OCCURRENCE OF TOXOPLASMA ANTIBODIES IN CAPRINE MILK AND SERUM IN EGYPT

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ABSTRACT

Received at:29/3/2012

Accepted: 22/4/2012

Toxoplasma specific antibodies were identified in both serum and milk of naturally infected goats using Indirect Hemagglutination Test (IHAT). Samples were collected from a total of 182 goats in different localities of Greater Cairo, Beni-Suif, and Zagazig regions, where both milk and serum samples were obtained from 73 lactating and only serum samples from109 non-lactating goats. Milk was investigated for the presence of *T. gondii* using experimentally infected cats. The results indicated that 77(42.30 %) out of 182 were seropositive, where 38 (34.86%) out of 109 non-lactating goats were seropositive for *T.gondii* antibodies using IHAT. Positive results were also indicated in 43(58.90 %), and 39(53.42 %) out of 73 lactating does in both milk and serum samples. Toxoplasma IgG and IgM antibodies were detected in 55(30.22%) and 22(12.09%) out of 77 samples respectively. All experimentally infected cats fed on milk from acutely infected goats shed *T.gondii* oocysts, whereas only one out of 4 cats fed on milk from chronically infected goats shed *T.gondii* oocysts in their faeces. The public health importance due to consumption of raw or unpasteurized goat milk was discussed.

تواجد الأجسام المضادة للتوكسوبلازما في لبن ودم الماعز بمصر

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تم تحديد الأجسام المضادة الخاصة بالتوكسوبلازما في كل من مصل وحليب الماعز المصابة طبيعيا وذلك باستخدام اختبار التلازن الدموي غير المباشر. وقدتم تجميع عينات من ١٨٢ من الماعز في مناطق مختلفة من القاهرة الكبرى، بني سويف، ومنطقة الزقازيق، حيث تم تجميع كل من عينات الحليب وعينات الأمصال لـ٧٣ من الماعز الحلابة وكذلك ١٠٩عينة مصل الدم من الماعز غير الحلابة. هذا وقد تم فحص الحليب لوجودطفيل التوكسوبلازما وذلك باستخدام القطط المصابة تجريبيا. وقد أشارت النتائج إلى أن ٧٧ (٤٢,٣٠٪) من أصل ١٨٢ كانت إيجابية ، حيث أن ٣٨ (٣٤,٨٦٪) (من أصل ١٠٩ من الماعز غير الحلابة تحتوي على الأجسام المضادة للطفيل باستخدام اختبار التلازن الدموي غير المباشر. قد كانت النتيجة ايجابية أيضا في٤٣ (٥٨,٩٠٪) و ٣٩ (٥٣,٤٢٪) من أصل ٧٣ الماعز الحلابة في كل من عينات الحليب ومصل الدم على التوالي 0وتم أيضا الكشف عن وجود الأجسام المناعية(IgM, IgG) في ٥٥ (٣٠,٢٢٪) و ٢٢ (١٢,٠٩٪) من أصل ٧٧ عينة على التوالي. وقد تم الكشف أيضا عن وجود حويصلات التوكسوبلازما في فضلات كل القطط التي تغذت تجريبيا على حليب الماعز المصابة بالتوكسوبلازما الحادة ، في حين أنه تم الكشف أيضا عن وجود هذه الحويصلات في قطة واحدة فقط من أصل ٤ من القطط التي تغذت على حليب الماعز المصابة بالتوكسوبلازما المزمنة . وقد تم مناقشة الأهمية الصحية العامة نظرا لاستهلاك حليب الماعز غير المبستر.

Key words: *Toxoplasma gondii*, Indirect Hemagglutination Test (IHAT), *Caprine milk*

INTRODUCTION

Toxoplasmosis, a coccidian infection, is caused by the obligate intracellular parasite *Toxoplasma gondii*. Although generally benign for healthy people, infection can result in stillbirth, blindness, mental retardation, and occasionally the death of congenitally infected infants (Frenkel, 1988). More recently, this disease has been observed in immunocompromised patients, particularly those with acquired immunodeficiency syndrome but also those with neoplastic disease and bone marrow or heart transplant recipients (Frenkel, 1988). In veterinary medicine, abortion and neonatal loss due to toxoplasmosis are of great economic importance in many parts of the world (Lautenslager, 1987). Goats infected by *T. gondii* represent an important source of human infection due to ingestion of meat and milk from infected animals. Such fact is extremely important concerning the disease control and mainly for public health, since the consumption of goat milk is elevated in children (Dubey, 1980). *T. gondii* tachyzoites were already isolated from milk of naturally infected goats as reported by Chiari and Neves (1984). Furthermore, an epidemiological survey showed that there was a statistically significant correlation between positive serology for *T. gondii* in humans and ingestion of goat milk (Chiari *et al.*, 1987). The seroprevalence of *T. gondii* in goats has been surveyed in many countries, and these worldwide reports were recently summarized (Dubey, 2009). *T.gondii*-specific antibodies were identified in milk using IFAT (Azab *et al.*, 1992) and ELISA (Haridy et al., 2010). However, IHAT was used by Masala *et al.* (2003), who detected that test is fast, accurate; and not expensive. Due to increasing exploration of goat herds and the risk for public health by ingestion of contaminated milk, the development of reliable and sensitive tests for diagnosing this zoonosis becomes very important. Thus, the aim of this study was to evaluate the IHAT to determine the seroprevalence of *T. gondii* infection in goat serum and milk as well as to demonstrate the presence of the organism in goat milk using experimentally infected cats.

MATERIALS and METHODS

Collection of samples:-

A total of 182goats over one year, were randomly selected from different herds in Greater Cairo, Beni-Suif, and Zagazig Governorates in Egypt. Blood samples were collected from all animals (lactating and non-lactating goats), where milk samples were collected from only 73 lactatin goats.

1- Blood samples:

About 3 ml of blood were obtained via a jugular vein, in clean and dry test tubes, left to clot at room temperature and then centrifuged at 2000 *r/m* for 5 minutes. The separated sera were kept in sterile labeled tubes and stored at -20°C until used.

2- Milk samples:

Milk samples were taken and collected in clean dry test tubes and rapidly frozen at -20°C. Before use, all milk samples were centrifuged at 2000 *r/m* for 20 min., and the interface between the lipid layer and the pelleted cellular debris was used (Grundy *et al.*, 1983).

Laboratory investigations:-

Antibodies to *T. gondii* were determined in sera using an Indirect Hemagglutination Test (IHAT) with a commercially available kit (Toxo-IHA-Fast) obtained from ABC Diagnostics, New Domietta, Egypt. IHAT is fast, accurate, and not expensive and used mainly for diagnosis of *T.gondii*. (Hove *et al.*, 2005). Antibodies titers of $\geq$ 1/80 were considered positive for *T.gondii*.

In brief, sera were added to 96 well U-bottomed polystyrene plates, and diluted in a

four-fold series from 1:40 to 1:2560, whereas, milk was used undiluted. The plates were shaken for 2 minutes and then incubated at 37°C for 2 hours without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:80 or higher, and positive and negative controls were included in each test. All reactive serum samples were treated with 2-mercaptoethanol (2-ME) in order to verify the presence of IgM antibodies (Camargo *et al.*, 1978).

Experimental infection:

Ten cats about one to two months of age were kept individually in cages and fed only boiled milk and bread. Faecal examinations were carried out daily for two weeks to insure that they were parasite free. Experimental animals were divided into three groups; four cats were fed fresh milk from acutely Toxoplasma infected goat, other four were fed fresh milk from chronically infected goat, and two were fed only boiled milk and bread as control group. Faecal samples from all animals were examined daily after five days of infection for one month using concentration flotation technique for detection of Toxoplasma oocysts according to Soulsby (1986). The detected oocysts were measured and kept in 2.5% potassium dichromate at 27°C for sporulation.

RESULTS

The prevalence of *T.gondii* in the serum and milk of examined goats were indicated in Table (1). The overall seroprevalence was 77(42.30 %) out of 182 goats where 38 (34.86%) out of 109 non-lactating goats and 39 (53.42 %) out of 73 lactating goats were positive for *T.gondii* using IHAT.

On the other hand, 43 milk samples (58.90 %) out of 73 had demonstrable *T.gondii* antibodies in the same lactating goats.

According to Table (2), the seropositive samples to *T.gondii* showed presence of IgG and IgM antibodies in 55(30.22%) and 22(12.09%), respectively.

The antibody titers of IHAT ranged from 1/80 to 1/2560 in the sera of examined goats (Table 3).

Experimental infection in cats showed that only one cat out of 4 given milk from IgG seropositive goats (chronically infected) shed *T.gondii* oocysts 5 days post infection (d.p.i.), whereas all 4 cats given milk from IgM seropositive goats (acutely infected) shed *T.gondii* oocysts in their faeces 5-7 d.p.i. (Table 4).

Freshly excreted *T.gondii* oocysts measured 9-11μm x 10-12μm. sporulation time was 3days at 27°C. Sporulated and nonsoprulated *T.gondii* oocysts were shown in fig.1.

Table 1: Prevalence of *T. gondii* in the serum and milk of goats as determined by IHAT.

Animal	Examined	Serum		Milk	
		(+ve)	(%)	(+ve)	
Non-lactating	109	38	34.86%	-	-
Lactating does	73	39	53.42%	43	58.90%
Total	192	77	42.30%	43	58.90%

Table 2: Seroprevalence of *T. gondii* antibodies in goats as determined byIHAT.

Examined	(+ve)	IgG	(%)	IgM	(%)
182	77	55	30.22	22	12.09

Table 3: Antibody titers in the serum of Toxoplasma infected goats using IHAT.

Examined	Total (+ve)	1/80	1/160	1/320	1/640	1/1280	1/2560
No.	77	16	29	13	10	6	3
(%)	42.30%	20.7%	37.7%	16.9%	13.0%	7.8%	3.9%

Table 4: *Toxoplasma gondii* oocysts excreted by cats experimentally infected by goat milk.

Source of milk	No. of cats	(+ve)	Prepatent Period (days)	Patent Period (days)
Chronic infection	4	1	5	8
Acute infection	4	4	5-7	8-10
Control (-ve)	2	-	-	-

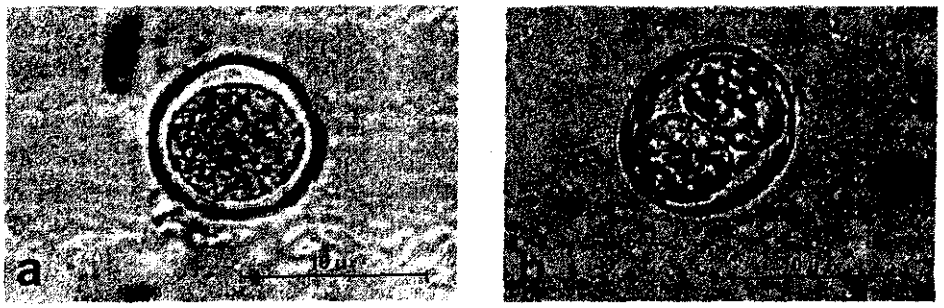

Fig.1: Toxoplasma gondii oocysts, nonsporulated (a), sporulated (a).

DISCUSSION

Toxoplasma gondii is an important zoonotic pathogen causing significant human and animal health problems. Infection in dairy goats not only results in significant reproductive losses, but also represents an important source of human infection due to consumption of infected meat and milk constituting zoonotic transmission (Sacks *et al.*, 1982).

Serologic prevalence data indicate that infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions. High prevalence of infection in France has been related to a preference for eating raw or undercooked meat, while high prevalence in Central America has been related to the frequency of stray cats in a climate favoring survival of oocysts (Ciamak, 2006).

Caprine Toxoplasmosis was reported worldwide (Dubey, 1980; Masala *et al.*, 2003 and Dubey, 2009). Even though few studies have been conducted on milk antibodies (Azab *et al.*, 1992; Haridy *et al.*, 2010),

nothing is known about the detection of *T.gondii* in caprine milk in Egypt.

In the present study, the overall seroprevalence of *T.gondii* infection in goats was 42.30 % using IHAT in Egypt. Similar observations were reported in Egypt (Ghoneim *et al.*, 2010; El-Manyawe *et al.*, 2001; Maronpot and Botros, 1972). The high positivity in IHAT suggest continuous exposure of goats to heavy environmental contamination with oocysts shed from stray cats, poor manage conditions, infected males, and the origin of the animals (Dubey, 1996).

The extent of toxoplasmosis in goat population varied from flock to flock around the world. Unlike our results, lower prevalence were recorded, 19% in Brazil (Josely *et al.*, 2001), 14.1% in China (Zhao *et al.*, 2011), 27% in Egypt (Abou Zeid *et al.*, 2010). Such differences in prevalence may be attributed to diagnostic technique, demographic of goat population, breeding conditions and management (Masala *et al.*, 2003), immune status, timing of infection and genetic composition of the host and the organism (Suzuki, 2000) or distribution and behavior of cats (Buxton, 1990).

Toxoplasma antibodies are detectable two weeks post infection and maintain a low level through the host's life (Lin and Bowman, 1991). Accordingly, testing for IgM antibodies which appear about one week post infection permitted recent infection. The results indicated that 55(30.22%) out of 77 goats had IgG, whereas 22(12.09%) had IgM. This result also agreed with similar study in Italy (Masala *et al.*, 2003). Such value of IgM indicates true infection with *T.gondii* (Camargo *et al.*, 1978).

The antibodies titers of IHAT ranged from 1/80to 1/2560 with the most frequent at 1/160. This result agrees with those reported by AbouZeid *et al.* (2010) and El-Manyawe *et al.* (2010). High values of antibody titers can be ascribed to active toxoplasma infection as well as reactivation of infection due to immunosupressor conditions (Robert *et al.*, 1981).

In the present study we confirmed IHAT on serum samples by application of the test on milk samples as milk antibodies were observed between 7-10 days post infection (Thierry *et al.*, 1990). Toxoplasma antibodies were detected in the milk of naturally infected lactating women (Azab *et al.*, 1992), in working donkeys (Haridy *et al.*, 2010) and experimentally in mice (Thierry *et al.*, 1990). The present study indicated that 53.42% of examined goats had demonstrable antibodies in both milk and serum samples. In fact, the majority of positive (58.9%) were reactive in milk samples.

It has been shown that milk antibodies reflect local antigenic stimuli to the infection, such antibody detection might be of assistance in studies of the endemicity of the disease (Grundy *et al.*, 1983). A comparison of milk and serum reactivity to *T.gondii*is further complicated by the differences in immunoglobulin concentrations between individual milk samples as well as between milk and serum. In endemic areas the systemic antibody response may be boosted by repeated infections, and high-titer serum antibodies may persist for years even in the absence of continuous infection. Whereas the systemic response has a longer-lasting immunological memory, the local immunological memory is short lasting, although reinfection may boost memory of a somewhat longer duration. Therefore, although in an endemic area serum antibodies indicate past or present invasive disease, milk antibodies are more likely to suggest present or recent infection (Grundy *et al.*, 1983). The presence of positive IgMin goat serum samples suggests recently acquired or active infection, demonstrating that these animals can constitute an important source of transmission to man, since they are able to present *T. gondii* tachyzoites in milk.

The organism was detected previously in the milk of experimentally infected cat (Powell *et al.*, 2001), and rat (Costa and Langoni, 2010) both in chronic and acute infections.

Regarding to the comparison between serological results and experimental infection in cats, this study demonstrated that *T. gondii* was found in the milk of both chronically and acutely infected goats. All experimentally

infected cats given milk from seropositive goats to IgM shed *T.gondii* oocysts, whereas only 1 out of 4 cats given milk from seropositive goats to IgG shed *T. gondii* oocysts in their feaces. So, there were complete concordance between IgM antibodies and experimental infection in cats.

The presence of the organism in the milk of chronically infected goats may be due to the resurgence of tissue *T. gondii* tachyzoites cysts which can circulate again and be excreted in the milk during the peripartum period. Similar results were obtained by Camossi *et al.* (2011).

Previous studies indicated that consumption of raw milk from infected goats represent a vehicle for transmission of toxoplasmosis as unpasteurized milk is considered an important food source in rural areas (Riemann *et al.*, 1975 and Sacks *et al.*, 1982). It is particularly important in infants than in adults. It has been shown that milk promoted higher infectivity and mortality, revealing probably better preservation or efficiency of *T.gondii* as milk nutrients maintains tachyzoites viable for longer time and protects them from gastric juice. So, milk could be considered a potential source of *T.gondii* transmission, especially in rural areas where there's no pasteurization (Gross *et al.*, 1996).

Therefore, caprine toxoplasmosis deserves special attention of the public health organizations in order to advise the population on the real situation of the infection in the country through frequent serological surveys, since this disease often occurs as subclinical form in man and animals.

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