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Toxoplasma gondii Infection in Free-Ranged (FR) and Caged Chickens and Turkey by Bioassay and Serologic Methods in Hamadan, Iran

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Original Article

Abstract

Introduction: Toxoplasmosis is a widely prevalent zoonotic disease, caused by *T. gondii*. Chickens, consumed widely in many countries, are considered as one of the most important hosts in the epidemiology of *T. gondii* infection because they could be a main source of infection for both cats and humans. There was no information regarding *T. gondii* infection in chickens in this area, therefore, this investigation carried out to determine the prevalence of *T. gondii* infections in poultry in Hamadan.

Methods: A total of 203 birds including free-ranged, caged chickens and 2 turkeys were studied. The poultries' sera tested by indirect hemagglutination test (IHA) for *T. gondii* antibody. The birds' brain tissue used for testing for *T. gondii* tissue cyst. Brain of each bird grinded and suspension were made by normal saline and inoculated to peritoneal cavity of five mice. Peritoneal aspirate examined for tachyzoites after 5-10 days. Data regarding kind of bird, age, gender and raising type were recorded and analyzed.

Results: Tissue cyst of *T. gondii* was detected by bioassay in the brain of 3 out of the 203 samples (one FR and one caged) by peritoneal inoculation (1%). Seropositivity for *T. gondii* antibody was 6.1% (12.196). Positive cases were 6 FR hens, 1 caged chicken and 5 roosters. No positive case found in the turkeys.

Conclusion: This study indicates that, both FR and caged chickens may have similar risk of infection to *T. gondii* and can transmit the parasite to humans.

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Introduction:

T. gondii is a protozoan of the Coccidia order that is an obligate intracellular parasites of humans and other warm-blooded vertebrates, including domesticated mammals and birds and, nearly one-third of the human population has been exposed to this infection (1). This parasite infects a large proportion of the world's population (perhaps one

third) but uncommonly causes clinically significant disease. The most acquired infections are asymptomatic and non-obvious (1,2). Definitive host of this parasite is domestic cats and other Felids and sexual cycle is completed in the small intestine of these animals leading to the formation of the oocyte that is excreted in the feces. Human infection can occur by ingestion of oocysts following the handling of contaminated soil or cat litter or through the consumption of contaminated water or food sources (eg unwashed garden vegetables). Infection can also occur via ingestion of tissue cysts (bradyzoites) in undercooked or uncooked meat of warm blooded animals, as well as the transmission from mother to fetus and organ transplantation containing tissue cysts (3). In people with normal immune system, toxoplasmosis is usually asymptomatic, however, if infection occurs early in pregnancy, it may have clinical symptoms such as physical or mental retardation and chorioretinitis with impaired vision and hearing (4).

In addition, infection with *T. gondii* can cause high mortality in immunocompromised patient, especially HIV positive individuals (5). This parasite has great importance in both veterinary and medicine because it may cause miscarriage or congenital disease in the host (6). Poultry is in close relation to humans, especially in rural areas and it may live three to four years in nature, and their infection can be used as a scale for the measurement of the oocyte concentration in the environment (soil, water, raw vegetables, …) (7).

Chicken is an important component of food in the world, and especially in Iran. As a result, people can be infected with *T. gondii* eating raw or under-cooked chicken having been infected with this parasite. Meat or other organs of hen infected with *Toxoplasma* may be used to produce salami paste. This can also be a risk factor for transmission *of T. gondii*. Poultry may be eaten by other animals, especially Felid carnivorous, and play an important role in the cycle of the parasite (8). Likely, traditional or domestic raised poultry, infected with *Toxoplasma* is due to higher exposure to environment and soil contaminated with cat feces (9). The results of previous studies done on humans and the number of animals in Iran showed that natural infection with this parasite is relatively high in different parts of country and different hosts.

In a study conducted by Gharavi and colleague in 1990, there were 29% of serologically positive in the examined birds (10). Seroprevalence of *T. gondii* infection in pigeons and poultry has been reported only in a few countries (7). Few studies have been conducted in Iran and especially in Hamadan on poultry. Therefore, the purpose of this study was to determine the contamination of poultry in Hamadan to *Toxoplasma* by serology and parasitology methods and also attracting more people and poultry farm owners' attention to control the *Toxoplasma* infection.

Methods:

This study was a descriptive cross- sectional study using serological and parasitological methods, conducted in Hamadan and its surrounding villages during 2014.

The birds include traditional and industrial raised poultry (broilers and laying hens, roosters and turkey). A total of 203 birds including 137 hens, 64 roosters and 2 turkeys enrolled that, 162 birds had been raised as traditional type (64 rooster, 96 hens and 2 turkeys) and 41 birds were from industrial farms (18 layer hens and 23 broilers).

The fowl purchased from traditional bird market in Ekbatan Street and suburbs of the city. Information such as age, sex and breeding type were recorded. Poultry over two years were considered as old and others in terms of size of the foot gaff and the appearance of the eye and the crown categorized as "young". Blood samples were taken from wing vein for indirect hemagglutination test (IHT). After the slaughter the birds, their heads were removed and along with blood samples carried to Research Laboratory of Parasitology, University of Medical Sciences, Hamadan. The sera provide by centrifugation (3500rpm/5miutes), and stored at -20C until use.

Parasitology test (bioassay)

Parasitology test was carried out using smears prepared from the brain of birds. Bioassay was performed by preparing the suspension of grinded brain material in PBS and inoculated intra peritoneally to mice as described previously (11). Briefly, poultrys' brain were removed from the skull and a suspension of 20% was prepared by

macerated brain and saline individually and inoculated intraperitoneally in a group of five mice. After 10 to 14 days, peritoneal exudates examined for presence of tachyzoites. After this time, the mice euthanized by chlorophorm anesthesia; and after cutting the abdominal skin aseptically, 2-3 ml of saline was injected into the abdominal cavity. After shaking, the peritoneal exudates aspirated and the smears were prepared, and were examined by microscope. The inoculated animals considered as *Toxoplasma* infection when tachyzoites or tissue cysts were found in peritoneal exudates wet smears or brain tissue respectively. The negative specimen air dried; fixed by methanol and stained by Giemsa staining. The poultry brains also examined for *T. gondii* tissue cysts. The small pieces of the brain tissue placed between two glass slides and impression smears were made, air dried, fixed by methanol and stained by Geimsa for microscope examination and detecting the tissue cysts.

Indirect Hemagglutination test (IHA)

Indirect hemagglutination test was used to detect anti-*Toxoplsma* antibodies in the sera of birds, according to method described by described by Gharavi 1991(12). In this experiment, the crude antigen was prepared from mice which were infected by *Toxoplasma* RH strain 3-4 days before. The antigen prepared by freeze and thaw of tachyzoites. Serial dilutions of sera were as: 1:20, 1:100, 1:200 through 1:12800 and, the titers $\geq$1:20 were considered as positive.

For data analyzing, SPSS software version 16 were used and $P<0.05$ was considered as significant.

Results:

A total of 194 sera samples were eligible for analysis and 9 samples were lost. We couldn't take a blood sample from turkeys for IHA but, the number of samples for parasitology (bioassay) test was 203.

The number of positive cases in bioassay was 2 specimens (0.98%) and serologically positive cases were 12 samples (6.18%).

These 12 cases were positive with the titers 1: 100 to 1: 3200. The majority of positive cases were at a titer of 1: 200. The details of findings according to method of infection detection and epidemiologic factors presented at the tables 1 through 5.

There wasn't observed a significant relationship between age, sex, breeding type (industrial or traditional) and the prevalence of anti-*Toxoplasma* antibodies. One of the two strains that isolated from a traditional raised rooster was pathogenic for mouse, and the mouse was died within a week. Another strain was isolated from a laying industry farm hen. Two cases that parasite was isolated from were also positive by serology.

Table 1. Serological prevalence of *T.gondii* infection in poultry of Hamadan

Inoculated mice	No.	%
Negative	201	99
Positive	2	1
Total	**203**	**100**

Table 2. Bioassay test results for *T. gondii* infection in the poultry of Hamadan

Serology	No.	%
Positive	12	6.18
Negative	182	93.8
Total	**194**	**100**

Table 3. Serological prevalence of *T. gondii* in poultry of Hamadan in terms of age

Age	Serology result		Total
	Positive (%)	Negqative (%)	
Young	6 (4.34)	132 (95.65)	138
Old	6 (10.7)	50 (89.3)	56
Total	**12 (6.18)**	**182 (93.8)**	**194**

Table 4. Serological prevalence of *T. gondii* in poultry of Hamadan in terms of gender

Genus	Serology result		Total
	Positive (%)	Negqative (%)	
Male	7 (5.34)	124 (94.65)	131
Female	5 (7.93)	58 (92)	63
Total	**12 (6.18)**	**182 (93.8)**	**194**

Table 5. Serological prevalence of *T. gondii* in poultry of Hamadan in terms of breeding type

Breeding type	Serology result		Total
	Positive (%)	Negqative (%)	
Traditional	11 (7.9)	144 (92.9)	155
Industrial	1 (2.56)	38 (97.4)	39
Total	**12 (6.18)**	**182 (93.8)**	**194**

Conclusion:

This study showed the *Toxoplasma* infection is not rare in the poultry of this area and if the people consume undercooked chicken, they will be at risk of infection to toxoplasmosis. Seroprevalence also varied amongst traditional and industrial poultry breeding types but, is not different between male and female fowls. Anti *Toxoplasma* antibodies were found only in 12 (6.1%) of 194 birds, whereas, the parasite isolated only from two seropositive chickens.

Toxoplasmosis is a widespread infection that has been reported from humans and animals, including domestic and wild birds, in all continents. Worldwide serological prevalence of toxoplasmosis in FR chickens varied from 2% to 100% depending on the source of chickens (13). The rate of human infection and some animals, such as cats, dogs, sheep, goats and cattle in some parts of Iran reported relatively high. Meanwhile, studies on the birds' toxoplasmosis are less than others and there was not complete information of the prevalence of toxoplasmosis in the birds in Iran, especially those consuming routinely. This is first report of bird toxoplasmosis with an acceptable sample size carried out in Hamadan.

The majority of studies carried out in Hamadan had been done on limited human populations, usually the women. Seroprevalence of toxoplasmosis reported 41.3% in the peoples admitted to Malayer health centers (14), 33.5% in primigravida women referred to a teaching university hospital (15), 38.9% in the women aged 15-45 years (16), and 30% in pregnant women (17). Report on the fowl toxoplasmosis in Hamadan is rare and first report in these birds is from Ghorbani and colleagues that carried out on the toxoplasmosis in the birds of Iran and a few birds also studied from Hamadan Province; they reported the strain with high virulence isolated from a rooster (12). They also isolated *T. gondii* from six of 109 (5.4%) chickens from other parts of Iran; 30% had IHA titers of 1:20 or higher and the parasite was isolated from five seropositive and one seronegative chicken. A report of toxoplasmosis of free-ranged chickens showed higher prevalence rate (36%) in the Shiraz, south of Iran (18) that is six-fold of ours. In addition to fowl, sheep also reported as an important reservoir of human toxoplasmosis in Iran (19) with high prevalence rate (61%). The studies on animal toxoplasmosis, carried out more often by serological methods and bioassay is rare in Iran and, this will be an advantage of present study. In addition, isolates from poultry can use for more studies on the molecular characteristics of parasite in this area.

The prevalence of *Toxoplsma* in FR chickens could be a good indicator for the environmental contamination of *T. gondii* oocysts, because FR chickens usually feed on the ground materials. Meanwhile, caged chicken has limit access to oocysts, but in this study one *Toxoplasma* strain isolated from a caged hen. This finding indicates that, even caged animals could be a potential source of *Toxoplasma* infection.

The sources of infection for humans worldwide vary greatly with culture, nutrition habits environmental conditions and geographical location and, ethnicgroups. The great importance of chickens as the main source of protein in Iranian people and cooking type of chickens as kebab, that is usually undercooked or semi-cooked, meat of poultry can considered as a major source of infection in humans.

In the most rural areas, chicken and eggs are used as common food and those usually consumed under cooked; as a consequent, they can be a potential risk for human *Toxoplasma* infection.

Results of the previous studies carried out in humans and some domesticated animals in Iran showed natural infection with this parasite are relatively high. The number of turkey in this study was not enough for correct justification about the infection rate of this bird.

The infection rate of *Toxoplasma* in eggs is usually very low; as a result, eating raw eggs should not be considered an important source for the transmission of toxoplasmosis (18).

The infection rate of *Toxoplasma* in this study was lower than those in previous studies in other parts of Iran. This difference may be due to precipitation decline in this area at the recent years, very cold weather in the Winter, Fall and Spring; and raising some poultry in the caged condition and probably little exposure to cats. In bioassay, we could isolate parasite only in the two birds of 12 seropositive cases; one from a layer hen and another from a rooster that had been raised in an

industrial farm. Recent studies showed some strain of *Toxoplasma* couldn't produce tissue cysts in the brain tissue but, the reasons for this phenomenon has not explained clearly (20), therefore, low negative rate in the bioassay could be explained by this hypothesis.

This study showed that, the prevalence of poultry toxoplasmosis in Hamadan, in contrast to other previous studies in the Iran, is low relatively.

Although no significant correlation was found between the prevalence of antibodies and breeding type; though the infection rate in traditional raised poultry was higher than industrial type.

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References:

1. Flegr J, Prandota J, Sovic kova Israili ZH. Toxoplasmosis - A Global Threat Correlation of Latent Toxoplasmosis wthi Specific Disease Burden in a Set of 88 Countries. PloS One. 2014;9(3):e90203.
2. Yan C, Yue CL, M, Qiu SB, Li HL, Zhang H, Song HQ, et al. Seroprevalence of T*oxoplasma gondii* infection in domestic pigeons (*Columba livia*) in Guangdong Province of southern China. Vet Parasitol. 2011;177(3–4):371-373.
3. Dubey JP. *Toxoplasma gondii* Infections in Chickens (*Gallus domesticus*): Prevalence, Clinical Disease, Diagnosis and Public Health Significance. Zoonoses Public Health. 2010;57(1):60-73.
4. Cook A, Gilbert R, Buffolano W, Zufferey J, Petersen E, Jenum P, et al. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control studyCommentary: Congenital toxoplasmosis—further thought for food. BMJ. 2000;321(7254):142-147.
5. Belanger F, Derouin F, Grangeot-Keros L, Meyer L. Incidence and Risk Factors of Toxoplasmosis in a Cohort of Human Immunodeficiency Virus-Infected Patients: 1988–1995. Clin Infec Dis. 1999;28(3):575-581.
6. Dubey JP. Toxoplasmosis of animals and humans. 2th ed. CRC Press; 2010,3:112.
7. Salant H, Landau DY, Baneth G. A cross-sectional survey of *Toxoplasma gondii* antibodies in Israeli pigeons. Vet Parasitol. 2009;165(1–2):145.
8. Yan C, Yue CL, Zhang H, Yin CC, He Y, Yuan ZG, et al. Serological Survey of *Toxoplasma gondii* Infection in the Domestic Goose (*Anser domestica*) in Southern China. Zoonoses Public Health. 2011;58(4):299-302.
9. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol. 2008; 38:1257–1278.
10. Ghorbani M, Gharavi MJ. Serological and Parasitological Investigations on *Toxoplsma* Infection in Domestic Fowls in Iran. Iran J Pub Health. 1990;19(1-4):9-18.
11. Godoi FS, Nishi SM, Pena HF, Gennari SM. Toxoplasma gondii: diagnosis of experimental and natural infection in pigeon (Columba livia) by serological, biological and molecular techniques. Rev Bras Parasitol Vet. 2010;19(4): 238-243.
12. Gharavi MJ. Toxoplasmosis in dirds of Iran [dissertation]. Tehran, Iran: Tarbiat Modares University; 1991. [Persian]
13. Dubey JP, *Toxoplasma gondii* Infections in Chickens (*Gallus domesticus*): Prevalence, Clinical Disease, Diagnosis and Public Health Significance. Zoonoses Public Health. 2010;57(1):60-73.
14. Shahmoradi A, Sardarian Kh, Fallah M. Seroepidemiologic study of toxoplsmosis in the patients admitted to Malayer urban health centers. Shaahed Medical Journal. 1994;2(5-6):70-73. [Persian]
15. Fallah M, Rabiee S. Matini M, Taherkhani H. Seroepidemiology of toxoplasmosis in primigravida women in Hamadan, Western

Iran. East Mediterr Health J. 2008;14(1):163-171.

16. Rabiee S, Fallah M, Shirmohammadi A, Serpoush H. Seroepidemiology of *Toxoplasma* infection in the women aged 15-45 years in Hamadan, west of Iran. J Res Health Sci. 2003;3(2),P:9-12.
17. Maghsoosd AH, Fallah M, Moradi Sardareh H, Shahnazi H, Hosseini-Zijood SM, Moradi Y. Seroepidemiology of toxoplsmosis in pregnant women referring to health centers in the city of Hamadan, 2012. Pajouhan Scientific Journal. 2012;11(2):3-8. [Persian]
18. Asgari Q, Farzaneh A, Kalantari M, Akrami Mohajeri F, Moazeni M, et al. Seroprevalence of Free-Ranged Chicken Toxoplsmosis in Sub-Urban Regions of Shiraz, Iran. International Journal of Poultry Science. 2006;5(3):262-264.
19. Shahmoradi A, Rezaeian M, Dalimi Asl A. Sheep an important reservoir of human toxoplasmosis in Iran. Medical Journal of Islamic Republic of Iran. 1993;7(3):173-177.
20. Asgari Q, Keshavarz H, Shojaee S, Motazedian MH, Mohebali M, Miri R, et al. In vitro and in vivo potential of RH strain of*Toxoplasma gondii* (Type I) in tissue cyst forming. Iran J Parasitol. 2013;8(3):367.