

**Original Article**

# **Histopathological and Serological Analysis of Aborted Ewes and Neonatal Death with *Toxoplasma gondii* in Duhok City, Kurdistan-Iraq**

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Received 05 November 2019; Accepted 21 December 2019

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## **ABSTRACT**

This study was carried out on seven flocks of ewes suffered from late abortion and neonatal mortality with the prevalence rate of infection reported as 13.95%. The blood and tissue samples were collected from the aborted ewes in several flocks of Duhok province, Kurdistan Region, Iraq. Serological analysis indicated that all the aborted ewes were confirmed positive for agglutination to *Toxoplasma gondii* (*T. gondii*) antibody. The investigation of the aborted fetuses showed the blood-stained fluid in the thoracic and abdominal cavity. Most of the aborted fetuses had also enlarged, congested, and friable livers and lungs. The placenta was swollen, reddish, and friable, and its cotyledons also spotted with whitish foci. *T. gondii* tachyzoites were also demonstrated in the placental sections of some aborted ewes. Severe congestion, necrosis, and infiltration of multinucleated cells were the most predominant histopathological changes of the aborted fetuses, as well as presented tissue cysts, tachyzoites, and bradyzoites in the liver, brain, heart, and lung. There were also several clusters of dark purple banana-shaped *T. gondii* tachyzoites within the brain and heart tissues in most of the examined aborted fetuses in different flocks. *T. gondii* tachyzoites were also detected from the peritoneal ascites of mice inoculated experimentally 12 days following the infection. Moreover, *T. gondii* tissue cysts were detected from the impression smears of the mice brains 32 days after the infection. Accordingly, the demonstration of *T. gondii* in Giemsa-stained impression smears associated with characteristic histopathological changes of different organs is a great fundamental method for the diagnosis of *T. gondii* in aborted cases.

**Keywords:** *Toxoplasma gondii*, Abortion, Ewes, Serology, Histopathology

## **Analyse Histopathologique et Sérologique des Brebis Avortées et de la Mort Néonatale Causée par *Toxoplasma gondii* dans la Ville de Duhok, Kurdistan-Irak**

**Résumé:** Cette étude a été réalisée sur sept troupeaux de brebis souffrant d'avortement tardif et de mortalité néonatale avec un taux de prévalence d'infection rapporté à 13,95%. Les échantillons de sang et de tissus ont été prélevés sur les brebis avortées dans plusieurs troupeaux de la province de Duhok, région du Kurdistan, en Irak. L'analyse sérologique a indiqué que toutes les brebis avortées étaient confirmées positives pour l'agglutination à l'anticorps *Toxoplasma gondii* (*T. gondii*). L'enquête sur les fœtus avortés a montré le liquide taché de sang dans la cavité thoracique et abdominale. La plupart des fœtus avortés avaient également des foies et des poumons agrandis, congestionnés et friables. Le placenta était enflé, rougeâtre et friable, et ses cotylédons étaient également tachetés de foyers blanchâtres. Des tachyzoïtes de *T. gondii* ont également été mis en évidence dans les coupes placentaires de certaines brebis avortées. Les changements histopathologiques les plus

prédominants chez les fœtus avortés étaient une congestion sévère, une nécrose et l'infiltration des cellules multinucléées ainsi que les kystes tissulaires, les tachyzoïtes et les bradyzoïtes présents dans le foie, le cerveau, le cœur et les poumons. Il y avait également plusieurs grappes de tachyzoïtes de *T. gondii* en forme de banane violette foncée dans les tissus du cerveau et du cœur dans la plupart des fœtus avortés examinés dans différents troupeaux. Des tachyzoïtes de *T. gondii* ont également été détectés dans l'ascite péritonéale de souris inoculées expérimentalement 12 jours après l'infection. De plus, des kystes tissulaires de *T. gondii* ont été détectés 32 jours après l'infection à partir des frottis du cerveau des souris. En conséquence, l'examen des frottis colorés au Giemsa associés, permettant la détection des changements histopathologiques caractéristiques aux différents organes, est une méthode de choix et fondamentale pour le diagnostic de *T. gondii* dans des cas avortés.

**Mots-clés:** *Toxoplasma gondii*, Avortement, Brebis, Sérologie, Histopathologie

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## INTRODUCTION

Toxoplasmosis is an important zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*), a ubiquitous protozoan parasite that uses felids as definitive hosts and several species of vertebrate animals as intermediate hosts (Dubey, 2009). Toxoplasmosis is a major cause of reproductive failure associated with abortion in sheep and goats (Tenter et al., 2000). In sheep, the abortion or prenatal mortality of lambs occurs when ewes suffer a primary infection during pregnancy (Tenter et al., 2000). Abortion and neonatal mortality are the most important economic losses of sheep and goat industries worldwide (Dubey, 1996). The lambs or kids may die in the uterus and after delivery (i.e., stillbirth) or succumb within a few days of birth. Sheep are globally considered important in the epidemiology of *T. gondii* infection, especially in Europe (Buxton et al., 2007). Sheep are also essential to the economy of most countries because they are considered sources of food for human consumption. The *T. gondii* infection of sheep and goat poses a risk to public health and causes economic losses due to reproductive failure (Edwards and Dubey, 2013). The diagnosis of toxoplasmosis may be established through a primary exposure history of animals to *Toxoplasma* infection during pregnancy or white foci or nodules visible on the cotyledons, livers, or tissues of aborted

fetuses (Schlafer and Miller, 2007). Histologically, necrotic foci are observed in the white matter of the brain and cotyledons. The results of serology indicate exposure to the parasites, and elevated immunoglobulin M (IgM) or rising immunoglobulin G (IgG) titers suggest *Toxoplasma* abortion. The serologic activity of the fetus shows the organisms that cross the placenta because the maternal antibody (i.e., IgG) does not cross the ruminant placenta; accordingly, the observation of the organism in tissue confirms infection. Several serological diagnostic tests, including indirect immunofluorescence (van der Puije et al., 2000), indirect haemagglutination test (Nieto and Melendez, 1998), latex agglutination test (Hashemi-Fesharki, 1996), and enzyme-linked immunosorbent assay (Subedi et al., 2018), are used to establish *Toxoplasma* infection. With this background in mind, the present study was conducted to estimate the extent to which abortion and neonatal mortality in ewes were associated with *T. gondii* infection using histopathological and serological investigations.

## MATERIAL AND METHODS

**Study Design and Sampling.** This study was carried out in different areas of Duhok province, Kurdistan Region, Iraq, within October 2016 to November 2017. The serum samples were collected from a total of 211 aborted ewes in seven different flocks of Duhok

province. The sampled ewes constituted about one-third of all the ewes in which abortion or stillbirth has recently occurred. For the separation of serum, a blood sample of 5 ml was obtained from each animal by jugular venipuncture in nonheparinized vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, N.J., USA). The sera were then separated and stored at -20°C for the analysis. Five of the aborted fetuses were necropsied from each flock of the sheep to examine the gross lesions; however, the samples of their placentae, brains, livers, and lungs were directly submitted for microscopic (i.e., Giemsa-stained impression smear) and histopathological examinations.

#### **Experimental infection of mice with *T. gondii*.**

Albino laboratory mice about 6 weeks old and weighing 20-25 g were investigated from the College of Veterinary Medicine, University of Duhok. All the mice were acclimatized under laboratory conditions for a week before the initiation of the experiment. The mice were kept in clean cages and fed on pelleted food and water. These mice were used for the bioassay of the aborted infected tissues in order to isolate *T. gondii* tachyzoites from the peritoneal cavity or obtain *T. gondii* tissue cysts containing bradyzoites from their brains, lungs, and livers. The inoculation was performed on the tissues taken from the small samples of the liver, lung, and brain of the aborted fetuses as described by Dubey et al. (2002), with several modifications. The tissues were homogenized in a sterile mortar and then suspended in normal saline solution at a ratio of 1 gm tissue: 2.5 ml normal saline. To prevent bacterial contamination, 1000 and 100 mg of penicillin and streptomycin were added per milliliter of tissue homogenate, respectively. The heavy material was removed by standing at room temperature at least for 1 hr, and supernatant fluid (0.5 ml) was intraperitoneally inoculated into every five mice. The mice were then daily monitored for illness. In the control group, every five mice were inoculated with 0.5 ml of phosphate-buffered saline. Afterward, the peritoneal ascites were examined by direct wet smears

from the infected mice for *T. gondii* isolates immediately following the observation of clear clinical signs. Imprinted smears were also obtained from the livers, lungs, or brains and hearts of slaughtered mice and then stained by 10% Giemsa in order to demonstrate tachyzoites and/or tissue cysts. The inoculated mice were considered infected with *T. gondii* since the observation of tachyzoites or tissue cysts in the examined tissues.

**Microscopic examination (i.e., tissue impression smears).** *T. gondii* infection was initially diagnosed by Giemsa-stained impression smears obtained from different organs of the fetus. The samples were collected from the cut surface of the brain, lung, heart, and liver of the aborted fetus for the identification of *T. gondii* infection stages, including cyst, tachyzoite, and bradyzoite (Soulsby, 1986). Briefly, the impression smears were fixed with 70% methanol for 5 min, directly stained with 10% Giemsa for 30 min, and then examined for the presence of *T. gondii* stages under oil immersion lens (100×).

**Serological examination (i.e., latex agglutination test).** *T. gondii* antibody was detected in the infected sera by latex agglutination test using a Toxoplasmosis Latex Test Kit (Plasmatec Laboratory Products Ltd, UK). The TOXO-Latex reagent is a suspension of polystyrene latex particles coated with soluble *T. gondii* antigen. Latex particles allow the observation of the antigen-antibody reaction. If specific anti-*Toxoplasma* antibodies are present in the tested sera, they will react to soluble *T. gondii* antigen in the latex reagent and be visualized by latex particles as a clear agglutination after 5 min of the slide rotation due to the presence of *Toxoplasma* antibodies > 4 IU/ml.

**Histopathological examination.** The portions of different organs (including the liver, lung, heart, and brain) obtained from the aborted fetuses were firstly fixed with 10% formalin and then dehydrated in ethyl alcohol with increasing concentration (from 70% to 100%). Afterward, the samples were embedded in paraffin wax using routine tissue processing. From each

block, the tissue sections of 4  $\mu\text{m}$  thicknesses were cut using a rotary microtome (Leica RM2235, Biosystems, Leica Biosystem Company, Germany), deparaffinized, rehydrated, and stained with hematoxylin and eosin in order to observe histopathological changes in the placenta, brain, heart, liver, and lungs. The entire slide was also investigated for *T. gondii* tissue cysts, including bradyzoites and tachyzoites, for the presence of histopathological section (Olympus BX40, objective 40X).

## RESULTS

**Clinical symptoms.** Within October to April 2017, the ewes in different flocks of Duhok province witnessed the successive waves of abortion and stillbirth in the late stage of gestation during the breeding seasons. Most abortion cases occurred during the late stage of gestation (>4 months) in the ewes of 1-3 years of age, and some of the aborted ewes suffered from depression, dyspnea, pyrexia, and vaginal discharge shortly before the abortion.

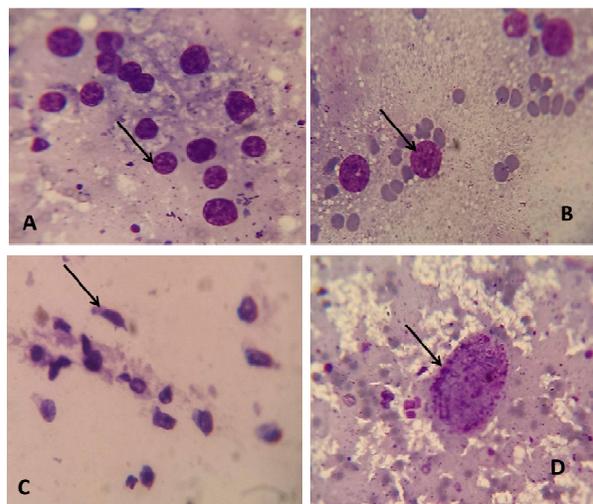
**Abortion rate due to toxoplasmosis in sheep of different flocks.** Out of 1,516 ewes, 211 (13.9%) sheep were aborted in seven different flocks of Duhok province (Table 1). This incidence indicated an infectious cause of active toxoplasmosis. All the aborted animals of different flocks were confirmed positive for *T. gondii* antibody infection immunologically in the primary diagnosis of toxoplasmosis using latex agglutination test but with different degrees of agglutination and microscopically by Giemsa-stained impression smears of the aborted organs.

**Microscopic examination.** The microscopic examination of Giemsa-stained impression smears obtained from the lungs, livers, brains, and hearts of the aborted fetuses in different flocks of sheep infected with toxoplasmosis showed the stages of *T. gondii* including cysts, bradyzoites, and tachyzoites (Figure 1). According to morphological characteristics, the isolated tachyzoites appeared dark-purple crescent or banana-shaped with nucleus and cytoplasm, pointed at one end and round or blunt at another one (Figure 1-C).

**Isolation of *T. gondii* infection stages in mice tissues.** The bioassay of the suspected infected tissues was conducted in mice to isolate *T. gondii* tachyzoites from the peritoneal cavity or obtain the *T. gondii* tissue cysts containing bradyzoites from their organs. *T. gondii* tachyzoites were successfully isolated from the peritoneal ascites of mice inoculated 12 days after the infection with the organs of the homogenized aborted ewe. However, *T. gondii* tissue cysts containing bradyzoites were detected in the brain tissues of the mice 32 days following the infection.

**Table 1.** Rate of abortion in infected sheep with toxoplasmosis in seven flocks

Flocks	Sheep (n)	Abortion (n)	Abortion (%)
F1	230	30	13.04
F2	156	45	28.85
F3	300	35	11.66
F4	200	40	20
F5	220	25	11.36
F6	235	26	11.06
F7	175	10	5.71
<b>Total</b>	<b>1516</b>	<b>211</b>	<b>13.9</b>



**Figure 1.** Giemsa staining of *Toxoplasma gondii*; A) and B) tissue cyst; C) tachyzoites (typically crescent-shaped with a prominent centrally-placed nucleus); D) bradyzoites (tissue cyst separated from host tissue by homogenization of infected brain, lung, and liver).

**Pathological and Histopathological Findings.** The postmortem examination of the aborted fetuses in the late gestational stage showed the generalized blood-

stained fluid in their thoracic and abdominal cavities. Most of the aborted fetuses were reported with enlarged, congested, and friable livers and lungs. Furthermore, the placenta was swollen, reddish, and friable, and its cotyledons were speckled with whitish foci or nodules of necrosis. In most flocks, the lambs born within 1 to 3 days were dead (i.e., stillbirth). Intracellular *T. gondii* tachyzoites were demonstrated in the placental sections of some aborted fetus. Histopathological examinations showed severe fatty change (i.e., fatty droplets) and congestion of the liver in aborted animals. There was also the infiltration of multinucleated inflammatory cells, including Kupffer cells surrounding the portal vein and artery. Moreover, the liver showed disseminated irregular multiple necrotic foci throughout the hepatic lobules associated with the dissociation of hepatic cords. Toxoplasmosis stages, especially tissue cyst, were also presented and distributed through the liver. Furthermore, the histopathological investigation also revealed severe congestion and necrosis of the brain, lung, and heart of the aborted fetus with the mild infiltration of inflammatory cells and presented different stages of toxoplasmosis, including tissue cysts.

## DISCUSSION

Toxoplasmosis is a global zoonosis caused by *T. gondii* as an intracellular apicomplexan parasite. Sheep are economically important in many countries due to the production of meat and milk for human consumption (Ragozo et al., 2010). Sheep rearing constitutes an important agricultural industry with a huge economy in Kurdistan Region. Sheep are commonly infected with tissue protozoa parasite; in this regard, the *T. gondii* infection of sheep and goat poses a risk to public health in the world and causes significant economic losses to farm animals due to reproductive failure (Buxton et al., 2007; Edwards and Dubey, 2013). Reproductive failure has a negative influence on animal production, welfare, health, and rural economy.

Abortion and neonatal mortality are among the most important economic losses to sheep and goat industries worldwide (Buxton et al., 2007). Such cases were observed among the studied area in different farm animals due to *T. gondii* infection. Therefore, the present study was performed to confirm toxoplasmosis as the main etiological cause of abortion in flocks of sheep using serological, microscopic, and histopathological examinations. In the present study, the abortion rate of different sheep flocks in Duhok province was 13.9% (n=211) due to *T. gondii* infection that showed a serious economic effect on the fertility and overall reproductive failure (i.e., abortion) of the involved sheep flocks. All the serum samples of the aborted ewes were also confirmed positive for agglutination to *T. gondii* antibodies by latex agglutination test and Giemsa-stained impression smears of the organs. Infection is commonly diagnosed by the presence of specific IgM and IgG antibodies to *T. gondii* antigens in the sera of infected animals. The IgM antibodies usually become detectable within several days after infection; nevertheless, IgG antibodies become identified after 1-2 weeks and may have lifelong persistence. The presence of IgM antibodies indicates acute infection; however, the presence of IgG antibodies demonstrates a chronic infection (Petersen et al., 2005). The general diagnosis of toxoplasmosis in animals is established through serological, histopathological, or molecular techniques or combination of the aforementioned methods. The clinical signs of toxoplasmosis are nonspecific and not adequately characteristic of a definite diagnosis. The diagnosis of toxoplasmosis can also be reached by the identification of the *T. gondii* stages in host tissue removed by biopsy or necropsy. A rapid diagnosis can be reached through the microscopic examination of the impression smears of the aborted fetus organs. After drying the smears, they are fixed in methanol and stained with Giemsa in order to identify the infection stages of *T. gondii*. Well-preserved *T. gondii* organisms are dark purple and crescent-shaped (or

banana-shaped). In tissue sections, the tachyzoites usually appear round to oval (Hill and Dubey, 2002). The stages of toxoplasmosis, predominantly tissue cysts, were presented in the livers, lungs, and brains of the aborted cases during the microscopic examination of Giemsa-stained impression smears. Several studies reported that the rate of abortion due to *T. gondii* infection may vary from 10-100% in different parts of northern Iraq (Issa and Omer, 2011; Al-Barwary and Mikail, 2014). This finding is partly similar to the results of the present study depending on the immunity of animals, dose of oocyst, and time of exposure to infection. Neonatal mortality in sheep is more likely due to the multiplication of *T. gondii* in the placenta rather than the invasion of the parasite (Dubey, 1996). When *T. gondii* infection starts in the placenta, the multiplication of tissue protozoa causes the necrotic foci of the placenta and some inflammatory changes which develop throughout the rest of the gestation up to birth or abortion (Buxton et al., 2007). The persistent multiplication of parasite in the fetus and placenta is more likely due to local immune mechanism suppression in the maternal placenta and maturity of the fetal immune system. It was previously reported that the ovine fetus is able to mount an immune response to *T. gondii* infection for about 2 months of gestation; however, the immunocompetence is not adequate to protect up to the last month before birth (Buxton et al., 2007). It was also noted that most of the aborted ewes in the abortion group subsequently conceived and lambed normally; accordingly, it was confirmed that ewes usually develop immunity against the parasite which protects them from disease during subsequent pregnancies (Dubey, 2009). Therefore, *T. gondii* infection before 40 days of gestation can cause embryonic mortality and fetal resorption. In addition, the infection within 40 to 120 days of gestation can lead to fetal mummification and maceration or may result in abortion; however, the infection after 120 days of gestation causes stillbirth and/or weak birth (Dubey, 2009). In the current study, most abortion cases among the ewes were observed during the late stage of

gestation (>4 months), and some of them suffered from dyspnea, depression, pyrexia, and vaginal discharge shortly before the abortion. In the present study, *T. gondii* tachyzoites were detected in the peritoneal fluids of the inoculated mice with the homogenized infected tissue of the aborted ewes 12 days following the infection. Nevertheless, *T. gondii* tissue cysts comprising bradyzoites were successfully isolated from the brains of the mice 32 days after the infection. These results are inconsistent with the findings of another study (Shaapan and Ghazy, 2007) which reported *T. gondii* tachyzoites in the impression smears were obtained from the peritoneal exudate on inoculated mice 6-8 days following the infection. They also observed that *T. gondii* tissue cysts containing bradyzoites in the impression smears were taken from the brains of mice 45 days after the inoculation. Dubey (1997) reported that *T. gondii* tachyzoites and cysts were noticed in the mesenteric lymph nodes, lungs, and brains of mice 3, 7, and 60 days after the infection, respectively. The discrepancy between expected and obtained results may be due to the differences in the route of inoculation, inoculum dose, strain of the parasite, age of inoculated mice, and development of their immune system. The present study also investigated the postmortem examination of the ovine aborted fetus and demonstrated widespread subcutaneous edema associated with the accumulation of the blood-stained fluid in the abdominal and thoracic cavities. Most of the aborted fetuses showed enlarged, congested, reddish, and friable livers; however, the lungs were also congested and hemorrhagic. In addition, the cotyledons of the aborted cases were spotted with whitish foci or nodules of necrosis. Consistent with the results of the current study, several previous studies reported naturally occurring and experimentally induced toxoplasmosis in fetal kids (Ahmed et al., 2008). The manifestation of such pathological changes and identification of *T. gondii* stages in fetal heart, brain, and liver indicated transplacental infection. In the current study, the placenta, lung, brain, and heart are the most commonly

affected organs in *Toxoplasma*-induced abortion among sheep. The presence of *T. gondii* cysts in these organs was also reported by other studies (Dubey and Jones, 2008). In the present study, it was also observed that necrotic foci followed by the infiltration of multinucleated inflammatory reactions were the most frequent changes in toxoplasmosis in organs severely infected with tissue cysts and tachyzoites; this finding seems to be commonly associated with the rapid intracellular replication of *T. gondii* in such organs. It was previously confirmed that the demonstration of the tissue parasites with the characteristic histopathological images in the placenta, liver, lung, and the brain of the aborted fetus is important for the diagnosis of ovine toxoplasmosis (Ahmed et al., 2008). It was also confirmed that interstitial nephritis, myocarditis, hepatitis, nonsupportive encephalitis, and diffuse interstitial pneumonia are the most significant histopathological changes of an aborted fetus. In conclusion, the infection by the protozoan parasite *T. gondii* is highly prevalent in animals. Toxoplasmosis in the sheep industry has a potential risk of transmission to humans through the consumption of contaminated meat with the tissue cysts of *T. gondii* in Iraq. Most of the sheep breeding farms in Kurdistan Region are traditional, and animals are directly in contact with cats. The presence of cats and history of abortion in farm sheep are the main risk factors associated with *T. gondii* infection. In another study (Dubey and Jones, 2008), it was reported that tissue oocyst-contaminated pastures and drinking water are the major sources of postnatal infection in sheep. The results of the present study provided evidence of the *T. gondii* involvement in abortion and neonatal mortality among the naturally infected ewes in Duhok province. Accordingly, it is essential to identify different stages of *T. gondii* infection with some characteristic histopathological changes in the organs of an aborted fetus and perform serological and microscopic examinations for the diagnosis of toxoplasmosis in ewes.

### Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Authors' Contribution

Study concept and design: Naqid, I.A.; Zangana, I.Q.  
 Acquisition of data: Khanamir, R.A.; Zangana, I.Q.  
 Analysis and interpretation of data: Naqid, I.A.; Khanamir, R.A.  
 Drafting of the manuscript: Naqid, I.A.  
 Critical revision of the manuscript for important intellectual content: Khanamir R.A.; Naqid, I.A.; Zangana, I.Q.  
 Statistical analysis: Naqid, I.A.  
 Administrative, technical, and material support: Khanamir, R.A.; Zangana, I.Q.

### Acknowledgment

The authors would like to extend their gratitude to Dr. Mahdi Ali Abdulah, Dr. Dijwar Haji, and Ahmed Sabri for their help with histopathological examination.

### References

- Ahmed, Y., Sokkar, S., Desouky, H., Soror, A., 2008. Abortion due to toxoplasmosis in small ruminants. *Global Vet* 2, 337-342.
- Al-Barwary, L., Mikail, F., 2014. Seroprevalence of toxoplasmosis in aborted ewes by using different immunologic tests in Duhok governorate, Kurdistan region, Iraq. *Iraqi J Vet Sci* 28, 11-15.
- Buxton, D., Maley, S.W., Wright, S.E., Rodger, S., Bartley, P., Innes, E.A., 2007. *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. *Vet Parasitol* 149, 25-28.
- Dubey, J., 1996. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. *Vet Parasitol* 64, 65-70.
- Dubey, J.P., 1997. Tissue cyst tropism in *Toxoplasma gondii*: a comparison of tissue cyst formation in organs of cats, and rodents fed oocysts. *Parasitology* 115, 15-20.
- Dubey, J.P., 2009. Toxoplasmosis in sheep--the last 20 years. *Vet Parasitol* 163, 1-14.

- Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M., *et al.*, 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil: unexpected findings. *Int J Parasitol* 32, 99-105.
- Dubey, J.P., Jones, J.L., 2008. *Toxoplasma gondii* infection in humans and animals in the United States. *Int J Parasitol* 38, 1257-1278.
- Edwards, J.F., Dubey, J.P., 2013. *Toxoplasma gondii* abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype *T. gondii* from an aborted lamb from a chronically infected ewe. *Vet Parasitol* 192, 129-136.
- Hashemi-Fesharki, R., 1996. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats in Iran. *Vet Parasitol* 61, 1-3.
- Hill, D., Dubey, J.P., 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin Microbiol Infect* 8, 634-640.
- Issa, N., Omer, L., 2011. Prevalence of *Toxoplasma gondii* in aborted ewes and does in Duhok province of Iraq. *Res Opin Anim Vet Sci* 1, 627-630.
- Nieto, S.O., Melendez, R.D., 1998. Seroprevalence of *Toxoplasma gondii* in goats from arid zones of Venezuela. *J Parasitol* 84, 190-191.
- Petersen, E., Borobio, M.V., Guy, E., Liesenfeld, O., Meroni, V., Naessens, A., *et al.*, 2005. European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *J Clin Microbiol* 43, 1570-1574.
- Ragozo, A.M., Pena, H.F., Yai, L.E., Su, C., Gennari, S.M., 2010. Genetic diversity among *Toxoplasma gondii* isolates of small ruminants from Brazil: novel genotypes revealed. *Vet Parasitol* 170, 307-312.
- Schlafer, D., Miller, R., 2007. *Palmer's pathology of domestic animals*, Saunders Elsevier, Philadelphia.
- Shaapan, R.M., Ghazy, A.A., 2007. Isolation of *Toxoplasma gondii* from horse meat in Egypt. *Pak J Biol Sci* 10, 174-177.
- Soulsby, E., 1986. *Helminths, arthropods and protozoa of domesticated animals.*, Baillière, Tindall & Cassell, London.
- Subedi, S., Sharma, B., Singh, S., Bindari, Y.R., 2018. Seroprevalence of *Toxoplasma gondii* in sheep in different geographical regions of Nepal. *Vet Anim Sci* 5, 7-9.
- Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 30, 1217-1258.
- van der Puije, W.N., Bosompem, K.M., Canacoo, E.A., Wastling, J.M., Akanmori, B.D., 2000. The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Trop* 76, 21-26.