Prevalence of *Toxoplasma gondii* antibodies in ostriches (*Struthio camelus*) from commercial breeding facilities in the state of São Paulo, Brazil

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Abstract

Toxoplasmosis is widespread zoonosis caused by *Toxoplasma gondii* ,a protozoan that may infect mammals and birds. The aim of the present study was to assess the prevalence of *T. gondii* in ostriches (*Struthio camelus*) from commercial breeding facilities in the state of São Paulo, Brazil, in a way to increase the knowledge on the behavior and importance of the parasite in this animal species. A total of 195 serum samples were collected from ostriches from Sorocaba, Campinas, São Carlos, Araçatuba, São Paulo, Vale do Ribeira, Botucatu and são José do Rio Preto, in the state of São Paulo. These samples were analyzed by means of the Modified Agglutination Test (MAT) in order to investigate the occurrence of *Toxoplasma gondii* antibodies. The test showed that 14.36% of the animals were seropositive to *Toxoplasma gondii*. Minimum titer was considered to be equal or greater than 1:16, and the greatest dilution observed was 1:16,384. No statistically significant differences were found between males and females. Seronegative animals occurred in only two regions (São Paulo and São José do Rio Preto). These results point out the importance of further studies on this infection in ostriches, and on management practices that may minimize the risk of toxoplasmosis transmission in these birds which would, in their turn, decrease the risk for the final consumer.

Key words:
Toxoplasma gondii.
Ostrich.
MAT.

Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii*, an obligate intracellular parasite, affecting men and all warm-blooded animals that have already been tested. It is spread in humans and many other homoeothermic animals, and it is considered to be one of the most commom parasites of human. In its life cycle, domestic and wild felines are definitive hosts and human, other mammals and birds are its intermediate hosts.[1,2,3,4,5,6,7]

Intermediate hosts acquire the infection by three main routes: 1. ingestion of oocysts in soil, sand or any other place where cats defecate, being disseminated by means of transport hosts such as flies, cockroaches and worms; 2. ingestion of cysts in raw or undercooked meat; 3. transplacentary infection.[4,7,8]

In one study on *Toxoplasma gondii* in ostriches, the autors studied *T. gondii* in the serum of 50 wild ostriches in Zimbabwe and observed that 48% of the animals were reactors, whereas another study, in Canada, found only 2.9% seropositive animals. The latter study, however, involved animals of commercial breeding facilities.[9,10]

The objective of the present study was to estimate the prevalence of anti-*Toxoplasma gondii* antibodies in commercial breeding facilities in the state of São Paulo, in order to better understand the behavior and importance of this parasite in this animal species.

Material and Method

The software EPIINFO[11] was used to determine the number of samples required for the study (n). Expected prevalence equal to 8% was used as a reference, significance level was set at α = 1%, confidence level at 99% and error margin at 5%, and n was determined to be equal to 195.[11,12]

Samples were collected in eight breeding regions of the state of São Paulo (Sorocaba, Campinas, São Carlos, Araçatuba, São Paulo, Vale do Ribeira, São José do Rio Preto and Botucatu). The cities of Sorocaba, Campinas and São Carlos account for 62% of the breeding facilities of the state of São Paulo. Therefore, the number of samples was divided in a way to represent the main breeding cities/regions, as seen in box 1. This division was based only in the number of breeders, for there was no data on the exact number of animals in each region. Age of the birds used in the trial ranged from 12 to 18 months, the minimum age recommended for slaughter. In each region, samples were collected from the same number of males and females.

A total of 102 blood samples were collected from females, and 93 from male ostriches. Collection of the samples was carried out by means of a puncture in the right jugular, brachial or metatarsal veins, using 10-mL syringes and 40x12 needles. Volume ranged from five to 10mL.

Samples were placed in sterile test tubes and left still for serum to be obtained. They were then sealed, identified and sent to the *Laboratório de Diagnóstico em Zoonoses* (LDZ), in a styrofoam cooler containing ice. After arriving there, they were centrifuged for a better separation of the serum, and were then placed again in sterile test tubes, which were identified and kept frozen until the moment of serological testing.

Modified direct agglutination (MAT) was used according to Desmonts and Remington[13]. MAT is recommended as the most convenient method in the detection of *T. gondii* in epidemiological surveys in birds. MAT has high sensitivity and specificity for the detection of anti-*Toxoplasma gondii* antibodies, and has the additional advantage of not requiring species-specific conjugates.[13, 14, 15, 16]

Results

Positive animals were observed in all regions studied. Among the 195 serum samples that were submitted to MAT, 28 samples showed titers equal or greater than 1:16, with 14.36% animals seroreactor to *Toxoplasma gondii*, as may be observed in table 1. For the interpretation of the results, samples were considered to be positive when titers were equal or greater than 1:16. Distribution of seroreactors according to MAT titers in shown in table 2.

Box 1 - Number of ostrich serum sample obtained in São Paulo state, representing the main breedin cities/ regions – 2005

City/Region	Number of samples	%
Sorocaba	20	10,26
Campinas	75	38,46
São Carlos	25	12,82
Araçatuba	20	10,26
São Paulo	10	5,12
Vale do Paraíba	10	5,12
Botucatu	15	7,7
São José do Rio Preto	20	10,26
Total	**195**	**100**

Positive results were observed in 14.70% females and 13.98% males (Table 3), with no statistically significant differences (ñ = 0.95).

The distribution of the results of Modified Agglutination Test for *Toxoplasma gondii* in 195 ostriches serum samples, from breeding proprieties of Sao Paulo state, according with the city/region, is showed in box 2.

Table 1 - Results of Modified Agglutinatin Test for *Toxoplasma gondii* in 195 ostriches serum samples, from breeding proprieties of Sao Paulo state, 2005, considering titers equal or greater than 1:16

Total Sample	Positives		Negatives	
	N	%	N	%
195	28	14,36	167	85,64

Table 2 - Results of Modified Agglutinatin Test for *Toxoplasma gondii* in 195 ostriches serum samples, from breeding proprieties of Sao Paulo state - 2005, considering all titers

Diluition	N	%
Negatives	167	85,64
1:16	5	2,56
1:64	11	5,64
1:256	5	2,56
1:1024	2	1,03
1:16384	2	1,03
Total	**195**	**100**

Table 3 - Results of Modified Agglutinatin Test for *Toxoplasma gondii* in 195 ostriches serum samples, from breeding proprieties of Sao Paulo state - 2005, considering the sex

	Number of samples	Positives	%	Negatives	%
Male	93	13	13,98	80	86,02
Female	102	15	14,70	87	85,29
Total	195	28	14,36	167	85,64

Box 2 - Distribution of the results of Modified Agglutinatin Test for *Toxoplasma gondii* in 195 ostriches serum samples, from breeding proprieties of Sao Paulo state, 2005, according with the city/region

City/Region	Positives		Negatives		Total	
	N	%	N	%	N	%
Sorocaba	03	15	17	85	20	10,26
Campinas	16	21,33	59	78,66	75	38,46
São Carlos	03	12	22	88	25	12,82
Araçatuba	03	15	17	85	20	10,26
São Paulo	00	00	10	100	10	5,12
Vale do Paraíba	01	10	09	90	10	5,12
Botucatu	02	13,33	13	86,66	15,	7,7
São J. R. Preto	00	00	20	100	20	10,26
TOTAL	**28**	**14,36**	**167**	**85,64**	**195**	**100**

Discussion

Prevalence of anti-*Toxoplasma gondii* was 14.36% in ostriches from commercial breeding facilities in the state of São Paulo. In Brazil, no studies on the species were found, but in Rio Grande do Sul a similar study was carried out in rheas from commercial breeding facilities submitted to similar management. The authors reported prevalence equal to 8.10% as assessed by passive hemagglutination. Another study, in Canada, involving 973 ostriches observed only 2.9% seropositive animals. As cited before, in wild ostriches from Zimbabwe prevalence was equal to 48%. As in the present study, these authors also used MAT.[10,12]

The clinic signs in birds are normally severe, with ocular and cerebral lesions, affecting from canaries and turkeys to penguins. In trials involving birds, little is known on clinical signs. Several authors studied the experimental infection in wild and domestic birds. However, this kind of report involves only the immunological response of the animals to *T. gondii*, for animals are euthanized soon after the immune response is recorded, leaving a lot to be studied.[17,18,19,20]

Sporulated *T. gondii* oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months. However, they do not survive long under cold or dry conditions. Oocysts of the parasite would have better conditions to survive in pastures of Brazil than in those of Canada, what would reinforce the hypothesis found in this study that the differences observed in the two studies are due to the climate, pasture contamination and sanitary and nutritional management to which the ostriches are submitted.[10,21]

In a study involving poultry, bovines, ovine and caprines, which took into account the management schemes of each species (extensive, semi-extensive and intensive), infection rates were lower in intensive breeding units. Technification of the breeding facilities and less contact of the birds with soil leads to less contaminated animals.[22,23]

Ostriches do not depend only on grains for their nutrition, but also on other sources of fiber. The diet of birds such as ostriches is mainly based on insects, other small invertebrates and forage plants. A large part of the water t hey need comes from plants.[24,25]

Little is known on the clinical symptoms of toxoplasmosis in ratites in general, and specifically in ostriches, and their implications to the breeding of these animals. Further studies are necessary on the infection of this animal species and their immune response to the different antigenic stimuli of this parasitosis.

Conclusion

The prevalence of antibodies anti-*Toxoplasma gondii* in the serum of ostriches from commercial breeding facilities from the state of São Paulo, as assessed by direct agglutination test, was 14.36%. Males and females showed very similar prevalence, with no significant differences between genders.

It is important to know and improve sanitary management of these birds, preventing environmental contamination, as well as that of feed and water supplied to them, in order to control and decrease the prevalence of animals contaminated by the parasite, consequently reducing the risk of infection to humans.

Ostrich meat may be safely consumed, provided it not undercooked, as indicated for any kind of meat. However, more specific studies should be carried out on the subject.

Prevalência de anticorpos anti — *Toxoplasma gondii* em avestruzes (*Struthio camelus*) de criatórios comerciais no estado de São Paulo

Resumo

A toxoplasmose é uma zoonose cosmopolita causada pelo protozoário *Toxoplasma gondii*, podendo acometer mamíferos e aves. O presente estudo teve como objetivo estimar a prevalência do

Palavras-chave:
Toxoplasma gondii.
Avestruz.
MAD.

Toxoplasma gondii em avestruzes (*Struthio camelus*) de criatórios comerciais do estado de São Paulo, como forma de auxiliar no conhecimento do comportamento e importância do parasito nesta espécie animal. Foram colhidas 195 amostras de soro de avestruzes, provenientes de Sorocaba, Campinas, São Carlos, Araçatuba, São Paulo, Vale do Ribeira, Botucatu e São José do Rio Preto, estado de São Paulo. As amostras foram analisadas pela Técnica de Aglutinação Direta Modificada (MAT), para a pesquisa de anticorpos anti – *Toxoplasma gondii*. Os exames sorológicos revelaram 14,36% de animais sororreagentes ao *T. gondii*. A titulação mínima considerada foi a diluição maior ou igual a 1:16, e a maior diluição encontrada foi 1:16384. Não foi constatada diferença significativa entre os sexos. Apenas duas regiões (São Paulo e São José do Rio Preto) não apresentaram animais sororreagentes. Esses resultados salientam a importância de um estudo mais aprofundado sobre a infecção em avestruzes, e também sobre as práticas de manejo que venham a minimizar o risco de transmissão da toxoplasmose para essas aves e, por conseqüência, para o consumidor final.

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