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Prevalence of Food-Borne *Toxoplasma gondii* in Free-Ranging Chickens Sold in Tunis, Tunisia

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Abstract

Background: *Toxoplasma gondii*, as an important food-borne protozoan parasite, occurs widely in humans and animals including domestic poultry throughout the world. The goal of this survey was to determine prevalence rate of *T. gondii* in free-ranging chickens sold in Tunis, Tunisia.
Methods: In the present study, the prevalence of *T. gondii* in 40 free-range chickens (*Gallus domesticus*) marketed in Tunis, Tunisia was assessed. Blood and brain samples from each chicken were examined for *T. gondii* infection using the Modified Agglutination Test (MAT) followed by mouse bioassay and Polymerase Chain Reaction (PCR).
Results: Antibodies against *T. gondii* were found in all 40 samples (100%) with the titer of 10 in 14 chickens, 20 in 9 chickens, 40 in 3 chickens, 80 in 5 chickens, 160 in 6 chickens, 320 in 2 chickens and ≥640 in one chicken. By the end of the bioassay, we detected *Toxoplasma* DNA in 15 mouse tissues (37.5%). *T. gondii* was detected from chicken tissues with different antibody titers.
Conclusion: Results of this study indicated very high environmental contamination with *T. gondii* oocysts around Tunis city. The high seroprevalence refers to the public health importance of chickens as source of zoonotic toxoplasmosis to human which suggests that consumption of poultry meat in Tunis city may pose a potential threat to pregnant women health and should be given attention.

Introduction

Toxoplasma gondii occurs widely in humans and animals, including domestic poultry throughout the world. The distribution of *T. gondii* is believed to be best detected using its levels of distribution in free-range chickens (Dubey, 2009; Dubey et al., 2005; Montoya and Liesenfeld, 2004; Tenter et al., 2000). Moreover, according to some traditional and ethnic believes, the meat of free-ranging chickens is one of the most three meat advised to pregnant women for healthier babies. Congenital toxoplasmosis occurs after acquiring infection of *Toxoplasma gondii* during pregnancy (Cook et al., 2000; Jones et al., 2001; Wallon et al., 2004). If the infection is transmitted to the fetus, variable clinical consequences can be observed from the benign forms to the drastic and fatal issues (Dubey, 2010; Dubey and Jones, 2008; Weiss and Dubey, 2009).

Infection is mainly observed via food-borne way by the consumption of unwashed fruits/vegetables, unclear water and/or undercooked/raw meat (Aspinall et al., 2002; Boughattas and Salehi, 2014; Villena et al., 2012). The severity of the infecting parasitic strain can be identified partially within mouse bioassay, strains can be regrouped mainly to fatal, chronic or sub-acute ones.

In our previous works, we reported that congenital toxoplasmosis can be a serious issue in some geographical

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areas with the identification of mixed strains of the parasite (Boughattas et al., 2010), known to lead to severe clinical manifestations (Boughattas et al., 2011a). We tried to identify the food-borne source of contamination by analyzing the prevalence among the three most consumed hosts including horse, sheep and free ranging-chicken. The analysis within horses (Boughattas et al., 2011b) showed a prevalence of 2.4%, in the same city from where clinical cases were reported, assuming that horse meat wasn't a major contamination source. When we investigated the analysis of sheep meat (Boughattas et al., 2014), high seroprevalence was reported within animals (about 50%). The molecular characterization of the isolates suggested the involvement of the sheep meat as an important risk factor. However, the final conclusion of the major source contamination by *Toxoplasma* when consuming meat cannot be retained without the analysis of the free-ranging chickens. Moreover, the prevalence of *Toxoplasma gondii* in free-ranging chickens (*Gallus domesticus*) is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground.

Therefore, we carried out this study to analyze the prevalence rate of *T. gondii* in free-ranging chickens sold in Tunis, Tunisia.

Materials and methods

Sampling

Forty free-range chickens (*Gallus domesticus*) marketed in Tunis, Tunisia was obtained. In the laboratory, brain and blood were sampled after euthanasia and then slaughtering of chickens.

Serology analysis

Blood samples were centrifuged at 800 xg for 10 min and sera were stored at -20 °C until use. Antibodies against *T. gondii* were determined using Modified Agglutination Test (MAT). Briefly, *Toxoplamsa* antigen was made by growing the parasite in mice intra-peritoneal followed by treatment with trypsin and fixation with formaldehyde. The whole antigen was used to coat 96 well U bottomed polystyrene plates.

The sera were screened first at two dilutions 1:10 and 1:100 in 2-mercaptoethanol/Phosphate Buffer Saline (PBS). The plates were shaken for 1 min and then covered and incubated at room temperature for at least 5 h free of any vibrations.

The test was considered positive when a layer of agglutinated antigen/serum was formed covering at least 50% of the bottom of the wells at one dilution at least. In negative wells, antigen precipitation is observed. The positive samples were then titrated by two fold dilution.

Tissues treatment

Tissues were collected in individual plastic bags and kept refrigerated (4 °C). When the corresponding blood sample showed positivity by serological analysis, brains were subjected to enzymatic treatment for the mouse bioassay. Briefly, tissue was ground in 2 volumes (w/v) of 0.9% NaCl and digested 2:3 (v/v) with 2.5% fresh trypsin solution in PBS. Lysis was achieved after incubation during 1 h at 37 °C. The product was then filtrated twice, pelleted by cold centrifugation 10 min at 800 xg, washed and resuspended in saline containing PenicillinG/Streptomycin.

Two milliliters of each pellet were then inoculated intraperitoneally to 3 or 4 *Toxoplasma*-free mice. Mice were followed for 40 days, then sacrificed by cervical dislocation and their tissues were sampled and analyzed for any eventual *Toxoplasma* infection.

Mice tissues were subjected to DNA extraction protocol as described by Boughattas et al. (2014). *T. gondii* diagnostic was carried out using B1-PCR by the B22/B23primers. Amplification program started with preliminary steps of 2 min at 50 °C then, 6 min at 95 °C and followed by 40 cycles of 30 s at 94 °C, 30 s at 57 °C and 1 min at 72 °C adding 1 s/cycle. The final elongation step was achieved after 7 min at 72 °C. Reaction products were resolved on 3% agarose gel.

Results

Antibodies against *T. gondii* were found in all 40 samples (100%) with the titer of 10 in 14 chickens, 20 in 9 chickens, 40 in 3 chickens, 80 in 5 chickens, 160 in 6 chickens, 320 in 2 chickens and ≥640 in one chicken. High titers were then retained in 35% of the samples.

By the end of the mouse bioassay, we detected *Toxoplasma* DNA in 15 mouse tissues (37.5%). *T. gondii* was detected from chicken tissues with different antibodies titer (Table 1).

Almost all chicken samples were asymptomatic to mice. Only one sample was virulent. From the 3 bioassayed mice by the sample, one of 3 experimented mousses died 3 days after inoculation without any ascite formation. The remaining two mice were both positive to *Toxoplasma* infection by serology, microscopy and molecular analysis. The chicken was diagnostic in acute toxoplasmic infection phase as its initial titer was ≥640.

Discussion

Surveys conducted worldwide reported variable prevalence of *T. gondii* infection in free-range chickens ranging from 0% in Japan (Matsuo et al., 2014), 6.9% in Mexico (Alvarado-Esquivel et al., 2012) to 90% in Australia (Chumpolbanchorn et al., 2013). In neighborhood countries

on African continent, prevalence can be 30.5% in Ethiopia (Gebremedhin et al., 2014), 47% in Uganda (Lindström et al., 2008) and 68.8% in Egypt (Barakat et al., 2012). High seroprevalence rate in free-range chickens in the present study also corroborates well with the high seroprevalence previously reported in sheep intended to human consumption (66%) from the same area (Boughattas et al., 2014).

The differences in the seroprevalence observed between the studies may be related to differences in relative climatic conditions, cat densities, study design, the number of chickens examined, sanitation condition, housing of chickens, type of serological tests used and the cut-off value reported. The choice of MAT as a diagnostic tool for the current study was made because of its high sensitivity (96.22%) and specificity (98.8%) in chickens compared to other serological tests (Dubey et al., 1993). The pathogenecity of the *Toxoplasma* strains was investigated by mouse bioassay. Strains are grouped mainly in three groups including 1) virulent strains which cause acute toxoplasmosis and the death of the mouse within 10 days of inoculation, 2) avirulent strains leading to chronic infection without any apparent symptoms and 3) strains with intermediate virulence causing sub-acute infection in mice. It seems that strains from the first and third group or a combination between groups are more associated with clinical toxoplasmosis.

In our present analysis, we detected one sample that led to one mouse death among the three inoculated and high positivity of the remaining two. This could suggest a mixture of strains and further molecular analysis should be investigated to determine its exact genotype.

Table 1: Overview of *T. gondi* infection in chickens sold in Tunis, Tunisia using Modified Agglutination Test (MAT) and Polymerase Chain Reaction (PCR)

	Antibody titer							
Tests	**10**	**20**	**40**	**80**	**160**	**320**	**≥640**	**Total**
MAT No. (%)	14 (35)	9 (22.5)	3 (7.5)	5 (12.5)	6 (15)	2 (5)	1 (2.5)	40 (100)
PCR positive No. (%)	5 (33.3)	5 (33.3)	2 (13.4)	0 (0)	2 (13.4)	0 (0)	1 (6.6)	15 (37.5)

Conclusion

Our previous analyses enable to discard the horse meat as a risk factor and to incriminate the sheep meat as an important risk factor in Tunis, Tunisia. Within the present study, we assessed that the free-ranging chickens can also be an important risk factor for pregnant women due to the high observed seroprevalence and especially with the new report of long parasite persistence in poultry meat. However, further genotyping investigations should be achieved to determine if the infecting strains in chickens are similar to those identified in congenital toxoplsamosis cases and hence assess if the free-ranging chicken should be avoided by pregnant women or not.

Conflicts of interest

The authors indicate that there is no conflict of interest in this work.

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