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

Oladele Ogunremi¹, Garry MacDonald, Stanny Geerts, Jef Brandt

Abstract. A new method of diagnosing cysticercous 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 solution³ 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 postmortem⁷,¹⁰ and are less easily recog-
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, 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. 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 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. In this study, tissue sections (5 mm) were placed on poly-l-lysine-coated slides 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% H2O2 in methanol for 12 minutes at room temperature. Tissue sections were digested with 0.05% protease solution prepared in phosphate-buffered saline (PBS, pH 7.4) 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 and blocked by sequential incubations in a humidified chamber with 4% horse serum and 1% nonfat skim milk made in PBS for 10 minutes each. Monoclonal anti-*T. saginata* 158C10 A10 (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 130I (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, 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 H2O2-activated 3,3'-diaminobenzidine tetrahydrochloride. 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. 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>
<thead>
<tr>
<th>Gross and histological identification of lesions and normal tissues</th>
<th>Immunohistochemical diagnosis of <em>T. saginata</em> cysticercus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable <em>T. saginata</em> cysticercus</td>
<td>87</td>
</tr>
<tr>
<td>Degenerate <em>T. saginata</em> cysticercus</td>
<td>115</td>
</tr>
<tr>
<td>Non-cysticercus</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
</tr>
</tbody>
</table>
Taenia ovis and Echinococcus granulosus. Taenia ovis is not known to infect humans, and the cisticercus 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. For practical purposes, the monoclonal antibody 158C11A9 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 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. In addition, the antibody bound to molecules present in a layer of densely packed reticular-type tissue, which interfaced 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 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 cisticercus lesion. Failure of a polymerase chain reaction
test to detect parasite DNA in 3 of 34 cysts identified as *T. saginata* 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. Histouse II, Simport Plastics, Beloosl, 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, Biomedra Corp, Calgary, Alberta, Canada.
h. BioRad, Hercules, CA.

**References**