

BIOLOGICAL ASPECTS OF *Toxoplasma gondii* IN SAMPLES FROM FREE-RANGE CHICKENS (*Gallus gallus domesticus*) FROM RURAL HOLDINGS IN ESPÍRITO SANTO STATE, BRAZIL

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Abstract: Background: *Toxoplasma gondii*, the causative agent of toxoplasmosis, can infect a broad range of hosts, including humans, and can lead to abortions, neonatal mortality and neurotoxoplasmosis. Thus, knowledge of the biological and molecular aspects of the parasite is important for better integration with epidemiological studies. **Methods:** A serologic screening of 58 free-range chickens (*Gallus gallus domesticus*) grown for human consumption in Espírito Santo State, Brazil, by means of indirect haemagglutination assay (IHA). Thirteen chickens tested positive for anti-*T. gondii* antibodies. Of these, five seropositive animals were selected, and their hearts and brains were extracted and individually used for isolation by mouse bioassay. Ten isolates were obtained and biologically characterised in BALB/c mice inoculated with 10^1 , 10^2 , 10^3 or 10^4 tachyzoites. All isolates were classified as virulent or intermediately virulent. **Results:** Among the isolates obtained in this study, none were considered avirulent, and only two of the heart samples (TgCkBrEs1h and TgCkBrEs5h) were considered intermediately virulent (Table 2), even though survival only occurred at the lowest dose (10^1 tachyzoites). **Conclusion:** These results contribute to the knowledge of the *T. gondii* strains circulating in the state of Espírito Santo and already identified in other parts of Brazil and the world.

Keywords: *Toxoplasma gondii*, Isolation, Virulence, Genotyping.

BACKGROUND

Toxoplasma gondii, a protozoan parasite that can infect any homoeothermic vertebrate, is a pathogenic agent with significant impacts in zoonoses and veterinary medicine. According to estimates, one-third of the human population is infected with the parasite (WEISS & DUBEY, 2009). Felids are the only known definitive hosts of *T. gondii*, which means that the sexual cycle of the parasite occurs in only these animals. Among the most important intermediate hosts are mammals, including humans, and birds. Free-range chickens (*Gallus gallus domesticus*) habitually scratch the soil in search of food and are thus prone to acquire infection in an environment potentially contaminated with oocysts, one of the infective forms of *T. gondii*. For this reason, this bird species is considered an excellent indicator of environmental contamination (DUBEY, 2007a).

T. gondii has a highly clonal population structure in the Northern Hemisphere (DARDÉ, 2008), whereas nonclonal strains, *i.e.*, atypical genotypes, are predominant in South America (SILVA *et al.*, 2014; SHWAB *et al.*, 2014). Thus, the genetic diversity of the parasite typically follows a geographical distribution. While few genotypes dominate in the Northern Hemisphere, hundreds of genotypes coexist in the Southern Hemisphere. Despite none being notably dominant over others, some genotypes have a higher relative frequency (SHWAB *et al.*, 2014). In Brazil, more atypical strains exist (DUBEY & SU, 2009; CARNEIRO *et al.*, 2013; SILVA *et al.*, 2014), a situation related to several factors, such as geographical range, tropical climate, rich fauna and diverse transmission routes (FERREIRA *et al.*, 2006). Furthermore, four genotypes with wide circulation and described in different hosts in Brazil have been proposed as Brazilian clonal lineages. These lineages are termed BRI, BRII, BRIII and BRIV and are distinct from the archetypal lineages types I, II and III (PENA *et al.*, 2008).

One classic means of categorising *T. gondii* virulence is by inoculating the parasite in experimental models. According to mortality in mice, *T. gondii* is classified as virulent, avirulent or intermediately virulent. Clonal strains of type I are virulent, independent of the inoculated dose, whereas the avirulent strains (type III) establish a chronic infection at doses below 10^3 tachyzoites (SILVA *et al.*, 2014).

Thus, the objective of the present study was to isolate *T. gondii* from the tissues of free-range chickens from Espírito Santo State (ES), Southeast Brazil, and to follow up by assessing the virulence.

I. METHODS

A total of 58 free-range chickens from the municipalities of Espírito Santo, Brazil, were evaluated in the period of January to October 2014. Samples were first submitted to indirect haemagglutination assays (IHAs) (Imuno-HAI Toxoplasmoses WAMA®, São Carlos, São Paulo, Brazil) according to the manufacturer's instructions. Seropositive chickens were sacrificed by cervical dislocation, and the brains and hearts were collected. Organs were stored at 4 °C until used.

T. gondii strains were isolated in a bioassay of the brains and hearts of five seropositive chickens. Isolation was not successful for the remaining chickens. Full organs were macerated, digested with pepsin, washed with sterile phosphate-buffered saline (PBS, pH 7.2), and inoculated intraperitoneally into two Swiss mice (DUBEY, 1998a). Cases of infection by *T. gondii* were confirmed when tachyzoites or cysts were found in the tissues under a light microscope. The present study has been approved by the Animal Ethics Committee of the Federal University of Espírito Santo (Comitê de Ética em Experimentação Animal, Universidade Federal do Espírito Santo – CEUA/UFES 080/2011).

Virulence of the isolated parasites was assessed by means of biological characterisation. Groups of five mice were infected intraperitoneally with increasing doses of 10^1 , 10^2 , 10^3 and 10^4 tachyzoites of each isolate. Animals were observed for 30 d and then sacrificed. Infection was confirmed by the presence of brain cysts and IgG antibodies detected by enzyme-linked immunosorbent assay (ELISA), as described by Brandão *et al.* (2009).

II. RESULTS

A. Serology

All birds from two localities of Espírito Santo exhibited negative IHA results. Of the 34 birds from other one, 13 (38 %) tested positive for anti-*T. gondii* IgG. (**Table 1**).

Table 1. Indirect haemagglutination antibody (IHA) test for toxoplasmosis in free-range chicken sera from Espírito Santo State, Brazil.

Location	Samples	Positive (%)
1	8	0 (0 %)
2	16	0 (0 %)
3	34	13 (38 %)
Total	58	13 (22 %)

B. Isolation and characterisation of *T. gondii* strains

The isolation of *T. gondii* was successful; all inoculated Swiss mice died and/or developed ascites with tachyzoites at 7 to 15 d post-inoculation. Five isolates were obtained from the brains and five from the hearts. Among the isolates obtained in this study, none were considered avirulent, and only two of the heart samples were considered intermediately virulent (Table 2), even though survival only occurred at the lowest dose (10^1 tachyzoites).

III. CONCLUSIONS

The HAI test can be considered reliable when compared to other methods. It was used, for the isolation, cardiac tissues of chickens, for being highly consumed in Brazil. Free-range chickens were successfully used as sentinels of environmental infection by *T. gondii*.

It was observed 100% of *T. gondii* isolation from the brain and heart of serologically positive chicken, collected in one of the regions. The animals exhibited high tissue parasitism, which facilitates isolation via bioassays in mice. The results of this study bring a greater knowledge about environmental

contamination and is a warning for an appropriate preparation of the meat of these animals for human consumption. Concern about toxoplasmosis is so focused on domestic cats that it is common for people to forget that consumption of meat contributes significantly to the cycle of toxoplasmosis transmission.

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