Usefulness of serological ELISA assay for *Taenia saginata* to detect naturally infected bovines

Utilização de teste sorológico ELISA para a detecção de bovinos naturalmente infectados por *Taenia saginata*

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Abstract

Bovine cysticercosis, a cosmopolitan disease caused by *Taenia saginata*, leads to economic losses due to carcass devaluation at slaughter. Sanitary inspection at slaughterhouses, the routine diagnostic method in Brazil, lacks the necessary sensitivity to detect the mildly infected cattle that are typically encoutered in Brazil. In this study we have tested cattle sera from animals diagnosed as positive and negative by veterianry inspection for (1) anti-parasite antibodies using metacestodes antigens (*T. solium* vesicular fluid and *T. saginata* secretions) and (2) the HP10 secreted antigen of viable metacestodes. The cut-off values were calculated by ROC curve for intense and mild infections conditions, and by the classical method ($\bar{x}$ + 2DP for negative samples). The sensitivity and specificity of these diagnostic tests were different depending on the assumed cut-off value and, importantly, whether the infection was mild or intense. In spite of these observations, however, such ELISA assays for serum antibodies and parasite antigens constitute an important tool for epidemiological porposes, and in establishing priorities for the control of bovine cysticercosis.

Keywords: Sanitary inspection, HP10 antigen assay, ELISA, vesicular fluid, excretion-secretion.

Resumo

A cisticercose bovina, uma doença cosmopolita causada pela *Taenia saginata*, resulta em perdas econômias devido à desvalorização de carcaças durante o abate. A inspeção sanitária nos frigoríficos, método de diagnóstico de rotina no Brasil, não possui sensibilidade necessária para detectar animais levemente infectados, os quais são tipicamente encontrados no Brasil. Neste estudo testou-se soro de animais diagnosticados positivos e negativos pela inspeção veterinária por (1) anticorpos anti-parasita usando antígenos de metacestóides (fluido vesicular de *T. solium* e secreções de *T. saginata*) e (2) antígeno secretado de metacestóides viáveis. Os pontos de corte foram calculados pela curva ROC, considerando condições de intensa e leve infeção, e pelo método clássico ($\bar{x}$ + 2DP das amostras negativas). A sensibilidade e a especificidade dos testes diagnósticos foram diferentes dependendo do valor de ponto de corte assumido e, sobretudo, se a infecção era intensa ou leve. Apesar destas observações, no entanto, tanto o ensaio ELISA para anticorpos séricos quanto para antígeno de parasita constituem importante ferramenta para propósitos epidemiológicos e no estabelecimento de prioridades no controle da cisticercose bovina.

Palavras-chave: Inspeção sanitária, ensaio para antígeno HP10, ELISA, fluido vesicular, excreção-secreção.

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Introduction

Cattle are intermediate hosts of the cestode parasite *Taenia saginata*, developing bovine cysticercosis, a cosmopolitan disease characterized by the localization of the parasitic larval form (metacestode) in the muscles of infected animals. *T. saginata* infected carcasses have reduced value due the treatment necessary to kill the parasite (freezing, heat or brine) or to its condemnation when the intensity of infection makes the carcasses unfit for human consumption (BRASIL, 1952; FERRER et al., 2007a; YAMANE et al., 2012).

Bovine cysticercosis is endemic in Brazil with a prevalence that ranges from 0.7% to 7.4% (UNGAR; GERMANO, 1992; MARQUES et al., 2008). Thus, with an infection rate of 5%, in the approximately 209 million of Brazilian cattle, there would be an economic loss equivalent to about 10 million infected animals (SOUZA et al., 2007; ABIEC, 2012).

There are two factors that play a negative role in the control of this disease in Brazil. First, infections are typically mild, raising the risk that some infected animals will escape veterinary inspection at the slaughterhouses. Second, ranches supplying the slaughterhouses usually do not breed the animals; these are supplied at about three months of age from other farms without any testing. After their arrival at the cattle ranches, the animals then spend the following twelve to eighteen months on pasture, and their final three months prior to slaughter in confinement feeding on silage. This is the typical scenario in the major beef producing States of Brazil like São Paulo and Mato Grosso do Sul.

Diagnosis of bovine cysticercosis in Brazil is based on meat inspection of the carcasses during slaughter by cuts in the predicted sites of infection such as masseter, heart and diaphragm, and tongue if palpation indicates the possible presence of metacestodes (BRASIL, 1952). However, this method presents low sensitivity, especially in mild infection where metacestodes can occur in other than the predicted sites, for example, shoulder clod, knuckle and back ribs (LOPES et al., 2010).

ELISA immunoassays for anti-parasite antibodies using *T. saginata* vesicular fluid (ONYANGO-ABUJE et al., 1996b), total soluble metacestode antigens (Minozzo et al., 2004), recombinant peptides (FERRER et al., 2007b) and excretion-secretion antigens (OGUNREMI; BENJAMIM, 2010) have been developed. Homology between *Taenia* species has been also explored and used in favor of antigen production for diagnostic purposes (GONZÁLEZ et al., 2007; OLIVEIRA et al., 2007; GONÇALVES et al., 2010). Such tests only demonstrate exposure of the animals to the infection and not necessarily a concomitant infection (HARRISON et al., 1989). Evidence for viable metacestodes through HP10 ELISA test detecting a selected parasite molecule in contrast, does identify cattle habouring living and thus transmissible parasites. Taken together, ELISA assays identifying anti-parasite antibodies and the HP10 metacestode antigen provide useful information relevant for epidemiological studies or for the selection of animals to be treated before slaughter.

In this study we have tested cattle sera from animals diagnose as positive and negative by veterinary inspection for (1) anti-parasite antibodies using metacestode antigens and (2) the secreted HP10 antigen of viable metacestodes. The results ilustrate the problem of diagnosing mild infections, but do reinforce the usefulness of such serological ELISA assays as tool for epidemiological studies and as an adjunct to meat inspection.

Materials and Methods

Biological samples

This research was approved by the Ethics Committee on Animal Experiments (CEUA 02017-2011).

Serum samples were obtained from 200 bovines during sanitary inspection divided in two groups: positive (n = 100) and negative bovines (n = 100) and conserved at –20 °C, until processing.

In all ELISA tests, serum from two *T. saginata* experimentally infected 8-month-old calves harboring 3.072 and 3.762 metacestodes each, and a 60-day-old calf non-cysticercotic from a non-endemic area were included as positive and negative controls, respectively, in order to normalize any day to day variation.

Antigens

T. solium cysticerci were dissected from skeletal muscles of naturally infected pigs during slaughter procedures and collected in phosphate buffered saline (PBS, pH 7.4). The metacestodes were ruptured with fine needle and vesicular fluid was collected. Calcium was removed by adding 50 μL of ammonium oxalate (0.3 M) and 25 μL ammonia (1:3, v/v, in water) to each mL of vesicular fluid, centrifuging (2,000 × g, 4 min, at 20 °C), and discarding all precipitated material. The supernatant was then collected and stored in liquid nitrogen (LARRALDE et al., 1986).

T. saginata metacestodes were excised from the skeletal muscle of naturally infected cattle from São Paulo State, Brazil, during slaughter procedures. After dissection, pooled metacestodes were washed in saline (NaCl 0.15 M) at 4 °C, in the presence of a protease inhibitor (PMSF 25 mM). The metacestodes were ruptured with fine needle and vesicular fluid was collected and centrifuged at 15.000 × g for 60 minutes at 4 °C. The supernatant was collected and a protease inhibitor (PMSF 25 mM 10%) was added. Afterwards, the fluid was centrifuged at 15.000 × g for 30 minutes at 4 °C and the supernatant was collected and stored at –80 °C (VAZ et al., 1997).

T. saginata excretory-secretory antigen (ES) was prepared with modifications according to Ogunremi and Benjamin (2010): groups of twenty viable metacestodes were washed in phosphate-buffered saline (NaCl 0.15 M) at 4 °C were cultured in RPMI 1640 medium (GIBCO®) supplemented with penicillin (500 units/mL) and streptomycin (500 μg/mL at 37 °C and 5% CO_2. After three days, RPMI 1640 supplemented with L-glutamine was substituted for the DMEM medium. Metacestodes were cultured up to 15 days, the supernatant was collected every 48 hours and stored at –20 °C until used. Pooled supernatant containing the ES antigen was centrifuged then dialyzed against PBS at 4 °C and lyophilized for concentration.

Detection of anti-parastie antibodies

Indirect ELISA was performed according to Ferrer et al. (2007b) with slight modifications. MaxiSorp (Nunc-Immuno™ Plates) polyestyrene microplates were coated with antigens extracts, at 10 µg/mL, diluted in carbonate-bicarbonate buffer (0.05M, pH 9.6), at 4 °C, overnight. Blocking was done by 5% nonfat milk diluted in carbonate-bicarbonate buffer (0.05M, pH 9.6), at 4 °C, for 2 hours. Assays were performed with bovine serum samples diluted 1:100 in phosphate solution (PBS 0.01M, pH 7.2, Tween 20® 0.05%) plus 5% nonfat milk, followed by incubation for 1 hour, at 37 °C. Peroxidase-labelled rabit IgG antibovine conjugate (Sigma®-A5295), diluted 1:8000 in PBS/ Tween 20® 0.05% / 5% nonfat milk, was added and incubated at 37 °C for 1 hour.

The 3, 3', 5, 5' tetramethyl benzidine (TMB) in citrate-phosphate buffer (pH 5.0) (Invitrogen® cat.00-2023) was used as substrate and the reaction was stopped by adding 3N HCl 20 minutes after its addition. Microplates were read by Multiskan EX (Labsystems®) photometer at 450 nm.

The test was performed in duplicate with a final volume of 100 µL/well and the microplates were washed 3 times, for 3 minutes with PBS-Tween 20® 0.05% between each immunoassay stages.

Detection of HP10 antigen

Sandwich ELISA was performed according to Harrison et al. (1989) with slight modification, using HP10 monoclonal antibody. MaxiSorp (Nunc-Immuno™ Plates) polystyrene microplates were coated with monoclonal antibody HP10 (10 µg/mL) diluted in carbonate-bicarbonate buffer (0.05M, pH 9.6), at 4 °C, for 18 hours. Microplates were blocked with 1% bovine serum albumin (BSA) (Sigma – A7906) diluted in phosphate buffer (PBS, 0.01M, pH 7.2) and incubated at 37 °C, for 1 hour.

Bovine serum samples were added undiluted and incubated for 1 hour, at 37 °C, followed by biotinylated monoclonal antibody HP10 (2,5 µg/mL in PBS 0.01M, pH 7.2), 1 hour, at 37 °C. As detection system, streptavidin labeled with horseradish peroxidase (Sigma®–S2438) was added (1:40.000) diluted in PBS (0.01M, pH 7.2) and incubated for 1 hour, at 37 °C. Samples were tested in duplicate with a final volume of 100 µL well.

Microplates were washed 4 times, for 3 minutes each, with PBS-Tween 20® 0.05%, between each immunoassay stage, except after incubation with biotinylated HP10 and before chromogen solution addition, when washing was done for 5 minutes each time.

The 3, 3', 5, 5' tetramethyl benzidine (TMB) in citrate-phosphate buffer, pH 5.0 (Invitrogen® - cat. n. 00-2023) was used as substrate. The color reaction was allowed to proceed for 15 minutes at 37 °C in the dark, before stopping by adding 100 µL of 0.2 M H_2SO_4 (Baker). Absorbance values were measured in a Multiskan EX (Labsystems®) photometer at 450 nm.

Statistical analysis

Optical density (O.D.) values were standardized for indirect ELISA by the formula described by Ogunremi and Benjamim (2010) modified to:

$$\frac{Sample/}{Positive} = \frac{\text{Mean O.D. sample} - \text{Mean O.D. negative sample control}}{\text{Mean O.D. positive sample control} - \text{Mean O.D. negative sample control}} \quad (1)$$

The ELISA cut-off for each antigen preparation and under intense and mild *T. saginata* infection was established based on the classical method, using the mean S/P values plus two standard deviations of the negative samples as well as by the Receiver Operating Characteristic Curve (ROC Curve). Tests performances were compared through the area under the curve (AUC) with its respective confidence interval of 95%, using GraphPad® Prism4 software.

In order to verify the difference among the antigens preparation for the indirect ELISA assay Cochran Q test ($\alpha = 0,05$) was performed by the BioEstat® 5.0 program.

To control for day to day variations, standard negative and positive control bovine sera were included, and the values obtained for the experimental samples were accordingly normalised.

Results

Cut-off values were calculated by the classical way ($\bar{x} + 2SD$) using 100 samples negative by inspection. Values were 0.655 for HP10 ELISA and 0.653, 0.628 and 1.559 for *T. solium* vesicular fluid, *T. saginata* vesicular fluid and *T. saginata* ES antigens in indirect ELISA, respectively.

As the majority of cases of bovine cysticercosis in Brazil are light infections with few metacestodes, cut-off values were also calculated by the ROC curve considering both, intense and mild infection condition. Thus for bovine harboring up to two viable metacestodes (n = 95) the cut-off values were 0.171 for HP10 ELISA and 0.240, 0.256 and 0.682 for *T. solium* vesicular fluid, *T. saginata* vesicular fluid and *T. saginata* ES indirect ELISA, respectively. For animals harboring more than 50 viable metacestodes (n = 5), the cut-off values were 0.349 for HP10 ELISA and 0.528, 0.615 and 1.560 for *T. solium* vesicular fluid, *T. saginata* vesicular fluid and *T. saginata* ES indirect ELISA, respectively.

The various assays and their established cut-off values were then applied to sera from bovines diagnosed as negative (n = 100) and positive (n = 100) by sanitary inspection. As can be seen, there were significant difference between the veterinary inspection and the four different serological (ELISA) analysis (Figure 1).

Sensitivity and specificity of HP10 ELISA and indirect ELISA varied according to the antigen preparations, the way cut-off values were established and due to intensity of infection (Table 1).

Values of the area under the curve (AUC) for intense and mild infection conditions were 0.905 and 0.681 for HP10 ELISA; 0.872 and 0.684 for *T. solium* vesicular fluid; 0.834 and 0.567 for *T. saginata* vesicular fluid; 0.794 and 0.530 for *T. saginata* ES indirect ELISA. The AUC performed best for both mild and intense infections using *T. solium* vesicular fluid antibody assay.

The Q Cochran test showed no significant difference between antigens preparations used in this study (P > 0.05) in indirect ELISA.

Best sensitivity was achieved by indirect ELISA using *T. solium* vesicular fluid in mild infection condition. Specificity was better with indirect ELISA using either *T. saginata* vesicular fluid or excretion-secretion antigen in intense infection conditions.

Discussion

In this study the possibility of serological identification of naturally *T. saginata* infected bovines in Brazil was explored by ELISA assays detecting (1) anti-metacestodes antibodies using three different target antigens and (2) viable metacestodes through detecting of HP10 antigen, a secreted product of viable metacestodes.

Reliable diagnosis of bovine cysticercosis in Brazil, either through veterinary inspection or serological procedures, is complicated by the fact that most of the bovines harbor few metacestodes.

A particular problem for serological diagnosis is that the negative control cut-off value is often close to the infected group in the ELISA assays. For example, Smith et al. (1991) performing indirect ELISA for bovines experimentally infected with a low dose of *T. saginata* eggs encountered precisely this problem.

As control of bovine cysticercosis is both a veterinary and public health problem, high sensitivity is essencial, and this ultimately depends on how the cut-off is established. In this study, cut-off values were established through the classical way ($\overline{X}$ + 2SD) and
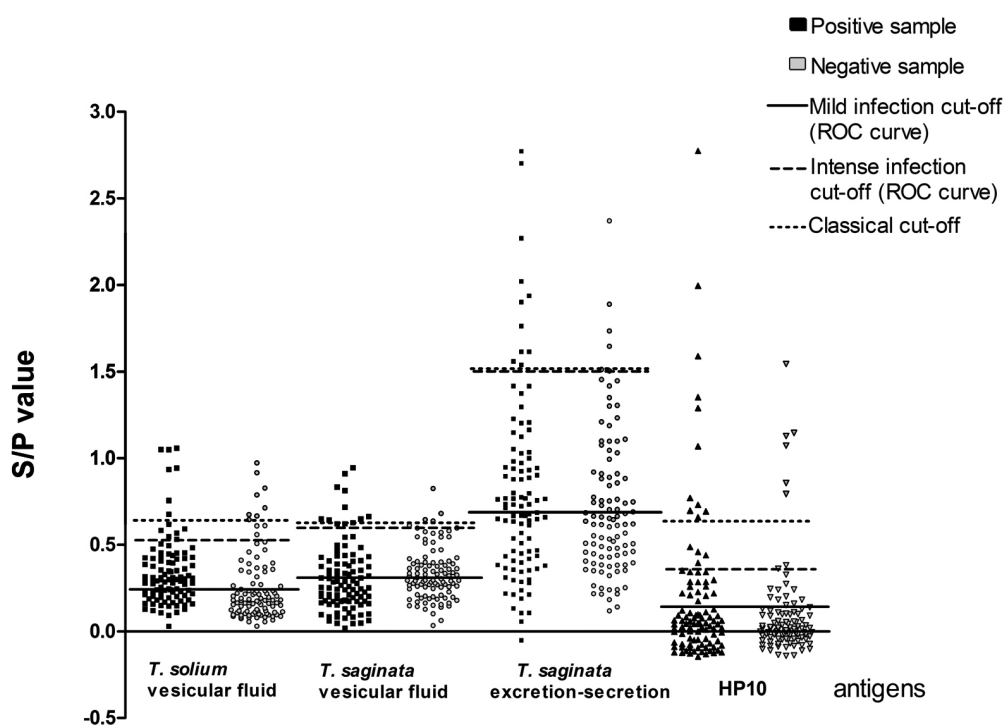

Figure 1. Detection of anti-*Taenia saginata* metacestodes IgG antibodies in serum samples from positive (n = 100) and negative bovines (n = 100) for cysticercosis by indirect ELISA, using *T. solium* and *T. saginata* vesicular fluid and *T. saginata* excretion-secretion antigen, and sandwich ELISA using HP10 monoclonal antigen.

Table 1. Sensitivity and specificity for bovine cysticercosis by indirect ELISA, using *T. solium* and *T. saginata* vesicular fluid and *T. saginata* excretion-secretion antigen, and sandwich antigen ELISA using HP10 monoclonal antigen.

	T. solium vesicular fluid	*T. saginata* vesicular fluid	*T. saginata* excretion-secretion	HP10
Sensitivity (%)				
Classical cut-off[a]	7	9	9	11
Intense infection[b]	12	12	9	16
Mild infection[b]	64	49	52	31
Specificity (%)				
Classical cut-off[a]	93	96	96	94
Intense infection[b]	89	96	96	92
Mild infection[b]	70	30	57	83

[a]Cut-off calculated by $\overline{X}$ + 2SD of negative samples. [b]Cut-off calculated by ROC curve.

for conditions of mild and intense infection, calculated by the ROC curve. The later has the advantage of permiting changes in the cut-off value depending on the purpose of the testing (GARDNER et al., 2010).

Although the antibody detection measures previous exposure and not necessarily a concomitant infection it does serve to identify infected herds. Applying the cut-off calculated by the ROC curve and considering mild infection condition, good sensitivity was observed for antibody detection using *T. solium* vesicular fluid (64%). Minozzo et al. (2004) also reported higher sensitivity using *T. solium* vesicular fuid antigen to detect infected bovines diagnosed as negative by sanitary inspection during slaughter in Brazil. *T. saginata* excretion-secretion antigens also showed good sensitivity (52%), although lower than the observed by Ogunremi and Benjamin (2010) that identified experimentally infected bovines with mild infection, using the same antigen and calculating the cut-off value by the ROC curve. Usually, the identification of positives is easier in experimental condition. Monteiro et al. (2006) observed higher number of false negative reactions on sera of naturally infected bovines compared to the experimentally infected animals, confirming the difficulties while testing field samples.

In contrast to antibody detection, the HP10 assay detects viable metacestodes and thus cattle with the potential to transmit taeniasis to humans. Clearly, the sensitivity in the HP10 assay depends on the number of viable metacestodes in an infected animal; the lower the number, the lower the signal, as has been pointed out in studies conducted in Spain Allepuz et al. (2012). Using the HP10 assay, better results were observed considering cut-off under mild infection condition (Table 1). One explanation is the higher possibility of antigen-antibody complexes in conditions of intense infection (DORNY et al., 2000). Another possibility is that the amount of ES-products released in the circulation might vary according to a differential permeability of the cysticercus on different times of development (BRANDT et al., 1992).

Onyango-Abuje et al. (1996a) reported a higher sensitivity for the detection of naturally infected bovines using the HP10 ELISA than with an indirect ELISA with *T. saginata* vesicular fluid antigen. Samples were taken at slaughterhouses in Kenya and so we can now make a direct comparison between their results and ours.

Thus, low infection condition as well as the stage of infection can influence the amount of excretion-secretion circulating antigen making more difficult the detection of a naturally infected bovine.

Conclusion

Measurement of anti-parasite antibodies, particularly with *T. solium* vesicular fluid, would be a recommended strategy for epidemiological purposes, whereas measurement of parasite antigens by HP10 sandwich ELISA would be a recommended strategy for identifying infected animals at slaughter. Overall, there is a need of improving the sensitivity of immunodiagnosis to identify infected cows with low level infections.

References

Associação Brasileira das Indústrias Exportadoras de Carne - ABIEC. [online]. 2012 [cited 2013 Jan 31]. Available from: http://www.abiec.com.br/3_rebanho.asp.

Allepuz A, Gabriel S, Dorny P, Napp S, Jansen F, Vilar MJ, et al. Comparison of bovine cysticercosis prevalence detected by antigen ELISA and visual inspection in the North East of Spain. *Res Vet Sci* 2012; 92(3): 393-395. PMid:21524428. http://dx.doi.org/10.1016/j.rvsc.2011.03.027

Brandt JRA, Geerts S, De Deken R, Kumar V, Ceulemans F, Brijs L, et al. A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. *Int J Parasitol* 1992; 22(4): 471-477. http://dx.doi.org/10.1016/0020-7519(92)90148-E

Brasil. Ministério da Agricultura e do Abastecimento Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. RIISPOA [online]. 1952 [cited 2013 Jan 31]. Available from: http://www.agricultura.gov.br/arq_editor/file/Aniamal/MercadoInterno/Requisitos/RegulamentoInspecaoIndustrial.pdf.

Dorny P, Vercammen F, Brandt J, Vansteenkiste W, Berkvens D, Geerts S. Sero-epidemiological study of *Taenia saginata* cysticercosis in Belgian cattle. *Vet Parasitol* 2000; 88(1-2): 43-49. http://dx.doi.org/10.1016/S0304-4017(99)00196-X

Ferrer E, Bonay P, Foster-Cuevas M, González LM, Dávila I, Cortéz MM, et al. Molecular cloning and characterisation of Ts8B1, Ts8B2 and Ts8B3, three new members of the *Taenia solium* metacestode 8 kDa diagnostic antigen family. *Mol Biochem Parasitol* 2007a; 152(1): 90-100. PMid:17210192. http://dx.doi.org/10.1016/j.molbiopara.2006.12.003

Ferrer E, González LM, Martínez-Escribano JA, González-Barderas ME, Cortéz MM, Dávila I, et al. Evaluation of recombinant HP6-Tsag, an 18 kDa *Taenia saginata* oncospheral adhesion protein, for the diagnosis of cysticercosis. *Parasitol Res* 2007b; 101(3): 517-525. PMid:17351832. http://dx.doi.org/10.1007/s00436-007-0507-x

Gardner IA, Greiner M, Dubey JP. Statistical evaluation of test accuracy studies for *Toxoplasma gondii* in food animal intermediate hosts. *Zoonoses Public Health* 2010; 57(1): 82-94. PMid:19744298. http://dx.doi.org/10.1111/j.1863-2378.2009.01281.x

Gonçalves FA, Machado GA, Oliveira HB, Rezende MTNP, Mineo JR, Costa-Cruz JM. Hydrophobic fraction of *Taenia saginata* metacestodes, rather than hydrophilic fraction, contains immunodominant markers for diagnosing human neurocysticercosis. *Rev Soc Bras MedTrop* 2010; 43(3): 254-259. http://dx.doi.org/10.1590/S0037-86822010000300008

González LM, Ferrer E, Spickett A, Michael LM, Vatta AF, Gárate T, et al. The *Taenia saginata* homologue of the major surface antigen of *Echinococcus* spp. is immunogenic and 97% identical to its *Taenia solium*

homologue. *Parasitol Res.* 2007; 101(6): 1541-1549. PMid:17674048. http://dx.doi.org/10.1007/s00436-007-0673-x

Harrison LJS, Joshua GWP, Wright SH, Parkhouse RME. Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. *Parasite Immunol* 1989; 11(4): 351-370. PMid:2674862. http://dx.doi.org/10.1111/j.1365-3024.1989. tb00673.x

Larralde C, Laclette JP, Owen CS, Madrazo I, Sandoval M, Bojalil R, et al. Reliable serology of *Taenia solium* cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am J Trop Med Hyg* 1986; 35(5): 965-973. PMid:3766855.

Lopes WDZ, Santos TR, Soares VE, Nunes JLN, Mendonça RP, Lima RCA, et al. Preferential infection sites of *Cysticercus bovis* in cattle experimentally infected with *Taenia saginata* eggs. *Res Vet Sci* 2010; 90(1): 84-88. PMid:20493507. http://dx.doi.org/10.1016/j. rvsc.2010.04.014

Marques GM, Buzi KA, Galindo LA, Baldini ED, Biondi GF. Avaliação dos registros de condenação por cisitecercose em bovinos abatidos em frigoríficos da região Centro Oeste do Estado de São Paulo – 1996 a 2000. *Vet Zootec* 2008; 15:114-120.

Minozzo JC, Thomaz-Soccol V, Olortegui CC, Soares VE, Costa AJ. Teste imunoenzimático (enzyme-linked immunosorbent assay) para diagnóstico da cisticercose bovina e estudo da cinética de produção de anticorpos contra-*Cysticercus bovis*. *Cienc Rural* 2004; 34(3): 857-864. http://dx.doi.org/10.1590/S0103-84782004000300031

Monteiro LL, Pinto PSA, Dias FS. Evaluation of the ELISA test for the antibody detection in cattle naturally and experimentally infected with *Cysticerccus bovis*. *Vet Parasitol* 2006; 141(3-4): 260-263. PMid:16806711. http://dx.doi.org/10.1016/j.vetpar.2006.05.017

Oliveira HB, Machado GA, Cabral DD, Costa-Cruz JM. Application of *Taenia saginata* metacestodes as an alternative antigen for the serological diagnosis of human neurocysticercosis. *Parasitol Res* 2007; 101(4): 1007-1013. PMid:17510761. http://dx.doi. org/10.1007/s00436-007-0578-8

Ogunremi O, Benjamin J. Development and field evaluation of a new serological test for *Taenia saginata* cysticercosis. *Vet Parasitol* 2010; 169(1-2): 93-101. PMid:20083357. http://dx.doi.org/10.1016/j. vetpar.2009.12.014

Onyango-Abuje JA, Nginyi JM, Rugutt MK, Wright SH, Lumumba P, Hughes G, et al. Seroepidemiological survey of *Taenia saginata* cysticercosis in Kenya. *Vet Parasitol* 1996a; 64(3): 177-185. http://dx.doi. org/10.1016/0304-4017(95)00915-9

Onyango-Abuje JA, Hughes G, Opicha M, Nginyi KM, Rugutt MK, Wright SH, et al. Diagnosis of *Taenia saginata* cysticercosis in Kenyan cattle by antibody and antigen ELISA. *Vet Parasitol* 1996b; 61(3-4): 221-230. http://dx.doi.org/10.1016/0304-4017(95)00840-3

Smith HJ, Snowdon KE, Finlay RC. Serological diagnosis of cysticercosis by an enzyme-linked immunosorbent assay in experimentally infected cattle. *Can J Vet Res* 1991; 55(3): 274-276. PMid:1889037 PMCid:1263464.

Souza VK, Pessoa-Silva MC, Minozzo JC, Thomaz-Soccol V. Prevalência da cisticercose bovina no Estado do Paraná, sul do Brasil: avaliação de 26.465 bovinos inspecionados no SIF 1710. *Sem Cienc Agr* 2007; 28(4): 675-684.

Ungar ML, Germano PML. Prevalência da cisticercose bovina no Estado de São Paulo (Brasil). *Rev Saúde Pública* 1992; 26(3): 167-172. PMid:1342497. http://dx.doi.org/10.1590/S0034-89101992000300007

Vaz AV, Nunes CM, Piazza RMF, Livramento JA, Silva MV, Nakamura PM, et al. Immunoblot with cerebrospinal fluid from patients with neurocysticercosis using antigen from cysticerci of *Taenia solium* and *Taenia crassiceps*. *Am J Trop Med Hyg* 1997; 57(3): 354-357. PMid:9311649.

Yamane K, Suzuki Y, Tachi E, Li T, Chen X, Nakao M, et al. Recent hybridization between *Taenia asiatica* and *Taenia saginata*. *Parasitol Int* 2012; 61(2): 351-355. PMid:22301089. http://dx.doi.org/10.1016/j. parint.2012.01.005