J Vet Diagn Invest 16:438–441 (2004)

Diagnosis of *Taenia saginata* cysticercosis by immunohistochemical test on formalin-fixed and paraffin-embedded bovine lesions

Oladele Ogunremi[1], Garry MacDonald, Stanny Geerts, Jef Brandt

Abstract. A new method of diagnosing cysticercus or larval stage of the human tapeworm, *Taenia saginata,* also known as *Cysticercus bovis,* in formalin-fixed bovine tissue was developed using a monoclonal antibody to *T. saginata* and avidin–biotin complex immunohistochemistry. Grossly recognizable viable and degenerate cysts were identifiable after immunohistochemical staining and could be differentiated from *Sarcocystis, Actinobacillus,* or non-cyst, normal bovine structures. The new test should permit laboratory confirmation of suspected *T. saginata* cysticercus lesions.

Infection of cattle with metacestodes of the human tapeworm, *Taenia saginata,* or *Cysticercus bovis,* also known as bovine cysticercosis or *T. saginata* cysticercosis occurs worldwide; the public health and economic consequences are considerable.[12] In countries like Canada, where *T. saginata* cysticercosis is a reportable disease, regulatory policies may require that important but costly trace-back investigations be done to identify the source of infection to cattle.[2] Humans are at a risk of infection when raw or undercooked beef harboring a viable cyst is consumed. On excision, fluid-filled viable cysts are more easily identified, and the scolex is often readily seen unlike degenerate cysts. Differentiation of a viable *Taenia* cyst from other similar looking lesions can be done by evagination in a 5–10% bile solution[3] and microscopic examination of the unarmed scolex or of hematoxylin-eosin–stained sections because of the characteristic histology of the metacestode, even when a scolex is absent.[13] Compared with viable cysts, degenerating parasites are more commonly found at postmortem[7,10] and are less easily recognizable because of the loss of familiar gross and microscopic architecture.[13] Demonstration of a scolex in a degenerate lesion is diagnostic of tapeworm infections; however, this is not always possible. Calcareous corpuscles that are dissolved during processing of the tissue for histology and are represented by small elliptical blank spaces have been found to be particularly useful for identifying degenerating cysts when the parenchymal tissue had been destroyed,[13] but they are not always observed. Cysticerci could be confused with lesions of actinobacillosis (especially in the tongue) and even adipose tissue.[8] Lesions of macroscopic *Sarcocystis,* which are found in cattle in some parts of the world, can lead to condemnation of beef during meat inspection[5] and may be confused with cysticerci of *T. saginata.* Typically, a suspected lesion is submitted to a diagnostic laboratory for confirmation because of the expertise required for a correct identification. In an attempt to develop a more reliable method of identifying *T. saginata* metacestodes in submitted bovine lesions, the monoclonal IgG1 antibody $158C_{11}A_{10}$, which is directed against a secretory product of *T. saginata* metacestodes,[3] was used to develop an immunohistochemical test using biotinylated rabbit anti-mouse IgG as a conjugate and the avidin–biotin complex peroxidase detection system.[a] Bovine lesions were obtained during postmortem examination of 7 calves inoculated with *T. saginata* in a different study. Identification and recovery of cysts were carried out after

From the Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon Laboratory, Saskatoon, Saskatchewan, Canada (Ogunremi, MacDonald), and the Institute of Tropical Medicine, Veterinary Department, B-2000 Antwerpen, Belgium (Geerts, Brandt).

[1]Correspondence Author: Oladele Ogunremi, Centre for Animal Parasitology, Canadian Food Inspection Agency, Saskatoon Laboratory, 116 Veterinary Road, Saskatoon, Saskatchewan S7N 2R3, Canada.

the dissection of skeletal muscle, heart, esophagus, and liver of infected cattle by technical personnel trained to recognize lesions caused by metacestodes. Inspection of each recovered cyst and designation as viable or degenerate were subsequently done by a veterinarian with experience in identifying metacestodes. Each cyst was visually examined, palpated, and classified as viable if soft to touch and had fluid, with or without a visible scolex, or as degenerate if hard to touch or had creamy, greenish, or yellowish discoloration. Individual cysts or groups of cysts were placed in tissue cassettes,[b] labeled, and immersed in 10% buffered formalin for 24–48 hours. The cassettes were then immersed in 70% ethanol, processed, and embedded in paraffin according to standard procedures.[1] Samples of normal adipose tissue, lymph nodes, hemal lymph nodes, and skeletal or cardiac muscles obtained from experimental animals during postmortem examination and during routine necropsy of cattle not infected with *T. saginata* were also processed and embedded in paraffin as described above. Paraffin-embedded lesions of actinobacillosis and microscopic sarcocystis previously diagnosed after examination of hematoxylin-eosin–stained sections of routine postmortem submissions were obtained[c] and included as controls in the immunohistochemical test. The avidin–biotin immunohistochemical technique and its application to the diagnosis of a viral pathogen have been described.[9] In this study, tissue sections (5 mm) were placed on poly-L-lysine–coated slides[d] and deparaffinized and rehydrated by sequential immersion in xylene, ethanol (absolute, 95%, and 70%), and tap water. Endogenous peroxidases were inactivated by immersing the slides in 1.2% H_2O_2 in methanol for 12 minutes at room temperature. Tissue sections were digested with 0.05% protease solution[e] prepared in phosphate-buffered saline (PBS, pH 7.4)[f] warmed to 37 C before incubation with the sections at the same temperature for 30 minutes. Sections were washed 3 times with a commercial automation buffer[g] and blocked by sequential incubations in a humidified chamber with 4% horse serum[f] and 1% nonfat skim milk[h] made in PBS for 10 minutes each. Monoclonal anti–*T. saginata* $158C_{11}A_{10}$ (1.0 mg/ml) was diluted 1:1,000 in PBS containing 4% horse serum (i.e., assay diluent) and incubated at 37 C for 2 hours. For antibody controls, monoclonal anti-*Trichinella* TSP 130[11] (IgG1 isotype, 1.2 mg/ml) or normal mouse serum was diluted at 1:1,200 or 1:1,000, respectively, and used on separate slides containing sections of the same lesions. Slides were washed 3 times in PBS, and biotinylated rabbit anti-mouse IgG diluted 1:800 in assay diluent was applied and allowed to react with the tissue section for 30 minutes at 37 C and was thereafter washed in PBS 3 times. Interactions between the antibody and epitopes on the tissue section were magnified by the use of avidin-biotinylated horseradish peroxidase complex,[a] after dilution according to the manufacturer's instructions, and incubation with the tissue section at 37 C for 45 minutes. Slides were then washed 3 times in automation buffer and exposed to H_2O_2-activated 3,3′-diaminobenzidine tetrahydrochloride.[d] Color development was allowed to proceed for 4 minutes at room temperature, and the substrate was washed off with PBS. Tissue sections were counterstained with hematoxylin, followed by dehydration with alcohol and subsequent removal of the alcohol by xylene. Sections were covered with coverslips before examination under the microscope at 40× for brown-colored deposits indicative of specific antigen–monoclonal antibody reaction and for histological features of a metacestode, e.g., distribution of color reaction, the presence of scolex, cestode cuticle, parenchyma, spiral canal, and bladder cavity, or other features attributable to bovine cysticercosis.[13] Eighty-seven cysts that were soft to touch and appeared fluid filled or had a visible scolex were scored as viable. Another 115 cysts were hard to the touch and, when incised, were observed to contain caseous material and thus were classified as degenerate cysts. The caseous material was creamy, greenish, or yellowish in color, and the discoloration was apparent before incision. Monoclonal anti–*T. saginata* reacted with all 87 viable and 115 degenerate cysts tested (Table 1) as shown by the development of a brown reaction in the vicinity of the lesion after addition of the color substrate and microscopic examination (Fig. 1a, 1b). Both the cuticle and the parenchyma surrounding the bladder cavity in a viable cyst as well as the epithelial lining of the scolex's sucker and the spiral canal reacted with the antibody. In addition, a layer of densely packed reticular-type tissue that interposes between the cuticle and the host cellular reaction was also strongly stained. This parasite–host interface is infiltrated by inflammatory cells during the early degenerative changes of the parasite. Also at this time, strongly stained cells originating from the multinucleated parenchyma begin to accumulate in the bladder cavity of the parasite. In con-

Table 1. Immunohistochemical staining of *Taenia saginata* metacestodes (viable, degenerate) and other noncyst tissues (normal bovine tissues, *Actinobacillus* and *Sarcocystis* lesions).

Gross and histological identification of lesions and normal tissues	Immunohistochemical diagnosis of *T.saginata* cysticercus	
	+	−
Viable *T. saginata* cysticercus	87	0
Degenerate *T. saginata* cysticercus	115	0
Non-cysticercus	0	46
Total	202	46

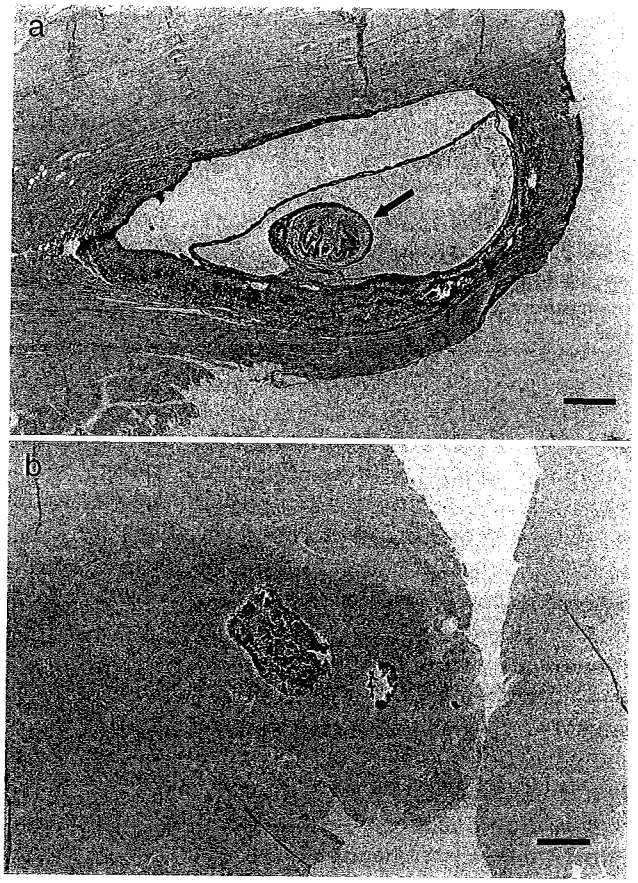

Figure 1. Immunohistochemical staining of a viable (**a**) and a degenerate (**b**) *Taenia saginata* metacestode using monoclonal antibody $158C_{11}A_{10}$ and avidin–biotin complex stain with diaminobenzidine chromagen and hematoxylin counterstain. Arrow indicates the scolex of the viable metacestode. Bar = 500 μm.

trast, the parasite architecture in the degenerate cysts was not usually discernible; nevertheless, significant staining was consistently observed in the middle of the lesion. Nearly all viable cysts (97%) and most of the degenerate cysts (72%) could be detected by examining the stained slide with the unaided eye. Among the viable cysts, calcareous corpuscles were seen in or around the scolex of 21 of 25 parasites but not in 2 degenerate parasites that each had a scolex. They were found in 7 of 113 degenerate cysts in which no scolex was discernible. The monoclonal anti–*T. saginata* did not immunohistochemically stain hemal lymph nodes (n = 8), adipose tissue (n = 12), skeletal muscle (n = 6), cardiac muscle (n = 5), or lymph node (n = 5) obtained from either the experimental or the normal animals, and it did not react with bovine *Sarcocystis* sp. (n = 5) or actinobacillosis lesion (n = 5). Anti-*Trichinella* monoclonal antibody or normal mouse serum failed to react with all the 13 viable or 4 degenerate cysts tested. The monoclonal anti–*T. saginata* antibody has been shown to cross-react occasionally with *Taenia ovis* and *Echinococcus granulosus*.[3,17] *Taenia ovis* is not known to infect humans, and the cysticercus is not found in cattle. *Echinococcus granulosus* can infect cattle but mostly produce infertile cysts that are usually restricted to the lungs and liver and may not be infectious to humans.[15] For practical purposes, the monoclonal antibody $158C_{11}A_{10}$ should afford a specific diagnosis of bovine cysticercosis in skeletal and cardiac muscles and aid in the control of human taeniasis. Indeed, when tested against other lesions (e.g., actinobacillosis and sarcocystosis) and normal organs (e.g., adipose tissue and hemal lymph node) that may be confused grossly or histologically with *T. saginata,* no cross-reactivity was observed. Antigenic relatedness between microscopic and macroscopic *Sarcocystis*[6] suggests that the monoclonal antibody may also fail to react with the latter. Further validation of the test using a larger number of samples including cases of naturally infected animals should allow for a comprehensive description of the diagnostic sensitivity and specificity of the test. The monoclonal antibody bound to molecules present in the cuticle, including the lining of the spiral canal, and to a lesser extent in the parenchyma or subcuticle of the metacestode.[13,14] In addition, the antibody bound to molecules present in a layer of densely packed reticular-type tissue, which interposed between the cuticle and the host cellular reaction. This parasite–host interface has not been described in the literature before, and further studies including the characterization of its cellular composition may provide insight into why the parasite undergoes degeneration faster in some tissues, e.g., cardiac muscle, and into the ability of the parasite to withstand the host's immunological onslaught for a considerable period of time in others, e.g., skeletal muscle. The increasing use of this monoclonal antibody, in serological diagnosis[4,17] and now in immunohistochemical detection, requires that information about the recognized ligand be pursued, and the knowledge could further help in the elucidation of the parasite's biology including its biochemistry and anatomy. Parasite antigen identified by the monoclonal antibody is widely distributed in the metacestode. However, a cyst showing an advanced stage of degeneration is typically characterized by a restricted and smaller area of antigen distribution, usually concentrated at the center of the lesion, although the overall lesion becomes bigger presumably because of increasing inflammatory infiltrate. Thus, it is advisable to test multiple sections prepared from each submitted lesion to increase the chances of sectioning through the tissue at a plane where the antigen can be detected. A prolonged, longitudinal study of experimentally infected animals should address how long the parasite antigen persists in a *T. saginata* cysticercus lesion. Failure of a polymerase chain reaction

test to detect parasite DNA in 3 of 34 cysts identified as *T. saginata*[16] may suggest that parasite molecules do not persist indefinitely in a degenerate cyst. In Canada, the regulatory direction is away from provision of compensation to owners of herds infected with *T. saginata.* This tendency is consistent with the practice in many countries, and underscores the need for a more reliable diagnostic test. The developed immunohistochemical test should provide an inexpensive means of reliably identifying viable and degenerate *T. saginata* metacestodes in submitted tissue sections and differentiating them from normal tissues and other pathogenic lesions. The test is expected to find wide use in improving the current meat inspection procedures. The highest demand for a standardized and validated test is expected to be for the diagnosis of recovered, degenerate *T. saginata* lesions that would otherwise not be amenable to identification by gross examination or microscopic examination of hematoxylin-eosin–stained sections.

Acknowledgements. Financial support for this study was provided by the Canadian Food Inspection Agency (CFIA). The authors thank Drs. Alvin Gajadhar and Brad Scandrett of the Centre for Animal Parasitology for experimental infection of cattle with *T. saginata* and Dr. John Allen of the Centre for Animal Parasitology, Drs. Debbie Haines and Baljit Singh of the University of Saskatchewan, and Dr. Keith West, Brian Chelack, and Phil Dillman of the Prairie Diagnostic Services Inc. (PDS) Saskatoon for helpful discussions and input. Dr. Gary Wobeser of the University of Saskatchewan and Ed Bueckert of PDS provided tissues and sections of normal bovine tissues, as well as *Sarcocystis* and *Actinobacillus* lesions. Postmortem dissection of cysts from infected cattle and other essential support were provided by the technical staff of Centre for Animal Parasitology, CFIA Saskatoon Laboratory.

Sources and manufacturers

a. Vector Laboratories, Burlingame, CA.
b. Histosette II, Simport Plastics, Beloeil, Quebec, Canada.
c. Electron Microscopy Sciences, Fort Washington, PA.
d. Prairie Diagnostic Services Inc., Saskatoon, Saskatchewan, Canada.
e. Sigma Chemical Co., St. Louis, MO.
f. GIBCO, Burlington, Ontario, Canada.
g. ESBE Labs, Biomeda Corp, Calgary, Alberta, Canada.
h. BioRad, Hercules, CA.

References

1. Anderson G, Gordon KC: 1996, Tissue processing, microtomy and paraffin sections. *In:* Theory and practice of histological techniques, ed. Bancroft JD, Stevens A, 4th ed., pp. 47–67. Churchill Livingstone, New York, NY.
2. Borman-Eby HAC, Ayim DS, Small C: 1994, *Cysticercus bovis* in cattle in two beef feedlots in southern Ontario. Can Vet J 35: 711–713.
3. Brandt JRA, Geerts S, De Deken R, et al.: 1992, Monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in *Taenia saginata* cysticercosis. Int J Parasitol 22:471–477.
4. Dorny P, Vercammen F, Brandt J, et al.: 2000, Sero-epidemiological study of *Taenia saginata* cysticercosis in Belgian cattle. Vet Parasitol 88:43–49.
5. Dubey JP, Udtujan RM, Cannon L, Lindsay DS: 1990, Condemnation of beef because of *Sarcocystis hirsuta* infection. J Am Vet Med Assoc 196:1095–1096.
6. Gasbarre LC, Suter P, Fayer R: 1984, Humoral and cellular immune responses in cattle and sheep inoculated with *Sarcocystis.* Am J Vet Res 45:1592–1596.
7. Geerts S, Kumar V, Van den Abbeele O: 1980, *Taenia saginata* cysticercosis in slaughter cattle in Belgium. Vlaams Diergeneeskd Tijdschr 49:365–374.
8. Gracey JF, Collins DS: 1992, Meat hygiene, 9th ed. Ballière Tindall, London, UK.
9. Haines DM, Chelack BJ: 1991, Technical considerations for developing enzyme immunohistochemical staining procedures on formalin-fixed paraffin-embedded tissues for diagnostic pathology. J Vet Diagn Invest 3:101–112.
10. McLauchlan I: 1981, Bovine cysticercosis survey. Vet Rec 109: 291.
11. Ortega-Pierres MG, Yepez-Mulia L, Homan W, et al.: 1996, Workshop on a detailed characterization of *Trichinella spiralis* antigens: a platform for future studies on antigens and antibodies to this parasite. Parasite Immunol 18:273–284.
12. Pawlowski ZS, Murrell KD: 2001, Taeniasis and cysticercosis. *In:* Foodborne disease handbook, ed. Hui YH, Sattar SA, Murrell KD, et al., 2nd ed., pp. 217–227. Marcel Dekker, New York, NY.
13. Silverman PH, Hulland TJ: 1961, Histological observation on bovine cysticercosis. Res Vet Sci 2:248–252.
14. Šlavis J: 1970, The morphology and pathogenicity of the bladder worms, *Cysticercus cellulosae* and *Cysticercus bovis.* Czechoslovak Academy of Sciences, Prague, Czech Republic.
15. Thompson RCA, Allsopp CE: 1992, Hydatidosis: veterinary perspectives and annotated bibliography. CAB International, Oxon, UK.
16. Van der Logt PB, Gottstein B: 2000, Unidentified parasitic cysts in cattle. Vet Rec 146:610–612.
17. Van Kerckhoven I, Vansteenkiste W, Claes M, et al.: 1998, Improved detection of circulating antigen in cattle infected with *Taenia saginata* metacestodes. Vet Parasitol 76:269–274.