Plant Archives Vol. 21, Supplement 1, 2021 pp. 1281-1286 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.202

MOLECULAR DETECTION OF *TOXOPLASMA GONDII* IN LOCAL BREED DOMESTIC CHICKENS (*GULLAS GULLAS DOMESTICUS*) IN BAGHDAD CITY, IRAQ

Enas Jaffer Kadhim Al-Hadad* and Haider Mohammed Ali Al-Rubaie
Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq.
*Email: enasalhadad7@gmail.com

ABSTRACT

This study aimed to detect the infection rate of *Toxoplasma gondii* in 100 local breed domesticated chickens (*Gullas gullas domesticus*) by Real Time-PCR based on B1 gene and determined the effects of some factors (sex, age and areas) in this rate during the period 1/ 10 / 2019 till 31/3/ 2020. The total infection rate was 11% and a higher infection rate (17.24%) was found in females than males (2.38 %) with significant (P≤ 0.01) difference. A higher infection rate (16.32%) was recorded in young (<6 months) females while no infection rate (0.00%) was found in young males (<6 months). The adult females (>6 months) were showed a higher infection rate (22.22%) than adult males 4.76% (>6 months) with significant (P≤0.01) difference. A higher infection rate (23.33%) was recorded in AL-Baia area followed by Al-Malhani (6.66%), while no infection rate (0.00%) was found in Baghdad AL-Jadida with significant (P≤ 0.01) difference.

Keyword: *Toxoplasma*, *gondii*, domestic chickens, *Gallus*, Real time PCR

Introduction

Toxoplasma gondii is one of the most world's common protozoan parasite (Dubey and Beattie, 1988) cause a systemic disease (Siim *et al*., 1963; Kaneto *et al*., 1997; Tenter *et al*., 2000; Dubey, 2002) .It is widespread zoonotic disease that infects all warm-blooded vertebrates (Smith and Reduck, 2000; Dubey, 2008; Dubey, 2010). The definitive hosts are domestic and wild cats (felids), which the sexual phase of the life cycle occurs in the epithelium of intestine ended by the shedding of unsporulated oocysts in the feces, while the intermediate hosts are animals or man (Dubey and Beattie, 1988; Hill and Dubey, 2002; Afonso *et al*., 2006; Dubey, 2010). The parasite has three infectious stages tachyzoites (rapidly multiplying and circulating), bradyzoites (tissue cysts) and sporozoites within oocysts (Hill and Dubey, 2002) .In additional to naturally occurring toxoplasmosis (cases) in domestic birds, experimental studies have been carried out in many species such as white quails, Japanese quails, chickens, broilers, pigeons, turkeys, and pheasants (Boch *et al*., 1966 ; Bianeifiori *et al*., 1986 ; Dubey *et al*., 1993, 1993a; Kaneto *et al*., 1997) . It has subclinical course in many avian species (Atasever *et al*., 2020).The clinical toxoplasmosis in chickens is consider as sciatic nerve neuritis, chorioretintis and encephalitis (Hepding, 1939) .

The definitive diagnosis of *T. gondii* is mainly established by parasitological, immunological and molecular tests (Villena *et al*., 2004) and the direct detection of parasite-specific DNA in biological samples using PCR-based molecular methods has gained popularity (Calderaro *et al*., 2006). These methods have proved to be simple, sensitive, reproducible , and have been applied to a variety of clinical samples from animals and humans (Bell and Ranford-Carteright, 2002; Contini *et al*., 2005 ; Bastien *et al*., 2007; Bessieres *et al*., 2009). Real-time PCR has been use to amplify and quantify DNA from the *T. gondii* B1 gene (Costa *et al*., 2000; Lin *et al*., 2000). It is a highly sensitive and specific method; but it is expensive, requires specialized detection systems; therefore may only be cost effective in laboratories where analysis of large numbers of samples are carried out (Nagy *et al*., 2007) . Due to the no data available in the information about molecular diagnosis by real Time PCR of *Toxoplasma gondii* the infection in local breed domestic chickens this study was designed.

Materials and Methods

Animals, area and period of the study

One hundred local breed domestic chickens (*Gullas gullas domesticus*) were brought from the local markets (Abu-Ghurib, Al-Malhani, Baghdad Al-Jadida and Al-Baia) in Baghdad city during the period from 1/ 10 / 2019 till 31/3/ 2020.

Blood samples collection

About 2ml of jugular vein blood was collected of each bird by a sterile syringe into and transfer to EDTA tubes (Hendrix and Robinson, 2006) and kept in -20 °C till used for Real-Time PCR analysis.

DNA extraction

The DNA extraction from blood was done by using G-spin DNA extraction kit (Intron Biotechnology, cat. no.

17045) and the steps were follows the manufacture procedure and the primers of B1 gene and conditions that used for reactions were illustrated in table (1).

Table 1 : The specific primers of B1gene.

Primers	Sequence	Tm (ºC)	GC (%)	Reference
Forward	5'-TCCCCTCTGCTGGCGAAAAGT-'3	61.3	57.1	Al-Nasrawi
Reverse	5'-AGCGTTCGTGGTCAACTATCGATTG-3'	59.1	48	*et al.* (2014)

Agarose gel electrophoresis of DNA

The agarose gel has been made 1% (Sambrook *et al.*, 1989). Three µl of the processor loading buffer (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis .The process of loading added to the holes of the gel. An electric current of 7 v\c2 had been exposed for 1-2 h till the tincture had reached to the other side of the gel. The gel had been tested by a source of the UV light with 336 nm after put the gel in pool contain on 3µl red safe nucleic acid staining solution.

Components and concentrations of mixture for diagnose gene

The components and concentrations of mixture for diagnose gene illustrated in table (2)

Table 2 : The components and concentration of mixture specific interaction for diagnoses the B 1 gene.

Components	20 µL (Final volume)	Final concentration
Sybrgreen Kappa Master Mix	10 µL	1X
Forward primer	1 µL	0.2µM
Reverse primer	1 µL	0.2µM
Nuclease free water	5µL	--------
DNA sample volume	3µL	1pg-100ng

RT- PCR analysis for B 1 gene

Amplification program for RT- PCR analysis (Lin *et al.*, 2000) was illustrated in table (3).

Table 3 : Amplification program of RT- PCR for B 1 gene.

No.	Amplification program
1	95.0°C-:02:00x
2	95.0° C-:00:30x
3	59.0° C -00:30x
4	72.0° C-01:00x
5	72° C-01:00x

Statistical analysis

Chi-square test (χ^2) was used to compare the significance difference between the parameters (SAS, 2012).

Results

The total infection rate

The total infection rate of *Toxoplasma gondii* by Real Time- PCR in the domesticated local breed chickens (*Gallus gallus domesticus*) was 11% (11/100) in Baghdad city (Table 4, Fig. 1).

Table 4 : Total infection rate of *Toxoplasma gondii* in local breed chickens.

Test	No. of samples examined	Positive	Percentage (%)
Real time PCR	**100**	**11**	**11**

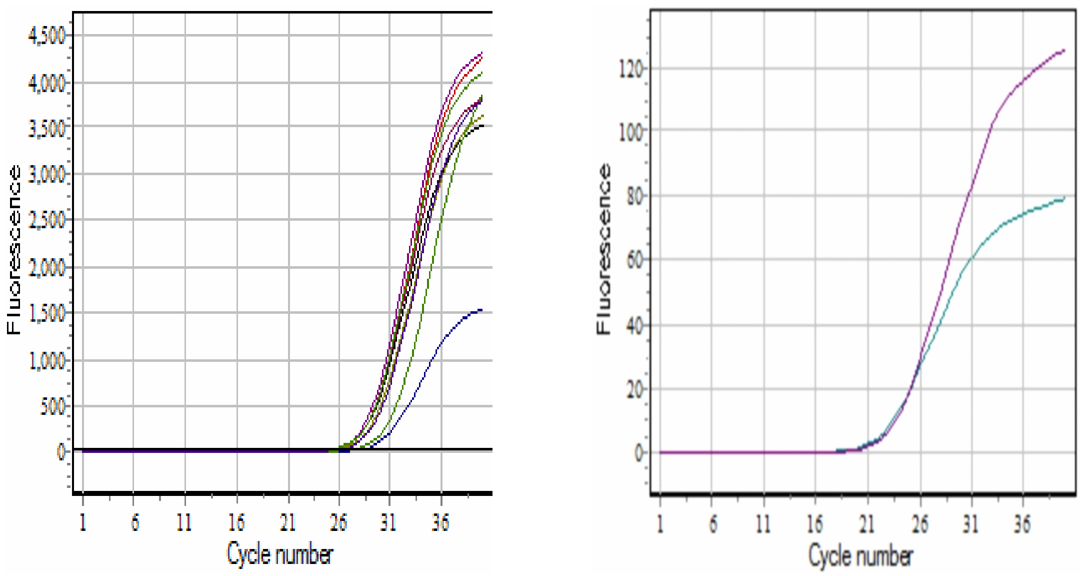

Fig. 1 : The Positive results of *Toxoplasma gondii* in chickens by Real Time PCR in Baghdad city.

The effect of sex in infection rate of *Toxoplasma gondii* in chickens

A higher infection rate 17.24 % (10/58) of *Toxoplasma gondii* was recorded in females, while the lower infection rate 2.38 % (1/42) was found in males with significant ($P\leq 0.01$) difference (Table 5)

Infection rate of *Toxoplasma gondii* in chickens according to sex and age

The infection rate of *Toxoplasma gondii* in domesticated local breed chickens (*Gallus gallus domesticus*) according to sex and age was showed a higher infection rate 16.32% (8/49) in young female chickens and a lower infection rate 0.00% (0/21) was found in young male chickens. Also, adult female chickens were showed a higher infection rate 22.22% (2/9), than adult male chickens which showed a lower infection rate 4.76% (1/21) with significant (P≤ 0.01) difference. (Tab. 6)

The effects of areas in the infection rate of *Toxoplasma gondii* in chickens.

There was an effect of areas on the infection rates of *Toxoplasma gondii* of domesticated local breed chickens (*Gallus gallus domesticus*). A higher infection rate23.33% (7/30) was recorded in AL-Baia followed by Al-Malhani 6.66% (2/30), while the lower infection rate 0.00 (0/15) was found in Baghdad AL-Jadida with significant (P≤ 0.01) difference (Table 7).

Table 5 : The effect of sex in infection rate of *Toxoplasma gondii* in chickens.

Sex	No. of samples examined	Positive	Percentage (%)
Males	42	1	2.38
Females	58	10	17.24
Total	100	11	11
Chi-Square (χ^2)	6.021 **		

** P≤ 0.01

Table 6 : Infection rate of *Toxoplasma gondii* in chickens according to sex and age by Real Time -PCR.

Age/sex		No. of samples examined	Positive	Percentage (%)
Young < 6 months	Males	21	0	0.00
	Females	49	8	16.32
Adults > 6 months	Males	21	1	4.76
	Females	9	2	22.22
Chi-Square (χ^2)		8.153**		

** P≤ 0.01

Table 7 : The infection rate of *Toxoplasma gondii* in chickens according to the areas.

Areas	No. of samples examined	Positive	Percentage (%)
Al-Baia	30	7	23.33
Baghdad Al- Jadida	15	0	0.00
Abu-Ghurib	25	2	8.00
Al-Malhani	30	2	6.66
Total	100	11	11
Chi-Square (χ2)	9.108**		

** P≤ 0.01

Discussion

Toxoplasmosis is one of the most common human infections throughout the world infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions (James, 2003). Gondim *et al.* (2010) was mentioned the detection of *T. gondii* DNA in seronegative birds that was confirmed the importance of other diagnostic techniques to complement serological examination of birds infected with this parasite. Generally, the routine diagnosis of infection is commonly performed by serological tests Modified Agglutination Test (MAT) and Indirect Fluorescent Antibody Test (IFAT) for detection of specific antibodies (Dubey *et al.*, 2002, 2003a, b, 2005a, 2007) .The molecular methods have are more sensitive, specific and take less time compared to other assays (Villena *et al.*, 2004).Real-time PCR has been use to amplify and quantify DNA from the B1 gene (Dubey and Beattie, 1988; Costa *et al.*, 2000; Lin *et al.*, 2000) . The result of the presents study were showed that all over infection rates in chickens by RT-PCR 11% that was agree or disagree with some previous studies in the world such as India 17.9% (Sreekumar *et al.*, 2003) ,Brazil 38% (Dubey *et al.*, 2007a), Nicaragua 85% (Dubey *et al.*, 2006), China 11.4% in free ranging chickens and 4.1% of caged chickens, Egypt 18.7% was found antibodies in chickens from the slaughterhouses by using MAT (Deyab and Hassanain , 2005) , and Thailand 64% in free ranging chickens by using fluorescent antibody test (IFAT) and 47.2% that was reported by El-Massary *et al.* (2000) and Zhu *et al.* (2008) . Zhao *et al.* (2012) was referred that the seropositive rates of infection very high from 30-50% in free-range chickens or maybe reached 100% in backyard chickens (Dubey, 2010; Dubey *et al.*, 2010),that difference in the infection rate of parasite was mentioned before in naturally infected poultry varies greatly (Siim *et al.*, 1963; Devada *et al.*, 1998; El-Massary *et al.*, 2000; Dubey, 2010) and Shokri *et al.* (2017) was found in the field the overall prevalence included chicken 20%, pigeons 8% and sparrows 15%.The high prevalence of *T. gondii* in all the areas showed that the free-range chickens are a major reservoir for *T. gondii* parasites. The free-range chicken in the study area had free access to habitats around homesteads where they scavenged for feed which mainly included left overs, grass, and insects. (Mose *et al.*, 2016) or in most developing countries, the free-range chickens are slaughtered at home or in unsupervised slaughterhouses and their viscera such as heads are left for scavengers that can include cats and other chickens (Dubey *et al.*, 2012). The variation in the infection rates may due to the distribution of domestic cats, which are the major source of contamination to the environment due to the greatest formation of oocysts in these animals, which excrete millions of them after ingesting only one bradyzoite or tissue cyst (Dubey and Frenkel, 1972;

Dubey, 2001). On the same way, the wall of oocyst is adapted very well to protect it from the damage to extreme makes the parasite resilient for the environment and it can be survived in the moist environment for more than one year (Mai *et al* ., 2009). The soil contamination with oocysts is an important factor in the development of disease in free-range poultry (Dubey, 2010). The prevalence of parasite in free-ranging chickens is a good indicator of the prevalence of parasite oocysts in the soil because they feed from the ground (Ruiz and Frenkel, 1980; Dubey *et al*., 2005). Free ranging chickens play an important role in the epidemiology of parasite in the rural environment because they are clinically resistant to it and live longer ; cats that fed naturally infected chickens tissues can shed millions of oocysts (Dubey, 2002a) ; the oocysts in soil may do note stay there ,but the invertebrates such as flies, cockroaches, dung beetles and earthworms can be mechanically spread and / or even carry them onto food, and the infection rate in cats reflects the infection in local avian and rodent population, because they are thought be infected by eating these animals and release more oocysts in the environment, more prey animals will become infected, and the result is increase the cats infection (Dubey and Beattie, 1988) . The sporogony of oocysts occurs outside the host and leads to the development of infectious oocysts that remain viable in the environment for months to years (Lelu *et al*., 2012). Sporulated oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months; but they don´t survive long under cold or dry conditions (Dubey *et al.*, 2000). Although, the oocysts disseminated throughout the premises (Levine, 1973) and ingesting of them in water, soil or feed is probably the most common route for infection in non-carnivorous mammals and birds; meat-producing animals can show a very high seroprevalence throughout the world may be up to 100% (Tenter *et al*., 2000), for that domestic breeding birds and poultries are less infected than free ranging or industrial breeding since they are not allowed to contact with infective oocysts or feline (Holsback *et al*.,2012). It is also known that felids are generally excellent predators, thus contributing to maintenance and success of *T. gondii* in the environment and birds can be considered as an important reservoirs of *T. gondii* as they are often hunted by felids (Dubey, 2006). Also the tissues of infected chickens are a source for cats infection (Ibrahim *et al.* 2009; Shokri *et al*., 2017) .In conclusion, *T. gondii* was distributed with a high infection rate in local breed domestic chickens in Baghdad city.

References

Afonso, E.; Thulliez, P.; and Gilot-Formont, E. (2006). Transmission of *Toxoplasma gondii* in an urban population of domestic cats (*Felis catus*). Int.J. Parasitol., 36:1373-1382.

AL-nasrawi, H.A.; Naser, H.H. and Kleaf, S. F. (2014). Molecular detection of *Toxoplasma gondii* in human and chicken by real-time PCR technique .Int. J. Adv., 23: 1023-1027.

Atasever, A.; Ekebas, G. and Gram, D.Y. (2020). Spontaneous toxoplasmosis in a chicken. Ankara. Üniv. Vet. Fak, Der.G., 67: 101-105.

Bell, A. and Ranford-Cartwright, L. (2002).Real-time quantitative PCR in parasitology. Trends Parasitol., 18: 337–342.

Boch,J.; Rommel, M. and Weiland, G. (1966): Experimentelle *Toxoplasma* infectionen bei Legehennen. Berl Munch Tierarztl Wochenschr, 79: 352-355.

Bianeifiori,F.; Rondini, C.; Grelloni, V. and Frescura, T.(1986).Avian toxoplasmosis: experimental infection of chicken and pigeon. Comp. Immunol. Microbiol. Infect. Dis., 9: 337-346.

Bastien, P.; Jumas-Bilak, E.; Varlet-Marie, E. and Marty, P. (2007). Three years of multi-laboratory external quality control for the molecular detection of Toxoplasma gondii in amniotic fluid in France. Clin. Microbiol. Infect., 13: 430–433.

Bessie`res, M. H.; Berrebi, A.; Cassaing, S.; Fillaux, J.;Cambus, J. P.; Berry, A.; Assouline, C.; Ayoubi, J. M. and Magnaval, J. F. (2009). Diagnosis of congenital toxoplasmosis: prenatal and neonatal evaluation of methods used in Toulouse University Hospital and incidence of congenital toxoplasmosis. Memorias do Instituto Oswaldo Cruz., 104: 389–392.

Costa, J.M.; Pautas, C.; Ernault, P.; Foulet, F.; Cordonnier, C. and Bretagen, S. (2000). Real-time PCR for diagnosis and follow-up of *Toxoplasma* reactivation after allogeneic stem cell transplantation using fluorescence resonance energy transfer hybridization probes. J. Clin. Microbiol., 38: 2929–2932.

Contini, C.; Seraceni, S.; Cultrera, R.; Incorvaia, C.; Sebastiani, A. and Picot, S. (2005). Evaluation of a real-time PCR-based assay using the light cycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis. Int. J. Parasitol., 35: 275–283.

Calderaro, A.; Piccolo, G.; Gorrini, C.; Peruzzi, S.; Zerbini, L.; Bommezzadri, S.; Dettori, G. and Chezzi, C. (2006). Comparison between two real-time PCR assays and a nested-PCR for the detection of *Toxoplasma gondii*. Acta Bio. Medica., 77: 75–80.

Devada, K.; Anandan, R. and Dubey, J.P. (1998): Serologic prevalence of Toxoplasma gondii in chickens in Madras, India. J. Parasitol., 84: 621-622.

Deyab, A.K. and Hassanein, R. (2005). Zoonotic toxoplasmosis in chicken. J. of the Egyptian Society of Parasitol., 35(1):341-350.

Dubey, J.P. (2001). Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. J. Parasitol., 87:215-219.

Dubey, J. P. (2002). A review of toxoplasmosis in wild birds. Vet. Parasitol., 106:121– 153.

Dubey, J.P. (2002a). Tachyzoite-induced life cycle of *Toxoplasma gondii* in cats. J. Parasitol., 88:713-717.

Dubey, J. P. (2006). Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gondii* for intermediate (mice) and definitive (cats) hosts. Vet. Parasitol., 140(1-2): 69-75

Dubey, J.P. (2008). The history of *Toxoplasma gondii*,The first100 years. J. Euk. Microbiol., 55(6):467-475.

Dubey, J.P. (2010). *Toxoplasma gondii* infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis, and public health significance. Zoon. Pub. Hlth., 57(1): 60-73.

Dubey, J.P. and Frenkel, J.K. (1972).Cyst- induced toxoplasmosis in cats. J. Protozool., 19:155-177.

Dubey, J.P. and Beattie, C.P. (1988). Toxoplasmosis of Animals and Man.CRC Press, Boca Raton, FL.

Dubey J.P.; Ruff, M.D. and Kwok, O.C.H. (1993). Experimental toxoplasmosis in bobwhite quail (*Colinus virginianus*). J. Parasitol., 79: 935-936.

Dubey, J. P.; Scandrett, W.B.; Kwork, O.C.H.; Gajadhar, A.A. (2000). Prevalence of antibodies to *Toxoplasma gondii* in ostriches Struthio camelus. *J. Parasit*., 86 (3): 623-624.

Dubey, J. P.; Sundar, N.; Gennari, S.M.and Minervino, A.H.H. (2007). Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations. Vet. Parasitol., 143(2): 182-188.

Dubey, D. H.; Graham, C.R.; DA Silva, D.S; Lehmann, T. and Bahla- Oliveira, L. M. G. (2003b). *Toxoplasma gondii* isolates from free ranging chickens from Rio de Janeiro, Brazil, Mouse mortality, genotype, and oocysts shedding by cats. J. Parasitol., 89:851-853.

Dubey, J.P.; Lago, E.G.; Gennari, S.M.; Su, C.; and Jones, J.L. (2010). "Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease and epidemiology. Parasitol,. 139 (11) : 1375–1424.

Dubey, J.P.; Bhaivat, M.I.; Macpherson, C.N.L.; de Allie, C.; Chikweto, A. and Kwok, O.C.H. (2006). Prevalence of *Toxoplasma gondii* in Rats (*Rattus norvegicus*) in Grenada, West Indies. J. Parasitol., 92 (2): 1107-1108.

Dubey, J.P.; Ruff, M.D.; Camargo, M.E.; Shen, S.K.; Wilkins, G.L.; Kwok, O.C. and Thulliez, P. (1993a). Serologic and parasitological responses of domestic chickens after oral inoculation with *Toxoplasma gondii* oocysts. Am. J .Vet. Res., 54:1668-1672.

Dubey, J.P. Rajapkse, R.P.V.J.; Wijesundera,R.R.M.K.K.; Sundar,N.; Velmurugan, G.V.; Kwok, O.C.H.and Su, C.(2007a). Prevalence of *Toxoplasma gondii* in dogs from Sri Lanka and genetic characterization of the parasite isolates. Vet. Parasitol., 146:341-346.

Dubey,J.P.; Hill, D.E.; Rozeboom, D.W.; Rajendran, C.; Choudhary, S.; Ferreira, L.R.; Kwok, O.C. and Su, C. (2012). High prevalence and genotypes of *Toxoplasma gondii* isolate from organic pigs in northern USA. Vet. Parasitol., 188(1-2):14-18.

Dubey, D.H.; Graham,C. R.; Dahl, E.;Hilali, M.; EL-Ghaysh, A.; Sreerumar, C.; Kwoe, O. C. H.; Shen, S. K. and Lehmann, T. (2003a). Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. Vet. Parasitol., 114:89-95.

Dubey, J.P.; Bhaiyat, M.I. ; Allie, C. D.E. ; Mcpherson, C. N. L. ; Sharma, R. N. ; Sreekumar, C.; Vlanna, M. C. B.; Shen, S. K. ; Kwok, O. C. H. and Lehmann, T. (2005). Isolation, tissue distribution, and molecular characterization of *Toxoplasma gondii* from chickens in Grenada, West Indies. J. Parasitol., 91:557- 560.

Dubey, J.P.; Graham, D. H.; Blackston, C. R.; Lehmann, T.; Gennari, S.M.; Ragozo, A.M. ; Nishi, S. M.; Shen, S.K. ; Kwok, O.C.H.; Hill, D.E. and Thulliez, P. (2002). Biological and genetic characterization of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil, unexpected findings. Int. J. Parasitol., 32:99-105.

Dubey, J.P.; Hill, D. E.; Jones, J.L.; Hightower, A.W.; E Kirkland,E.; Roberts, J.M.; Marcet, P.L.; Lehmann, T.; Vianna, M.C.B.; Miska, K.; Sreekumar, C.; Kwok, O.C.H.; Shen, S.K.and Gamble, H.R. (2005a). "Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States, Risk assessment to consumers. J. Parasitol., 91 (5): 1082–1093.

El-Massary, A.; Mahdy, O.A.; El-Ghaysh, A. and Dubey, J.P.(2000). Prevalence of *Toxoplasma gondii* antibodies in sera of turkeys, chickens, and ducks from Egypt. J. Parasitol., 86(3): 627-628.

Gondim, L. S.; Abe-Sandes, K.; Uzêda, R.S.; Silva, M.S.; Santos, S.L.; Mota, R.A.; Vilela, S.M. and Gondim, L.F.(2010). *Toxoplasma gondii* and *Neospora caninum* in sparrows (*Passer domesticus*) in the Northeast of Brazil. Vet. Parasitol., 168(1-2):121-124.

Hepding, L. (1939). Ueber *Toxoplasma (Toxoplasma gallinarum*) n. sp in der Retina eines Huhnes und u.. ber deren Beziehung zur Hu..Hnerla..Hmung. Zeitschrirt fur Infektions krankheiten usw. der Haustiere., 55:109-116.

Hendrix, C. M. and Robinson, E. (2006). Diagnostic Parasitology for Veterinary Technicians. 3rd ed., Elsevier Mosby, Edinburgh.

Hill. D. and Dubey, J.P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbial. Infect., 10: 634–40.

Holsback, L.; De, H.F.;Pena, J.; Ragozo, A.; Lopes, E.G.; Gennari, S.M. and Soares, R.M. (2012).Serologic and molecular diagnostic and bioassay in mice for detection of *Toxoplasma gondii* in free ranges chickens from Pantanal of Mato Grosso do Sul. Pesq. Vet. Bras., 32(8): 721-726.

Ibrahim, H.M.; Huang, P.; Salem, T.A.; Talaat, R.M.; Nasr, M.I.; Xuan, X. and Nishikawa, Y. (2009). Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in northern Egypt. Am. J. Trop. Med. Hyg., 80(2): 263–267.

James, D. (2003). Determination of *Toxoplasma gondii* Antibody Prevalence in Midwest Investigator. McKean Institution: Iowa State University.

Kaneto, C.N.;Costa,A.J.; Paulillo,A.C.; Moraes, F.R.; Murakami, T.O. and Meireles, M.V. (1997). Experimental toxoplasmosis in broiler chicks. Vet. Parasitol., 69: 203-210.

Levin, M.E (1973). Adaptation and assessment a rationale for nursing intervention. Am .J. Nursing, 66 (11): 2450-2453.

Lélu , M.; Villena, I.; Dardé, M.L.; Aubert ,D.; Geers, R.; Dupuis, E.; Marnef ,F.; Poulle, M.L.; Gotteland, C.; Dumetre, A. and Gilot-Fromont, E.(2012). Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil. Appl. Environ. Microbiol., 78(15): 5127–5132.

Lin, M.H.; Chen, T.C.; Kuo, T.; Tesng, C.C. and Tesng, C.P. (2000). Real-Time PCR for quantitative detection of *Toxoplasma gondii*. J. Clin. Microbiol., 38:4121–4125.

Mai, k.; Sharman, P.A.;Walker, R.A.; Katrib, M.;Souza, D.D.; McConville, M.J., Wallach, M.G.; Belli, S.I.; Ferguson, D.J.P. and Smith, N.C. (2009). Oocyst wall formation and composition in coccidian parasite. Mem. Inst. Oswaldo. Cruz, 104 (2):281-289.

Mose, J.M.; Kagira, J. M.; Karanja, S. M.; Ngotho, M.; Kamau, D. M.; Njuguna, A. N. and Maina, N. W. (2016). Detection of natural *Toxoplasma gondii* infection in chicken in Thika region of Kenya using nested polymerase chain reaction. Hindawi Bio .Med. Res. Int., ID 7589278. https://doi.org/10.1155/ 2016/ 7589278.

Nagy, B.; Lazar, L.; Nagy, G.; Ban, Z. and Papp, Z. (2007). Detection of *Toxoplasma gondii* in amniotic fluid using quantitative real-time PCR method. Orv. Hetil., 148: 935–938.

Ruiz, A. and Frenkel, J.K. (1980). Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. Am. J. Trop. Med. Hyg., 29: 1161-1166.

SAS. 2012. Statistical Analysis System, user's guide statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sambrook, J.; Fritschi, E.F. and Maniatis, T. (1989). Molecular cloning: A laboratory manual.2nd ed. Cold Spring Harbor Laboratory Press, New York. University of Texas, South Western Medical Center, USA.

Smith, J.E. and Reduck, N.R. (2000). *Toxoplasma gondii* strain variation and pathogenicity. In: Cary, J.W., Lin, J.E., Bhatnagar, B. (Eds).In: Microbial foodborne disease: Mechanisms of pathogenesis and toxin synthesis. Tech. Pub. Lancaster, PA: 405-431.

Siim, J.C.; Biering-Sorensen, U. and Moller, T. (1963). Toxoplasmosis in domestic animals. Adv. Vet. Sci., 8: 335-337.

Shokri, A.; Sharif ,M.; Teshnizi , S. H.; Sarvi ,Sh.; Rahimi ,M.T.; Mizani, A.; Ahmadpour ,E.; Montazeri, M. and Daryani ,A. (2017). Birds and poultries toxoplasmosis in Iran: A systematic review and meta-analysis. Asian Pacif. J. Trop. Med.,10 (7) : 635–642.

Sreekumar, T.V. Liu, T.; Kumar, S.; Ericson, L.M.; Hauge, R.H. and Smalley, R.E. (2003). Crystallization and oriention studies in polypropylene single wall carbon nanotube composite. J. Vet. Parasitol., 44:2373-2377.

Tenter, A.M.; Heckeroth, A.R. and Weiss, L.M. (2000). *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol., 30:1217–1258.

Villena, I.; Marle, M.; Dardé, M.-L.; Pinon, J.-M. and Aubert, D. (2004) .*Toxoplasma* strain type and human disease: Risk of bias during parasite isolation. Trends Parasitol., 20(4):160–162

Zhao, G.W.; Shen, B.; Xie, Q.; Xu, L.X.; Yan, R.F.; Song, X.K.; Hassan, I.A. and Li, X.R. (2012) . Detection of *Toxoplasma gondii* in free-range chickens in China based on circulating antigens and antibodies. Vet. Parasitol., 185(2-4):72–77.

Zhu, J.; Yin, J.; Xiao, Y.; Jiang, N.; Ankarlev, J.; Lindh, J. and Chen, Q. A. (2008). Sero-epidemiological survey of *Toxoplasma gondii* infection in free-range and caged chickens in northeast China. Vet. Parasitol., 158:360–363.