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Risk of infection by the consumption of liver of chickens inoculated with low doses of *Toxocara canis* eggs

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ABSTRACT

Experimental studies and registries of cases of human toxocariasis have shown that the consumption of raw or undercooked offal of the paratenic host of *Toxocara canis* may pose a risk of infection. Thus, we evaluated the risk of infection due to the consumption of liver of chickens inoculated with different doses of embryonated *T. canis* eggs. Doses were 5–100 times smaller than the ones previously employed in this type of study. Groups of five chickens were inoculated with 5000 (control), 1000, 500, 300 or 50 eggs of *T. canis*, and at 72 h post-inoculation, the liver of each bird was consumed by a BALB/c receptor mouse. Forty-eight hours after consumption, we examined the organs and carcasses of the mice for larvae of *T. canis*. All mice were positive for larvae, except the group that consumed the chicken liver inoculated with 50 eggs. This group contained only one positive mouse, in which the larva was lodged in the brain. In mice that consumed livers of chickens inoculated with ≥300 eggs, larvae concentration was primarily in the liver and lungs, characterizing the initial phase of infection. We conclude that the consumption of raw poultry liver, under the studied conditions, poses a risk of infection even with a low number of infected *T. canis* eggs.

1. Introduction

Visceral toxocariasis is a parasitic zoonosis that is widely distributed, but its worldwide prevalence is likely underestimated, primarily due to the difficulty of obtaining a correct diagnosis (Smith et al., 2009). Most cases are associated with the *Toxocara canis* and *Toxocara cati* nematodes, which are intestinal parasite of dogs and cats, respectively (Azizi et al., 2007; Yoon et al., 2009). The mode of infection most frequently recorded is by accidental ingestion of embryonated eggs from the environment (Despommier, 2003). However, the consumption of raw or undercooked offal and meat from birds and mammals, which are paratenic hosts of *T. canis*, can also transmit the parasite to humans, especially given that this is a common practice in some cultures (Hoffmeister et al., 2007).

There have been several reported cases of human toxocariasis acquired by this type of transmission, particularly after the consumption of raw chicken, duck, and ostrich

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liver (Ito et al., 1986; Nagakura et al., 1989; Morimatsu et al., 2006; Hoffmeister et al., 2007; Noh et al., 2012). Moreover, experimental studies have also demonstrated the risk of infection due to the consumption of poultry liver inoculated with eggs of *T. canis* (Pahari and Sasmal, 1990a,b; Tüzer et al., 2002; Taira et al., 2003, 2004). However, all these previous studies used high infectious doses of *T. canis* eggs (≥5000), which does not reflect what likely occurs during the natural infection of such paratenic hosts. The objective of this study was to evaluate the risk of infection by *T. canis* through the consumption of chicken livers inoculated with smaller doses of embryonated eggs than those hitherto used, with the aim of increasing our knowledge of the transmission by the consumption of offal from paratenic hosts of *T. canis*.

2. Materials and methods

This study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande (CEPAS n° P021/2011), and all of the experiments were performed according to Brazilian legislation on animal care.

2.1. Mice

Groups of BALB/c female mice aged between 6 and 7 weeks were used. The animals were kept in an acclimatized environment at 22 °C (±2 °C) with a bright–dark cycle of 12 h, and food and water were available *ad libitum*.

2.2. Broilers

Male broilers were of the Sussex breed and were 15 days old. They were kept in cages with 0.32 m × 0.38 m × 0.16 m, respectively, in length, breadth and height, with a maximum of three birds and received food and water *ad libitum* in the Biotery Center – FURG.

2.3. Incubation with T. canis eggs

T. canis eggs were collected directly from the uterine tubes of 50 adult female parasites following the treatment of five young dogs with pyrantel pamoate (15 mg/kg). Afterwards, the eggs were incubated in an 80 ml of 2% formalin solution at 28 °C for 30 days (De Avila et al., 2012).

2.4. Inoculation of T. canis eggs in chickens

Embryonated eggs were inoculated through a stomach tube into five groups (G) of five chickens each. One group (G1) was given 5000 eggs (control), according to the methodology used by Gargili et al. (1999), and the other groups received lower doses of *T. canis* eggs: 1000 (G2), 500 (G3), 300 (G4) and 50 (G5). The birds were euthanized 72 h post egg-inoculation (Taira et al., 2003, 2004).

2.5. Consumption of chicken livers by mice

The liver of each donor chicken, weighing an average of 4 g was offered to a receptor mouse, which was euthanized 48 h after the consumption of the all viscera. Next, the search for *T. canis* larvae was conducted by individual tissue digestion of skeletal muscle (carcass), brain, liver, lungs, kidneys, heart and eyes, in a 1% hydrochloric acid solution and 1% pepsin, under constant agitation for 12 h at 37 °C (Xi and Jin, 1998). After, morphological identification and quantification of larvae in an optical microscope (400 times increase) was taken (Dutra et al., 2013).

2.6. Statistical analysis

The number of larvae recovered in the mice was subjected to mathematical transformation, represented by the following equation: log n° of larvae + 1. Then, variance analysis was performed, and the means of different organs were compared using the Tukey test, at a significance level of 5%.

3. Results

All recipient mice that were fed chicken livers inoculated with ≥300 *T. canis* eggs were positive for *T. canis* larvae (Table 1). The only group that was not 100% positive for larvae was the group that consumed liver from G5 chickens, which were inoculated with 50 *T. canis* eggs. Furthermore, the average recovery of larvae from this group was lower than in groups that consumed livers from the G1, G2, G3 and G4 groups, inoculated with 5000, 1000, 500 and 300 *T. canis* eggs, respectively. Although there was no significant difference with respect to the recovery of larvae in mice of the G1, G2, G3 and G4 groups ($p > 0.05$), there was a decrease in the number of recovered larvae as the dose of inoculated eggs decreased in donor chickens.

In the group of mice that consumed the G1 chicken liver, 86% of total larvae were recovered by the sum of the liver, lungs and carcass. From mice that consumed chicken liver from the G2, G3 and G4 chickens, 85.4%, 76.7% and 86.8% of larvae, respectively, were recovered in the lungs and liver (Table 2). One mouse that consumed a chicken liver from the G5 group was positive for one larva of *T. canis*, which was found in the brain, but like the others mice in the study, no clinical change was perceived.

4. Discussion

Experimental studies (Taira et al., 2004; Dutra et al., 2013) and cases of human toxocariasis associated with the consumption of raw livers from birds infected with *Toxocara* spp. (Nagakura et al., 1989; Morimatsu et al., 2006; Hoffmeister et al., 2007; Noh et al., 2012) have provided evidence of this type of transmission. Moreover, considering that dogs can eliminate 15,000 parasite eggs per gram of feces (Acha and Szyfres, 1986) and the presence of eggs on dog's hair (Roddie et al., 2008; Amaral et al., 2010), is frequent exposure of paratenic hosts.

In this study, we found *T. canis* larvae in mice that consumed the liver of chickens inoculated with 1000, 500, 300 or 50 *T. canis* eggs, which are infective doses that are 5–100 times lower than those previously used for this transmission model (Pahari and Sasmal, 1990a; Taira et al., 2003, 2004). These results allow us to conclude that the consumption of the raw liver of this paratenic host, even if infected with a low number of eggs, poses a risk of infection for

Table 1
Mean number of *Toxocara canis* larvae recovered in mice ($n = 5$) 48 h after consuming livers of chicken inoculated with embryonated eggs ($n = 5$).

Chicken group/N° of inoculated eggs	Mice post-consumption of liver				
	Mean number of larvae	Standard deviation	Total larvae	Positive mice	*P* value
G1: 5.000	42.2A	31.3	211	05	0.0001
G2: 1.000	19.0A	29.6	95	05	0.0155
G3: 500	17.2A	14.6	86	05	0.0028
G4: 300	10.6A	3.0	53	05	0.0033
G5: 50 (control)	0.2B	0.4	01	01	

Different letters between the lines indicate significant differences at the 5% level.

Table 2
Percentage of *Toxocara canis* larvae recovered in organs and carcasses of mice ($n = 5$) 48 h after consumption of the liver of chickens inoculated with different doses of embryonated parasite eggs ($n = 5$).

Chicken group/N°of inoculated eggs	Mice post-consumption of liver							Total larvae
	Recovery larvae (%)							
	Liver	Lungs	Carcass	Brain	Heart	Eyes	Kidneys	
G1: 5000	28.8	35.8	21.4	7.9	3.7	0.5	1.9	211
G2: 1000	26.5	58.8	2.1	12.6	0	0	0	95
G3: 500	43.0	33.7	9.3	10.5	0	0	3.5	86
G4: 300	45.3	41.5	Zero	Zero	1.9	1.9	9.4	53
G5: 50	Zero	Zero	Zero	100.00	0	0	0	1

humans. Moreover, the presence of the infection is more important than the intensity of the infection, as unlike in most previous studies, which have involved direct inoculation of the eggs, in this study, the infection was obtained from the consumption of an organ (liver) of a paratenic host (El-Shazly et al., 2002; Al-Saeed and Mahmood, 2011).

A total of 100% of the receptor mice fed with liver of chickens inoculated with a dose ≥300 eggs of *T. canis* were positive for *T. canis* larvae. The concentration of *T. canis* larvae in organs such as the liver and lungs of the mice 48 h after consumption of the chicken liver demonstrates that the larvae were migrating in the body of the paratenic host, which characterizes the initial phase of infection (Dunsmore et al., 1983).

In addition to the larvae found in the lungs and liver, larvae were found in the carcasses of mice that consumed livers from poultry inoculated with 5000 eggs. However, all examined organs of mice from this group were positive for larvae, which was likely due to the migration of larvae. These results are in agreement with those of previous studies (Tüzer et al., 2002; Taira et al., 2003, 2004).

In the group of mice that consumed chicken livers from the G5, chickens that were inoculated with 50 *T. canis* eggs, only one mouse was positive, and the larva was recovered from the brain. Even though only one mouse was positive for a larva of *T. canis* in this group, this result indicates the risk of migration to organs such as the brain in infections with low numbers of larvae. According to Magnaval et al. (2000), the ingestion of a few eggs can cause infection in humans, even if asymptomatic, and can result in the migration of the larvae to the eyes or brain. It is important to initiate treatment in these cases.

Although *T. canis* is the most common cause of visceral larva *migrans*, other species of *Toxocara* and even other parasites can trigger the infection. Azizi et al. (2007) and Taira et al. (2011) indicated that the use of viscera of poultry infected with *T. cati* can cause toxocariasis. In addition, there have been reports of visceral larva *migrans* caused by *Baylisascaris procyonis*, the raccoon roundworm (Park et al., 2000; Perlman et al., 2010). Thus, due to difficulty in making the differential diagnosis, it is possible that the infection can be caused by other species of *Toxocara*, as well as other parasites.

We found *T. canis* infection in all groups of mice that consumed donor chicken livers, even when using low doses of embryonated *T. canis* eggs, which simulates what may occur in the transmission of human toxocariasis.

Conflict of interest statement

The authors declare no conflict of interest.

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