.

Human infections by *Toxoplasma gondii* are primarily asymptomatic but lymphadenopathy or ocular toxoplasmosis may occur in some patients. *Toxoplasma gondii* infection in pregnant women may lead to abortion, stillbirth or other serious consequences in newborns. The surviving infected infants suffer from progressive mental retardation, hydrocephalus or microcephalus, chorioretinitis and jaundice and neonatal death (Joynson and Wreghitt, 2001; Dubey, 2004; Montoya and Liesenfeld, 2004; Sharif *et al.,* 2007; Tenter, 2009 and Higa *et al.,* 2010). In immunocompromised patients, clinical toxoplasmosis ranges from asymptomatic reactivation to severe disseminated disease with encephalitis, meningoencephalitis or more commonly tumor lesions. Also, life-threatening cases of pneumonia have recently been described. Motor syndrome, consciousness disturbances, seizures and focal signs are common manifestations that are clinically indistinguishable from other CNS complications. A disseminated disease with detection of *Toxoplasma* in blood and bone marrow may occur. The reactivated disease may be fatal if not recognized and treated early (Mele *et al.,* 2002; Schurmann *et al.,* 2002 and Leal *et al.,* 2007).

Pettersen (1984) showed that the transmission of *Toxoplasma gondii* tachyzoit in the milk was attributed to suckling trauma and tissue cyst excretion. While, Deyrup-Olsen and Luchtel (1998) described that excretion of *Toxoplasma gondii* tachyzoites in mammary gland is facilitated by cellular exocytosis of milk secretion. During pre-lactation, relatively stable mammary cells could harbor *Toxoplasma gondii* cysts. Therefore, those silent cysts could be secreted from the mammary gland cells by exocytosis then coated by host cell membranes similar to milk fat globules secretion allowing milk contamination inside the gland.

Although tachyzoites are sensitive to proteolytic enzymes and are destroyed by gastric digestion, it can be survived for up to two hours in acid pepsin solutions and that oral application of tachyzoites might have resulted in an infection (Dubey, 1998). However, infants who are more susceptible to toxoplasmosis than adults have a lower concentration of proteolytic enzymes in their gastrointestinal tract. This may explain one report of toxoplasmosis in a breast-fed infant whose mother acquired a primary infection with *Toxoplasma gondii* (Bonametti *et al.,* 1997).

Asgari *et al.* (2011) demonstrated that *Toxoplasma* tachyzoites are resistant to milk media and conserve their infectivity for up to 30 min due to its oral

transmission. Moreover, Walsh *et al.* (1999) indicated the survival of *Toxoplasma* tachyzoites in goat milk at 4°C for 3-7 days. Therefore, reinforcing the importance of milk pasteurization before any processing or ingestion. Infectivity of bradyzoite in milk was maintained even after storage for 20 days at refrigerator temperatures (Hiramoto *et al.,* 2001).

Riemann *et al.* (1975); Räisänen (1978) and Sacks *et al*. (1982) reviewed that *Toxoplasma gondii* trophozoites survive and remain infectious for a long time in biological liquid media (24 h in saline, 3 days in 3.5% serum albumen, 3 days in colostrum, and 17-43 days in serum solutions) and can easily penetrate mucous membranes, so can enter the host through mucosal tissue and thereby gain access to the host circulation or lymphatic system before reaching the stomach.

The present study aimed to investigate the incidence of *Toxoplasma gondii* infection in sheep and goats raw milk in Assiut, Egypt as an indication of the parasite excretion in milk and evaluation the possibility of using milk as a valid tool for the diagnosis of toxoplasmosis in small ruminants.

MATERIALS and METHODS

Collection of samples: A total of 105 milk samples were collected from 58 sheep and 47 goats randomly selected from various regions Aboteeg, Al-Qusiya, Al-Mateya in Assiut, Egypt. Milk samples were taken manually after disinfection of the teats with 70% ethyl alcohol and collected in clean dry and sterile test tubes. Samples were kept under refrigeration until arrival to the laboratory. Milk smears were prepared for microscopic examination and milk samples rapidly frozen at -20^{o}C until used for serological examination and PCR assay.

Preparation and staining of milk smear: Fresh milk samples centrifuged and milk smears were made from the cells deposited and clean slide. One drop of deposit of each centrifuged fresh milk samples was taken to slide and spread into an even thin film by a second clean slide held at a 45^{o} angle and immediately dried by the air. The slide was labeled and kept in upright position in a special box. The prepared microscopic smears were fixed by absolute methyl alcohol then allow drying. The films stained with freshly diluted Giemsa stain solution for 20 minutes. The films washed by distilled water to remove the excess of stain. The slide placed upright for draining and drying. Then the slides were thoroughly examined with microscope using oil immersion lens (1000×) for presence of *Toxoplasma* tachyzoites (Duran-Jorda, 1944; Schalm *et al.,* 1971 and Lindmark-Månsson *et al.,* 2006).

Serological examination: Milk samples thawed at room temperature and centrifuged at 2000 rpm for 20 minutes and the interface between the lipid layer and the pelleted cellular debris was used for detection of *Toxoplasma gondii* antibodies using undiluted milk (Grundy *et al.,* 1983).

Latex agglutination test (Toxo Latex Kit, Cam Tech Medical, U. K.): Bring milk samples and reagents to room temperature and a serological pipette used to apply 40µl of milk samples to slide. The vial of latex agglutination was shaked well and added 20 µl of toxo latex to the milk sample, then mixed well with stirring sticks and the slide rotated slowly. After 4-6 minutes the sample was checked for agglutination (Syamala *et al.,* 2008).

Polymerase Chain Reaction (PCR): Milk samples from 50 (40 positive and 10 negative by latex agglutination test) were tested with PCR assay.

1. Extraction of DNA from 50 collected milk samples: Milk samples thawed at room temperature then centrifuged at 2200 rpm for 5 minutes to be concentrated (Murphy *et al.,* 2002). Further 1 ml of the sediment was re-suspended in 200µl TE (1mM EDTA, 10mlM Tris-HCl with pH 7.6 and 300µl of 0.5M EDTA with pH 8.0) and centrifuged at 3000 rpm for 10 minutes to avoid the interference with casein (Psifidi *et al.,* 2010). Thus, the milk pullet was diluted in 200 µl of PBS and the extraction of DNA was done by using Genomic DNA Purification Kit (Biotools, Madrid, Spain), according to the manufacturer instructions. After extraction, DNA was stored at -20°C until use.

2. DNA amplification reaction: The extracted DNA was amplified by PCR into 35-fold repetitive B1 gene specified for the characterization of *Toxoplasma gondii* (Bezerra *et al.,* 2013) using primers B1 gene (Biosearch Technologies, Inc., USA)

Toxo B22 (F) 5′AACGGGCGAGTAGCACCTGAGGAG′3
Toxo B23 (R) 5′TGGGTCTACGTCGATGGCATGACAAC′3

with 115 bp (Bretagne *et al.,* 1993). The reaction was applied in 20µl reaction volume containing 10µl of 2x power Dream Tag Green PCR master mix, 20µl pmol B1 each primer and 2µl of the purified DNA.

3. The reaction conditions of PCR: The reaction conditions consisted of one cycle of 95°C for 5 minutes followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute and the final extension at 72°C for 10 minutes (Master cycler, Eppendorf, Hamburg, Germany). The agarose gel was prepared from agarose powder (Bioshop^{R}, Canada inc. lot No: OE16323**)** in concentration 2% in 1x TBE (89 mM Tris-Borate, 2 mM EDTA at pH 8.3) and run in 1 x TBE, 5 µM ethidium bromide (Bioshop ® Canada Inc, Lot No: 0A14667). The amplification products were analyzed by 1.2% of agarose gel electrophoresis stained with ethidium bromide. PCR

product was electrophoresed at 45 minutes at 100 volts and examined using UV transilluminator and photography. 100 bp DNA ladder (Fermentas, lot No: 00052518) was used as a marker.

Statistical analysis: The resulting data were analyzed using SPSS (2007) for Windows (SPSS, version 16, Inc., Chicago, IL). Chi-square was performed, differences were considered significant at values of $P < 0.05$.

RESULTS

A total of 105 sheep and goat milk samples were tested by microscopic examination, Latex agglutination test and 50 samples (40 positive and 10 negative by Latex agglutination test) were tested with PCR. Incidence of *Toxoplasma gondii* in sheep and goat milk samples was 10.48% (11/105) by microscopic examination and 39% (41/105) by Latex agglutination test. Positive *Toxoplasma gondii* DNA were detected in 16% (8/50) sheep and goat's milk samples (Table 3).

Incidence of *Toxoplasma* had high significant differences in raw sheep milk samples between micropscopic examination, latex agglutination test and PCR assay. Microscopic examination of milk smears stained with Giemsa stain showed banana shape tachyzoites of *Toxoplasma gondii* (Figure 2, 3, 4). Incidence of *Toxoplasma gondii* in raw sheep milk samples was 8.62% (5/58)) by microscopic examination and 39.66% (23/58) by Latex agglutination test (Table 1). The B1 gene of *Toxoplasma gondii* was detected by PCR in 3 sheep milk samples (Figure 7, 8). The results showed that 10.71% (3/28) of sheep were excreting *Toxoplasma gondii* DNA in their milk (Table 1). While, significance differences were found in incidence of *Toxoplasma* in raw goat milk by micropscopic examination, Latex agglutination test and PCR assay. Incidence of *Toxoplasma gondii* in raw goat milk samples was 12.77% (6/47) by microscopic examination and 38.30% (18/47)) by Latex agglutination test (Figure 1). The B1 gene of *Toxoplasma gondii* was detected by PCR in 5 goat milk samples (Figure 5, 6). The results showed that 22.73% (5/22) goats were excreting *T. gondii* DNA in their milk (Table 2).

Table 1: Incidence of *Toxoplasma gondii* in raw sheep milk samples based on microscopic examination, Latex agglutination test and PCR assay.

Used test	Number of examined samples	Result			
		Positive		Negative	
		No.	%	No.	%
Microscopic examination	58	5	8.62**	53	91.38
Latex agglutination	58	23	39.66**	35	60.34
Polymerase chain reaction (PCR)	28	3	10.71**	25	89.29

** Means high significance differences ($\chi^2 = 18.94$ and $P < 0.01$).

Table 2: Incidence of *Toxoplasma gondii* in raw goat milk samples based on microscopic examination, Latex agglutination test and PCR assay.

Used test	Number of examined samples	Result			
		Positive		Negative	
		No.	%	No.	%
Microscopic examination	47	6	12.77*	41	87.23
Latex agglutination	47	18	38.30*	29	61.70
Polymerase chain reaction (PCR)	22	5	22.73*	17	77.27

* Means significance differences ($\chi^2 = 8.245$ and $P < 0.05$).

Table 3: Comparison of microscopic examination, Latex agglutination test and Polymerase chain reaction (PCR) for detecting *Toxoplasma* in sheep and goat milk samples.

Detection methods	Number	Positive samples	Incidence (%)
Microscopic examination	105	11	10.48%
Latex agglutination test	105	41	39%
Polymerase chain reaction (PCR)	50	8	16%

Figure (1): Showing latex agglutination test of *Toxoplasma gondii* with positive agglutination in well (1) while wells (2, 3, 4, 5, 6) were negative milk samples.

Figure (2): Showing banana shape *Toxoplasma gondii* tachyzoite (arrow) in sheep milk smear stained with giemsa stain (Lens, 1000x).

Figure (3): Showing banana shape *Toxoplasma gondii* tachyzoite (arrow) in sheep milk smear stained with giemsa stain (Lens, 1000x).

Figure (4): Showing banana shape *Toxoplasma gondii* tachyzoite (arrow) in goat milk smear stained with giemsa stain (Lens, 1000x).

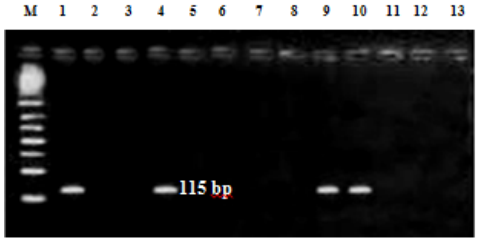

Figure (5): The electrophoresis pattern of PCR amplicon on goat milk samples using B1 gene of *Toxoplasma gondii* primer (115 bp PCR product). M: 100 bp DNA ladder; 1: Control positive; 2: Control negative; 4, 9 and 10: Positive samples for *Toxoplasma gondii*; 3, 5, 6, 7, 8, 11, 12 and 13: Negative samples.

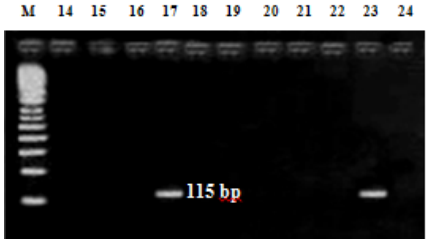

Figure (6): The electrophoresis pattern of PCR amplicon on goat milk samples using B1 gene of *Toxoplasma gondii* primer (115 bp PCR product). M: 100 bp DNA ladder; 17 and 23: Positive samples for *Toxoplasma gondii*; 14, 15, 16, 18, 19, 20, 21, 22 and 24: Negative samples.

Figure (7): The electrophoresis pattern of PCR amplicon on sheep milk samples using B1 gene of *T. gondii* primer (115 bp PCR product). M: 100 bp DNA ladder; 5: Positive samples for *Toxoplasma gondii*; 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13 and 14: Negative samples.

Figure (8): The electrophoresis pattern of PCR amplicon on sheep milk samples using B1 gene of *T. gondii* primer (115 bp PCR product). M: 100 bp DNA ladder; 16 and 19: Positive samples for *Toxoplasma gondii*; 15, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27and 28: Negative samples.

DISCUSSION

Ghoneim *et al.* (2009) and Abdel-Rahman *et al.* (2012) recorded high prevalence of *Toxoplasma gondii* infection in sheep and goats in Egypt due to wide spread of large number of infected stray cats which is in favor of a higher prevalence of oocysts in humid environment and farming animal rearing are also common. Egyptian rural areas have the habit of eating homemade cheese (Kareish cheese) which is made from raw milk. Such habit has a relation to *Toxoplasma gondii* (El Deeb *et al.,* 2012). In addition, (Fusco *et al.,* 2007) showed that local homemade cheese from raw milk is considered a risk factor for public health.

Toxoplasmosis plays an important role in public health worldwide particularly in rural areas that use unpasteurized milk. Contamination of milk with *Toxoplasma gondii* could be originated from the infected animal or contamination of milk with cats' feces due to presence of stray cats around and within farms. Cats are the important risk factor for *Toxoplasma gondii* infection in small ruminants (Chiari *et al.,* 1987; Cordeiro, 2006; Jittapalapong *et al.,* 2008 and Bezerra *et al.,* 2013). *Toxoplasma* oocysts shed continuously in the cat's feces from 4 until 14 days after infection with an expected peak output of tens of millions at 6-8 days. Thus, fifty grams of infected cat feces may contain as many as 10 million oocysts (Dubey and Frenkel, 1972). Infected cats shed *Toxoplasma gondii* oocysts that after sporulation become infective for men and animals, preserving this capability for up to 18 months in the environment (Gorbani *et al.,* 1983).

In the present study, the incidence of *Toxoplasma gondii* in sheep and goat milk samples was 10.48% by microscopic examination, 39% % by Latex agglutination test and 16% by PCR assay (Table 3). The differences observed could be due to the diagnostic technique or breeding condition and management. The high incidence of *Toxoplasma gondii* infection indicated continuous exposure of sheep and goats to infection due to heavy environmental contamination with oocysts shed form the observed stray cats in the farms with poor management conditions (Masala *et al.,* 2003).

Caprine toxoplasmosis was reported worldwide (Dubey, 1980; Masala *et al.,* 2003 and Dubey, 2009b). *Toxoplasma* antibodies are detectable two weeks post infection and maintain in the host a low level through all life (Lin and Bowman, 1991). Serum antibodies was recorded in an endemic area indicating past or present invasive disease while presence of antibodies in milk indicate present or recent infection which reflect local antigenic stimuli to infection, such antibody detection may help in studies of the endemicity of the disease (Grundy *et al.,* 1983).

Testing of milk samples is easier and less expensive than testing of blood samples (Schares *et al.,* 2004) and can be considered a good diagnostic tool for toxoplasmosis at flock level. The level of antibodies in milk is in general lower than that in serum (Elvander *et al.,* 1995). *Toxoplasma* antibodies were detected in the milk of naturally infected lactating women (Azab *et al.,* 1992).

Incidence of *Toxoplasma gondii* in sheep and goat milk samples was 39% (41/105) by Latex agglutination test. Sheep milk samples showed higher percent of anti-*Toxoplasma* IgG (39.66%) than goats milk samples (38.30%) (Table 1, 2). Lower infection rates in goats compared to those in sheep may be attributed to the differences in susceptibility to *Toxoplasma gondii* and the feeding habits of the animals (Bahrieni *et al.,* 2008).

In this study, incidence of *Toxoplasma gondii* antibodies was 38.30% in goat milk samples by Latex agglutination test (Table 2). In comparison to our results, higher result was recorded by (Abdel-Rahman *et al.,* 2012) who found that 58.90% of the examined goats milk samples in Egypt had *Toxoplasma gondii* antibodies. While, lower results were reported by Dehkordi *et al.* (2013) who recorded that the prevalence *Toxoplasma gondii* antibodies was 8.88% in caprine milk samples by ELISA test in Iran and (Da Silva *et al.,* 2015) who estimated that anti-*Toxoplasma gondii* IgG antibodies was 5.78% in raw goat milk samples by IFAT in Brazil. The variation in these results may be attributed to the difference in the diagnostic technique used, study locality, breeding conditions and management (Masala *et al.,* 2003).

Abdel-Rahman *et al.* (2012) found *Toxoplasma gondii* tachyzoites in the milk of both chronically and acutely infected goats. They explained the presence of *Toxoplasma gondii* tachyzoites in the milk of chronically infected goats was due to the resurgence of tissue *Toxoplasma gondii* tachyzoites cysts which can circulate again and be excreted in the milk during physiological decrease in the peripartum immunity.

The obtained results revealed that the incidence of *Toxoplasma gondii* antibodies by Latex agglutination test in sheep milk samples was (39.66%) (Table 1). Lower results were observed by Dehkordi *et al.* (2013) who showed that the prevalence *Toxoplasma gondii* was 5.94 % in ovine milk samples by ELISA test in Iran and (Da Silva *et al.,* 2015) who found anti-*Toxoplasma gondii* IgG antibodies in raw milk of sheep was 3.76% by the IFAT in Brazil. It worth to mention that, our climate favors the survival of oocysts and has contributed to high *Toxoplasma* prevalence (Ciamak, 2006).

PCR assay is a major breakthrough for the diagnosis of *Toxoplasma gondii* infection and have higher

accuracy, sensitivity and specificity than traditional diagnostic methods (Held *et al.,* 2000; Martins *et al.,* 2000 and Kompalic-Cristo *et al.,* 2007). Several PCR assays have been developed for the detection of *Toxoplasma gondii* DNA by amplification of target as B1 gene which is a 35- fold repetitive gene sequence; PCR amplifying this gene target has shown high specificity for *Toxoplasma gondii* DNA detection (Jones *et al.,* 2000 and Reischl *et al.,* 2003).

Powell *et al.* (2001) recorded lactational transmission of *Toxoplasma gondii* from experimentally infected cats to their kittens since the organism detected in feline milk by either bioassay or PCR. While (Costa and Langoni, 2010) confirmed the presence of tachyzoites of *Toxoplasma gondii* in the milk secreted from experimentally infected rats by PCR as well as the seroconversion of offspring after breastfeeding, both in chronic and acute infections with intermittent parasite elimination, corroborating the results of Pettersen (1984). Also, Dehkordi *et al.* (2013) showed higher sensitivity of PCR for detection *Toxoplasma gondii* DNA from milk samples in Iran.

Data presented showed that *Toxoplasma gondii* DNA was detected in 22.73% (5/22) of goat milk samples by PCR assay (Table 2). This result is in agreement with that pointed out by Ghoneim *et al.* (2009) who recorded *Toxoplasma* genomic DNA in 25% of goats in El-Fayoum Governorate, Egypt. Unlike the obtained result, lower results were estimated by Dehkordi *et al.* (2013) and Tavassoli *et al.* (2013) who found that *Toxoplasma gondii* DNA was 9.44% and 1.07% of goats milk samples by PCR in Iran, respectively, (Bezerra *et al.,* 2013 and Da Silva *et al.,* 2015) who detected DNA of *Toxoplasma gondii* in 6.05 and 2.7% of the raw goat milk samples by PCR technique in Brazil, respectively, (Mancianti *et al.,* 2013) who documented that prevalence of *Toxoplasma gondii* DNA was found in 13% of milk samples naturally infected in Italy and Ahmed *et al.* (2014) who estimated that *Toxoplasma gondii* DNA was 8% of goat from rural settings at Sharkia, Egypt. While, higher prevalence was recorded by Spišák *et al.* (2010) who found that the presence of *Toxoplasma gondii* DNA was 32.56% in goats milk from a farm in Slovakia. The variation in these results may be attributed to the immune status of animals, timing of infection and genetic composition of the host and the organism (Suzuki, 2000).

Moreover, the results of this research showed that *Toxoplasma gondii* DNA was detected in 10.71% (3/28) of sheep milk samples by PCR assay (Table1). Lower results were indicated by Fusco *et al.* (2007) who found that the prevalence of *Toxoplasma gondii* DNA was (3.4%) by PCR in ovine milk samples from Italy, (Camossi *et al.,* 2011) who identified *Toxoplasma gondii* DNA in the milk of naturally infected ewes in 5% milk samples by PCR in Brazil, (Tavassoli *et al.,* 2013 and Dehkordi *et al.,* 2013) who recorded *Toxoplasma gondii* DNA was 4.63 and 6.48% of sheep milk samples by polymerase chain reaction (PCR) in Iran, respectively and (Ahmed *et al.,* 2014) who found that 2% of sheep milk samples had *Toxoplasma gondii* DNA in the rural settings at Sharkia, Egypt. This variation may be due to the difference in the study locality, management and distribution and behavior of cats (Buxton, 1990 and Tenter *et al.,* 2000). On the other hand, higher results were found by Ghoneim *et al.* (2009) who detected *Toxoplasma* genomic DNA in 67.7% of sheep in El-Fayoum and Luptakova *et al.* (2015) who found *Toxoplasma gondii* DNA in ewes' milk samples with real-time PCR was 28%. This variation may be attributed to frequency of felines on farms and climatic variations from one region to another (Dubey, 2004).

In the present study, serological results do not coincide with the PCR results because not all serologically positive animals eliminate the parasite in their milk. Several milk samples positive serologically revealed negative PCR results. This elimination depends on the animal infection phase and their immunity. This in agreement with Bezerra *et al.* (2013) and Camossi *et al.* (2011) who noticed that compromised immunity in sheep during the peripartum period may favour the reactivation of cystic forms of *Toxoplasma gondii* , thus eliminating tachyzoite in milk.

The obtained results revealed that 2 goat milk samples were positive in PCR and negative by Latex agglutination test. These results may be due to that animals were in the initial phase of the infection, in which the quantity of antibodies is not yet sufficient to be detected by serological tests. These biological characteristics could explain the prevalence of animals that were positive in the PCR and negative in the serology (Bezerra *et al.,* 2013).

Furthermore, the present work showed that sheep and goats were excreting *Toxoplasma gondii* DNA in their milk as first recording in Assiut Governorate, Egypt. These results are in agreement with Spišák *et al.* (2010) and Tavassoli *et al.* (2013) who demonstrated that presence of *Toxoplasma gondii* DNA in milk of sheep and goats refers to the risk of human infection through consumption raw milk. Also, Ahmed *et al.* (2014) showed a significant correlation between the seroprevalence of *Toxoplasma gondii* in pregnant women and the contact with cats and consumption of raw milk and homemade cheese at Sharkia, Egypt.

CONCLUSION

From the obtained results, it was concluded that there is high incidence of toxoplasmosis among sheep and

goat milk samples in Assiut Governorate, Egypt. Presence of *Toxoplasma gondii* DNA in the milk of sheep and goats raises the possibility that the parasite could be transmitted to human through consumption of raw milk. From the result achieved, it can suggest that boiling or pasteurization of milk before human consumption to eliminate the risk of parasite transmission to milk consumers. Moreover, further investigations are necessary to detect the incidence of *Toxoplasma gondii* DNA in other milk-producing animals and apply effective control strategies against toxoplasmosis.

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الكشف الجزيئى عن جين توكسوبلازما جونديای فى اللبن الخام للماعز و الأغنام مع مناقشة اهميتة للصحة العامة فى محافظة أسيوط

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أجريت هذه الدراسة لتحديد نسبة الاصابه بطفيل التوكسوبلازما ومدى انتشاره فى عينات الألبان الخام للأغنام والماعز فى محافظة أسيوط. وقد تم تجميع عدد ١٠٥من العينات في مناطق مختلفة من محافظة اسيوط حيث تم تجميع عدد ٤٧ عينه من البان الماعز و٥٨ عينه من البان الاغنام وقد تم فحص العينات باستخدام الفحص المجهري لمسحات الحليب وإختبار تراص اللاتكس لتحديد الأجسام المضادة. وتم عمل إختبار تفاعل البلمرة المتسلسل لعدد ٥٠ عينه. حيث وجد أن نسبة الاصابه كانت ١٠.٤٨ ٪ (١٠٥/١١) بالفحص المجهري و ٣٩٪ (١٠٥/٤١) باستخدام إختبار تراص اللاتكس و ١٦٪ (٥٠/٨) باستخدام إختبار تفاعل البلمرة المتسلسل. وسجلت فروق معنويه عالية في إصابة البان الاغنام بطفيل التوكسوبلازما في ٨.٦٢٪ (٥٨/٥) بالفحص المجهري و(٥٨/٢٣)٦٦, ٣٩٪ باستخدام إختبار تراص اللاتكس مع الكشف عن جين B1 للتوكسوبلازما في ١٠.٧١٪ (٢٨/٣) من خلال إختبار تفاعل البلمرة المتسلسل. وسجلت فروق معنويه للإصابة بالتوكسوبلازما في البان الماعز فى ١٢.٧٧٪ (٤٧/٦) بالفحص المجهري و ٣٨.٣٠٪ (٤٧/١٨) باستخدام إختبار تراص اللاتكس مع الكشف عن جين B1 للتوكسوبلازما في ٢٢.٧٣٪ (٢٢/٥) بواسطة إختبار تفاعل البلمرة المتسلسل. حيث أظهرت هذه الدراسه وجود الحمض النووى للتوكسوبلازما فى ألبان الأغنام و الماعز. وأوضحت نتائج هذه الدراسة أنه يمكن الاستفاده من إختبار تراص اللاتكس وإختبار تفاعل البلمرة المتسلسل فى تشخيص الاصابه بعدوى طفيل التوكسوبلازما فى ألبان الأغنام والماعز. وتعد هذه الدراسة هي أول دراسه للكشف المباشر عن طفيل التوكسوبلازما في عينات ألبان الأغنام والماعز في محافظة أسيوط. وقد تمت في هذه الدراسة مناقشة أهمية الطفيل الصحية وطرق الوقاية منه.