ATROFIA NEURONAL MIENTÉRICA NO ÍLEO DE RATOS INFECTADOS CRONICAMENTE POR UMA CEPA GENÓTIPO I DE *Toxoplasma gondii*

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BARBOSA[1], B. J. P; ARAÚJO[2], E. J. A; SILVA[3], A. V; SANT'ANA[4], D. M. G. Atrofia neuronal mientérica no íleo de ratos infectados cronicamente por uma cepa genótipo I de *Toxoplasma gondii*. **Arq. Ciênc. Vet. Zool. UNIPAR**, Umuarama, v. 12, n. 2, p. 101-108, jul./dez. 2009.

RESUMO: A toxoplasmose é uma zoonose que desperta grande preocupação na saúde pública em nível mundial. No Brasil, os índices de soropositividade para *Toxoplasma gondii* variam entre 54% e 75% da população. Neste estudo objetivou-se avaliar os efeitos da infecção crônica, causada por uma cepa genótipo I de *T. gondii*, sobre o número e a morfometria de neurônios mientéricos do íleo terminal de ratos. Foram utilizados oito ratos (*Rattus norvegicus*) Wistar machos. O grupo experimental foi inoculado oralmente com 10^5 taquizoítos de uma cepa genótipo I de *T. gondii*. Após 30 dias de infecção, os animais foram submetidos à eutanásia, e por meio de laparotomia, o jejuno-íleo foi retirado, mensurado em seu comprimento e largura para cálculo de área. O íleo terminal de cada animal foi dissecado para confecção de preparados totais, os quais foram corados pela técnica de Giemsa. Não foram observadas alterações no peso corporal, comprimento ou área intestinal, apenas a largura do jejuno-íleo sofreu aumento. Não houve alteração significativa em relação à análise quantitativa dos neurônios. Na análise morfométrica, observou-se redução da área do pericário, do núcleo e do citoplasma dos neurônios mientéricos do grupo experimental. No que se refere à frequência de neurônios nas diferentes classes de intervalo da área do pericário, não houve alterações significativas. Entretanto, houve um aumento do número de neurônios cujos núcleos representavam de 21 a 30% de seu pericário e uma redução no número daqueles em que o núcleo representava 51-60% e mais que 71%.
PALAVRAS-CHAVE: Toxoplasmose. Íleo. Plexo mientérico. Neurônios entéricos. Análise quantitativa. Morfometria.

MYENTERIC NEURONAL ATROPHY INDUCED BY CHRONIC INFECTION CAUSED BY A GENOTYPE I *Toxoplasma gondii* STRAIN IN THE RAT ILEUM

ABSTRACT: Toxoplasmosis is a zoonosis which causes a great worldwide public health concern. In Brazil, *T.gondii* seropositivity indexes range from 54% to 75% of the population. This study assesses the effects of chronic infection caused by a genotype I *Toxoplasma gondii* strain over the amount and morphometrics of myenteric neurons of rat terminal ileum. Eight male Wistar rats (*Rattus norvegicus*) were used. The experimental group was orally inoculated with 10^5 tachyzoites from a genotype I strain of *Toxoplasma gondii*. The animals were submitted to euthanasia 30 days after the infection. Blood sampling was carried out by punctioning the retro-orbital plexus in order to detect *anti-T.gondii* seric antibodies. The ileum-jejunum was removed, measured with respect to its total length and width for the calculation of its area through laparotomy. The ileum from each animal was collected, dissected and stained with Giemsa. Alterations concerning body weight, length or intestinal area were not observed, however, the width of the ileum-jejunum had increased. There were no significant alterations with respect to the quantitative analysis. Reduction of the area of the perikarium, nucleus, and cytoplasm of the myenteric neurons from the experimental group were noticed through morphometric analysis. There were no significant alterations with respect to the incidence of neurons within the different interval classes of the perikarium area. However, there was an increase of the amount of neurons whose nuclei presented 21-30% of their perikarium as well as the reduction of those in which the nucleus presented 51-60%, and higher than 71%.
KEYWORDS: Toxoplasmosis. Ileum. Quantitative analysis. Morphometric analysis.

ATROFIA NEURONAL MIENTÉRICA EN EL ÍLEON DE RATAS INFECTADAS CRONICAMENTE POR UNA CEPA GENOTIPO I DE *Toxoplasma gondii*

RESUMEN: La toxoplasmosis es una zoonosis que despierta mucha preocupación en la salud pública a nivel mundial. En Brasil, los índices de soropositividad para *Toxoplasma gondii* varían entre 54% y 75% de la población. El objetivo de este

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Hypertrophy of the neurons in the ileum of rats infected with cysts of *Toxoplasma gondii* (genotype II)

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ABSTRACT. This paper verified possible alterations caused by a genotype II *Toxoplasma gondii* strain with respect to the total number and morphometry of the myenteric neurons in the terminal ileum and descending colon of rats. Eight rats were divided into two groups: control (n = 4) and experimental (n = 4). This group was inoculated orally with 20 tissue cysts of *T. gondii* from a genotype II strain (ME-49). Whole mounted from the terminal ileum and the descending colon were stained with Giemsa. There was not any neuronal loss on both organs. The neurons became hypertrophied in the terminal ileum, whereas morphometric alterations were not observed for the neurons in the descending colon.

Key words: enteric nervous system, toxoplasmosis, morphology.

RESUMO. Hipertrofia de neurônios do íleo de ratos infectados com cistos de *Toxoplasma gondii* (genótipo II). Objetivou-se verificar as possíveis alterações causadas por uma cepa genótipo II de *Toxoplasma gondii*, sobre o número total e a morfometria de neurônios do plexo mientérico, do íleo terminal e do cólon descendente de ratos. Oito ratos foram divididos em dois grupos: controle (n = 4) e experimental (n = 4), sendo este inoculado, por via oral, com 20 cistos teciduais de *T. gondii* de uma cepa genótipo II (ME-49). Preparados totais do íleo terminal e cólon descendente foram corados com Giemsa. Não houve perda neuronal em ambos os órgãos. Os neurônios se tornaram hipertróficos no íleo terminal, enquanto nenhuma alteração morfométrica foi observada nos neurônios do cólon descendente.

Palavras-chave: sistema nervoso entérico, toxoplasmose, morfologia.

Introduction

Toxoplasmosis is cause for concern in public health and animal production. It is usually asymptomatic in humans; however, it may cause blindness, severe neurological disorders, hepatitis, and pneumonia in immunocompromised patients, as well as severe damage to fetuses (ASCENZI et al., 2005). On animals such as dogs, toxoplasmosis may cause fever associated with lassitude, anorexia, diarrhea, pneumonia, and neurological manifestations. In small ruminants, clinical toxoplasmosis is associated with fever, dyspnea, nervous symptomatology, and abortion, generally in sheep and perinatal mortality in lambs (URQUHART, 1998) whereas it presents diarrhea, cough, and dyspnea in pigs (WINGSTRAND et al., 1997).

This disease is caused by an obligate intracellular protozoan parasite named *Toxoplasma gondii*, which is scattered worldwide (DUBEY; BEATTIE, 1988). Its life cycle is facultatively heteroxenous: Felidae are definite hosts where the sexual reproduction of the parasite occurs with the formation of oocytes, which are eliminated via feces. All homoeothermic animals can be intermediary hosts, where the asexual reproduction of the parasite occurs with the formation of tachyzoites and bradyzoites (JACOBS, 1967; DUBEY, 1994). Tachyzoites present a high proliferation rate, what favors their dissemination within the host, characterizing the acute phase of the disease. Tissue cysts, full of bradyzoites, last for a long period of time, sometimes the entire life of the host, which characterizes the chronic phase of the disease (FERREIRA et al., 2003).

Infection by *T. gondii* may occur as a result of: (1) ingestion of food or water contaminated with oocytes from cat feces, (2) ingestion of tissue cysts in raw or undercooked meat, (3) via transplacentary (BARRAGAN; SIBLEY, 2003), (4) blood transfusion (DUBEY; BEATTIE, 1988), or (5) accidental inoculation during laboratorial manipulation. When oral infection occurs, the parasite crosses the intestinal epithelium, disseminates to other tissues, and may cross other biological barriers such as the placenta and the hematoencephalic barrier, and reach

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Hypertrophy of NADH-diaphorase positive myenteric neurons in rat jejunum after acute infection caused by *Toxoplasma gondii*

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ABSTRACT: Toxoplasmosis, a globally distributed feline-associated zoonosis caused by the protozoan *Toxoplasma gondii*, affects birds and mammals, including humans. This study assesses the consequences of acute *T. gondii* infection for NADH-diaphorase positive myenteric neurons in rat jejunum. Ten male Wistar rats (*Rattus norvegicus*) were divided into two groups: G1 (n = 5) and G2 (n = 5). Animals from G2 were orally inoculated with 500 genotype III (M7741) *T. gondii* oocysts. Twenty-four hours after inoculation, the animals were euthanized and had their jejuna removed, through laparotomy, and measured (length and width) to calculate their areas. Intestinal segments were submitted to NADH-diaphorase histochemistry to evidence the most metabolically active subpopulation of myenteric neurons. No changes were found in body weight; intestinal length, width or area; or neuron population density. Increase of body cell area and cytoplasm and decrease of nuclear area of the myenteric neurons of infected animals were observed by morphometric analysis.

KEY WORDS: *Toxoplasma gondii*, jejunum, myenteric plexus.

CONFLICTS OF INTEREST: There is no conflict.

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Toxoplasma gondii Induces Death of Gastric Myenteric Neurons in Rats

Toxoplasma gondii Induce la Muerte de Neuronas del Plexo Mientérico Gástrico en Ratas

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ALVES, M. S.; SILVA, A. V.; BIANCHI, L. R. O.; ARAÚJO, E. J. A. & SANT'ANA, D. M. G. *Toxoplasma gondii* induces death of gastric myenteric neurons in rats. ***Int. J. Morphol., 29(1)***:293-298, 2011.

SUMMARY: The aim of the study was quantifying and morphologically analyzing the myenteric neurons of the small and large gastric curvatures of the glandular stomach of rats infected the tachyzoites of the *Toxoplasma gondii* for 30 days. Ten male rats were assigned into two groups: Control Group (CG) and Experimental Group (EG). The animals from the CG received saline solution orally whereas the EG animals received 104 tachyzoites of the *T. gondii* genotype III strain (BTU II). After 30 days, euthanasia was conducted for the removal of the stomach, which was dissected under the stereomicroscope for removal of the tunica mucosa and the tela submucosa. Whole mounts were stained with Giemsa. Quantification of the myenteric neurons was conducted by using a 40X-objective microscope in 40 microscopic fields for the region of the small gastric curvature and 40 fields for the large gastric curvature of the glandular stomach of the animals from both groups. The cell body of 50 myenteric neurons from each region was measured for each animal. Chronic experimental infection caused by the genotype III strain of *Toxoplasma gondii* was verified to reduce myenteric neuron density only in the small gastric curvature region of the glandular stomach, not resulting in significant changes in the size of the neurons.

KEY WORDS: Stomach; Toxoplasmosis; Enteric nervous system; Myenteric plexus.

INTRODUCTION

Gastric lesions caused by *T. gondii* are described for humans and animals (Alpert *et al*., 1996; Schreiner & Liesenfeld, 2009). In immunodepressed humans, they involve ulceration, thickening of gastric wall and folds, thickening and necrosis of tunica mucosa and inflammatory infiltrates (Alpert *et al*.; Kofman *et al*., 1996; Smart *et al*., 1990). Several animals species also present inflammatory infiltrates and necrosis on the gastric wall after infection with *T. gondii* (Canfield *et al*., 1990; Cunningham *et al.*, 1992; Hartley *et al*., 1990; Inskeep *et al*., 1990; Schreiner & Liesenfeld).

Studies on experimental gastric toxoplasmosis are rare. Stoicov *et al.* (2004) infected BALB/c mice with *T. gondii* for 20 weeks and occasionally verified tachyzoites on the gastric mucosa, even though they induced no alterations on its structure, causing little inflammation and maintaining the gastric wall thickness.

Confirming gastric toxoplasmosis can be performed by demonstrating the presence of tachyzoites and cysts of *T. gondii* by histopathologic methods, immunohistochemical and ultrastructural (Alpert *et al*.; Kofman *et al.*; Smart *et al.*). The parasite was found inside the epithelial, muscle and endothelial cells, macrophages (Alpert *et al*.) and inside intracellular vacuoles in glandular cells on the gastric wall (Florêncio *et al.*, 1992).

The presence of gastric changes indicates that the stomach myenteric plexus is subjected to direct and indirect lesions by *T. gondii* and the lack of reports with respect to the myenteric plexus of these organs led us to propose this study. The aim of this study was quantifying and morphologically analyzing the myenteric neurons of the small and large gastric curvatures of the glandular stomach of rats infected the tachyzoites from this parasite for 30 days.

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Direct Agglutination Test for Diagnosis of *Toxoplasma* Infection: Method for Increasing Sensitivity and Specificity

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A method that increases the sensitivity and specificity of the direct agglutination (AG) test for diagnosis of *Toxoplasma gondii* infection is described. Qualitative results in the Sabin-Feldman dye test (DT) and AG test were in excellent agreement (98%). Differences in titers between these two tests often related to the length of time the individual was infected. The AG test titer was most often lower than the DT titer during acute (recent) infection; the AG test titer was most often higher than the DT titer in older or chronic infection. If the AG test antigen described here can be made available, the AG test would be ideal for use as a screening test and would provide a simple and inexpensive means for the surveillance of seronegative women during pregnancy and for detection of seroconversions.

At present, the diagnosis of acute acquired infection with *Toxoplasma gondii* depends on serological test results. A number of problems associated with available methods for serodiagnosis of this infection have been an impetus to search for alternative methods. The routine methods presently available to physicians in practice are often expensive to perform, time consuming, not readily adaptable to screening programs, or not sensitive enough to be useful in early diagnosis of the infection (e.g., the hemagglutination test).

The toxoplasma agglutination (AG) test was first described by Fulton et al. (5-8). This method uses whole killed organisms and is now routinely performed in France with an antigen that has been prepared as described by Ardoin et al. (1) and that is commercially available (bioMérieux, Charbonnières-les-Bains, France). The method is very simple and useful but as presently used has two drawbacks. First, it lacks sensitivity; the titer in the AG test is usually much lower than that in the dye test (DT) or the conventional immunofluorescent-antibody (IFA) test. As a consequence, some sera that are positive in the latter two tests are reported as negative in the AG test. Second, it lacks specificity; some sera that are negative in the DT and IFA test are reported as positive in the AG test (2). This has been shown to be due to the binding of normal immunoglobulin M (IgM) ("natural IgM antibody") to the surface of the parasite (3).

We describe here a method for preparing antigen which increases the sensitivity of the AG test and a method for suppressing nonspecific agglutination by the use of a buffer containing 2-mercaptoethanol (2ME). When the AG test is modified by these two methods, its specificity and sensitivity parallel those of the DT. The technique and the reading are so simple and accurate that, if the antigen is made available, the modified AG test method would be convenient for laboratories that perform serology only occasionally as well as for those that perform large-scale surveys.

MATERIALS AND METHODS

Preparation of the antigen. The antigen is prepared using the RH strain of toxoplasma cultivated along with mouse TG 180 sarcoma cells in the peritoneal cavities of mice. As many as 2×10^8 to 5×10^8 toxoplasma per mouse may be obtained with this method. This is approximately 10 times more than can be obtained when the conventional method of inoculating toxoplasma alone into the peritoneal cavities of mice is used.

Mice inoculated with the mixture of sarcoma cells and parasites develop an exudate containing both sarcoma cells and parasites, the proportion of which depends on the ratio of parasites to sarcoma cells inoculated and on the time elapsed after inoculation of the mixture. If one examines these exudates microscopically (400×, phase contrast), six different stages may be noted. In stage I, most cells are not infected, and those few (one in every two or three fields) that are infected contain only a few parasites. In stage II, 5 to 10% of cells are infected, each containing only a few parasites. In stage III, approximately half of the cells are infected, and most of these contain only a few parasites. A few cells are heavily infected. There are few extracellular toxoplasma. In stage IV, almost every

Tobacco Mosaic Virus Inhibition by Bark Extracts

Several plant virus inhibitors have been reported to be present in the different parts of plants[1] but no attention was paid to the inhibitory activity of juices from plant barks. This investigation is concerned with the inhibition of tobacco mosaic virus with the bark extracts of some plants.

Culture of tobacco mosaic virus used was maintained in systemically infected *Nicotiana tabacum* L. var. White Burley plants were kept in insect-proof cages. Inoculum was prepared by grinding diseased tobacco leaves in a mortar and the juice was expressed by squeezing the pulp through muslin cloth and diluted 1:10 with distilled water and kept at 0 °C. The inhibitor preparations were made by homogenizing small pieces of fresh and cleaned barks with an equal weight of distilled water in a waring Blender. Juices from these homogenates were expressed through several layers of muslin cloth and tested as inhibitors. The inhibitory effect of bark extracts was determined by comparing the infectivity of equal volumes of tobacco mosaic virus and distilled water (control) with equal volumes of tobacco mosaic virus and inhibitor, by the local lesion method. The mixtures were rubbed after 10 min of mixing on the *Chenopodium amaranticolor* Coste et Reyn leaves. 5 replicates were taken for each treatment. Each mixture was inoculated on 17 half-leaves and the half-leaves alloted to each treatment were distributed among plants so as to form a randomized block. Carborundum powder was dusted on leaves before inoculation and inoculations were made with fore-finger wet with inoculum. The results of tobacco mosaic virus inhibition by the bark extracts are indicated in the Table.

Effect of bark extracts on the infection by tobacco mosaic virus

Treatments of tobacco mosaic virus with bark extracts	Average No. of lesions/half-leaf
Artocarpus heterophyllus Lamk.	36.4
A. lakoocha Roxb.	0[a]
Azadirachta indica L.	0[a]
Butea monosperma (Lam.) Kuntze	61.5
Callistemon lanceolatus D C.	2.2[a]
Ficus bengalensis L.	1[a]
F. elastica Roxb.	0[a]
F. rumphii Blum.	0[a]
Eriobotrya japonica Lindle.	0[a]
Mangifera indica L.	35.6
Morus alba Bureau.	52.6
Plumeria rubra L.	2.7[a]
Psidium guayava L.	0[a]
Pyrus communis L.	2[a]
Syzygium jambolana F.B.I.	0[a]
Tamarindus indica L.	0[a]
Distilled water (control)	56.7

[a] Significant at 0.1 level.

Results of the Table indicate that the extracts from the barks of *Artocarpus lakoocha, Azadirachta indica, Ficus elastica, F. rumphii, Eriobotrya japonica, Psidium guayava, Syzygium jambolana, Tamarindus indica,* inactivates the activity of tobacco mosaic virus totally, while the extracts of other barks are also inhibitory up to a certain percentage. Further work on the nature and activity of inhibitors in the bark extracts is in progress[2].

Zusammenfassung. Die bekannte Hemmwirkung von Gewebeextrakten aus Pflanzenteilen auf die Virusentwicklung (Infektiosität?) wird ergänzt durch Untersuchungen über hemmende Wirkungen von Borkenextrakten. Es wurden Pflanzen mit und solche ohne hemmende Wirkung ihrer Borkenextrakte gefunden.

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Department of Botany, The University, Gorakhpur (India), 24 July 1968.

[1] F. C. Bawden, Adv. Virus Res. *2*, 31 (1954).

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PRO EXPERIMENTIS

Detection of Nerve Cells by a Histochemical Technique

A special staining technique has been designed in order to obtain a specific and constant demonstration of nerve cells in the autonomic nervous system. This method is based upon the histochemical reaction for the detection of NADH-diaphorase activity[1] with nitro-BT as electron acceptor.

Thin-walled organs (e.g. mouse urinary bladder) or organs whose nervous structures are not far below the external surface (e.g. the intestinal canal of small animals, as regards the Auerbach's plexus) can be stained in toto. In order to get a thinner wall, it may be useful to stretch the organs during sampling by injecting saline solution under low pressure. After sampling, the organs must be frozen as soon as possible on dry ice for several minutes; in airtight vessels the samples may be stored a few days either at dry ice temperature or in a freezer at −70 °C. Afterwards the specimen is allowed to reach room temperature and kept stretched during thawing under saturated moisture. The organ is then dipped directly into the substrate solution[2], and incubated for 10–30 min at room temperature with gentle agitation. The reaction, which gives direct staining, may be visually

[1] A. G. E. Pearse, *Theoretical and Applied Histochemistry* (Churchill, London 1960).

[2] The simplest and one of the most suitable incubating medium was prepared as follows: Nitro-BT (0.5 mg/ml) 5 ml, 0.1 *M* phosphate buffer at pH 7.3 5 ml, H_2O 10 ml, NADH 10 mg. This must be prepared immediately before use.

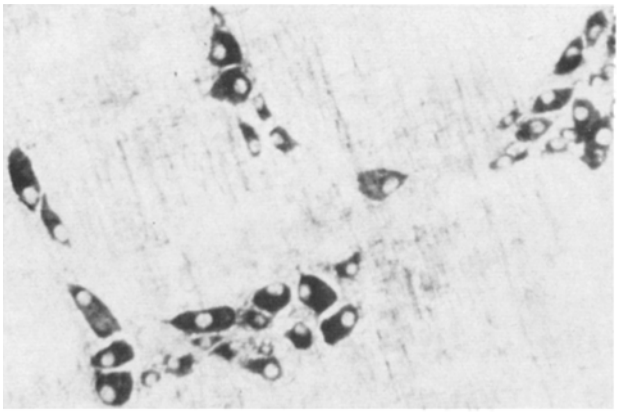

Fig. 1. Adult rat cecum. Auerbach's plexus. Nerve cells of various size are heavily stained in this in toto preparation of the muscle coat. × 140.

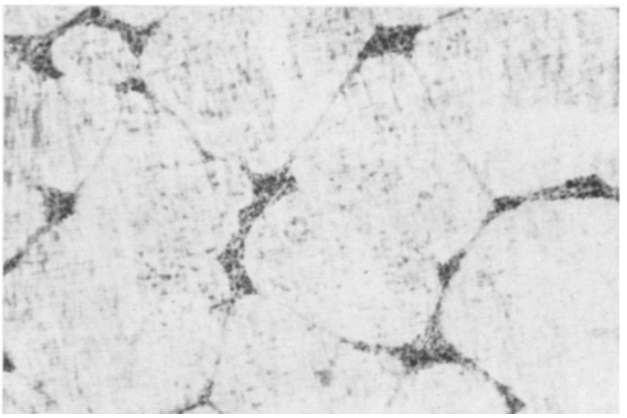

Fig. 3. Adult guinea-pig rectum. In toto preparation of the muscle coat. The ganglia of the Auerbach's plexus, each containing several nerve cells, are easily detected. × 39.

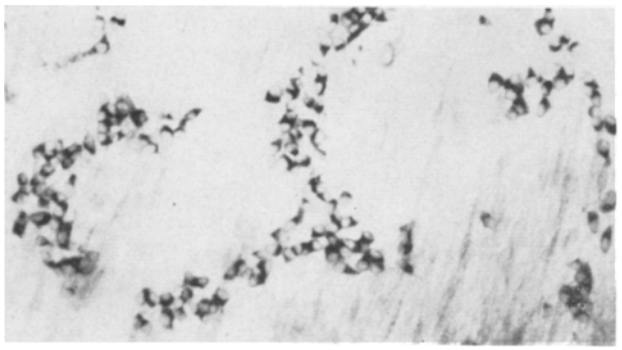

Fig. 2. New-born rat stomach. A number of small-sized nerve cells of the Auerbach's plexus are apparent in this in toto preparation of the muscle coat. × 125.

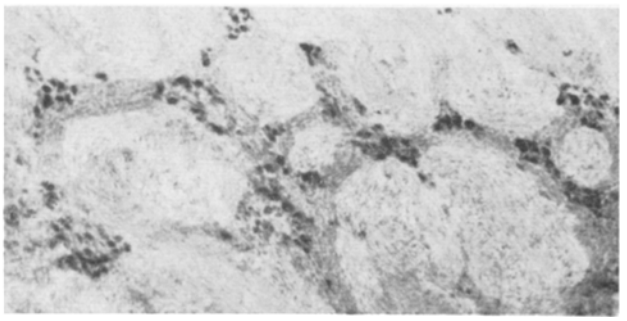

Fig. 4. Human rectum. Diagnostic intraoperative test. In this section of the muscle coat, tangential to the external surface, a large number of nerve cells is observed. × 45.

controlled under the dissection microscope, and it is stopped by immersion of the sample in 10% neutralized formalin. After a 24-h fixation in formalin, the specimen is trimmed into fragments, delaminated when necessary, and mounted in glycerine jelly.

The enzyme activity is so high within nerve cell bodies that they stain always much earlier than any other elements of the surrounding tissues (Figure 1). Not only mature nerve cell bodies of large volume and typical outline are stained but also small nerve cells with scarse cytoplasm, as observed e.g. in the autonomic nervous system of newborn animals (Figure 2). The substrate penetration, within the incubation time given above and after the freezing treatment, is in the range of more than 100 μ of thickness.

Organs with too thick walls, which cannot be treated in toto, are processed as follows: after freezing on dry ice, followed by partial thawing, microtomic sections are prepared; due to the high penetration of the substrate, these sections may well be 100–200 μ thick; the sections are then dipped into the medium where the reaction can be visually pursued, and further treated as above indicated.

To summarize, this technique allows a specific and reliable demonstration of the nerve cells of the autonomic nervous system. When employed on organs in toto, it allows counting of the nerve cells[3] over large surfaces (Figure 3); moreover, a very precise measurement of the outline of each nerve cell can be achieved which leads to easy evaluation of the cell volume. Such a method, easy and quick to prepare, can be proposed as a diagnostic intraoperative test, when the presence or the absence of nerve cells must be ascertained (Figure 4)[4].

Riassunto. Viene descritto un procedimento istochimico di utile impiego per l'evidenziazione e il conteggio di cellule nervose, particolarmente in alcuni distretti del sistema nervoso autonomo. Per l'alta penetrabilità del substrato è possibile applicare il metodo su preparazioni in toto ed avere quindi una elevata precisione nelle determinazioni del numero e del volume delle cellule nervose.

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[3] G. GABELLA, Experientia *23*, 52 (1967).

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Análise morfométrica da parede intestinal e dinâmica de mucinas secretadas no íleo de frangos infectados por *Toxoplasma gondii*

Morphometric analysis of the intestinal wall and the dynamic of mucins secreted in the chicken ileum infected with *Toxoplasma gondii*

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RESUMO

*Objetivou-se analisar os efeitos da toxoplasmose sobre a morfometria da parede intestinal e a dinâmica de mucinas secretadas no íleo de frangos. Foram utilizados 16 frangos de corte machos (**Gallus gallus**) da linhagem Cobb, com 26 dias de idade. As aves foram divididas aleatoriamente em três grupos (G1, G2 e G3). O G1 não recebeu nenhum inóculo caracterizando o grupo controle, o G2 foi inoculado com cistos teciduais da cepa ME49 de **Toxoplasma gondii**, e o G3 foi inoculado com oocistos da cepa M7741 de **T. gondii**. Após 60 dias da inoculação, os animais foram mortos, e coletou-se o íleo, o qual foi submetido à rotina de processamento histológico. Cortes transversais de 4mm foram corados com Hematoxilina-Eosina (HE), Periodic Acid Schiff (PAS), Alcian Blue pH 2,5 e Alcian Blue (AB) pH 1,0. Nos animais do G2, observou-se aumento da parede intestinal, sobretudo a túnica muscular, muscular da mucosa e túnica mucosa, além de aumento na secreção de mucinas neutras. Já nos animais do G3, houve uma atrofia da parede intestinal, sobretudo para a túnica mucosa, e aumento na secreção de mucinas neutras.*

Palavras-chave: *toxoplasmose, **Gallus gallus**, intestino delgado, histologia, células caliciformes, morfometria.*

ABSTRACT

*The effects of toxoplasmosis on the intestinal wall morphometry and the dynamic of mucins secreted in the chicken ileum were analyzed. Sixteen 26-day-old, male, Cobb broiler chicks (**Gallus gallus**) were used and randomly divided into three groups (G1, G2 and G3). G1 received no inoculum characterizing the control group, G2 was inoculated with tissue cysts of ME49 strain of **Toxoplasma gondii**, and G3 was inoculated with oocytes of M7741 strain of **T. gondii**. After 60 days of inoculation, the animals were killed and had their ileum collected and submitted to histological processing. Tranversal cuts (4mm) were stained with Hematoxilin-Eosine (HE), Periodic Acid Schiff (PAS), Alcian Blue pH 2.5 and Alcian Blue (AB) pH 1.0. Intestinal wall increase was noticed for the animals from G2 – mostly the muscle tunic, the muscularis mucosae, and the mucous tunic, including the increase of secretion of neutral mucins. The animals from G3 presented atrophy of the intestinal wall – mostly the mucous tunic, and increase of the secretion of neutral mucins.*

Key words: ***toxoplasmosis, Gallus gallus**, small intestine, histology, goblet cells, morphometry.*

INTRODUÇÃO

A toxoplasmose é uma das zoonoses parasitárias mais comuns, tanto no homem, como nos animais, apresentando ampla distribuição geográfica. Estima-se que mais de um terço da população humana já foi exposta à infecção pelo ***Toxoplasma gondii***, o causador dessa doença. Trata-se de um protozoário do filo Apicomplexa, parasita obrigatoriamente intracelular, que possui um ciclo de vida heteroxeno, caracterizado por uma ampla diversidade de hospedeiros, incluindo somente os felídeos como definitivos e várias espécies de mamíferos e de aves como intermediários (TENTER et al., 2000).

A infecção por ***T. gondii*** pode ocorrer pela (1) ingestão de oocistos liberados pelas fezes de felídeos, que podem estar presentes no solo, na água ou nos alimentos; (2) ingestão de carne crua ou malcozida, contendo cistos teciduais, e (3) transmissão de taquizoítos por via transplacentária (transmissão

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Effects of infection with *Toxoplasma gondii* oocysts on the intestinal wall and the myenteric plexus of chicken (*Gallus gallus*)[1]

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This paper aims to analyze the effects of the *Toxoplasma gondii* infection in the intestinal wall and myenteric plexus of chicken (*Gallus gallus*). Ten 36-day-old chickens were separated into two groups: control and experimental, orally inoculated with oocysts of the *T. gondii* strain M7741 genotype III. After 60 days the birds were submitted to euthanasia and had their duodenum removed. Part of the intestinal segments was submitted to histological routine, HE staining, PAS histochemical technique, and Alcian Blue. Qualitative analysis of the intestinal wall and comparative measurements among the groups with respect to total wall thickness, muscle tunic, mucosa, and tunica mucosa were carried out. Caliciform cells were quantified. The other part of the intestinal segments was fixed in formol acetic acid and dissected having the tunica mucosa and the tela submucosa removed. Neurons were stained with Giemsa, counted, and measured. Chickens from the experimental group presented diarrhea and inflammatory infiltrates in the tunica mucosa, thickness reduction of all the parameters assessed in the intestinal wall, and an increase of the number of caliciform cells. There was a ~70% reduction regarding the intensity of myenteric neurons; and the remaining cells presented a reduction of ~2.4% of the perikarion and ~40.5% of the nucleus ($p<0.05$). Chronic infection induced by *T. gondii* oocysts resulted in intestinal wall atrophy, mucin secretion increase, death and atrophy of chicken myenteric plexus neurons. Death and atrophy of myenteric plexus neurons may be related with the causes of diarrhea observed in chickens with toxoplasmosis.

INDEX TERMS: Chicken, toxoplasmosis, duodenum, enteric nervous system, experimental infection.

RESUMO.- [**Efeitos da infecção por oocistos de *Toxoplasma gondii* sobre a parede intestinal e o plexo mientérico de *Gallus gallus*.**] O objetivo deste trabalho foi analisar os efeitos da infecção pelo *Toxoplasma gondii* sobre a parede intestinal e o plexo mientérico de *Gallus gallus*. Dez galinhas de 36 dias de idade separadas em dois grupos: controle e experimental inoculado com oocistos da cepa M7741 de *T. gondii* (genótipo III) pela via oral. Após 60 dias os animais foram submetidos à eutanásia e o duodeno coletado. Parte dos segmentos intestinais foi submetida à rotina histológica, coloração por HE e técnica histoquímica de PAS e Alcian Blue. Realizou-se uma avaliação qualitativa da parede intestinal e medidas comparativas entre os grupos da espessura da parede total, túnica muscular, muscular da mucosa e túnica mucosa. As células caliciformes foram quantificadas. Outra parte dos seg-

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Infecção toxoplásmica causa hipertrofia da parede do cólon de frangos

[*Toxoplasmic infecction causes hipertrophy of the chicken colon wall*]

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RESUMO

Estudaram-se os efeitos da infecção toxoplásmica sobre a morfometria da parede intestinal, a distribuição de fibras colágenas e a dinâmica de mucinas secretadas no cólon de frangos. Foram utilizados 16 frangos machos de linhagem comercial, com 26 dias de idade. As aves foram distribuídas, aleatoriamente, em três grupos (G). As do G1 não receberam inóculo e se caracterizaram como grupo-controle; nas do G2, foram inoculados cistos teciduais da cepa ME49 de *Toxoplasma gondii*; e nos G3, oocistos da cepa M7741 de *T. gondii*. Após 60 dias da inoculação, os animais foram sacrificados para coleta do cólon, o qual foi submetido à rotina de processamento histológico. Em G2 e G3, observou-se hipertrofia da parede do cólon, contudo não houve alteração na proporção do número de células caliciformes e de enterócitos presentes no epitélio intestinal.

Palavras-chave: aves, toxoplasmose, intestino grosso

ABSTRACT

*The effects of toxoplasmosis on the intestinal wall morphometry, the distribution of collagen fibers, and the dynamic of mucins secreted in the chicken colon were analyzed. Sixteen 26-day-old male Cobb chicks (*Gallus gallus*) were randomly distributed into three groups (G1, G2, and G3). G1 received no inoculums and characterized the control group, G2 was inoculated with tissue cysts of ME49 strain of* Toxoplasma gondii*, and G3 was inoculated with oocytes of M7741 strain of* T. gondii*. After 60 days of inoculation, the animals were slaughtered and had their colon collected and submitted to histological processing. Transversal cuts (4μm) were stained with Hematoxylin-Eosin (HE), Periodic Acid Schiff (PAS), Alcian Blue pH 2.5, Alcian Blue (AB) pH 1.0, and Azan. G2 and G3 animals showed hypertrophy of the colon wall, but the proportion between the number of goblet cells and enterocytes present in the intestinal epithelium was not altered.*

Keywords: birds, toxoplasmosis, bowel intestine

INTRODUÇÃO

A toxoplasmose é uma zoonose com grande disseminação geográfica, causada por um protozoário denominado *Toxoplasma gondii*. É um parasito intracelular obrigatório, de ciclo de vida heteroxeno, que se caracteriza por uma ampla diversidade de hospedeiros, incluindo somente felídeos como hospedeiros definitivos e várias espécies de mamíferos e de aves como intermediários. Os animais infectam-se, principalmente, por ingestão de água ou alimentos contaminados com oocistos, ou pelo consumo de carne crua ou malcozida, contendo cistos teciduais, os quais geralmente estão repletos de bradizoítos (Weiss e Kim, 2007). Vale destacar que as aves se infectam quando ingerem oocistos presentes no solo, alimento e água contaminados, por isso são vistas como importantes marcadores ambientais da disseminação de *T. gondii* (Dubey et al., 2006; Galli et al., 2008).

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