

Distribution of *Taenia saginata* metacestodes: a comparison of routine meat inspection and carcass dissection results in experimentally infected calves

V. E. SOARES^{*,†}, M. A. DE ANDRADE BELO^{*,†}, P. C. B. REZENDE[‡], V. T. SOCCOL[§], R. T. FUKUDA[¶], G. P. DE OLIVEIRA[†] and A. J. DA COSTA[†]

^{*}UNICASTELO, Camilo Castelo Branco University, Descalvado, SP, Brazil

[†]CPPAR, Center of Research in Animal Health, Department of Veterinary Pathology, São Paulo State University, UNESP, Jaboticabal, SP, Brazil

[‡]Centro Universitário Barão de Mauá, Ribeirão Preto, SP, Brazil

[§]Department of Basic Pathology, Federal University of Paraná, Curitiba, PR, Brazil

[¶]Brazilian Federal Beef Inspection Service, S.I.F., Ribeirão Preto, SP, Brazil

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A comparison of techniques for detecting the presence of *Cysticercus bovis* in bovine carcasses was made by using carcass dissection and routine beef inspection guidelines. In the study, 28 calves were used after they were tested and found to be negative for the presence of anti-*C. bovis* serum antibodies and were inoculated orally with aliquots containing 6×10^4 *Taenia saginata* eggs. One hundred and twenty days after inoculation, the animals were slaughtered and a *post mortem* evaluation was done following Brazilian Federal Beef Inspection guidelines. This routine meat inspection was able to identify 71.42% of the assessed infected carcasses as being parasitized. This result implies that 28.58% of the infected carcasses would have been released as fit for human consumption since they would have been considered as free of *C. bovis* infection when using this method for carcass assessment. Only 3.07% of the total 2311 metacestodes present in the carcasses were identified by the conventional procedures of sanitary inspection. The assessment of different parts of the carcasses showed high infestation rates in shoulder clod (14.37%), head (11.21%), neck+chuck roll (8.05%), heart (7.75%) and top (inside) round (7.18%) which, together, were responsible for housing 48.51% of all the cysts found in the 24 beef cuts assessed. These numbers contrasted to the low incidence of cysts found in organs such as tongue (3.12%), diaphragm (1.69%) and esophagus (1.60%) which are usually described as predilection sites for the parasite.

INTRODUCTION

Bovine cysticercosis, like other metacestode infestations in livestock, is a zoonosis of great socioeconomic and public health importance (FAO, 2005; OIE, 2006). The disease is defined as a parasitosis in which the parasite requires the participation of two different animal species as hosts for its survival in

nature, and one of these hosts is necessarily a human being (Fukuda, 2003). Transmission of this parasite to animals occurs upon contamination of their food or water by feces of infested humans (Geysen *et al.*, 2007). The contaminated material can derive directly from human feces or via sewage plants after flooding or sewage sediment distributed on pastures (Abuseir *et al.*, 2007). In Brazil, epidemiological control of the taeniasis/cysticercosis complex represents a very important challenge for sanitary inspectors, especially in areas with intense livestock and agricultural production and which present high population flux with overpopulated areas where, many times, basic

Reprint requests to: Vando Edésio Soares, UNICASTELO, Camilo Castelo Branco University, 950 Hilário da Silva Passos Ave., Descalvado City, São Paulo State, Brazil. E-mail: soaresvando@gmail.com

sanitary infrastructure is not available (Fukuda, 2003).

Taeniasis/cysticercosis is a parasitic infestation with worldwide occurrence, although it usually presents higher prevalences in underdeveloped countries due to poor sanitation and low social and economic conditions. According to Takayanagui and Leite (2001), the situation regarding this disease in Latin America is comparable to that occurring in Germany at the end of the nineteenth century, when sanitary conditions were very poor. In an epidemiological study conducted in abattoirs that follow Brazilian Federal Laws for beef inspection in the State of São Paulo during the period of 1980–2001, Fukuda (2003) observed a 4.28% increase in the occurrence of bovine cysticercosis. The occurrence of this parasitosis in developed countries, however, is also a cause for concern. In an epidemiological survey realized from March 2005 to December 2007, *Taenia saginata* metacestodes were detected in 284 animals from 67 cattle farms (24 dairy and 43 beef) in Catalonia, North-Eastern Spain (Allepuz *et al.*, 2009).

In 2001, Fernandes and Buzetti verified that in 97.46% of bovine cysticercosis infections identified, cysts were found in heart and cheek muscles (masseters). According to Kearney (1970), the predilection of the cysts for these specific muscles is due to their high rates of myoglobin and intense respiratory metabolism. Brazilian guidelines for beef inspection in abattoirs (Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal — RIISPOA) indicate (article 176, fifth paragraph) that routine inspection procedures should include in the final inspection the identification of parasitic lesions that were observed initially and also that the predilection sites of the cysts, specifically the cheek muscles, the heart, the muscular portion of the diaphragm including its pillars, should all be examined, avoiding, however, unnecessary slicing that would cause further carcass depreciation. On the other hand, most of the bovine cysticercosis prevalence studies were based on results found in routine carcass inspection in abattoirs, where

these were found to be not sensitive enough for the diagnosis of cysticercosis, in spite of the conscientious attempt of the inspectors in locating existing cysticerci at predilection sites. At the same time, the true prevalence of bovine cysticercosis as detected by classical meat inspection techniques (when carried out properly) is underestimated by at least a factor of 3–10 (Asaava *et al.*, 2009).

The presence of cysticerci in bovine carcasses and viscera leads to the condemnation of the beef originated from the infected animals with its taxation as unfit for human consumption. If not necessarily discarded, the beef resulting from the infected carcasses should receive treatment by heat, cold or salting as a condition for its human consumption. These necessary treatments increase production costs, cause depreciation in the beef's market value and generate restrictions in its commercialization with associated loss of revenue for the industry, besides becoming an obstacle for the exportation of beef to other countries (Fukuda, 2003). A HACCP-based approach to an on-farm food safety program for the Australian red meat industry identified *C. bovis* infection (beef measles) as an important and critical aspect to be considered in beef inspection (Horchner *et al.*, 2006). Due to the sanitary importance of the taeniasis/cysticercosis complex as well as Brazil's position as an important producer and exporter of beef, the present study was done to assess carcass distribution of metacestode stages (*C. bovis*) harbored in tissue of experimentally infected bovines, by comparing total carcass dissection and routine beef inspection procedures following the guidelines from Brazilian Federal Inspection Services for identifying cysticercosis in bovine carcasses.

MATERIALS AND METHODS

Study Location

All the animals used in the study were housed at the Center for Research in Animal Health 'Centro de Pesquisas em Sanidade Animal — CPPAR/FCAV/UNESP' for the

entire experimental period, where they received feed and water *ad libitum*. The laboratory testings were all conducted at the CPPAR/FCAV/UNESP and at the Department of Basic Pathology of the Federal University of the Paraná State (Departamento de Patologia Básica da Universidade Federal do Paraná) — UFPR.

Animal Selection

Bovines of 3 months of age in average were selected from the cattle farm 'Fazenda Palmital' located at Cássia county, MG state, a Brazilian region which normally presents extremely low prevalence rates for bovine cysticercosis. The calves were submitted to an enzyme-linked immunosorbent assay (ELISA) indirect sera test for detection of cysticercosis infection and then 28 of the animals which presented negative results from these tests were selected for the study. All the selected animals were then transported to the CPPAR/FCAV/UNESP facility, where they remained housed in individual stalls for the duration of the experiment.

Inoculum Preparation

T. saginata proglottids were collected at the county laboratory of parasitology from Curitiba county, PR state, from human excrement collected from patients which had not undergone de-worming treatments. The proglottids were collected with sieves for 7 days and then stored at 4°C. Identification procedures included proglottid compression between glass slides for observation with an optical microscope after which the proglottids were dissected for release of egg content. Egg count was obtained using a hemocytometer and these were then divided into aliquots of 60.000 eggs each. Dilution to the required concentration was done using a 0.09% NaCl solution, in 20 ml of volume. Experimental infection of the animals was obtained by oral inoculation of the aliquots.

Indirect ELISA Test

The indirect ELISA test used was developed specifically for the detection of anti-*C. bovis*

antibodies (Minozzo *et al.*, 2002). The test was standardized with antigen at a 250 ng/well concentration, with sera and the conjugate diluted 100 and 400 times respectively. Cutoff titers were determined by the mean value of optic density plus two times the standard deviation, resulting in a value of 0.059.

Post mortem Examination — Federal Inspection

All the infected calves were slaughtered 120 days after administration of the inoculum at an abattoir which officially operates following Brazilian Federal Inspection guidelines (Frigorífico Minerva, Barretos, SP). These animals' carcasses were submitted to inspection by meat inspectors from the Agricultural Brazilian Ministry and the investigation of possible metacestode (*C. bovis*) infection was done according to the guidelines from Federal Inspection service. After inspection, the carcasses and their respective organs were all packaged, identified and then sent to the CPPAR for continuation of the assessment.

Serial Examination — Carcass Dissection

The carcasses were transported in a refrigerated truck from the official slaughterhouse to the CPPAR facilities where the larger bones were separated from the meat and then divided into conventional meat cuts. All these parts and also the bovines' organs were then distributed on separate surfaces, maintaining separate the products from each individual animal. Muscles and organs were inspected for the presence of cysts after each being entirely sliced into serial cuts of approximately 5 mm each for a thorough assessment. The fragments of tissue which were found to harbour cysts were then placed in Petri dishes where, after careful dissection, the cysts were evaginated from the cyst membrane.

Correlation of Brazilian Commercial Beef Cuts to Anatomic Structures

In Brazil, anatomical bovine parts and commercial beef cuts do not correspond. For better understanding of the results found in the study and assessed considering presence of cysts found in different Brazilian cuts of beef, the correspondence between bovine anatomy and traditional Brazilian beef cuts, compared to American and Australian denominations are described in Table 1.

Statistical Analysis

The number of *T. saginata* metacestodes found in the study were submitted to a descriptive analysis and then transformed into logarithms to attend the requisites for normal distribution. All data were analyzed by an entirely random design and the comparison of mean values was done by Tukey's test ($P \geq 0.05$), assisted by SAS programs (SAS Institute Inc., 2001).

RESULTS

The occurrence of *C. bovis* (in percentages) found in the different bovine parts as well as the total number of infected animals listed according to tissue type where the cysts detected in the carcasses is presented in Table 2. The *post mortem* results showed high incidence of cysticerci in shoulder clod (14.37%), head (11.21%), neck + chuck roll (8.05%), heart (7.75%) and top (inside) round (7.18%) which, together, totaled 48.51% of the cysts found in the 24 beef cuts assessed (Table 2). On the other hand, sites normally described as of high predilection for the parasite such as the tongue (3.12%), diaphragm (1.69%) and the esophagus (1.60%) presented low incidence of cysts (Table 2).

Infection levels found in the assessment of the experimentally infected animals showed more than 30% of *C. bovis* in the following meat cuts: head (46.43%), shoulder

clod (42.86%), heart (39.29%), strip loin (35.71%), liver (35.71%), knuckle (35.71%) and tongue (32.14%) (Table 2).

When comparing the two different inspection methods, the number of *C. bovis* found by serial slicing of the carcasses was higher ($P < 0.05$) than the number of cysts detected by the federal routine beef inspection (Table 3). These results highlight the efficiency of total slicing of the carcass for detecting cysts in beef. Table 3 presents the comparison of the number of animals found to be positive for cysticercosis by detection of *C. bovis* cysts in their carcass when assessed by the federal routine beef inspection compared to the results obtained by the serial examination of the carcasses. This assessment showed that only 71.42% of the infected carcasses were identified by the standard meat inspection. Therefore, 28.58% of the infected carcasses would have been released for human consumption as free of *C. bovis*. Statistical analysis of these results showed that these errors can occur in the same proportion when the inspection of the carcasses is done according to routine beef inspection procedure (Table 3).

Table 4 presents the incidence of live *T. saginata* metacestodes found in the carcasses, with 70.53% of the total of cysts identified. Of these, only 1.25% was detected by routine beef inspection procedures. It was also observed that only 3.07% of the 2311 metacestodes that were present in the carcasses of the 28 animals were identified using the conventional procedure of meat inspection.

DISCUSSION

According to Pawlowski and Schultz (1992) and Maeda *et al.* (1996), the distribution of *T. saginata* cysts in bovine tissue can vary according to many factors such as breed, age and area from where the cattle is from. However, most of the guidelines for carcass inspection in abattoirs regarding *C. bovis* detection consider the heart, internal and

TABLE 1. Nominal correlation of the Brazilian, Australian and American (USA) commercial beef cuts¹ to the anatomic structures

	Australia	USA	Muscles ²
Alcatra (miolo)	Rostbiff	Top sirloin butt (Round eye)	Gluteus medius, Gluteus accessorius, Gluteus superficialis, Gluteus profundus
Contra filé	Outside flat	Strip loin / ribeye	Gluteus medius, Retractor costae, Interspinales, Spinalis dorsi. Caudal part of: Longissimus dorsi, Multifidus dorsi, Intertransversales colli, Levatores costarum, Iliocostalis, Intercostales externi and interni.
Coxão duro	Striploin	Outside round (flat)	Biceps femoris
Coxão mole	Topside / inside - cap off	Top (inside) round, cap off	Semimembranosus, Gracilis, Adductor femoris, Sartorius, Pectineus, Quadratus femoris, Obturator internus, Obturator externus, Gemellus
Filé mignon	Tenderloin	Full Tenderloin	Psoas major, Psoas minor, Ileo-psoas, Quadratus lumborum
Lagarto	Eye round	Eye of round	Semitendinosus
Maminha	Bottom sirloin triangle (Tri-tip)	Bottom sirloin butt (Tri-tip)	Tensor fascia latae
Músculo (braço + traseiro)	(Shin / Shank) + (Shin-special trim)	Foreshank + Shank	Anterior: Biceps brachii, Extensor carpi obliquus, Extensor carpi radialis, Extensor carpi ulnaris, Flexor carpi radialis, Músculo (Shin / Shank) Flexor carpi ulnaris, Pronator teres, Coracobrachialis, Common digital extensor: (braço + traseiro) + Posterior: Gastrocnemius, Soleus, Popliteus, Tibialis anterior, Tibialis cranialis, Tibialis caudalis, Peroneus longus, (Shin-special trim) Peroneus tertius. Flexor digitorum superficialis-pelvic, Lateral digital extensor-pelvic, Extensor digiti primi longus, Deep digital flexor-pelvic, Long digital extensor, Long digital flexor-pelvic.
Paleta	Blade (clod)	Shoulder clod	Triceps brachii, Deltoideus, Supraspinatus, Infraspinatus, Teres major, Subscapularis, Teres minor, Anconeus, Tensor fasciae antibrachii, Cutaneous omo-brachialis, Cranial part of Longissimus dorsi.
Patinho	Knuckle	Knuckle, peeled (Ball tip)	Rectus femoris, Vastus lateralis, Vastus medialis, Vastus intermedius
Pescoço + acém	Chuck	Chuck	Trapezius cervicis, Omotransversarius, Brachiocephalicus, Sternoccephalicus, Longus colli, Scalenus ventralis, Splenius, Semispinalis capitis, Rectus capitis dorsalis, Rectus capitis lateralis, Rectus capitis ventralis-major, Rectus capitis ventralis-minor, Obliquus capitis caudalis, Obliquus capitis cranialis. Cranial part of Intercostales externi, Intercostales interni, Intertransversales colli, Levatores costarum, Multifidus dorsi, Longissimus dorsi, Iliocostalis, Serratus dorsalis-anterior, Serratus dorsalis -posterior, Serratus ventralis.
Picanha	Rump cap	Top sirloin cap	Dorso-medial part of biceps femoris
Ponta de agulha + peito	Brisket-deckle off) + (Brisket point end plate)	(Inside skirt - plate) + Brisket, deckle-off, boneless)	Obliquus abdominis externus, Obliquus abdominis internus, Rectus abdominis, Subclavius, Cutaneous trunci, Transversus abdominis, Transversus thoracis, Pectoralis profundi, Pectoralis superficialis. Ventral part of Serratus ventralis, Longissimus dorsi, Serratus dorsalis-posterior, Intercostales externi, Intercostales interni.

¹ Adapted from Yokoo *et al.* (2003)² According to regulamentation from MAPA - Ministry of Agriculture, Livestock and Food Supply - Brazil (1988).

TABLE 2. Distribution of *Cysticercus bovis* in carcasses and viscera of the 28 experimental animals

Cuts	Number of <i>Cysticercus bovis</i> (Per beef cuts)		No. of infected animals (n=28)	
	Total	%	Total	%
Shoulder clod	331	14.32	12	42.86
Head	259	11.21	13	46.43
Chuck roll + neck	186	8.05	7	25.00
Heart	179	7.75	11	39.29
Top (inside) round	166	7.18	8	28.57
Liver	154	6.66	10	35.71
Knuckle	147	6.36	10	35.71
Ribs	139	6.01	4	14.29
Strip loin	134	5.80	10	35.71
Full tenderloin	106	4.59	6	21.43
Shank	97	4.20	8	28.57
Top sirloin butt	91	3.94	6	21.43
Tongue	72	3.12	9	32.14
Outside round	60	2.60	3	10.71
Diaphragm	39	1.69	7	25.00
Top sirloin cap	39	1.69	3	10.71
Esophagus	37	1.60	2	7.14
Lungs	26	1.13	3	10.71
Eye of round	16	0.69	4	14.29
Kidneys	12	0.52	2	7.14
Botton sirloin butt	8	0.35	2	7.14
Flank	6	0.26	4	14.29
Pilar	7	0.30	3	10.71
Tail	0	0.00	0	0.00
Total	2311	100.00	—	—

external masseter muscles, tongue, esophagus and diaphragm as predilection sites for cysts and, therefore, are cited as of obligatory inspection (Kyvsgaard *et al.*, 1990).

In an assessment by Fukuda (2003) in Brazil, the methodology used considered the

findings of many other authors, and therefore examined carcass points examined by these, finding a very high incidence of cysts in the masseter muscles, the heart, shoulder clod and in the neck. These results were in accordance to the results found in the

TABLE 3. Statistical analysis of the results from *Cysticercus bovis** investigation, data transformed into log (x+5), of the carcasses of calves artificially infected with *Taenia saginata* eggs

Type of inspection	<i>Cysticercus bovis</i> live	<i>Cysticercus bovis</i> dead	<i>Cysticercus bovis</i> total	No. of positive animals (% efficacy)
Serial slicing [†]	1.1093 ^A	0.9199 ^A	1.2515 ^A	28 (100%)
Routine inspection [‡]	0.7850 ^B	0.8216 ^A	0.8757 ^B	20 (71.42%)
TESTE F	5.63	1.09	7.19	—
$Pr > F^{\S}$	0.0226	0.3018	0.0106	—
CV% [§]	46.78	34.97	42.7	—

*Mean values (n=28) compared inside a column, with at least one letter (^A or ^B) in common do not differ by Tukeys test ($P > 0.05$).

[†]Slicing of carcass in serial examination of 0.5 mm.

[‡]Slaughter done by the Brazilian Federal Meat Inspection service (SIF).

[§]Probability of significance associated to the value of *F*; coefficient of variation (CV).

TABLE 4. Numbers (percentage) of live and of calcified *Cysticercus bovis* found by routine beef inspection and by serial slicing of carcasses of calves artificially infected with *Taenia saginata* eggs

Kind of inspection	Life cysts		Calcified cysts		Total (<i>Cysticercus bovis</i>)	
	No.	%	No.	%	No.	%
Serial slicing*	1601	69.28	639	27.65	2240	96.93
Routine inspection†	29	1.25	42	1.82	71	3.07
Total	1630	70.53	681	29.47	2311	100.00

*Slicing in 0.5 mm sections.

†Slaughter done according to Brazilian Federal Beef Inspection service (SIF) guidelines.

present study, with similar results in both assessments regarding these sites. Several studies have shown the same predilection sites regarding cysticercosis in bovines (Kebede, 2008; Kebede *et al.*, 2009; Scandrett *et al.*, 2009; Lopes *et al.*, 2011). However, the serial slicing of the diaphragm and the tongues in this study, although detecting small numbers of cysts in both, showed the high levels of cysticercosis infection in the bovines assessed. These included the inspection of the diaphragm and the tongue in the *post mortem* procedure (Fernandes and Buzetti, 2001).

Carcass inspection by serial slicing of the different beef cuts and organs detected low numbers of cysts in the esophagus. Such findings are in accordance to Scandrett *et al.* (2009) and Lopes *et al.* (2011) who observed low rates of infection at this site after inoculating 42 and 26 bovines with *T. saginata* eggs respectively. Both these results found by these authors and those from the present study considering number of cysts found in the esophagus, differ from those described in other studies. This difference could possibly be due to varying factors such as smaller number of animals assessed, methods used in husbandry practices and area where the animals were raised (Kearney, 1970).

Results from the present study showed the same tendency as those found by other Brazilian authors who studied the occurrence of *T. saginata* metacestode cysts in bovine carcasses (Fernandes and Buzetti, 2001; Fukuda, 2003). These authors found

high numbers of cysts in the strip loin, knuckle and shoulder clod when examining these beef cut parts by serial slicing. However, these tissues are not usually examined when inspection is done according to the routine federal beef inspection guidelines since slicing causes loss of commercial value. Fukuda (2003) concluded that it was possible that routine beef inspection would not detect all cysts embedded in inspected muscle parts and, therefore, the carcasses would harbor undetected cysts.

This occurrence would be an important problem when considering the incidence of *C. bovis* cysts in skeletal striated bovine muscle. Minozzo *et al.* (2002) verified that *C. bovis* in experimentally infected bovines was distributed throughout the carcasses with 85.19% of the cysts harbored in skeletal muscle and only 14.81% in other tissues and organs such as heart, diaphragm, lungs, kidneys, liver and tongue. These results were in accordance to those found in the present study where 77.78% of the cysts were found in skeletal muscle and 22.22% in other tissue and organs. Studies carried out in Ethiopia showed high incidence of *C. bovis* in thigh and triceps muscles (Kebede, 2008; Kebede *et al.*, 2009). These occurrences represent a problem for zoonotic control of *T. saginata*, since consumption of cyst infected meat by humans implies exposure of the human population to this zoonotic disease, even after the beef underwent inspection by the

competent official parties. Geerts *et al.* (1980) assessed the hearts of 100 bovines by serial slicing of these, after the organs had undergone inspection in Belgium and had been designated free of cysts. This second inspection detected *T. saginata* metacystodes in 25 of these hearts.

Kyvsgaard *et al.* (1990) concluded in their studies that beef inspection is more likely to detect cysts in bovines that present high levels of infection, with cyst detection much reduced in low levels of cysticercosis. Still according to these authors, only 4% of cysts present in bovine carcasses could be found on the surface of the organs that are routinely inspected and only 15% of the animals parasitized by *C. bovis* could be detected as infected by routine beef inspection. In the present study, the results were similar to those found by these authors, with 250 live cysts found in animals presenting low levels of infection, a fact that shows the sanitary and epidemiological importance of low infection levels of cysticercosis in cattle which, undetected, could originate teniasis infections in humans after consumption of beef originated from these animals.

Walther and Koske (1980) assessed the efficiency of routine beef inspection procedures for the detection of *T. saginata* metacystodes in 60 infected animals. Of these, the authors found that only 38.3% were identified as harbouring cysts by these procedures. Serial slicing of the same carcasses, however, showed that the other animals were also infected by *C. bovis* and their tissues harboured cysts. In another study, Minozzo *et al.* (2002) reported that 85.9% of the cysts detected by serial slicing of the carcasses would not have been detected by using the federal routine beef inspection guidelines (SIF). In a study by Fukuda (2003), some cysts were not detected by visual inspection and the author reported that, of 4366 infected animals assessed, 96.7% presented low levels of infection, with only one cyst being found at the examined predilection sites. These reports sustain the opinion of various

authors on the great importance and epidemiological issue of animals infected by monocysticercosis. Animals which are positive for such infections can go undetected, especially when suffering low levels of infection, a condition which is very frequent in bovine cysticercosis (Fukuda, 2003).

The numbers of live *T. saginata* metacystodes found in the present study reaffirm the epidemiological importance of this infection, due to the fact that live cysts found in viscera and carcass parts of beef cattle which are destined for human consumption could cause infection in these hosts. Asaava *et al.* (2009) described the importance of live *T. saginata* metacystodes in bovine tissue regarding public health, as a source of infection and maintenance of this parasite in the human population. In a study by Minozzo *et al.* (2002), the same tendency was found, since of 702 cysts found, 570 (81.20%) were alive and infectious while only 132 (18.80%) were degenerated.

The results found in the present study could lead to the conclusion that the choice of which viscera and which parts of bovine carcasses should be examined for detection of *C. bovis*, and the depreciation in beef value when excessively sliced, are both limiting factors in the diagnosis of bovine cysticercosis (Fukuda, 2003). These results demonstrate that great effort should be made and new guidelines should be instituted in beef inspection to obtain sustainable levels of teniasis/cysticercosis infections, especially regarding areas in which these infections are endemic. Suggested actions could be: establishment of efficient treatment programs using drugs which are effective in eliminating infective metacystodes harboured in bovine tissue; improvement of serological tests for detection of infected animals at the slaughterhouse/abattoir; and development of accessible cost vaccines which are capable of inducing immunological protection bovine hosts.

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