

Frequency of anti-*Toxocara* antibodies in broiler chickens in southern Brazil

Frequência de anticorpos anti-*Toxocara* em frangos de corte no Sul do Brasil

Adilson Cardoso de Oliveira¹; Guita Rubinsky-Elefant²; Yslla Fernanda Fitz Balo Meriguetti¹; Aline da Silveira Batista¹; Vamilton Alvares Santarém^{3*}

¹ Programa de Pós-graduação em Ciência Animal, Universidade do Oeste Paulista – UNOESTE, Presidente Prudente, SP, Brasil

² Laboratório de Soroepidemiologia e Imunobiologia, Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo – USP, São Paulo, SP, Brasil

³ Programa de Pós-graduação em Ciência Animal, Laboratório de Medicina Veterinária Preventiva (Parasitologia Veterinária), Hospital Veterinário, Universidade do Oeste Paulista – UNOESTE, Presidente Prudente, SP, Brasil

Received August 21, 2017

Accepted March 14, 2018

Abstract

The aim of this study was to evaluate the presence of anti-*Toxocara* antibodies in naturally infected broiler chickens (n = 189) from the state of Paraná, southern Brazil. The chickens were reared in a semi-intensive system by small family farmers (n = 7). An enzyme-linked immunosorbent assay (ELISA) was performed to detect the presence of anti-*Toxocara* spp. IgY after serum adsorption with *Ascaridia galli* antigens. An overall seroprevalence of 67.7% (128/189; 95% CI = 61.1-74.4) was observed. The frequency of positive animals by farm ranged from 29.6% to 100%. The optical density and reactivity index values observed in ELISA test indicated the possible chronicity of infection of the evaluated chickens. Associations between the presence of antibodies and the area where the chickens were reared (p = 0.382) or the population density of dogs on the farm (p = 0.785) were not observed. This study shows a high prevalence of *Toxocara* spp. antibodies in broiler chickens reared in semi-intensive systems and provides evidence that chickens are a good indicator of environmental contamination by larva migrans agents. Further studies are necessary to assess the risk factors associated with poultry infection and the likelihood of toxocarasis transmission to humans via the ingestion of free-range chicken meat.

Keywords: ELISA, *Gallus gallus domesticus*, seroprevalence, toxocarasis, zoonosis.

Resumo

A finalidade do presente estudo foi avaliar a presença de anticorpos anti-*Toxocara*, em frangos de corte naturalmente infectados (n = 189), no Norte do Paraná, Sul do Brasil. Os frangos foram criados em sistema semi-intensivo, em pequenas propriedades rurais (n = 7). Os testes sorológicos foram realizados pela técnica de ELISA, para detecção de anticorpos IgY (IgG), com pré-adsorção do soro com antígenos de *Ascaridia galli*. Foi observada uma prevalência de 67,7% (128/189; IC 95% = 61,1-74,4). A frequência de animais soropositivos por propriedade variou de 29,6% a 100%. Os valores da Densidade Ótica e do Índice de Reatividade observados no teste de ELISA indicaram uma possível cronicidade de infecção dos frangos avaliados. Não foi observada correlação entre a positividade dos animais, quando comparada a área (p = 0,382) e a densidade populacional de cães por propriedade (p = 0,785). O presente estudo verificou uma alta prevalência de anticorpos anti-*Toxocara* em frangos de corte criados em sistema semi-intensivo e oferece dados que apontam esses animais como bons indicadores de contaminação ambiental por agentes de *larva migrans*. Estudos futuros são necessários para avaliar os fatores de risco associados e a possibilidade da transmissão de toxocaríase ao ser humano pela ingestão de carne de frango.

Palavras-chave: ELISA, *Gallus gallus domesticus*, soroprevalência, toxocaríase, zoonose.

*Corresponding author: Vamilton Alvares Santarém. Laboratório de Medicina Veterinária Preventiva (Parasitologia Veterinária), Hospital Veterinário, Universidade do Oeste Paulista – UNOESTE, Rodovia Raposo Tavares, Km 572, Limoeiro, CEP 19067-175, Presidente Prudente, SP, Brasil.
e-mail: vamilton@unoeste.br



Introduction

Poultry, one of the most important and richest sources of protein for humans, are reared both intensively in fully automated, environmentally controlled systems and semi-intensively in backyards (FAO, 2014).

Backyard production methods imply low biosafety measures and a high risk of infectious diseases (CONAN et al., 2012; IBRAHIM et al., 2016). Chickens reared in backyards, for instance, harbour a wide variety of geo-parasites, becoming highly infected during feeding (BEN SLIMANE, 2016; FERDUSHY et al., 2016; JAVAREGOWDA et al., 2016). In Bangladesh, chickens reared in backyards were significantly more susceptible to infections by parasites than those reared in intensive systems (RABBI et al., 2006).

Chickens have been considered indicators for environmental contamination by soil-borne parasitic diseases (CARDOSO & YAMAMURA, 2004), especially toxoplasmosis, a zoonosis that may be transmitted to humans via the ingestion of undercooked, infected chicken meat (DUBEY, 2010).

Chickens are also considered an indicator for soil contamination by the eggs of dog/cat *Toxocara* spp. (CAMPOS-DA-SILVA et al., 2015; VON SÖHSTEN et al., 2017), a nematode responsible for human toxocarasis, one of the most prevalent helminthiasis in the world (RUBINSKY-ELEFANT et al., 2010). Toxocarasis is primarily transmitted to humans via the ingestion of soil containing embryonated eggs; however, the consumption of raw or undercooked meat from chickens may also represent an alternative route of transmission to humans (NAGAKURA et al., 1989; TAIRA et al., 2004; MORIMATSU et al., 2006).

Recent studies on natural infections in chickens reared in semi-intensive system/free-range chickens have shown a high prevalence of anti-*Toxocara* antibodies in poultry commercialized in street markets (VON SÖHSTEN et al., 2017) and from different origins (CAMPOS-DA-SILVA et al., 2015), along with the presence of *Toxocara* larvae (*T. canis* and *T. cati*) in the tissues of the animals (ZIBAEI et al., 2017).

Despite the high prevalence of anti-*Toxocara* antibodies in chickens, there is a lack of information regarding the factors associated with the infection.

In this study, we aimed to evaluate the presence of anti-*Toxocara* IgY antibodies in slaughtered broiler chickens, in addition to other factors associated with the infection.

Materials and Methods

Study area and farms

The serum samples of the chickens evaluated in this study were obtained in a slaughterhouse located in the municipality of Barra do Jacaré, state of Paraná, southern Brazil (23° 06' 54" S; 50° 10' 53" W).

The slaughterhouse was selected based on their management of an association of small farms (n = 7) that were located in the same municipality and were characterized by family maintenance, covered a small area (average = eight hectares), and produced broiler chickens as the major source of family income.

Animal rearing

In this association system, the slaughterhouse obtained, per week, approximately 1000 Label Rouge chicks at one day of age from a chick industry.

The chicks were maintained in a concrete shed base (250 m²) for a 30-day adaptive period and fed food based on corn (*Zea mays*) and water *ad libitum*.

Following the adaptive period, the chickens were then transferred to the seven associated farms (n = 100-150 chickens/farm). On the farms, the chickens were reared (cycles of 60-90 days) in a semi-intensive system (area from 1200 to 5000 m²) and given corn-based food (*Zea mays*) and water *ad libitum*.

All the information regarding the farm properties (number of dogs, total area of the farm) was provided by the slaughterhouse manager.

The study was approved by the Ethics Committee for Animal Use (CEUA) of the University of Western São Paulo (UNOESTE), Presidente Prudente, state of São Paulo, Brazil, under protocol 3114/2016.

Sample collection

Our study was carried out from May to July 2016 and was designed to randomly collect 27 samples of animals belonging to the same group of chicks (n = 100 animals/farm). Therefore, a total of 189 chickens, aged 60-90 days, were included in this study.

Following the slaughter of the chickens, blood samples were collected in vacuum tubes. The tubes were centrifuged (1,650 g, 10 min) to obtain the serum samples, which were transferred into microtubes and stored at -20 °C for processing.

Antigen preparation

Toxocara canis excretory-secretory L₃ antigens (TES) were obtained according to a previously described method (ELEFANT et al., 2006). Briefly, *T. canis* eggs were collected from the uteri of female *T. canis* adult worms and incubated (28°C) for a period of approximately one month in 2% formalin. Next, to induce the rupture of the mamilonated membrane of the embryonated eggs, the suspension was treated with a 5% sodium hypochlorite solution for 5 min at room temperature, and the second-stage larvae were recovered using a Baermann apparatus. The obtained larvae were maintained in serum-free Eagle's medium (37 °C), and the culture supernatant containing the TES was transferred to sterile flasks each week. Then, the supernatant was treated with 200 mM phenyl-methyl-sulfonyl fluoride, a protease inhibitor (Sigma®, St. Louis, MO, USA), concentrated with Amicon Ultrafiltration units (Millipore®, Danvers, MA, USA), dialysed against distilled water, centrifuged (18,500 × g, 60 min, 4 °C), and filtered using 0.2-µm Millipore membranes.

Protein concentrations were measured using the Lowry method (LOWRY et al., 1951).

Ascaridia galli adult worm extract (AWE)

Birds living in direct contact with soil are susceptible to infection via the transmission of other soil-borne helminths, in addition to *Toxocara* spp. Before performing the enzyme-linked immunosorbent assay (ELISA), each chicken serum sample was treated with an *A. galli* antigen suspension to minimize the cross-reactivity with *Toxocara* antibodies and, consequently, to increase the specificity of the ELISA (CAMPOS-DA-SILVA et al., 2015; VON SÖHSTEN et al., 2017).

To obtain the AWE, we followed a previously described protocol (ROMASANTA et al., 2003; ELEFANT et al., 2006). First, adult *A. galli* were recovered from the bowel of a naturally infected chicken. The worms were then macerated in distilled water, and NaOH was added until a final concentration of 0.15 M was obtained. The material was then incubated (room temperature, 2 h) and neutralized with 6 M HCl. Thereafter, the extract was treated with ether to remove the lipids and centrifuged (18,500 g, 20 min, 4 °C). The aqueous phase was removed and filtered (0.22-µm Millipore membrane). The sera were pre-incubated at a final concentration of 25 µg/mL AWE in 0.01 M phosphate-buffered saline (PBS, pH 7.2) with 0.05% Tween 20 (PBS-T) (Sigma®, St. Louis) (37 °C, 30 min).

Indirect ELISA for anti-*Toxocara* IgY detection

An indirect ELISA was performed to evaluate the anti-*Toxocara* IgY (IgG) antibodies according to the previously described protocol (SAVIGNY et al., 1979) with some modifications (ELEFANT et al., 2006). Briefly, 96-well polystyrene plates (Corning, Costar, New York, NY) were coated (100 µL/well) with TES diluted in 0.01 M PBS (pH 7.2) to a concentration of 1.9 µg/mL. Following incubation (37 °C, 2 h followed by 4 °C, 18 h), the wells were washed, and thereafter, each well was blocked with 200 µL of 1% bovine serum albumin (BSA, Sigma, St. Louis, USA) in PBS-T 20 (0.05%) with an incubation at 37 °C for 1 h.

After washing, the serum samples (100 µL/well), diluted 1:200, were incubated in duplicate (37 °C, 40 min). The plates were then washed and incubated (37 °C, 40 min) with peroxidase-conjugated anti-chicken IgY antibodies (IgG) produced in rabbit (A9046, Sigma, Birmingham, AL, USA) (100 µL/well), diluted 1:40,000 in 0.05% PBS-T. The material was washed, and tetramethylbenzidine (TMB-BD®, San Diego, CA, USA) was added as the chromogenic substrate (100 µL/well). Following incubation (37 °C, 6 min), the reaction was stopped using a 2 N H₂SO₄ solution (50 µL/well). The absorbance readings were performed at 450 nm (Titertek Multiskan MCC/340, Lab-System, Finland).

The positive and negative control sera used in our study were the same as those used in an experimental trial, in which three groups of chickens were infected with different infective doses of *T. canis* eggs (100, 1000, and 5000), and another group was employed as a negative control (SILVA RAPOSO et al., 2016). According to these authors, an ELISA had 96.8% sensitivity and 100% specificity in the detection of IgY on the 60th day post-infection, independent of the infective dose.

All the samples were evaluated in duplicate, and the final OD was determined as the average of the duplicates. Antibody levels were expressed as reactivity indices (RIs) and were calculated from the ratio between the absorbance values of each tested sample and the cut-off value (RI = OD sample / OD cut-off). The samples with RIs greater than 1 were considered positive. The cut-off calculation was based on the OD value of the negative control (0.087) plus 4 SDs ($4 \times 0.0194 = 0.078$), resulting in a value of 0.165.

Statistical analysis

The Pearson correlation test was performed to evaluate the relationship between the density of dogs per farm (m²) and the frequency of ELISA positivity. A 95% confidence interval (CI) was obtained using the Wilson method according to Sundar Dorai-Raj (2014). All analyses were performed using R software (R DEVELOPMENT CORE TEAM, 2014), and the results were interpreted using a significance level of 0.05.

Results

Our screening obtained positive results for anti-*Toxocara* IgY antibodies in 67.7% (128/189; 95% CI = 61.1-74.4) of the free-range chickens according to the results of the indirect ELISA.

Table 1 summarizes the ODs, RIs, and frequencies of poultry that tested positive per farm. The ELISA positivity ranged from 29.6% to 100%.

We compared the ODs of the positive animal samples with the ODs of the positive and negative controls. It was observed that 91.4% (n = 117) had ODs higher than the cut-off and lower than the positive control (PC), while in 8.6% (n = 11), the OD was equal to or higher than the PC.

The RIs ranged from 0.187 to 0.993 and from 1.0 to 3.431, respectively, for the negative and positive samples (RI mean = 1.358). For chickens that tested positive, 44.5% had RIs between 1.0 and 1.49, while 47.7% had RIs from 1.500 to 2.499, and the RIs for 7.8% were higher than 2.500 (Figure 1).

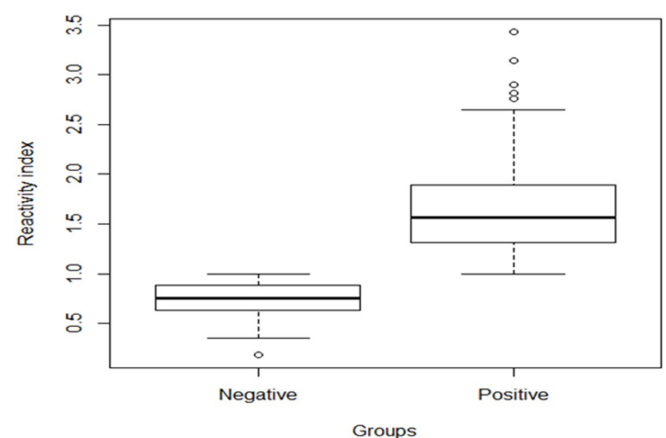


Figure 1. Box plot representing the distribution of the reactivity indexes of anti-*Toxocara* antibodies (IgY) in free-range broiler chickens detected by indirect ELISA. State of Paraná, southern Brazil - 2017.

Table 1. Frequencies of anti-*Toxocara* antibodies (IgY) in free-range broiler chickens detected by indirect ELISA. State of Paraná, southern Brazil. 2017.

Farm	Area (m ²)	Examined samples	Positive		RI ± sd
			N	% (95% CI)	
I	1,250	27	26	96.3 (81.7-99.3)	1.881 ± 0.466
II	1,200	27	27	100.0 (87.5-100.0)	1.826 ± 0.515
III	2,400	27	25	92.6 (76.6-97.4)	1.641 ± 0.570
IV	2,500	27	21	77.8 (59.2-89.4)	1.288 ± 0.478
V	5,000	27	11	40.7 (24.5-59.3)	1.009 ± 0.379
VI	1,200	27	8	29.6 (15.6-48.5)	0.942 ± 0.297
VII	2,500	27	10	37.0 (21.5-55.8)	0.919 ± 0.330
Total	2,293*	189	128	67.7 (60.8-74.0)	1.092 ± 0.434

*mean; CI: confidence interval; N: number; RI: reactivity index; sd: standard deviation.

The number of dogs by farm ranged from 1 to 12 (mean = 3.9), and the area where the chickens were reared varied from 1,200 to 5,000 m² (mean = 2,293 m²). Considering the frequency of the positive ELISA results, no correlations were observed when the area (p value = 0.382) and the density of dogs (p value = 0.785) were compared.

Discussion

We screened for the presence of anti-*Toxocara* spp. antibodies in free-range chickens slaughtered in an abattoir in southern Brazil. Our data revealed a high seroprevalence of the antibodies (67.7%). In Brazil, a high frequency of anti-*Toxocara* IgY antibodies has also been detected by ELISA in the sera of free-range chickens of different origins (CAMPOS-DA-SILVA et al., 2015) and in broilers commercialized in street markets (VON SÖHSTEN et al., 2017), with positive results obtained in 58.5% and 89.9% of samples, respectively.

In this study, serum pre-absorption with *A. galli* antigen suspensions was performed to minimize cross-reactivity with *Toxocara* (ELEFANT et al., 2006; CAMPOS-DA-SILVA et al., 2015; VON SÖHSTEN et al., 2017). These authors observed that the pretreatment of chicken serum with *A. galli* antigens reduced the OD values obtained in the ELISA by approximately 15%.

We evaluated samples of chickens that were 1) of the same breed, 2) exposed to the same rearing system, and 3) exposed during a defined period of time (60-90 days), thereby excluding certain confounding variables associated with the infection, particularly during the period of exposure. We observed that majority (91.4%) of the chicken samples had an OD higher than the cut-off value and lower than the PC. Campos-da-Silva et al. (2015) considered samples from chickens with an OD higher than the cut-off and lower than the PC to be in three possible groups: infected, with past infection, or infected with another nematode (cross-infection). In this study, given the RI and OD values, the evaluated chickens could not have had past infections; due to the short lifespans of the evaluated chickens, the presence of antibodies was likely a consequence of chronic infection by *Toxocara* spp.

In this study, the frequencies of seropositive chickens ranged from 29.6% to 100%. This variation in the seropositivity for anti-*Toxocara* antibodies has been considered to be dependent on the origin of the chickens. Campos-da-Silva et al. (2015) observed

differences in the prevalence rates of antibodies in evaluated samples of chickens from small farms (78.6%), backyards (57.0%), and slaughterhouses (42.8%), and those necropsied at a university (42.5%). Another study (VON SÖHSTEN et al., 2017) reported similar findings (50.0% to 100%) and analysed samples of birds sold at street markets across eight different months.

Some risk factors have been associated with toxocariasis. In humans, toxocariasis tends to be more prevalent in rural settings, where the presence of dogs and high soil contamination (RUBINSKY-ELEFANT et al., 2010; MOREIRA et al., 2014). Therefore, it is likely that free-range chickens reared in a rural environment are commonly exposed to *Toxocara* eggs.

The poultry evaluated in our study lived in environments that were cohabitated by pets, particularly dogs. However, the frequency of positive ELISA results was not associated with the density of dogs on the farm or with the area where the poultry were reared. Our study evaluated a small number of farms (n = 7), which likely limited the evaluation of risk factors associated with the infection.

Campos-da-Silva et al. (2015) stated that free-range chickens may represent an indicator of soil contamination by *Toxocara* eggs, which may be reinforced by our results. These animals are also considered one of the best indicators of environmental contamination by *Toxoplasma gondii* oocysts (DUBEY et al., 2006; DUBEY, 2010).

In our study, it was not possible to evaluate the presence of larvae in the tissues of chickens. Zibaei et al. (2017) demonstrated the presence of *Toxocara* larvae (83.3% *T. canis* and 16.7% *T. cati*) in the tissue of broiler chickens by digesting the tissues in pepsin followed by a PCR assay. The authors concluded that broiler chickens can be natural paratenic hosts for the larvae of *Toxocara* spp., and in poultry, these larvae may be agents of human toxocariasis when humans consume raw, undercooked chicken meat, as observed by Nagakura et al. (1989) and Morimatsu et al. (2006).

Based on the conditions where chickens are reared and the high level of antibodies observed in our study and by others (CAMPOS-DA-SILVA et al., 2015; VON SÖHSTEN et al., 2017), it is important to consider that the free-range environment may be a source of infection for humans and animals, and special attention should be given to farmers who are involved in free-range chicken-rearing activities.

In conclusion, the results of this study demonstrate that broiler chickens reared under semi-intensive conditions can be naturally infected by *Toxocara* species, thus serving as an indicator of soil contamination by *Toxocara* eggs. Further studies are necessary to

evaluate the risk factors associated with the infection of chickens by *Toxocara* spp. and to determine the amount of cross-reactivity in ELISA.

Acknowledgements

A portion of our research funds was provided by Dr. Aristeu Vieira da Silva, supported by the National Council for Scientific and Technological Development (CNPq grant #477764/2012-6) and by the Research Productivity Grant #308093/2013-5 to Aristeu V. Silva, Grupo de Pesquisa em Zoonoses e Saúde Pública, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Bahia, Brazil). Thanks to Dr. Rogério Giufrida (Unoeste) by statistical support.

References

- Ben Slimane B. Prevalence of the gastro-intestinal parasites of domestic chicken *Gallus domesticus* Linnaeus, 1758 in Tunisia according to the agro-ecological zones. *J Parasit Dis* 2016; 40(3): 774-778. <http://dx.doi.org/10.1007/s12639-014-0577-5>. PMID:27605783.
- Campos-da-Silva DR, Paz JS, Fortunato VR, Beltrame MAV, Valli LCP, Pereira FEL. Natural infection of free-range chickens with the ascarid nematode *Toxocara* sp. *Parasitol Res* 2015; 114(11): 4289-4293. <http://dx.doi.org/10.1007/s00436-015-4669-7>. PMID:26319520.
- Cardoso SP, Yamamura MH. Parasitas em produção de frangos no sistema de criação tipo colonial/caipira no Brasil. *Semina: Ciênc Agrár* 2004; 25(1): 63-74. <http://dx.doi.org/10.5433/1679-0359.2004v25n1p63>.
- Conan A, Goutard FL, Sorn S, Vong S. Biosecurity measures for backyard poultry in developing countries: a systematic review. *BMC Vet Res* 2012; 8(1): 240. <http://dx.doi.org/10.1186/1746-6148-8-240>. PMID:23216706.
- Dorai-Raj, S. *binom: binomial confidence intervals for several parameterizations. R package version 1.1-1* [online]. Vienna: R Foundation for Statistical Computing; 2014 [cited 2017 May 22] Available from: <https://CRAN.R-project.org/package=binom>
- Dubey JP, Gennari SM, Labruna MB, Camargo LM, Vianna MC, Marcet PL, et al. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon, Brazil. *J Parasitol* 2006; 92(1): 36-40. <http://dx.doi.org/10.1645/GE-655R.1>. PMID:16629312.
- Dubey JP. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. *Zoonoses Public Health* 2010; 57(1): 60-73. <http://dx.doi.org/10.1111/j.1863-2378.2009.01274.x>. PMID:19744305.
- Elefant GR, Shimizu SH, Sanchez MCA, Jacob CMA, Ferreira AW. A serological follow-up of toxocariasis patients after chemotherapy based on the detection of IgG, IgA and IgE antibodies by enzyme-linked immunosorbent assay. *J Clin Lab Anal* 2006; 20(4): 164-172. <http://dx.doi.org/10.1002/jcla.20126>. PMID:16874812.
- Ferdushy T, Hasan MT, Golam Kadir AK. Cross sectional epidemiological investigation on the prevalence of gastrointestinal helminths in free range chickens in Narsingdi district, Bangladesh. *J Parasit Dis* 2016; 40(3): 818-822. <http://dx.doi.org/10.1007/s12639-014-0585-5>. PMID:27605790.
- Food and Agriculture Organization of the United Nations – FAO. *Poultry and animal production* [online]. Rome: FAO; 2014 [cited 2017 Apr 18]. Available from: <http://www.fao.org/ag/againfo/themes/en/poultry/production.html>
- Ibrahim HM, Abdel-Ghaffar F, Osman GY, El-Shourbagy SH, Nishikawa Y, Khattab RA. Prevalence of *Toxoplasma gondii* in chicken samples from delta of Egypt using ELISA, histopathology and immunohistochemistry. *J Parasit Dis* 2016; 40(2): 485-490. <http://dx.doi.org/10.1007/s12639-014-0530-7>. PMID:27413325.
- Javaregowda AK, Kavitha Rani B, Revanna SP, Udupa G. Prevalence of gastro-intestinal parasites of backyard chickens (*Gallus domesticus*) in and around Shimoga. *J Parasit Dis* 2016; 40(3): 986-990. <http://dx.doi.org/10.1007/s12639-014-0620-6>. PMID:27605824.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193(1): 265-275. PMID:14907713.
- Moreira GM, Telmo PL, Mendonça M, Moreira AN, McBride AJ, Scaini CJ, et al. Human toxocariasis: current advances in diagnostics, treatment, and interventions. *Trends Parasitol* 2014; 30(9): 456-464. <http://dx.doi.org/10.1016/j.pt.2014.07.003>. PMID:25089038.
- Morimatsu Y, Akao N, Akiyoshi H, Kawazu T, Okabe Y, Aizawa H. A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. *Am J Trop Med Hyg* 2006; 75(2): 303-306. PMID:16896137.
- Nagakura K, Tachibana H, Kaneda Y, Kato Y. Toxocariasis possibly caused by ingesting raw chicken. *J Infect Dis* 1989; 160(4): 735-736. <http://dx.doi.org/10.1093/infdis/160.4.735>. PMID:2794572.
- R Development Core Team. *R software: a language and environment for statistical computing* [online]. Vienna: R Foundation for Statistical Computing; 2014 [cited 2017 May 22]. Available from: <http://www.r-project.org>
- Rabbi AKMA, Islam A, Majumder S, Anisuzzaman A, Rahman MH. Gastrointestinal helminths infection in different types of poultry. *Bangl J Vet Med* 2006; 4(1): 13-18. <http://dx.doi.org/10.3329/bjvm.v4i1.1519>.
- Romasanta A, Romero JL, Arias M, Sánchez-Andrade R, López C, Suárez JL, et al. Diagnosis of parasitic zoonoses by immunoenzymatic assays-analysis of cross-reactivity among the excretory/secretory antigens of *Fasciola hepatica*, *Toxocara canis*, and *Ascaris suum*. *Immunol Invest* 2003; 32(3): 131-142. <http://dx.doi.org/10.1081/IMM-120022974>. PMID:12916704.
- Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Ann Trop Med Parasitol* 2010; 104(1): 3-23. <http://dx.doi.org/10.1179/136485910X12607012373957>. PMID:20149289.
- Savigny DH, Voller A, Woodruff AW. Toxocariasis: serological diagnosis by enzyme immunoassay. *J Clin Pathol* 1979; 32(3): 284-288. <http://dx.doi.org/10.1136/jcp.32.3.284>. PMID:372253.
- Silva Raposo R, Santarém VA, Meriguetti YF, Rubinsky-Elefant G, de Lima Cerazo LM, Pereira L, et al. Kinetic and avidity of IgY anti-*Toxocara* antibodies in experimentally infected chickens. *Exp Parasitol* 2016; 171: 33-41. <http://dx.doi.org/10.1016/j.exppara.2016.09.009>. PMID:27746165.
- Taira K, Saeed I, Permin A, Kapel CMO. Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. *Vet Parasitol* 2004; 121(1-2): 115-124. <http://dx.doi.org/10.1016/j.vetpar.2004.01.018>. PMID:15110409.
- Von Söhsten AL, Silva AV, Rubinsky-Elefant G, Macedo LMS, Guerra M. Anti-*Toxocara* spp. IgY antibodies in poultry sold in street markets from Feira de Santana, Bahia, Northeastern Brazil. *Vet Parasitol: Reg Stud Rep* 2017; 8: 86-89. <http://dx.doi.org/10.1016/j.vprsr.2017.02.006>.
- Zibaei M, Sadjjadi SM, Maraghi S. The occurrence of *Toxocara* species in naturally infected broiler chickens revealed by molecular approaches. *J Helminthol* 2017; 91(5): 633-636. <http://dx.doi.org/10.1017/S0022149X16000559>. PMID:27571878.