# Diagnostic criteria of chronic lesions caused by *Taenia saginata* metacestodes in bovine myocardium

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## SUMMARY

**Introduction** - Humans are the only definitive host of the cestode *Taenia saginata* (beef tapeworm). The intermediate host of the larval form of *T. saginata* is mainly cattle. In cattle the eggs develop into the larval stage, *Cysticercus bovis*. Non-viable cysticerci undergoing degeneration can vary in appearance depending on the degree of inflammation, necrosis, and mineralization in the resulting lesion bovine cysticercosis can inflict serious economic losses to the cattle industry. These are mainly due to condemnation, refrigeration and downgrading of infected carcasses. histological evaluation of *T. saginata* cysticerci suspect lesions is still considered the only laboratory diagnostic tool suitable for routine confirmation of undiagnosed lesions during inspection procedures at slaughterhouse. There have been many studies to characterize the histopathology of cysticercosis, however none of them gave a practical and unbiased set of criteria in order to come to a sound based diagnosis.

**Materials and methods** - 117 specimens of bovine ventricular myocardium positive for chronic lesions, presumptive of *T. saginata* metacestodes at visual inspection at the abattoir, have been analyzed by histological examination and molecular methods.

**Results and discussion** - All the samples resulted positive for the presence of *T.saginata* Dna. Lymphocytes, granulocytes and necrosis were the elements observed with higher frequency. In our study, histological observations of 9 out of 117 chronic lesions found in bovine myocardium did not show typical cellularity of a parasitic granuloma. Statistical analysis demonstrate that the presence of lymphocytes and granulocytes is the most significant evidence of a chronic lesion left by the degeneration of the larval stage of the parasite, while a fibrotic capsule, though often present, shows a lower significance.

**Conclusions** - The aim of this study was to demonstrate the practical use of a set of histological criteria for the identification of chronic lesions caused by *T. saginata* metacestodes with the support of highly specific molecular methods.

## KEY WORDS

*Taenia saginata* metacestodes, Bovine cysticercosis, Histopathology.

## INTRODUCTION

Humans are the only definitive host of the cestode *Taenia saginata* (beef tapeworm). The intermediate host of the larval form (metacestode) of *T. saginata* is mainly cattle. In cattle the eggs develop into the larval stage, *Cysticercus bovis*. Bovine cysticercosis does not usually manifest itself clinically. In cattle the cysticerci degeneration takes place in several weeks and after one year many of them are dead or calcified[1]. The life cycle is completed with humans ingesting raw or inadequately cooked beef that contains viable cysticerci.

*T. saginata* has a cosmopolitan distribution and is found widely in most countries that raise cattle[2]. bovine cysticercosis, although, according to meat inspecton data, in Europe the prevalence ranges from 0.007% to 6.8%[3,4], the real prevalence is considered to be at least ten times higher[5,6].

In contrast to *T. solium*, the clinical importance of *T. saginata* is very limited. The presence of the tapeworm in the intestine can cause some abdominal discomfort, mild diarrhoea and weight loss. In cattle, cysticercosis is usually subclinical. However, bovine cysticercosis can inflict serious economic losses to the cattle industry[7]. These are mainly due to condemnation, refrigeration and downgrading of infected carcasses. According to the Regulation (EC) no. 854/2004 all bovines of over 6 weeks of age have to be inspected for cysticercosis by incision and visual examination in masseter muscles and heart. If an animal has a generalised infestation, the carcass and offal are declared unfit for human consumption. If the infestation is localised, however, the parts not infected may be declared fit for human consumption after having undergone a cold treatment.

Although it appears that there are no true predilection sites for this parasite, those sites traditionally examined are amongst the best for detecting infection. Of these, the heart is preferred for detection based on the relatively high numbers and frequencies with which cysticerci infect this organ, and the increased visibility of lesions due to the earlier degeneration of cysticerci in cardiac muscle and concomitant in-

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flammatory response[8]. Non-viable cysticerci undergoing degeneration can vary in appearance depending on the degree of inflammation, necrosis, and mineralization in the resulting lesion[9]. Cattle can harbor both viable (infective) and degenerate cysts concurrently[5]; thus recovery of only degenerate cysts does not imply absence of infective cysts in the carcass, or in herdmates. As well, viable cysts can persist in cattle for at least two to three years, and possibly for the life of the host[10]. Therefore, it is important to confirm cysticercosis even in cases where suspect lesions are obviously degenerated and non-infective.

Definitive diagnosis of degenerated lesions is often considered difficult using gross, stereoscopic or histological examination methods, on the grounds that the lesions no longer contain identifiable parasite features, and are deemed to be consistent histologically with chronic lesions of other etiologies[9].

True indeed is the fact that in bovine cysticercosis, because of the absence of rostellar hooks in *T. saginata* cysticerci, and standing the possibility that cysticerci in the heart and other tissues may not have developed fully prior to the onset of degeneration, the histological pattern available for the diagnosis is singularly vague[11].

The onset of degeneration in mature cysts depends on the inflammatory response of the host but generally consists of the breakdown of the cyst wall and cuticle, followed by the disappearance of following layers and eventually in the dissolution of parasite remnants[11].

There have been many studies to characterize the histopathology of cysticercosis, some of which describe grading criteria based on characteristic histological features for bovine or porcine cysticerci at various stages of degeneration[11,12]. However none of them gave a practical and unbiased set of criteria in order to come to a sound based diagnosis. In the recent past Polymerase chain reaction (PCR)-based methods have been developed for the molecular identification of a number of cestodes and larval stages thereof, among which *T. saginata*[1,9,13,14]. The aim of this study was to demonstrate the practical use of a set of histological criteria for the identification of chronic lesions caused by *T. saginata* metacestodes with the support of molecular methods. A number of histological elements, characteristic of chronic granulomatous lesions, have been taken in account and their presence has been statistically associated with positivity to a specific *T. saginata* PCR.

## MATERIALS AND METHODS

117 specimens of bovine ventricular myocardium positive for chronic lesions, presumptive of *T. saginata* metacestodes at visual inspection at the abattoir, were sampled for light microscopic examination. Samples have been fixed in 10% buffered formalin for 24-48 hours, and processed for routine paraffin embedding. Sections of 3-µm thickness were stained with hematoxylin and eosin (HE).

For each lesion, up to 25 mg of material was collected from a single excised cyst; effort was made to collect only the content of the cyst, avoiding the cyst wall to reduce the amount of non target bovine DNA.

DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used for DNA extraction from all samples. Some minor modification in the protocols consisted of an overnight incubation with Proteinase K and a decrease in the volume of the final elution step (100 mL).

Extracted DNA was quantified with a NanoDrop 2000 spectrophotometer (ThermoScientific, Wilmington, NC).

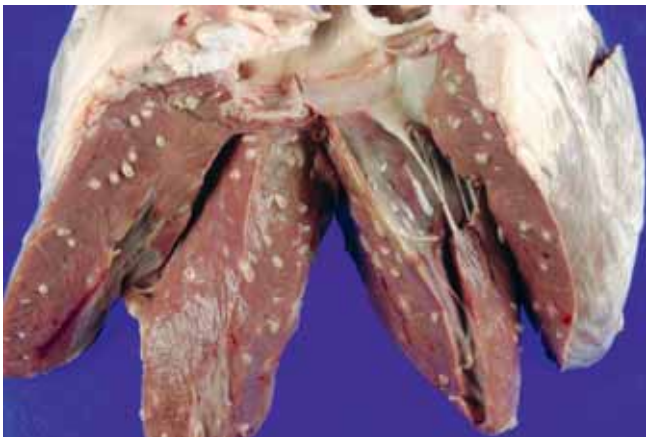

**Figure 1** - Heart with multifocal whitish nodules at the level of the left ventricular wall and the interventricular septum.

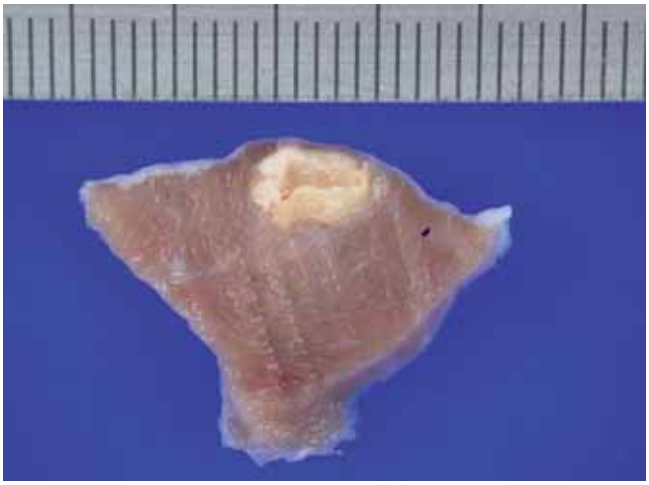

**Figure 2** - Particular of the heart wall with yellowish-white nodule partially friable.

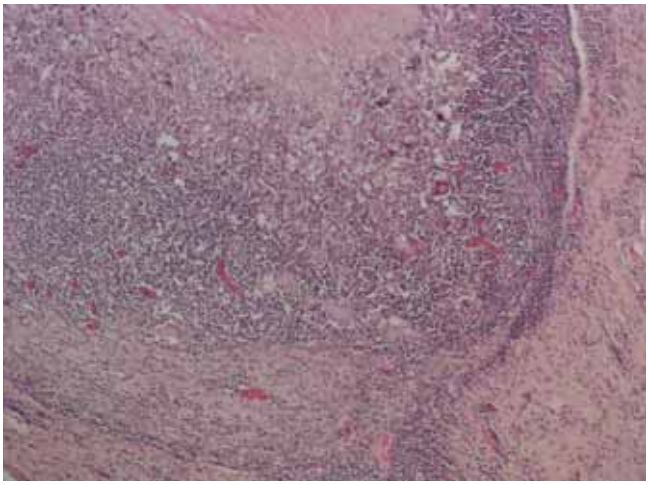

**Figure 3** - Histology of a parasitic granuloma in which it is possible to detect a connective capsule and a mixed inflammatory infiltrate with giant cells and a central necrotic area. Hematoxylin and eosin stain, 10 X.

PCR reactions were performed according to the protocol published by Chiesa et al (2010). Amplimers were resolved by electrophoresis on a 2% agarose gel (Invitrogen), run in Tris-acetate-ethylenediaminetetraacetic acid buffer for 70 min at 110 V, and stained with ethidium bromide (0.4 ng=mL) for 20 min.
All statistical analyses were conducted using SAS software. Results were expressed as unadjusted odds ratios (OR) and corresponding 95% confidence intervals and P values.

## RESULTS

All the samples resulted positive for the presence of *T.saginata* Dna. Out of 117 samples, 9 did not demonstrate the presence of histological elements normally occurring in parasitic granuloma; PCR, however, was found to be positive for 5 of them.
Lymphocytes, granulocytes and necrosis were the elements observed with higher frequency, as shown in Table 1.
In Table 2 are shown highly significant positive associations for each histological element and associations thereof expressed as OR.
The higher probability of the lesion being positive for *T. saginata* DNA presence has been in case of presence of granulocytes: the probability that a chronic lesion found in the bovine myocardium containing granulocytes would be positive at the PCR is 16 folds higher than the probability that such a lesion would yield a negative result at the molecular test.

**Tabella 1**

| Histological elements | Presence | Absence |
|---|---|---|
| Lynphocytes | 110 | 7 |
| Granulocytes | 108 | 9 |
| Necrosis | 97 | 20 |
| Macrophages | 92 | 25 |
| Fibrous capsule | 81 | 36 |
| Fibroblasts | 33 | 84 |

**Table 2** - Odds Ratios for association between the presence of histological elements and PCR positivity.

| Histological elements observed | OR | p value |
|---|---|---|
| Lymphocites + granulocytes | 16 | 0,002* |
| Granulocytes | 16 | 0,002* |
| Lymphocites | 13 | 0,01* |
| Fib. capsule + granulocytes | 9 | 0,005* |
| Fib. capsule + necrosis | 8 | 0,005* |
| Fib.capsule+macrophages + lymphocites + granulocytes | 7 | 0,01* |
| Fib. capsule + macrophages + lymphocites | 7 | 0,01* |
| Fib. capsule + macrophages | 7 | 0,01* |
| Fib. capsule | 5 | 0,02 |
| *Highly significant. | | |

## DISCUSSION

Several different diagnostic tests have been described to support visual diagnosis of *T. saginata* cysticercosis. Serological methods, such as detecting circulating antigens or antibodies, both in live and slaughtered animals have been studied[15,16]. However, this technique failed to detect many light infections[5], and the possibility of cross reaction with other parasites indicates the need for further investigation[17]. Also, an immunohistochemical method has been proposed as a promising alternative by Ogunremi et al. (2004), but results show that cross reaction with other taeniid species may occur. Therefore, histological evaluation of *T. saginata* cysticerci suspect lesions is still considered the only laboratory diagnostic tool suitable for routine confirmation of undiagnosed lesions during inspection procedures at slaughterhouse. The morphology and cellularity of these putative lesions can be used to distinguish them from other commonly encountered and somewhat similar lesions. Demonstration of a cysticercus- specific host structure is therefore necessary for a definitive diagnosis[11], but an accurate identification cannot always be made, as such features are often not obvious in degenerated specimens and especially if the sample contains calcified tissues[18].
No standard criteria have been established for the histological evaluation of C. bovis-suspect lesions.
In our study, histological observations of 9 out of 117 chronic lesions found in bovine myocardium did not show typical cellularity or only fibroblasts or capsule was present. 5 of those samples yield positive result to the PCR. This fact is explained by the localization of the histological examination within the lesion: in order to test the same lesion with both the histological and the molecular method, the sample was split before being formalin fixed. It may occur that the part of the lesion undergoing to histological examination would not be representative of the whole cellular population of the sample and that only the fibrous capsule was present.
The presence of the capsule alone, though significantly associated with PCR positivity, is, somewhat surprising, less indicative of *T. saginata* metacestodes: presence of lymphocytes and granulocytes is a much more strong evidence of a chronic lesion left by the degeneration of the larval stage of the parasite. Chronic lesions are the remnants of degenerated cysticerci undergone to host inflammatory response for months and possibly many years; earlier degeneration of cysticerci in cardiac muscle and concomitant inflammatory response lead, with every probability, to an histological features pattern dominated by those two inflammatory cells, while macrophages, present in the earlier stage of the inflammatory response, tend to disappear with time.
In conclusion we demonstrated that simple hematoxylin and eosin stain can be reliably used for the diagnosis of chronic lesions in bovine muscle suspected for *T. saginata* metacestodes, even in case of lesions no longer containing identifiable parasite features, if histological patterns of parasitic granuloma is observed. Even though the quality of the slides produced by frozen section is of lower quality than formalin fixed paraffin embedded tissue processing, the rapidity of the cryosection method would be of greater importance for the diagnosis of lesions in case of doubtful macroscopic interpretation at slaughterhouse.

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