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# Research Article
# Detection of *Toxoplasma gondii* from Wastage Nourished Small Ruminant and Poultry: Zoonotic Significance

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## Abstract

**Background:** The wastages nourished small ruminants and poultry are still free fed on street wastages and possibly exposed to *T. gondii* oocysts through feces of outdoor shedder cats and they are regarded as high prevalent sources for human toxoplasmosis via their meat containing viable *T. gondii* tissue cysts. **Materials and Methods:** A total No. of 859 samples of both blood and their matching tissue were collected from wastages nourished 455 sheep, 237 goats, 124 chickens and 43 ducks respectively from Giza governorate, Egypt. All animals were assayed serologically using Latex Agglutination Test (LAT) as a screen test and the results were confirmed by ELISA. Tissue samples which were identical to seropositive sera were digested and microscopically examined and exposed to DNA confirmation. The microscopic definite bradyzoites containing sera were bio-assayed through intra-peritoneal passage in mice as viability test to determine both $LD_{50}$ and $LD_{100}$ for each species isolate. Histopathological examination was done on symptomatic morbid and dead mice. **Results:** Corresponding to small ruminants and poultry, results of seropositive percentages were 47.5 and 29.3%, total microscopic 30.1 and 32.7%, DNA detection 74.8 and 71.4% and the total percentages of mice viability test 39.4 and 31.3%. In Addition, the total percentages of $LD_{50}$ were 30.3 and 31.3%, while the $LD_{100}$ were 9.1 and 0% in small ruminants and poultry respectively. The histopathological examination of inoculated mice signified cyst forming *T. gondii* in acute and chronic lesions within vital organs. **Conclusion:** The wastages nourished small ruminants and poultry is of zoonotic impact and significance and must be directed for incriminate this animal feeding pattern and for avoiding consumption under cooked meat of animals or birds.

Key words: *Toxoplasma gondii*, Egyptian sheep and goat, histopathology, ELISA, seropositive

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Postnatal human's toxoplasmosis is mainly through consuming undercooked meat containing tissue cysts plus to food or water contaminated with environmental oocysts shed in cat feces[1]. Also, opportunistic toxoplasmosis during pregnancy is possibly series to estradiol hormonal shift[2] or due to temporary gravidity hyperglycemia[3] in addition to anti-inflammatory corticosteroids therapy[4].

Meat tissue cysts signify the main source for human toxoplasmosis, attributable to its persistence viability in under cooked tissues[5]. Consequence to the high cost of animal ration in growing countries, some animals and poultry flocks are still free nourished on street wastages of human residuals and share habitats with the *T. gondii* oocysts shedder cats in their subset. So, the wastages nourished animals or poultry possible represent higher prevalent source of human toxoplasmosis through eating inadequately cooked meat containing tissue cysts[6].

Little is known of the biological characters of isolates from the Middle East and Africa[7]. Moreover, there have been relatively few recent reports on Egyptian small ruminants and poultry concerning toxoplasmosis[8-10]. Serological assay is not enough for confirming *T. gondii* tissue cysts harboring animals. However, the present study aimed to evaluate the zoonotic bio-hazard of seropositive wastages nourished animals and poultry through mice viability test and DNA recognition along with histopathological examination of inoculated mice reflecting to how extent the possible human infection via meat harboring virulent tissue cysts.

## MATERIALS AND METHODS

**Blood and tissue samples:** A total number of 859 blood samples plus its corresponding tissue samples were taken from wastage nourished Egyptian sheep (455), goats (237), chickens (124) and duck flocks (43) in different rural areas in Giza governorate, Egypt.

**Serological assays:** Sera were separated, labeled and kept at -20°C until examined serologically against toxoplasma infection by Latex Agglutination Test (LAT) according to the manufacturer's instructions (Toxocheck-MT, Eiken Chemical, Tokyo, Japan), results were considered positive when agglutination observed at dilutions of 1:64 and greater. Also, enzyme linked immunosorbent assay (ELISA) was carried out according to the method described by Aubert *et al.*[11] using soluble crude antigen prepared from *Toxoplasma* RH strain tachyzoites.

**Preparation of meat samples and digestion:** Tissue samples were prepared as described elsewhere[12,13]. Twenty grams of the equivalent tissue samples from diaphragm and thigh muscles were collected from small ruminants during individual slaughtering outside abattoirs and from various batches of free range chickens and ducks from diverse villages in rural areas in Giza governorate, Egypt. The tissues were cut into small cubes about 5×5×5 cm and classified into two groups, the 1st was frozen at -80°C for further DNA extraction while the 2nd group was exposed to pepsin digestion and then bio-assayed for both microscopic examination and mice viability test.

**Viability test with $LD_{50}$ and $LD_{100}$ determination in mice:** The test procedures were done according to Elfadaly *et al.*[2], a total number of 115 seronegative Swiss Webster Albino mice obtained from Laboratory Animals House, National Research Centre, Egypt were classified corresponding to animals and poultry isolates as 74 for sheep, 25 for goats, 11 for chickens and 5 for ducks. The inoculated mice were followed up daily for any apparent clinical signs of febrile response or acute toxoplasmosis with the exclusion of dead mice before 48 h of inoculation. The predicted signs may be varied between ascites, roughcast hairs, tottering gait, hunched appearance, with evidence of early emaciation and dehydration or death usually within 72-84 h (with highly virulent types) or nervous manifestations and partial off food (with moderately virulent types). Depending on the virulence of the isolate, the procedure will be continued. If ascites occurred, peritoneal exudates were collected from ascetic mice within 72-84 h DPI for microscopic examination for tachyzoites. If mice didn't show ascites they were sacrificed within 15th day post inoculation by cervical dislocation, samples were collected from heart, lung, liver, spleen and kidney for histopathological examination according to Ajzenberg *et al.*[14]. The $LD_{50}$ and $LD_{100}$ characters are differentiate the dissimilar virulent among varied isolates which could kill 50 or 100% of inoculated mice, respectively were recorded for each species isolates.

**DNA detection:** Preparation of specimens and isolation of DNA for PCR was carried out according to Burg *et al.*[15].

**Extraction of genomic DNA of *T. gondii* from the collected tissue:** Samples were extracted by a kit (ViVantis Co., Malaysia).

**PCR amplification of B1 gene:** The B1 gene was amplified[16], using primers 1 (5'-TCG GAG AGA GAA GTT CGTCGC AT-3') and 2 (5'-AGC CTC TCT CTT CAA GCA GCG TA-3'). The following reaction mixture was added in a 0.2 mL PCR tubes: DNA

template (100 ng $\mu L^{-1}$) 10 µL, Taq polymerase (5 µ $\mu L^{-1}$) 1 µL, 10x enzyme buffer 2 µL, dNTPs 0.8 µL, each primer 1 µL and Bidest water to 20 µL. The mixture was briefly spine and placed in the thermal cycler (T-gradient, Biometra, Germany), which was programmed as follow: Initial denaturing 95°C/2 min and 40 cycles consisting of denaturing 95°C/1 min, annealing 55°C/30 sec, extension 72°C/45 sec and final extension 72°C/10 min. The PCR products were electrophoresed at 80 v/15 min and finally examined using UV trans-illuminator. About 100 bp DNA ladder (Finnzymes) was used as a marker.

**Histopathological methods:** Tissue specimens about 0.5 $cm^3$ were individually obtained from lung, liver, spleen, brain, kidney and cardiac muscles of inoculated mice, fixed in 10% neutral buffered formal saline solution, sectioned and further processed according to conventional routine histological technique as described by Biancifiori *et al.*[17], including cutting at 5 µm and staining with hematoxylin and eosin (H and E), Periodic Acid-Schiff (PAS) and toluidine blue stains. The stained tissue sections were examined by optical microscope at power magnification of x400 and the protozoal associated lesions, pathological alterations and the *T. gondii* bradyzoites were scored.

**Ethical approval:** The study was approved ethically by the Medical Research Ethics Committee, National Research Centre, Egypt under registration No. 1-2 /0-2-1.2012.

## RESULTS

The serologic, microscopic, DNA, mice viability and $LD_{50}$ and $LD_{100}$ results were displayed in Table 1.

Seropositive percentages of *T. gondii* infection were recorded as 51.9, 39.2, 29 and 30.2% corresponding to sheep, goats, chickens and ducks with total seropositive percentages within small ruminants and poultry 47.5 and 29.3%, respectively.

Histopathological lesions of various vital organs of acute infected mice within 12 DPI through *T. gondii* isolates from small ruminants or poultry were recorded. The mice organs showed tachyzoites infiltration: Kidney, peritoneal exudates tachyzoites and leukocytes at acute phase 72 HPI, spleen, liver, cardiac muscles and lung (Fig. 1).

Table 1: Seropositive, microscopic examination, DNA detection, mice viability and $LD_{50}$ and $LD_{100}$ of small ruminants and poultry examined samples

| Groups | No. of samples | Seropositive/ total (%) | Microscopic/ seropositive (%) | DNA detection/ seropositive (%) | Mice viability/ microscopic (%) | $LD_{50}$ (%) | $LD_{100}$ (%) | Geno-typing |
|---|---|---|---|---|---|---|---|---|
| Sheep | 455 | 236/455 (51.9) | 74/236 (31.4) | 178/236 (75.4) | 28/74 (37.8) | 23 (31.1) | 5 (6.7) | I and II |
| Goat | 237 | 93/237 (39.2) | 25/93 (26.9) | 68/93 (73.1) | 11/25 (44) | 7 (28) | 4 (16) | I and II |
| Total small ruminants | 692 | 329/692 (47.5) | 99/329 (30.1) | 246/329 (74.8) | 39/99 (39.4) | 30 (30.3) | 9 (9.1) | - |
| Chickens | 124 | 36/124 (29) | 11/36 (30.5) | 26/36 (72.2) | 3/11 (27.3) | 3 (27.3) | - | II |
| Ducks | 43 | 13/43 (30.2) | 5 /13 (38.5) | 9/13 (69.2) | 2/5 (40) | 2 (40) | - | II |
| Total poultry | 167 | 49/167 (29.3) | 16/49 (32.7) | 35/49 (71.4) | 5/16 (31.3) | 5 (31.3) | - | - |

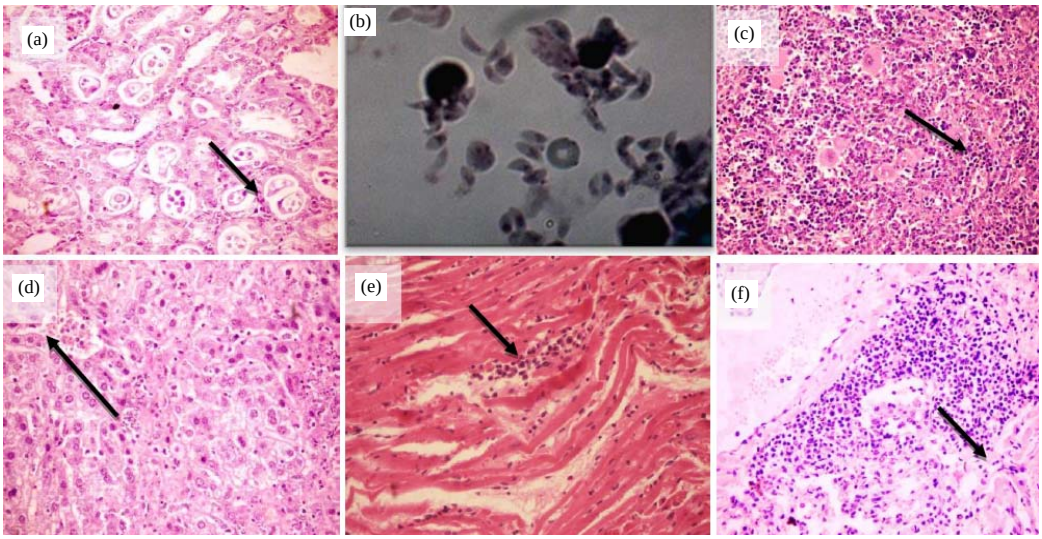


Fig. 1(a-f): Histopathological lesions of various vital organs of acute infected mice within12 DPI with *T. gondii* isolates from small ruminants or poultry. The mice organs show tachyzoites infiltration, (a) Kidney, (b) Mice peritoneal exudates tachyzoites and leukocytes at acute phase 72 HPI, (c) Spleen, (d) Liver, (e) Cardiac muscles and (f) Lung (X400)

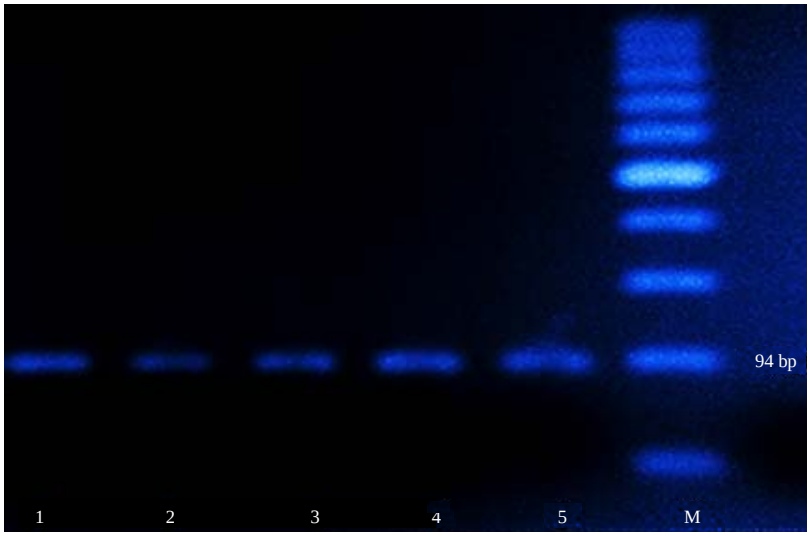


Fig. 2: An agarose gel electrophoresis showing PCR amplification product of *T. gondii* using B1 gene. Lane M: Marker, Lane 1: Positive control, Lanes 2, 3: Positive *T. gondii* DNA Egyptian isolates at 94 bp corresponding to sheep and goat, Lanes 4, 5: Positive samples of isolated chicken and ducks isolates at 94 bp

The microscopic examination and DNA detection of *T. gondii* were only performed on the equivalent digested tissue samples of positive sera, the percentage values were 31.4 and 75.4, 26.9 and 73.1, 30.5 and 72.2 and 38.5 and 69.2 corresponding to sheep, goats, chickens and ducks, respectively and the total microscopic and DNA detection percentages within small ruminants and poultry were 30.1, 74.8 and 32.7, 71.4%, respectively (Fig. 2).

The mice viability test of *T. gondii* was only concerned with the microscopic positive tissue samples which were showing bradyzoites similar protozoa, the recorded values and percentages were 28/74 (37.8) sheep, 11/25 (44) goat, 3/11 (27.3) chickens and 2/5 (40) ducks (Table 1, Fig. 1, 2), the total percentages of mice viability test were 39.4 and 31.3 corresponding to small ruminants and poultry, respectively.

The *T. gondii* $LD_{50}$ and $LD_{100}$ were recorded varied percentage values 31.1 and 6.7, 28 and 16, 27.3 and 0 and 40 and 0 with the corresponding to sheep, goats, chickens and ducks, respectively with total percentages within small ruminants and poultry 30.3, 9.1 and 31.3, 0, respectively.

## DISCUSSION

Up to 60% of Egyptians are *T. gondii* seropositive[3]. This higher ratio may be linked to consuming undercooked mutton or poultry meat rather than oocysts contaminated food or water. The current study supports this concept through definite wastages nourished small ruminants and poultry as a great source of toxoplasmosis through overall higher seropositive ratios 47.5 and 29.3, respectively. While in the previous Egyptian study; sheep was recorded lower seropositive value 34%[18], the higher difference between the two studies possible contributed to the wastage nourished feeding behavior, which usually maximize exposure with sporulated *T. gondii* oocysts[13]. Also, the results agree with Dubey *et al*.[8] who were run with our results and overcome high prevalence in Egyptian free range chickens 40.4 and ducks 15% from rural area surrounding Giza. This probably due to free range poultry mainly infected via environmental oocysts. Moreover, they like omnivore's species, possible feed on tissue cyst through engulfing raw meat remaining[19].

The obtained results denote that not all seropositive animals or birds were harboring virulent *T. gondii* bradyzoites in their tissues. Accurately; serological assay does not reveal tissue cyst harboring hosts. So, the current study evaluated the zoonotic bio-hazard of seropositive small ruminants and poultry through mouse viability test and histopathology of inoculated mice along with DNA recognition, act as specific *T. gondii* bio-indicators for the possible human infection. However, the main significant factors shared in non-synchronized results between seropositive hosts and their tissues containing cysts probably due to the transferred maternal antibodies or sequence to incompatibility between the collected examined samples with the carcasses/tissue cyst distribution[2], besides the possible cross-reactivity with other parasites as *Trichinella spiralis*[20]. However, mouse viability test is accurate and sensitive than DNA or microscopic tests, it

firmly excludes the possible presence of other *T. gondii* cyst forming protozoa or *T. gondii* non virulent strains which could be exciting PCR cycle through DNA similar sequence but they could not induce morbid mice.

According to mice viability and histopathology tests, goats were recorded the highest species harboring *T. gondii* tissue cysts (44%), followed by sheep (37.8%), while poultry was recorded the lowest value (31.3%). The results agree with most studies which signified sheep and goats as the most *T. gondii* susceptible species[21,22]. The equivalent digested tissue samples of small ruminants and poultry were confirmed DNA positive and reflect higher PCR sensitivity than the inferior microscopic ones.

The $LD_{50}$ and $LD_{100}$ are the lethal dosages that differentiate the dissimilar virulent among varied isolates which could kill 50 or 100% of inoculated mice respectively. It has been suggested that type I and II are more prevalent in clinical toxoplasmosis[16]. The results in current study signified a total 39.4% of small ruminants and 31.3% of poultry isolates were successfully passed in to mice with dissimilar morbidity and mortality ratios. Also, small ruminants were set higher in total $LD_{50}$ and $LD_{100}$ 30.3 and 9.1% than poultry 31.3 and 0%. Our results are corporate other studies which validated the vast majority of virulent Egyptian sheep and poultry isolates were corresponding to type II strains which the predominant human types[6]. Also, from São Paulo state, Brazil, *T. gondii* was isolated from tissue homogenates of 16 sheep, where six of the 16 isolates killed 100% of infected mice[23].

Mixed infection in the same meat sample may be involved but higher virulent types hides the biological properties of less virulent ones[24]. Therefore, the parasite isolation practice could results in a single strain even several strains were found in the inoculum.

## CONCLUSION

The bio-viability and histopathology along with DNA confirm of seropositive hosts are on target *T. gondii* tissue cysts bio-indicators, signify zoonotic impact of wastages nourished small ruminants and poultry, must be directed for incriminate this animal feeding pattern and for avoiding consumption under cooked meat of animals or birds.

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## REFERENCES

1. Tenter, A.M., A.R. Heckeroth and L.M. Weiss, 2000. *Toxoplasma gondii*: From animals to humans. Int. J. Parasitol., 30: 1217-1258.
2. Elfadaly, H.A., M.A. Hassanain, R.M. Shaapan, A.M. Barakat and N.I. Toaleb, 2012. Serological and hormonal assays of murine materno-fetal *Toxoplasma gondii* infection with emphasis on virulent strains. World J. Med. Sci., 7: 248-254.
3. Hassanain, M.A., H.A. El-Fadaly and N.A. Hassanain, 2014. *Toxoplasma gondii* parasite load elevation in diabetic rats as latent opportunistic character. Ann. Trop. Med. Pubblic Health, 7: 110-115.
4. Elfadaly, H.A., M.A. Hassanain, R.M. Shaapan, N.A. Hassanain and A.M. Barakat, 2015. Corticosteroids opportunist higher *Toxoplasma gondii* brain cysts in latent infected mice. Int. J. Zool. Res., 11: 169-176.
5. Fromont, E.G., B. Riche and M. Rabilloud, 2009. *Toxoplasma* seroprevalence in a rural population in France: Detection of a household effect. BMC Infect Dis., Vol. 9. 10.1186/1471-2334-9-76.
6. Hassanain, M.A., H.A. Elfadaly, R.M. Shaapan, N.A. Hassanain and A.M. Barakat, 2011. Biological assay of *Toxoplasma gondii* Egyptian mutton isolates. Int. J. Zool. Res., 7: 330-337.
7. Dubey, J.P., 2009. Toxoplasmosis in sheep-The last 20 years. Vet. Parasitol., 163: 1-14.
8. Dubey, J.P., D.H. Graham, E. Dahl, M. Hilali and A. El-Ghaysh *et al*., 2003. Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol., 114: 89-95.
9. Barakat, A.M.A., M.M. Abd Elaziz and H.A. Fadaly, 2009. Comparative diagnosis of toxoplasmosis in Egyptian small ruminants by indirect hemagglutination assay and ELISA. Global Veterinaria, 3: 9-14.
10. Ghoneim, N.H., S.I. Shalaby, N.A. Hassanain, G.S.G. Zeedan, Y.A. Soliman and A.M. Abdalhamed, 2010. Comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt. Foodborne Pathog. Dis., 7: 17-22.
11. Aubert, D., G.T. Maine, I. Villena, J.C. Hunt and L. Howard *et al*., 2000. Recombinant antigens to detect *Toxoplasma gondii*-specific immunoglobulin G and immunoglobulin M in human sera by enzyme immunoassay. J. Clin. Microbiol., 38: 1144-1150.
12. Sharma, S.P. and J.P. Dubey, 1981. Quantitative survival of *Toxoplasma gondii* tachyzoites and bradyzoites in pepsin and in trypsin solutions. Am. J. Vet. Res., 42: 128-130.
13. Shaapan, R.M. and H.A. Elfadaly, 2015. Latency in Toxoplasmosis. LAP Lambert Academic Publishing, Germany, ISBN-13: 978-3659761348, Pages: 72.

14. Ajzenberg, D., A.L. Banuls, M. Tibayrenc and M.L. Darde, 2002. Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int. J. Parasitol., 32: 27-38.
15. Burg, J.L., C.M. Grover, P. Pouletty and J.C. Boothroyd, 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbiol., 27: 1787-1792.
16. Howe, D.K., S. Honore, F. Derouin and L.D. Sibley, 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J. Clin. Microbiol., 35: 1411-1414.
17. Biancifiori, F., C. Rondini, V. Grelloni and T. Frescura, 1986. Avian toxoplasmosis: Experimental infection of chicken and pigeon. Comp. Immunol. Microbiol. Infect. Dis., 9: 337-346.
18. Shaapan, R.M., F.A. El-Nawawi and M.A.A. Tawfik, 2008. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Vet. Parasitol., 153: 359-362.
19. Cook, A.J., R.E. Gilbert, W. Buffolano, J. Zufferey and E. Petersen *et al*., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. Br. Med. J., 321: 142-147.
20. Hassanain, M.A., N.A Hassanain, R.M. Shaapan, H.A. El Fadaly and F.M. El Mogazy, 2009. Problem of toxoplasmosis and detection of possible cross-reactivity with *Trichinella spiralis*. Proceedings of the 14th Congress of the International Society for Animal Hygiene, July 19-23, 2009, Vechta, Germany, pp: 97-100.
21. Aspinall, T.V., D. Marlee, J.E. Hyde and P.F.G. Sims, 2002. Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction-food for thought? Int. J. Parasitol., 32: 1193-1199.
22. Dubey, J.P., N. Sundar, D. Hill, G.V. Velmurugan and L.A. Bandini *et al*., 2008. High prevalence and abundant atypical genotypes of *Toxoplasma gondii* isolated from lambs destined for human consumption in the USA. Int. J. Parasitol., 38: 999-1006.
23. Boyer, K.M., E. Holfels, N. Roizen, C. Swisher and D. Mack *et al*., 2005. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. Am. J. Obstet. Gynecol., 192: 564-571.
24. Aspinall, T.V., E.C. Guy, K.E. Roberts, D.H.M. Joynson, J.E. Hyde and P.F.G. Sims, 2003. Molecular evidence for multiple *Toxoplasma gondii* infections in individual patients in England and Wales: Public health implications. Int. J. Parasitol., 33: 97-103.