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RESEARCH ARTICLE

Seroepidemiology of *Toxoplasma gondii* infection and parasite DNA in free-range chickens in Aguascalientes, Mexico

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ABSTRACT

The objectives of the study were to estimate the prevalence of anti-*Toxoplasma gondii* antibodies in free-range chickens in Aguascalientes, Mexico, its association with certain risk factors, and the frequency of parasite DNA in the heart. Eighty-one small rural family farms were included, and blood and heart samples were taken from 150 clinically healthy, adult, free-range chickens. Serum samples were processed by indirect immunofluorescence antibody test considering a dilution of 1:16 as a positive reaction, while the DNA detection was done by PCR. The correlation between the presence of antibodies and the potential risk factors was estimated with logistic regression. The overall seroprevalence in the examined populations was 67%, with a range from 33% to 100% among municipalities, and 78% of the farms having at least one seropositive bird. The PCR test identified the presence of parasite DNA for one case only. Among the variables, the presence of cats and the presence of other animal species on the farm were the ones that had the highest values, while the presence of feral cats and the presence of noxious fauna were also identified as potential risk factors. The results indicate high soil contamination by *T. gondii* oocysts, which implies the active role of cats living on the farms.

KEYWORDS

Toxoplasma gondii, chickens, seroprevalence, risk factors, DNA, Mexico, soil contamination

INTRODUCTION

Toxoplasma gondii is a zoonotic obligate intracellular protozoan that is distributed worldwide. Domestic cats and wild felids are its definitive hosts, whereas a wide range of domestic and wild animals, as well as humans, can act as intermediate hosts (Dubey, 2021). As a result of the enteric cycle of *T. gondii* that takes place in their gut, cats are capable of producing millions of oocysts and excreting them into the environment where they can remain viable for several months (Dubey, 2021). Domestic free-range chickens (*Gallus domesticus*) take part in the spread of the disease as intermediate hosts although they rarely develop clinical signs after infection. However, they represent an excellent indicator of the presence of *T. gondii* oocysts in the environment because their behaviour during grazing allows them to feed at ground level, ingesting grains, plant material, insects, soil or water that may be contaminated with, or contain, sporulated oocysts (Dubey, 2010, 2021), which provokes an immune response resulting in the presence of circulating antibodies. Viable *T. gondii* cysts have been found in tissues of infected free-range chickens, especially the heart (Dubey, 2010, 2021). The worldwide prevalence of anti-*T. gondii* antibodies in chickens has recently been reviewed (Dubey et al., 2020). There is a wide

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diversity in its values depending on the study design and the geographic region, which makes their comparison difficult, but provides evidence of the wide distribution of this parasitosis. In Mexico, the seroprevalence of *T. gondii* infection in free-range chickens has been documented in two studies only (Dubey et al., 2004; Alvarado-Esquivel et al., 2012), finding 6.2% prevalence in the state of Mexico and Mexico City and 25.5% in the state of Durango, respectively.

The objectives of the present study were to estimate the prevalence of anti-*T. gondii* antibodies in free-range chickens in Aguascalientes, Mexico, its association with certain risk factors, and the prevalence of parasite DNA in the heart.

MATERIALS AND METHODS

Study site

The study was conducted in the state of Aguascalientes, Mexico, located in the north-central region of the country (21°52′54″N, 102°17′28″W), at an altitude between 1,860 and 2,010 m above sea level, with an average annual temperature of 18 °C, with a semi-dry weather and rainy regime in the summer. The state is divided into eleven municipalities, and samples were collected in all of them.

Birds and samples

Chickens were selected by convenience from 81 small family farms distributed in different rural communities in the state. The farmers who agreed to participate in the study had given away a different number of animals according to their personal situation, and thus a total of 150 clinically healthy adult free-range chickens were included in the work, regardless of sex. In every case, a blood sample was taken by cardiac puncture, after which the birds were euthanised and the heart was aseptically collected during necropsy and kept at −20 °C until use. The blood samples were centrifuged (1,000×*g*/15 min) in order to obtain the serum, which was placed in polystyrene vials for storage at −20 °C until use. The Committee on the Use and Care of Animals of the Instituto Tecnológico El Llano Aguascalientes and the farm owners approved this project (ITEL-CUCA 011/20).

Serological test

Sera were analysed with a commercial indirect immunofluorescence antibody test (IFAT) (VDRM Inc., USA), according to the manufacturer's recommendations. The tachyzoites of *T. gondii* from the RH reference strain, fixed on a slide, were used as antigens and fluorescein-labelled anti-IgY chicken conjugate (Merck/Sigma-Aldrich, USA), was used to detect captured specific antibodies. Adequate positive and negative controls were included in all reactions. The slides were examined under an epifluorescense microscope at 400 × magnification. The sample was considered positive at a cut-off 1:16 (Braz et al., 2020; Minutti et al., 2021). All samples were processed twice.

Questionnaire

In order to identify possible risk factors associated with seroprevalence, the farmers completed a questionnaire survey. Data regarding the general characteristics of the property where the chickens are kept as well as the general and sanitary farm management were recorded, in addition to investigating the presence of other animal species, especially domestic and feral cats, among others, rodents.

Molecular detection

DNA extraction from the heart was done under aseptic conditions using the commercial kit Quick-DNA Miniprep Plus Kit (Zymo Research, USA), following the manufacturer's instructions. The parasite DNA was detected with a PCR test to amplify an approximately 450 bp fragment of the 529 bp repetitive sequence of *T. gondii* using the specific primer pairs TOX5/TOX8 (5′-CGC TGC AGA CAC AGT GCA TCT GGA TT-3′) and (5′- CCC AGC TGC GTC TGT CGG GAT-3′), including positive and negative controls (Homan et al., 2000; Reischl et al., 2003; Feng et al., 2016). All samples were processed in duplicate. The DNA concentration of every sample was measured with Nanodrop spectrophotometer. The amplification protocol was as follows: initial denaturation at 94 °C for 2 min, followed by 35 cycles of amplification (94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min) and then final extension at 72 °C for 10 min (Feng et al., 2016). The amplified products were electrophoresed in 2% agarose gel in the presence of DNA ladder (GeneRuler 100 bp DNA Ladder, Thermo Fisher Scientific, USA) to estimate the product's weight; gels were stained with ethidium bromide and visualised using UV light. The PCR was considered positive if a product of 450 bp was visualised.

Data analysis

The prevalence of anti-*T. gondii* antibodies was calculated in all populations examined by municipality and according to the different variables identified in the survey with its respective Confidence Interval (C.I.). The prevalence of parasite DNA in heart samples was also calculated. To establish the risk of infection by *T. gondii*, a logistic regression analysis was performed, where the dependent variable was the serological status. The independent variables were selected through the elimination method 'backward step by step', in which the variables that did not result in significant differences ($P < 0.05$) according to the Chi square test, were excluded one by one. The odds ratios (OR) were estimated using the independent variables that showed statistical significance in the multivariate analysis ($P < 0.05$). The analysis was carried out using the Statistics Data Analysis software (STATA) v. 10.1.

RESULTS

The overall seroprevalence of anti-*T. gondii* antibodies in the study population was 67% (101/150; C.I. 95%, 59–74).

Table 1. Seroprevalence of anti-*Toxoplasma gondii* antibodies in free-range chickens in the state of Aguascalientes, Mexico

Municipality	Examined		Positive		Seroprevalence (%)	95% C.I.
	Farms (*n*)	Samples (*n*)	samples (*n*)	farms (*n*)		
Aguascalientes	8	20	7	6	35	16–59
Asientos	12	21	14	8	67	43–84
Calvillo	7	14	13	7	93	64–99
Cosío	1	3	1	1	33	1–87
El Llano	15	21	15	9	71	47–87
Jesús María	8	12	8	7	67	35–88
Pabellón de Arteaga	4	8	3	3	37	10–74
Rincón de Romos	2	4	4	2	100	39–1
San Francisco de los Romo	8	17	12	5	70	44–88
San José de Gracia	6	9	7	5	78	40–96
Tepezalá	10	20	17	9	85	61–96
Total	81	150	101	63	67	59–74

*C.I. = Confidence Interval.

Table 2. Variables associated with the seroprevalence of anti-*Toxoplasma gondii* antibodies in free-range chickens of Aguascalientes, Mexico

Variable	Seroprevalence (%)	OR*	C.I. 95%**	*P* value
Presence of cats	70	6.37	1.54–26.36	0.011
Presence of other animal species	67	6.21	1.40–27.48	0.016
Presence of feral cats	62	4.44	1.10–17.88	0.036
Presence of noxious fauna	69	4.15	1.11–15.49	0.034

*OR = Odds Ratios; **95% Confidence Interval.

All municipalities had seropositive chickens, with a range of seroprevalence from 33% to 100%. In 78% of the farms, at least one seropositive bird was found (Table 1). The seroprevalence was 70% (78/111; C.I. 95%, 60–78) in hens and 59% (23/39; C.I. 95%, 42–74) in roosters.

Four variables were identified as potential risk factors for *T. gondii* infection (Table 2). The presence of cats (OR = 6.38) and other animal species on the farm (OR = 6.21) had the highest values, while the presence of feral cats (OR = 4.4) and noxious fauna (OR = 4.15) were also identified as risk factors. It was not possible to identify a correlation between seroprevalence and other factors considered in the risk analysis, such as the number of cats on the farm, the origin of chickens, the sex of the birds and the absence of a chicken coop to house the birds.

The PCR test performed on the heart samples gave positive result in one case only (0.66%). This sample originated from a farm in the municipality of El Llano.

DISCUSSION

A high overall prevalence of anti-*T. gondii* antibodies was identified in the examined populations (67%). The positive cases were widely distributed in the state territory, indicating that these animals live in environments contaminated with the oocysts of *T. gondii*. Domestic free-range chickens are considered an excellent sentinel of environmental contamination by *T. gondii* oocysts excreted by felines due to their feeding habits and the fact that vertical transmission is very low in these animals, and the infection is established after birth in the majority of cases (Dubey et al., 2020; Dubey, 2021). It has been reported that under experimental conditions, birds can show significant specific antibody levels as early as four weeks after ingesting infective oocysts, and that re-infections can help in maintaining a detectable immune response (Geuthner et al., 2019). The seroprevalence found in this study was higher than that previously reported in free-range chickens in the country, which was 6.2% in the state of Mexico and Mexico City and 25.5% in the state of Durango (Dubey et al., 2004; Alvarado-Esquivel et al., 2012). The overall prevalence was also much higher than that found in broiler chickens from the states of Nayarit and Sinaloa (4.9%) (Alvarado-Esquivel et al., 2012). Worldwide, widely different seroprevalence values have been documented in different countries and regions, depending on factors such as geographic region, climate, farm management, presence of the definitive host, serodiagnostic technique and the determined cut-off value. At a country level, the seroprevalence was up to 90% and the infection was higher in free-range chickens than in caged chickens (Dubey et al., 2020; Nie et al., 2022). The IFAT technique has been used in different epidemiological studies as a screening tool for *T. gondii* antibodies in chickens due to its high sensitivity and specificity. A cut-off point of ≥16 has been used as reference in this species (Braz et al., 2020), in the same way as in this study.

The high seroprevalence identified in the present study can be explained by the housing conditions, in which hens can graze, and with this activity they may ingest infective oocysts released into the soil by the definitive host. Besides that, in this type of farms the coexistence with other domestic and wild species acting as potential hosts of *T. gondii* is common and may contribute to the circulation of the parasite in the environment.

Chickens themselves play an important role in the epidemiology of *T. gondii* in rural areas, because they are clinically resistant and live longer than other intermediate hosts such as mice and birds; in addition, cats fed infected chicken offal can catch the infection and shed millions of oocysts (Dubey, 2010, 2021).

The presence of *T. gondii* tissue cysts in chickens makes it possible to estimate the potential capacity of the tissues to act as a source of infection for carnivores. Data of the literature indicate that muscle tissue, especially the heart, represents a site where these cysts can most commonly be identified (Schares et al., 2017; Minutti et al., 2021). In the present work, parasite DNA was detected in one case; in a sample that was also seropositive. The success of PCR detection of parasite DNA may vary according to the origin of the hens, the type and quantity of the sample, the amount of parasite in the sample, as well as the PCR protocol used. According to some literature reports, the load of the parasite in backyard hens tends to be low (Dubey, 2021), whereas in other studies, DNA has been detected in the heart with moderate prevalence; however, the presence of DNA is not indicative of the viability of the parasite (Dubey et al., 2020).

In this study, the presence of domestic cats as well as feral cats on farms was identified as a risk factor for infection. Cats represent an important link in the chain of transmission and maintenance of the infection, since they are the only ones that can produce oocysts as definitive hosts, and spread them in the environment so that other animals can become infected orally by contaminated soil, forage and drinking water (Shapiro et al., 2019; Dubey, 2021). In the present study, it was possible to verify not only the presence of pet and stray cats, but also their behavioural habits that include wandering freely on the property, accessing the chickens' drinking troughs and defecating in different places. In addition, they retain their habits of hunting small prey, such as birds and rodents, and in some cases they are even used for the natural control of mice. All of these are elements that help the distribution and maintenance of *T. gondii* infection on farms (Schares et al., 2017; Dubey, 2021). However, *T. gondii* is also able to circulate between intermediate hosts, without the presence of cats (Gilot-Fromont et al., 2012).

The presence of other animal species on the farm was identified as an important risk factor. Small family farms keep not only chickens in the backyard but, generally, the farmer also owns other domestic animals, such as cows, pigs, dogs and horses, as happened in several farms in our study, which live together and/or share the property and sometimes also the sources of water and food. Backyard farms with a small population of chicken may have a higher prevalence of infection, tend to be seropositive in a higher ratio and it has also been found that the presence of other animal species represents a risk factor for infection (Bawm et al., 2016; Schares et al., 2017; Stelzer et al., 2019).

The presence of noxious fauna was also identified as a risk factor. The presence of rodents, particularly mice, is common in any farm since they find shelter and food there, and the farms examined in the study do not have an established biosecurity program or control of noxious fauna that would help reduce the health risk they pose. The use of cats as a natural control is counterproductive and represents a risk factor for infection since the ingestion of infected rodents may result in oocyst shedding and increased contamination of the environment (Schares et al., 2017). Other fauna, such as of earthworms, cockroaches and fly larvae as well as other arthropods present in the environment, which can act as mechanical vectors of *T. gondii* oocysts, may be ingested by hens during the grazing and may cause the infection of these birds (Shapiro et al., 2019).

The ingestion of undercooked chicken meat containing *T. gondii* tissue cysts is a potential risk of infection to humans. Different studies indicate that this possibility is lower in commercial chickens raised in confinement than in free-range chickens and in organically grown free-range poultry (Jones and Dubey, 2012). The ingestion of uncooked eggs is not considered an important risk factor for toxoplasmosis (Dubey, 2010).

The results of the present study show high seroprevalence of *T. gondii* in the population under study and a wide geographical distribution of the infection throughout the state of Aguascalientes, Mexico, indicating high levels of soil contamination with oocysts. The identification of cats as a risk factor provides further evidence of their active role in the persistence of *T. gondii* on farms. The high seroprevalence detected in hens also suggests that the infection may also be significantly present in other animals for human consumption, which is a risk for human health.

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REFERENCES

Alvarado-Esquivel, C., González-Salazar, A., Alvarado-Esquivel, D., Ontiveros-Vázquez, F., Vitela-Corrales, J., Villena, I. and Dubey, J. P. (2012): Seroprevalence of *Toxoplasma gondii* infection in chickens in Durango state, Mexico. J. Parasitol. **98,** 431–432.

Bawm, S., Maung, W. Y., Win, M. Y., Thu, M. J., Chel, H. M., Khaing, T. A., Wai, S. S., Htun, L. L., Myaing, T. T., Tiwananthagorn, S., Igarashi, M. and Katakura, K. (2016): Serological survey and factors associated with *Toxoplasma gondii* infection in domestic goats in Myanmar. Scientifica (Cairo) **2016,** 4794318.

Braz, B. M. A., Feitosa, B. C. O., Romão, E. A., Silva, E. M., Pinheiro Júnior, J. W, Mota, R. A., Azevedo, S. S. and Porto, W. J. (2020): Cross-sectional survey for toxoplasmosis in free-range chickens (*Gallus gallus domesticus*) from the Atlantic Forest area in Alagoas state, Northeastern Brazil. Braz. J. Vet. Parasitol. **29,** e014120.

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

Dubey, J. P. (2021): Toxoplasmosis of Animals and Humans. 3rd ed. CRC Press. pp. 17–25, 33–47, 181.

Dubey, J., Morales, E. and Lehmann, T. (2004): Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico. J. Parasitol. **90,** 411–413.

Dubey, J. P., Pena, H. F. J., Cerqueira-Cézar, C. K., Murata, F. H. A., Kwok, O. C. H., Yang, Y. R., Gennari, S. M. and Su, C. (2020): Epidemiologic significance of *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): the past decade. Parasitology **147,** 1263–1289.

Feng, Y., Lu, Y., Wang, Y., Lui, J., Zhang, L. and Yang, Y. (2016): *Toxoplasma gondii* and *Neospora caninum* in free-range chickens in Henan Province of China. Biomed. Res. Int. 8290536.

Geuthner, A., Koethe, M., Ludewig, M., Pott, S., Schares, G., Maksimov, P., Daugschies, A. and Bangoura, B. (2019): Development of an *in vivo* model for *Toxoplasma gondii* infections in chickens and turkeys simulating natural routes of infection. Vet. Parasitol. **276,** 108956.

Gilot-Fromont, E., Lélu, M., Dardé, M., Richomme, C., Aubert, D., Afonso, E., Mercier, A., Gotteland, C. and Villena, I. (2012): The life cycle of *Toxoplasma gondii* in the natural environment. In: Djurković-Djaković, O. (ed.) Toxoplasmosis – Recent Advances. IntechOpen, London, UK. pp. 3–36.

Homan, W. L., Vercammen, M., De Braekeleer, J. and Verschueren, H. (2000): Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int. J. Parasitol. **30,** 69–75.

Jones, J. L. and Dubey, J. P. (2012): Foodborne toxoplasmosis. Clin. Infect. Dis. **55,** 845–851.

Minutti, A. F., Vieira, G. F. E., Sasse, J. S., Martins, T. A., de Seixas, M., Cardim, S. T., de Barros, L. D. and Garcia, J. L. (2021): Comparison of serological and molecular techniques to detect *Toxoplasma gondii* in free-range chickens (*Gallus gallus domesticus*). Vet. Parasitol. **296,** 109515.

Nie, L. B., Gong, Q. L., Wang, Q., Zhang, R., Shi, J. F., Yang, Y., Li, J. M., Zhu, X. Q., Shi, K. and Du, R. (2022): Prevalence of *Toxoplasma gondii* infection in chickens in China during 1993–2021: a systematic review and meta-analysis. Parasitol. Res. **121,** 287–301.

Reischl, U., Bretagne, S., Kruger, D., Ernault, P. and Costa, J. M. (2003): Comparison of two DNA targets for the diagnosis of toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infect. Dis. **3,** 7.

Schares, G., Bangoura, B., Randau, F., Goroll, T., Ludewig, M., Maksimov, P., Matzkeit, B., Sens, M., Bärwald, A., Conraths, F. J., Opsteegh, M. and Van der Giessen, J. (2017): High seroprevalence of *Toxoplasma gondii* and probability of detecting tissue cysts in backyard laying hens compared with hens from large free-range farms. Int. J. Parasitol. **47,** 765–777.

Shapiro, K., Bahia-Oliveira, L., Dixon, B., Dumètre, A., de Wit, L., VanWormer, E. and Villena, I. (2019): Environmental transmission of *Toxoplasma gondii*: oocysts in water, soil and food. Food Waterborne Parasitol. **15,** e00049.

Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L., Maksimov, P., Gethmann, J., Conraths, F. J. and Schares, G. (2019): *Toxoplasma gondii* infection and toxoplasmosis in farm animals: risk factors and economic impact. Food Waterborne Parasitol. **12,** e00037.