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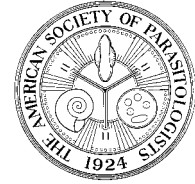
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PREVALENCE OF ANTI-*TOXOPLASMA GONDII* ANTIBODIES IN RATITES FROM BRAZIL

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KEY WORDS ABSTRACT

Toxoplasmosis The prevalence of anti-*Toxoplasma gondii* antibodies was investigated among 104 ratites: 68 rheas
Rhea (*Rhea americana*), 16 emus (*Dromaius novaehollandiae*), and 20 ostriches (*Struthio camelus*) in 4
Emu Brazilian states. The prevalence in rheas was 26.5% (18 of 68), and titers were 1:100 (n = 8), 1:200 (n
Ostrich = 1), 1:400 (n = 4), 1:800 (n = 4), and 1:1,600 (n = 1). In emus, the prevalence was 50% (8 of 16),
MAT and titers were 1:50 (n = 1) and 1:100 (n = 7). The ostriches were slaughtered for human
consumption, and 80% (16 of 20) were seropositive with titers of 1:200 (n = 1), 1:400 (n = 9), and
1:800 (n = 6). Sera were tested with a modified agglutination test, and the results confirmed the
distribution of the parasite in ratite species from Brazil. The data obtained in this study show that *T.*
gondii is prevalent among ratites from Brazil, and therefore ratite meat should also be considered a
potential source of human infection. This is the first report of *T. gondii* seroprevalence in emus.

Toxoplasma gondii is an intracellular protozoan that infects warm-blooded animals, including humans. The infection can be severe and is of great importance for human and veterinary medicine (Dubey, 2010). The parasite has been detected in farm animals worldwide, from which the meat might be a source of human infection (Tenter et al., 2000). Domesticated and wild avian species have been found to carry *T. gondii* (Hejlíček et al., 1981; Uterak et al., 1992; Leite et al., 2007; Dubey, 2010), but there is little information on the infection among ratites.

The meat from ratites has higher nutritional value than beef and chicken meat (Charuta et al., 2015). In this group of birds, 3 species are commercially reared in Brazil: rhea (*Rhea americana*), emu (*Dromaius novaehollandiae*), and ostrich (*Struthio camelus*). Rhea is a ratite from South America adapted to the climatic conditions of this continent and has the potential to be bred for consumption and exportation. After the species was successfully bred in North America and Europe, Brazil began to demonstrate an interest in it as a meat source (Filho and Lucio, 2006). Emu is a species native to Australia and was recently introduced in Brazil. The commercial interest in these birds is related to the tenderness and the low level of fat present in the meat—most of an emu's fat is stored on its back—resulting in both high nutritional value and health benefits (Sales, 2007). Although ostriches are African birds, they have also been successfully bred in Brazil, and meat/derivative exports are the main interest of breeders (Filho and Lucio, 2006).

The global ratite meat market meat is gaining popularity due to health-conscious consumers, who are becoming increasingly careful in choosing lean alternatives over traditional red meats (Naveena and Kiran, 2014). Considering the use of these animals as meat sources, it is important to investigate hygiene conditions and parasite occurrence because they can serve as sources of human infection by *T. gondii*. Therefore, we aimed to evaluate the frequency of anti-*T. gondii* antibodies in the serum of ratites from 4 Brazilian states and discuss the possibility for future consumption of the meat to influence human rates of infection in the country.

MATERIALS AND METHODS

Blood samples of 104 ratites (68 rheas, 16 emus, and 20 ostriches) were collected during technical visits through brachial vein puncture using a 22 G needle (rheas and emus) and during animal slaughtering from the neck (ostriches). Then the blood was placed directly into 15-ml centrifuge tubes without anticoagulant, refrigerated at 10 °C, transported to the laboratory, and centrifuged at 200 g for 10 min. Serum samples were stored at –20 °C until serological analysis for anti-*T. gondii* antibodies using the modified agglutination test (MAT).

Of the 68 blood samples of rheas, 48 were collected from a conservation farm in Cachoeiro do Itapemirim, Espírito Santo state, 10 from an ecological farm in São Carlos, São Paulo state, and another 10 from birds bred at the Universidade Estadual do

Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro state. Emu blood samples were also collected from animals residing at UENF. All samples collected at UENF were from newly acquired birds at a farm in Rio Verde, Goiás state. Ostrich blood samples were collected from 20 animals slaughtered for human consumption in a slaughterhouse from Quissamã, Rio de Janeiro state.

Samples were stored in 8 ml tubes and transported at -4 C to Núcleo de Pesquisas Avançadas em Parasitologia (NUPAP) located at UENF in Campos dos Goytacazes, Rio de Janeiro state, Brazil, where serological testing was performed through MAT according to Dubey and Desmots (1987). In short, sera were diluted 2-fold in 0.01 M phosphate-buffered saline (PBS) pH 7.2 from 1:25 to 1:3,200, and all samples with agglutination activity at a dilution of 1:25 were considered positive. The antigen consisted of formalin-fixed *T. gondii* tachyzoites of the RH strain, and the test was performed in U-shaped 96-well plates, where 25 μl of the antigen solution was mixed with 25 μl of the previously diluted sera. Positive and negative controls were also added and incubated at 37 C for 12 hr. The positive control was serum from a mouse experimentally infected with *T. gondii*. The negative control was serum from a mouse known to be negative for *T. gondii* infection. The results were based on the sedimentation profile of the tachyzoite suspension, where the formation of a blue bottom is negative, and a clear bottom is positive.

Analysis of the influence of gender, species of ratite, and locality on the presence of anti-*T. gondii* antibodies in sera were made using Fisher's exact test and a chi-square test, with a confidence interval of 95%.

RESULTS

Of the 104 ratites examined, 42 were seropositive, which indicated an overall prevalence of 40%. Specific prevalences were 27% (18/68), 50% (8/16), and 80% (16/20) for rheas, emus, and ostriches, respectively. These prevalences when compared were statistically significant ($P = 0$ [$\chi^2 = 19.12$ (CI 95%)]). Using Fisher's exact test no statistical difference was found between the prevalence of rheas and emus ($P = 0.08$) or between emus and ostriches ($P = 0.08$). However, a significant difference ($P = 0$) was seen between rheas and ostriches (Table I).

The highest titer observed was 1:1,600 ($n = 1$) in 1 rhea, and maximum terminal titers of 1:100 ($n = 7$) and 1:800 ($n = 6$) were observed in emus and ostriches, respectively. The complete range of titers for all birds analyzed is detailed in Table II.

Of the 68 rheas, 33 (49%) were males and 35 (51%) were females, with 7 seropositive males (39%) and 11 seropositive females (61%) (Table III). There was no significant difference between genders using Fisher's exact test ($P = 0.41$). Information on the gender of the emus and ostriches was not available at the time of sampling.

Of the 48 rheas from Espírito Santo state, 12 (25%) were seropositive, of which 5 (41.7%) were male and 7 (58.3%) were female. Of the 10 rheas from the property in São Paulo state, five (50%) were seropositive, of which 2 (40%) were male and 3 (60%) were female. Of the 10 rheas of Goiás state, only 1 (10%) young female was seropositive. No statistical difference was observed among the prevalences of rheas from these 3 states ($P = 0.12$ [$\chi^2 = 4.29$ (CI 95%)]).

Table I. Research of anti-*Toxoplasma gondii* antibodies in ratites from Brazil.

Ratites*	Seroprevalence			χ^2	P value†
	Positive (%)	Negative (%)	Total (%)		
Rheas (A)	18 (17.3)	50 (48.1)	68 (65.4)	19.12	0.0
Emus (AB)	8 (7.7)	8 (7.7)	16 (15.4)		
Ostriches (B)	16 (15.4)	4 (3.8)	20 (19.2)		
Total	42 (40.4)	62 (59.6)	104 (100)		

* Equal letters in the column do not differ statistically ($P < 0.05$) by Fisher's exact test with 95% significance.

† For 95% confidence interval.

DISCUSSION

The first report of seroprevalence in ratites was made by Orosz et al. (1992), who reported anti-*T. gondii* antibodies in 1 cassowary (1:1,024) and 1 rhea (1:4,096), both presenting with anorexia and gastrointestinal disturbance. A young female in the present study also presented a high antibody titer (1:1,600), and according to Soares et al. (2010), titers for *T. gondii* in birds are high at the beginning of the infection and become low or undetectable after infection, which is different from what occurs in mammals. Although Orosz et al. (1992) associated high titers of anti-*T. gondii* antibodies with clinical signs of severe infection in their research, including a favorable response to treatment, the high-titer rhea from our research did not present any clinical manifestations. Therefore, we infer that even in recent infections ratites may have different clinical responses.

Two out of 5 Brazilian regions have been tested for the seroprevalence of anti-*T. gondii* antibodies in rheas: the Northeast, with 4.3% prevalence (Soares et al., 2010), and the South, with 8.1% (Marobin et al., 2004) and 50% (Almeida et al., 2013) prevalence. In our study, we present outcomes for rhea bred in 2 other regions: the Midwest (Goiás state), with 10% prevalence, and the Southeast, with 25% (Espírito Santo state) and 50% prevalence (São Paulo state). This prevalence range is in accordance with that of previous studies and confirms that *T. gondii* is present in rheas throughout the vast Brazilian territory. Additionally, given the high *T. gondii* seroprevalence history of Southeast Brazil, the outcomes of the present research reinforce the hypothesis that this region is an important spot on the map of toxoplasmosis epidemiology, including for ratites.

It is important to consider that due to the use of different diagnostic methods, comparisons of prevalence values should be made with caution. The 2 studies performed in South Brazil (Marobin et al., 2004; Almeida et al., 2013) used different techniques—indirect hemagglutination test and MAT—and presented completely different prevalence levels (8.1% and 50%, respectively). However, considering all prevalence levels analyzed by MAT in Brazil, including ours, a wide range of results can still be observed. MAT has been extensively used for the diagnosis of toxoplasmosis in animals, with high specificity and sensitivity (Dubey, 2010).

This is the first study on the presence of anti-*T. gondii* antibodies in emus (50%), and the prevalence was found to be higher than those of all studies performed with rheas (Orosz et al., 1992; Marobin et al., 2004; Soares et al., 2010; Almeida et al., 2013) and ostriches (Dubey et al., 2000; Martínez-Díaz et al.,

Table II. Seroprevalence of anti-*Toxoplasma gondii* antibodies in ratites from Brazil with the modified agglutination test (cutoff = 1:25).

Species	State and municipality	n	No. of samples (%) with terminal titers of:							Total (%)
			25	50	100	200	400	800	≥1,600	
<i>Rhea americana</i>	Espirito Santo									
	Cachoeiro do Itapemirim	48	—	—	6 (13)	—	2 (4)	4 (8)	—	12 (25)
	Goiás									
	Rio Verde	10	—	—	—	—	—	—	1 (10)	1(10)
	São Paulo									
	São Carlos	10	—	—	2 (20)	1 (10)	2 (20)	—	—	5 (50)
<i>Dromaius novaehollandiae</i>	Goiás									
	Rio Verde	16	—	1 (6)	7 (44)	—	—	—	—	8 (50)
<i>Struthio camelus</i>	Rio de Janeiro									
	Quissamã	20	—	—	—	1 (5)	9 (45)	6 (30)	—	16 (80)
Total		104	0 (0)	1 (1)	15 (14)	2 (2)	13 (12)	10 (10)	1 (1)	42 (40)

2002; Hove and Mukaratirwa, 2005; Contente et al., 2009; Almeida et al., 2013; El-Madawy and Metawea, 2013; Rahimi et al., 2014; Silva and Langoni, 2016; Feng et al., 2017). Little is known about the clinical signs of toxoplasmosis in ratites, especially emus, and their influence on breeding. Further studies are necessary on *T. gondii* infection in this animal species and its immune response to the different antigenic stimuli of the parasite.

Seroprevalence rates for *T. gondii* in ostriches have been found to range from 0.8 to 48% (Dubey et al., 2000; Martínez-Díaz et al., 2002; Hove and Mukaratirwa, 2005; Contente et al., 2009; Almeida et al., 2013; El-Madawy and Metawea, 2013; Rahimi et al., 2014; Silva and Langoni, 2016; Feng et al., 2017), contrasting with the 80% seroprevalence detected in this research. In contrast to the natural environment of ostriches, Brazilian farms present a vast range of vegetation. Ostriches eat almost anything they can find, including green grasses, seeds, plants, small insects, fecal material, stones, and other objects (Aganga et al., 2003), and the high prevalence found in our research can be explained by this nonselective feeding habit that increases the possibility of ingesting pathogens. Since the ostriches tested came from a slaughterhouse, it should be taken into account that this meat is a potential source for *T. gondii* infection in humans.

There are 3 studies on the occurrence of anti-*T. gondii* antibodies in ostriches: 2 in the southeastern region (Contente et al., 2009; Silva and Langoni, 2016) and 1 in the southern region of the country (Almeida et al., 2013). Contente et al. (2009) and Silva and Langoni (2016) studied many areas of São Paulo state and detected seroprevalence rates of 14.4% in 195 and 11% in 344 ostriches, respectively. In the state of Rio Grande do Sul, 46 ostriches were tested, and a 17.4% seroprevalence was observed (Almeida et al., 2013). All these prevalence levels are well below

the 80% detected in the present study (Table I), indicating that the birds herein were probably bred in a farm with a high contamination rate by *T. gondii* oocysts in soil, water, or food.

The first research performed outside Brazil studied Canadian ostriches, and the infection rate was 2.9% in 28 animals tested (Dubey et al., 2000). In contrast, in Zimbabwe, a country with climatic conditions very similar to Brazil, the infection rate was high, 48% in 24 ostriches evaluated (Hove and Mukaratirwa, 2005). In Spain, Martínez-Díaz et al. (2002) analyzed 117 serum samples and found only 1 positive (0.8%) result. In Egypt, El-Madawy and Metawea (2013) analyzed 120 sera of ostriches and found a prevalence of 12.5%. Feng et al. (2017) analyzed 32 sera of ostriches from China and found a prevalence of 18.7%, and when analyzing sera and meat together from a total of 315 samples, a prevalence of 6% was found. In Iran, 20 samples of ostrich meat were analyzed, and 6 positive samples (21%) were found (Rahimi et al., 2014). Most likely, prevalence differences have occurred due to differences in climate since sporulated *T. gondii* oocysts do not survive for long in cold or dry climatic conditions (Contente et al., 2009). Although the prevalences found in several countries are low, the risk of toxoplasmosis cannot be ruled out by the consumption of meat from these birds. Consumption of undercooked and cured meat is a potential source of infection for humans and other animals (Hove and Mukaratirwa, 2005).

The antibody titers for rheas of the present study ranged from 100 to 1,600 (Table II). Marobin et al. (2004) observed a range of 8 to 128, Soares et al. (2010) observed a range of 50 to 200, and a range of 25 to 6,400 was observed by Almeida et al. (2013). The antibody titers for ostriches in the present study ranged from 200 to 800 (Table II). Dubey et al. (2000) observed a range of 25 to

Table III. Prevalence of anti-*Toxoplasma gondii* antibodies in sera of rheas of 3 Brazilian states and association with gender.

State	n (%)	Positive			Negative			P value*
		Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	
Espirito Santo	48 (70)	5 (8)	7 (10)	12 (18)	20 (29)	16 (24)	36 (53)	0.51
São Paulo	10 (15)	2 (3)	3 (4)	5 (7)	2 (3)	3 (4)	5 (7)	1.00
Goiás	10 (15)	0 (0)	1 (2)	1 (2)	4 (6)	5 (7)	9 (13)	1.00
Total	68 (100)	7 (11)	11 (16)	18 (27)	26 (38)	24 (35)	50 (73)	0.41

* Difference between sexes measured by Fisher's exact test with 95% confidence interval.

500, unlike Contente et al. (2009), who observed a larger interval of 16 to 16,384. Almeida et al. (2013) observed a range of 16 to 256, whereas Silva and Langoni (2016) observed a similar range (8 to 256). The only positive sample found by Martínez-Díaz et al. (2002) presented a titer of 1:25, and Feng et al. (2017) observed a range of 25 to 100. *Toxoplasma gondii* titers remain detectable for months or years, and high titers can occur during continuous exposure to the source of infection (Gallas-Lindemann et al., 2013), which may be occurring with the rheas in our research. Additionally, the low titers found in the cited works, including the emus of the present research, maybe due to a single or non-continuous contact that these animals had with *T. gondii*. According to Marobin et al. (2004), animals with low antibody titers showing no clinical signs cannot be considered a source of transmission to humans. Most likely, the emus came into contact with *T. gondii* and mounted an immune response, which remains only as immunological memory.

No studies were found in the literature that associated the prevalence of anti-*T. gondii* antibodies with sex in rheas, and our analysis did not detect significance in this association (Table III).

The rheas and emus of the present research were bred in large areas and, therefore, had contact with other animals, including wild animals. One hypothesis for the infection of the birds in the present study is the probable contact with infected domestic or wild felids, which strongly contribute to the dissemination of the infection by shedding oocysts in the soil (Gallas-Lindemann et al., 2013). Furthermore, it is important to understand and improve the sanitary management of ratites to prevent environmental contamination, as well as that of the feed and water supplied to the birds, to control and decrease the prevalence of infected animals, and consequently to reduce the risk of infection to humans (Contente et al., 2009).

MAT is a validated test for the detection of *T. gondii* in many animals and birds, including ratites (Feng et al., 2017); therefore, we could say that there is a high potential for human infection through the consumption of meat from high-titer animals. As the trade in ratite meat is likely to rise in the near future in Brazil, more studies, especially those on the isolation of *T. gondii* from meat samples, should be conducted. The virulence and genetic characteristics of the strains must be recorded and will be an important addition to the study of this important zoonosis of Brazil and the world. With the increase in the consumption of ratite meat, it is necessary for more extensive studies to be conducted to allow us to take preventive measures to control *T. gondii* infections and thus avoid transmission to consumers.

We conclude that *T. gondii* is prevalent among ratites in Brazil and that their meat should also be considered a potential source of human infection. In addition, this is the first report of *T. gondii* seroprevalence in emus.

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