CONTRIBUTION TO THE KNOWLEDGE OF THE RELIABILITY OF SOME SEROLOGICAL TESTS IN DETECTING SPONTANEOUS BOVINE CYSTICERCOSIS


Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, *District Veterinary Centre, Nymburk, ** Institute of Postgraduate Studies of Surgeons and Pharmacists, Prague, *** Faculty of Veterinary Medicine, Humboldt University, Berlin, and **** Regional Hygienic Inspection, Berlin

Abstract. A total of 110 bulls coming from a farm where cysticercosis repeatedly occurred were examined by means of indirect haemagglutination (IHA), micropreципitation in agar (MPA), and indirect immunofluorescence reaction (IFR). Cysticercosis (isolated cysts with live or dead cysticerci) was detected in 29 of them at post-mortem examination. Among the 29 infected animals, positive reactions were obtained in 37.9% with IHA, 31.7% with MPA and 40.3% with IFR. The remaining animals were negative. Of 82 animals giving negative reactions with IHA, 28% harboured cysticerci at post-mortem examination. Of 70 animals negative with MPA, 20% were infected with cysticerci and of 71 animals negative with IFR, 29.5% were positive at the slaughter. Consequently, none of the used serological methods is reliable for the detection of a very light spontaneous infection.

The occurrence of taeniasis in man is still increasing in the last years, even in the countries with a high-level communal hygiene. Besides other reasons, this is due to the fact that there is impossible to detect all cases of bovine cysticercosis during veterinary inspection of slaughtered animals. For this reason attempts at intravelal detection have been made by means of serological tests. If some of the tested methods proves to be reliable for the detection of cysticercosis in a live animal, then the possibility of man infection would be lowered and also the losses caused by unprofitable further breeding of the infected animals would be diminished. It was found that in experimental infection, the cysticercosis can be detected by means of haemagglutination, precipitation (Dewhurst et al. 1960, Walthier and Grossklaus 1972, Blažek et al. 1980), immuno-fluorescence (Hiepe and Buchwalder 1978, Flentje et al. 1978) and ELISA test (Albert and Hörechner 1978, Walthier and Sanitz 1979, Craig and Rickard 1980). However, the problem of applicability of serological methods in spontaneous and particularly very-light infection still remains unsolved. We have therefore decided to examine systematically young cattle from a fattening station with notorious occurrence of cysticercosis by means of IHA, MPA and IFR and evaluate the possible application of these methods for the diagnosis by comparing the results of serological examinations with post-mortem examination of the animals.

MATERIAL AND METHODS

a) Animals. The experimental animals came from a fattening station where cysticercosis had been detected for a long time. The blood samples were taken from groups of bulls (20—25 animals) one week before slaughter. The bulls were marked on ears for their later identification. The results of IHA
RESULTS

Cysticercosis was detected at post-mortem examination at the abattoir in 29 (26.3%) of 110 bulls which had been previously examined by serological methods (IHA, MPA, IFR). There occurred isolated cysticerci, sometimes only 1–2 cysts or solid, calcified foci. The cysticerci were found in the following organs: in heart (16 x), simultaneously in heart and masseter (5 x), in masseter (3 x), in lung (1 x), simultaneously in lung and masseter (1 x) and simultaneously in tongue and heart (1 x). Transparent cysts with live cysticerci were found in 15 cases. The cysts were always at the "resting" stage, with morphologically fully differentiated cysticerci. In the remaining cases the cysticerci were dead and mostly calcified. Parts of larva were detected even in completely calcified nodules after decalcification. Around the dead cysticerci occurred eosinophilic, large macrophages, giant multinuclear cells, fibroplasia and mostly a large number of lymphoid cells. Eosinophilic myositis (massater) was found in one case and eosinophilic myocarditis in another one.

Among the 29 animals in which cysticercosis was detected at post-mortem examination, only 11 (37.9%) had a positive antibody titre in indirect haemagglutination reaction (IHA). In 18 animals the antibody titres were below the positivity level (titre lower than 1 : 16) or the reaction was quite negative. Of the 82 serologically negative (IHA) animals, 18 (21.6%) harboured cysticerci at the inspection at the abattoir (Fig. 1). Out of 28 serologically positive bulls, 11 (39.2%) were positive at pathological-anatomical examination.

![Fig. 1. Ratio of number of bulls with cysticercosis (negative at serological examination) and total number of bulls negative at serological examination.](image)
The microprecipitation reaction (MPA) was positive in 15 (51.7%) of the 29 bulls. Among 70 animals which were negative at precipitation, 14 (20.0%) harbourcd cysticerci (Fig. 1). Among 40 animals positive at precipitation, 15 (37.5%) were positive also at post-mortem examination.

The results of indirect immunofluorescence reaction (IFR) were as follows: a marked positive reaction was found only in one of the 29 bulls suffering from cysticercosis and a weak positive reaction occurred in two of them (i.e., positive reaction was found in 3 (10.3%) animals). A dubious reaction was found in 3 (10.3%) animals. The remaining 23 (79.3%) animals gave negative reaction. At post-mortem examination, cysticerci were detected in 21 (29.5%) of 71 animals with negative IFR (Fig. 1) and in 3 (18.7%) of 16 animals weakly positive in IFR. Among 8 strongly positive animals (IFR) only one (12.5%) was positive also at post-mortem examination. A cysticercus was found also in 3 (20.0%) of 15 animals with dubious reaction.

In no case the results of the reactions examined were concordant (Table 1). The character of pathological changes in some of the examined animals is documented in Plates I–IV.

Discussion

In agreement with other authors, in our previous papers we provided evidence that experimental cysticercosis can be detected by means of indirect haemagglutination reaction, precipitation and immunofluorescence. We proved the suitability of these methods as well as of the antigens used. However, in all these cases a heavy infection was involved, whereas in the present work, the infection was very light, with single cysticerci, some of which were dead and calcified.

Of importance is the fact that among the animals harbouring cysticerci at post-mortem examination, only 37.9% were positive with IHA, 51.7% with MPA and 10.3% with IFR. Moreover, some of the animals negative at serological examination (22% with IHA, 20% with MPA and 29.5% with IFR) were found to be positive at post-mortem examination. The results revealed that none of the methods, which were successfully used in experimental infection (Blažek et al. 1960a) and partly also for the diagnosis of spontaneous infections of moderate intensity (unpublished), is reliable in case of a very light spontaneous infection. Similar results were achieved by Craig and Rickard (1980) and some other authors studying the applicability of ELISA test, which is generally considered to be very sensitive, for the diagnosis of cysticercosis. For example, Albert and Höhrener (1978) consider ELISA a sensitive test for studying the course of C. bovis infection, but in their opinion, this test is unsuitable for the detection of spontaneous cysticercosis, because the basic antibody titres of non-infected animals can be at the same level as those of animals in which the cysticercosis was proved. According to Walther and Sanitz (1979), a wide extinction spectrum should be awaited if ELISA test is used for the diagnosis of spontaneous cysticercosis and they conclude that for this reason the detection of light spontaneous infection would be very difficult by means of single examination. Fleitjen et al. (1979) and in a number of animals only single and calcified cysticerci, but the immunofluorescence reaction was positive (1:40–1:80). With regard to the light infection and the state of cysticerci, this finding is very interesting. However, Gathuma et al. (1978) regard the indirect immunofluorescence reaction as unreliable for the diagnosis of spontaneous infection with a low number of cysts.

The presented results showing the unreliability of the used methods are not due to a methodological failure or application of an unsuitable antigen. The reason lies in the peculiarities of the pathological process and the immune response in helminth infections in general. Some authors did not find any relation between the level of antibody titre and number of cysticerci (Albert and Höhrener 1978), whereas others assume that the level of the titre of humoral antibodies is directly proportional to the intensity of infection (Gallie and Sewell 1974). This follows also from the results obtained while detecting spontaneous cysticercosis by means of ELISA test (Walther and Sanitz 1979). We have ascertained that the level of humoral antibody titre need not be very high during cysticercosis, even in case of massive infection (Blažek et al. 1980a). According to our results, an important role in the immune response during bovine cysticercosis is played by the visible cellular reaction to the presence of young larva rapidly developing during the first weeks of infection. At that time, the formation of humoral antibodies and the phenomenon of hypersensitivity take place (Blažek et al. 1980a). If the animal is infected by germs which do not multiply (as in case of infection with cestode onchospheres), it may be supposed that the production of antibodies will really be proportional to the number of these parasites. In case that a quite small number of onchospheres penetrate into the organism, the cellular reaction is limited to solitary foci. Although even in this case the cellular immunity develops well due to the formation of clones of memory cells (own observations, Lloyed 1980), the humoral antibodies are not produced in such amount which could be detected by the above-men tioned methods.

Another reason for the failure of the studied serological methods may be the limited duration of antibody titres at a sufficient level in case of a light infection, since this level can be very low as early as in the beginning. In a heavy experimental infection, positive titres were found even over 250 days p.i. (when the titre of MPA was 40–1:320 and higher), whereas MPA was positive no longer than 70–85 days p.i. (Blažek et al. 1980a). However, Dewhirst et al. (1969) obtained positive precipitation reaction 24 weeks after infection. Soulé et al. (1972) found negative IFR after 28 weeks and Flen tje et al. (1978) after 18–23 weeks. The fattening of bulls, however, lasts much longer than the positivity of antibody titres, 18 months on the average.

The results of these studies and of the previous ones on the pathogenesis of bovine cysticercosis and antibody response show that the serological methods are unsuitable for the detection of a light or very light spontaneous infection, which is most important from the epidemiological viewpoint, and that other tests should be searched for this purpose.
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K. B., Parasitologický ústav ČSAV,
Flemingovo n. 2, 166 32 Praha 6,
ČSSR
Fig. 1. Node situated subperipherally. At the periphery, lymphocytes with eosinophils. In centre, calcification (Ca) and giant multinuclear cells at its margin (arrow). The cysticercus probably died at an early stage of development. Bull 10/IV, IHA +; MPA +; IFR —; HE, (150×).

Fig. 1. Cyst with dying cysticercus (C). Extensive dystrophic foci (DF) with calcium salt deposition (arrow) in the cyst wall. Thin infiltration of lymphocytes. Bull 7/IV, IHA +; MPA +; IFR —; HE, (150×).
Fig. 1. Node with dead cysticercus. In the centre, in addition to calcification, multifocal liquefaction (Li), leucocytes and macrophages with watery plasma (arrow). Bull 12/III, IHA +, MPA —, IFB +; HE, (100 x). Fig. 2. Detail from Fig. 1. Macrophages with voluminous and watery plasma. HE, (600 x).

Fig. 1. Detail of warped cyst wall. Histological examination revealed that a transformed vessel is involved. Histocytes, lymphocytes and plasma cells present in the thickened wall. Endothelial cells visible on the inner surface (arrows). Bull 13/IV, IHA +, MPA +, IFB —; HE, (600 x). Fig. 2. Detail of giant multinuclear cells (GC) on the border of calcified centre (Ca) of node from Plate I, Fig. 1. HE, (600 x).