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Short communication

Antibodies to *Toxoplasma gondii* in slaughtered free-range and broiler chickens

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ABSTRACT

The consumption of undercooked infected chicken can be a source of infection for humans and carnivores regarding the zoonotic protozoan parasite *Toxoplasma gondii*. Furthermore, free-range chickens are sentinels for the presence of *T. gondii* oocysts in the environment because they feed from the ground. By using the modified agglutination test (MAT), we investigated the presence of antibodies to *T. gondii* in 178 free-range and 170 broiler chickens raised indoors and slaughtered in Portugal. Prevalence of specific antibodies was 5.6% in free-range and 0.0% in broiler chickens raised indoors ($p = 0.002$).

1. Introduction

Infection by *Toxoplasma gondii* is prevalent in animals and humans worldwide (Dubey, 2010a). Wild and domestic felids are the definitive hosts of *T. gondii*, while several other homoeothermic animals are the intermediate hosts. *T. gondii* infection is generally transmitted by ingestion of undercooked or raw meat infected with tissue cysts, by ingestion of food or water contaminated with oocysts excreted by infected felids, or transplacentally (Dubey, 2010b).

Most infections in immunocompetent humans are asymptomatic, although in up to 10% of the infected individuals cervical lymphadenopathy or ocular disease may occur (Robert-Gangneux and Dardé, 2012). Nevertheless, toxoplasmosis can be a severe disease in immunosuppressed people and neonates whose mothers have acquired acute infections during pregnancy (Dubey, 2010a).

Poultry meat is currently an important source of protein, but consumption of undercooked infected chicken can be a source of infection with *T. gondii* for humans and other animals (Dubey, 2010b). Free-range chickens, which have access to the outdoors, are considered an epidemiological marker of *T. gondii* for soil contamination with oocysts (Ruiz and Frenkel, 1980; Dubey, 2010a; Yang et al., 2012) Additionally, chickens occasionally develop clinical toxoplasmosis signs, such as encephalitis, chorioretinitis and neuritis (Dubey, 2010a). Chickens raised in confinement have a lower risk of infection compared with the free-ranging ones, due to the fact that containment, management and hygienic procedures can reduce or even prevent the contact of animals with the sources of *T. gondii* (Millar et al., 2012).

In Portugal, previous studies have shown a widespread distribution of antibodies to *T. gondii* in humans and animals species (Lopes et al., 2014; Rodrigues et al., 2019) including free-range chickens (Dubey et al., 2006). In the previous study from Portugal by Dubey et al. (2006), backyard chickens were purchased from individual owners for the main purpose of isolation and genetic characterization of *T. gondii*. Currently, there are no data concerning the prevalence of *T. gondii* in chickens destined for human consumption in the country. This study aimed at determining the presence of antibodies to *T. gondii* in chickens raised under different production systems and slaughtered in the Centre region of Portugal.

2. Materials and methods

In order to determine the appropriate sample size for detecting a difference between two proportions, an expected prevalence of 5.2% for the free-range chickens (Esteves et al., 2012) and 0.0% for the broiler chickens (Dubey et al., 2016), a confidence level of 95% and a statistical power of 85% were adopted. A sample size of 165 units was calculated to be compared.

A total of 348 blood samples were collected from 178 free-range

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male chickens and from 170 conventional broiler chickens (both sexes) in two slaughterhouses in the Centre of Portugal. The 36-day-old conventional broiler chickens were raised in 15 farms under a confinement system, while the 90-day free-range chickens had access to outdoors from 4 weeks of age in another 15 farms. Free-range chickens were housed in 16-hectare parks with 20,000 birds, while broiler chickens were confined in groups of 40,000. Parks for the former were surrounded by fences and cats are not allowed. When a cat is seen there is first an attempt to ward it off; in case this is not effective, the competent authorities in Portugal are alerted. For rodents, there is a structured plan of rodenticide bait stations. For sylvatic birds, taking into account that food and water are placed indoors, anti-bird nets are placed on the windows to minimize access. Insecticides are used between successive flocks to control insect populations.

Ten to 12 chickens were sampled per each farm. Serum samples were separated from clots and stored at -25 °C until use. Sera were assessed for IgG antibodies to *T. gondii* at the dilutions of 1:10 (cut-off titre), 1:20 and 1:40, by a modified agglutination test (MAT) commercial kit (Toxo-Screen DA® bioMérieux, Lyon, France), as per the manufacturer´s instructions. Positive and negative controls supplied with the kit were included in each plate. MAT positive results had visible agglutination (at least 50% of the well´s diameter) after 5–18 hours of incubation at room temperature. The MAT was used because of its sensitivity and specificity for the detection of antibodies to *T. gondii* in chickens (Casartelli-Alves et al., 2014; Hamilton et al., 2017), and has recently been validated for this species (Dubey et al., 2016).

The proportions of chickens seropositive to *T. gondii* were compared using the Fisher's exact test. A p value of 0.05 was considered as statistically significant.

This study complied with the Portuguese legislation on the protection of animals used for scientific purposes (Decree-Law nº 113/2013, of 7 August), which transposes Directive 2010/63/EU of the European Parliament and of the Council, of 22 September.

3. Results

A total of 10 (5.6%) serum samples from free-range chickens were positive, with antibody titres of 1:10 in six and 1:20 in four chickens (Table 1). No statistically significant differences were found between seropositivity values in the 15 free-range chicken flocks ($p > 0.100$). Antibodies to *T. gondii* were not found in the group of broiler chickens raised indoors. A statistically significant difference ($p = 0.002$) was found between the percentages of free-range and that of broiler chickens found seropositive.

4. Discussion

In the present study, the prevalence of antibodies to *T. gondii* in free-range chickens (5.6%) was lower than the 27.1% in the previous study from Portugal (Dubey et al., 2006). There could be several reasons for this variability, including environmental conditions, presence of cats, and the serological test. Although the same MAT test was used in both surveys, the cut-off titres were different. In the previous report, the cut-off titre was 1:5 (Dubey et al., 2006), whereas a cut-off titre of 1:10 was used here to conserve sera. The MAT is considered the most sensitive and specific test for the detection of *T. gondii* antibodies in chickens. In a previous study, 2066 free-range (backyard-raised) chickens from 19 countries were serologically tested and all were bioassayed for the isolation of viable *T. gondii*. These chickens would have been exposed to other infections, including *T. gondii*. Viable *T. gondii* was not isolated from 802 seronegative (MAT < 1:5) chickens, supporting the specificity of MAT. Viable *T. gondii* was isolated from 16 of 105 chickens with MAT titre of 1:5, nine of 79 chickens with MAT titre of 1:10, and the isolation rate increased with an increase in titre (Dubey et al., 2016). Thus, seropositivity in the present study would be indicative of *T. gondii* infection, also by taking into account the statistically significant differences between titres, i.e. 10 (cut-off) and 20 (Table 1).

Table 1
Titres of antibodies to *Toxoplasma gondii* in chickens tested by means of the modified agglutination test (MAT).

Chickens	No. tested	No. of seropositive (%)	No. of sera (%)	
			Titre of 10	Titre of 20
Broilers	170	0 (0.0)[a]	0 (0.0)[b]	0 (0.0)[c]
Free-range	178	10 (5.6)[a]	6 (3.4)[b]	4 (2.2)[c]

[a] $p = 0.002$.
[b] $p = 0.016$.
[c] $p = 0.049$.

A review of worldwide *T. gondii* infection in free-range (including backyard) chickens indicated that up to 100% (range: 0.0–100%) of these birds were infected with *T. gondii*, and some of them developed clinical toxoplasmosis (Dubey et al., 2007, 2010b). No distinction was made between free range or backyard chickens for matters of positivity.

The farms in the present study were controlled properties, having regular veterinary assistance and standardised management (i.e. according to international recommendations by the breeder companies), which probably contributed to maintain a low level of seropositive birds. Although they are classified as being free-range, by using outdoors runs, food and warmth are available indoors. The chance of infected cats gaining access to the fenced runs is unlikely but possible, and the use of contaminated feed or water cannot be ruled out (Pereira et al., 2010; Chumpolbanchorn et al., 2013). Earthworms and arthropods should also be taken into account as paratenic or transport hosts of *T. gondii* oocysts acquired from contaminated soil and transmissible to chickens through the ingestion of those invertebrates (Graczyk et al., 2005). Chickens play an important role in the epidemiology of *T. gondii* in the rural environment, because they are resistant to disease, and infected chickens may serve as source of infection for cats that excrete environmentally resistant oocysts (Ruiz and Frenkel, 1980).

In the present study, the absence of antibodies to *T. gondii* in the broiler is due to the confinement, no contact with cats, rigorous management and hygienic measures, and shorter production cycle (average 36 days). At slaughtering, the free-range chickens were older than the confined broiler chickens, a circumstance which may increase the chance that they come into contact with potential sources of infection. In several previous studies, the prevalence of *T. gondii* in broiler chickens (raised indoors) was found to be low (Dubey, 2010b; Matsuo et al., 2014). In general, commercially-raised boiler chickens are not a likely source of *T. gondii* for humans and viable parasite was not isolated from 100 g of breast meat from each of 2094 chickens from grocery stores in USA nationwide (Dubey et al., 2005).

The present study revealed seropositivity in free-range chickens, which is indicative of *T. gondii* infection. One must also keep in mind that in developing countries chickens (i.e. free-range) in general are slaughtered at home or in unsupervised slaughter facilities and their viscera are left for scavengers or are improperly disposed of, thus potentially becoming a source of infection for cats and for further environment contamination via oocysts excreted by the latter (Ruiz and Frenkel, 1980; Dubey et al., 2016).

Although most of the chickens consumed in Portugal are raised indoor in facilities with little or no exposure to cats and oocysts, there is an increasing consumer demand for free-range chicken meat worldwide, which has boosted the production of this kind of poultry (Chumpolbanchorn et al., 2013; Ying et al., 2017). Because a distinction between boiler chickens and free range chickens may not be evident at grocery stores, all poultry must be properly cooked, not only to prevent *T. gondii* infection but other microbial infections, including salmonellosis.

In conclusion, the results presented in this paper show that chicken contacted with sources of *T. gondii*, and their meat should be considered a potential risk for humans and other animals. Larger scale studies are necessary to investigate the seroepidemiology, isolation and characterization of *T. gondii* strains in chickens in Portugal.

CRediT authorship contribution statement

Filipa T. Rodrigues: Investigation, Methodology, Writing - original draft. **Fernando A. Moreira:** Conceptualization, Resources, Writing - review & editing. **Teresa Coutinho:** Methodology, Writing - review & editing. **Jitender P. Dubey:** Formal analysis, Writing - review & editing. **Luís Cardoso:** Supervision, Writing - review & editing. **Ana Patrícia Lopes:** Supervision, Methodology, Writing - review & editing.

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