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COMPARATIVE EVALUATION OF IMMUNOELECTROPHORESIS, COUNTERIMMUNOELECTROPHORESIS AND ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DIAGNOSIS OF *TAENIA SAGINATA* CYSTICERCOSIS

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ABSTRACT

Geerts, S., Kumar, V., Aerts, N. and Ceulemans, F., 1981. Comparative evaluation of immunoelectrophoresis, counterimmunoelectrophoresis and enzyme linked immunosorbent assay for the diagnosis of *Taenia saginata* cysticercosis. *Vet. Parasitol.*, 8: 299—307.

The ante-mortem diagnosis of *Taenia saginata* cysticercosis remaining largely unresolved, the efficiency of immunoelectrophoresis (IEP), counterimmunoelectrophoresis (CIEP) and the enzyme linked immunosorbent assay (ELISA), has been compared. Of 32 experimentally infected cattle, these procedures could detect, respectively, 28, 30 and 31 of them. IEP and ELISA gave quite specific results whereas CIEP was relatively less specific.

When applied on 24 proven cases of natural cysticercosis in conventionally raised cattle, harbouring relatively light infections, IEP, CIEP and ELISA could detect, respectively, only 25, 54.2 and 37.5 per cent of the animals.

On 100 slaughtered cattle, which were declared free of cysticercosis by the abattoir authorities, 3, 8 and 6 per cent of the animals showed false positive reactions by the respective procedures. Evidence is presented that at least 2 of these false positive reactions were due to *T. saginata* metacestodes, which escaped detection by the abattoir authorities.

These data show that none of the serological tests discussed above are sufficiently reliable to make a diagnosis on an individual basis although these can be useful for a diagnosis on a herd basis.

INTRODUCTION

Recently, some of the newer serodiagnostic tests, such as counterimmunoelectrophoresis (CIEP) and enzyme linked immunosorbent assay (ELISA), have been standardized for the in-vivo diagnosis of *Taenia saginata* (=*Taeniarhynchus saginatus*) cysticercosis (Geerts et al., 1980a, 1981). Immunoelectrophoretic (IEP) studies have also produced valuable results for the diagnosis of this condition and for the study of the antigenic com-

ponents (Geerts et al., 1979). The purpose of the present study was to evaluate the merits of these three diagnostic procedures for the detection of *T. saginata* cysticercosis in conventionally-raised cattle. It has been pointed out by Kagan (1974) that the specificity of a laboratory standardized serodiagnostic method is rather poor when applied on the naturally-infected field hosts. Accordingly, in this paper the three above mentioned procedures were first compared for their own merits under experimental conditions, and then evaluated for their use on conventionally-raised cattle.

MATERIALS AND METHODS

Antigens

The hydrosoluble extract of the proglottides of *T. saginata*, as described elsewhere (Geerts et al., 1980a), was used as the antigen source for IEP and CIEP studies. For ELISA, the metacestodes of *T. crassiceps* were used as the antigen source (Geerts et al., 1981). Earlier, preliminary experiments had shown that for the purpose of ELISA the use of the proglottides of *T. saginata* or the metacestodes of *T. crassiceps* as the antigen source, yielded similar results. The latter source, however, has an advantage in that the metacestodes can be propagated in the laboratory through serial passages in mice, which allows a better standardization of the antigen quality.

Serum sources

Thirty-two healthy and parasite-free calves, maintained under strict hygienic conditions, were infected with 4000—150000 *T. saginata* eggs. They were bled periodically after the infection and necropsied between 7 and 20 weeks post-infection. In each case the metacestode burden was determined by slicing half, or the entire musculature of the carcass carefully, in 5 mm slices and counting the recovered metacestodes. Based on these figures the three grades of infection were scored as follows: (1) light infection — where less than 100 metacestodes were present, (2) moderate infection — where 100 to 1000 metacestodes were present and (3) heavy infection — where the number of metacestodes exceeded 1000.

The serum samples of the following groups of animals were included as controls: four healthy uninfected calves, one calf harbouring experimental *Fasciola hepatica*, 4 calves experimentally-infected with *Dictyocaulus viviparus*, sheep harbouring experimental *F. hepatica* (3 animals), *Moniezia expansa* (2 animals), *Cysticercus tenuicollis* (1 animal) and *Echinococcus granulosus* (10 animals) and cattle naturally-infected with *F. hepatica* (9 animals) and various gastro-intestinal nematodes (69 animals).

Serum samples of 24 naturally-infected cattle which were raised conventionally and were part of a herd which had an outbreak of *T. saginata* cysticercosis on a farm in the Netherlands were obtained. These animals

were subsequently confirmed to have cysticercosis on meat inspection by the abattoir authorities.

Serum samples of 100 slaughtered cattle which were found negative for cysticercosis by the meat inspection authorities of the abattoir were also included. These authorities usually examine the carcasses by making a few incisions in the heart, diaphragm and masseter muscles and by palpating the tongue and oesophagus. As an additional check, the hearts of all these animals were examined scrupulously in the laboratory by thorough and complete slicing.

Serodiagnostic procedures

The procedural details of IEP, CIEP and ELISA were described by Geerts et al. (1979), Geerts et al. (1980a) and Geerts et al. (1981), respectively. For interpretation of ELISA results, the extinction values of the uninfected cattle were also determined to conclude the results at the 95% limit of confidence as described previously (Geerts et al. 1981).

RESULTS AND DISCUSSION

Sensitivity

Table I shows the time of first appearance of positive serological reactions by IEP, CIEP and ELISA procedures among the calves harbouring three different grades of *T. saginata* cysticercosis and maintained under experimental conditions. Of the 32 experimental animal sera, these procedures could detect, respectively, 28, 30 and 31 of them. IEP was not very sensitive, as it failed to detect the two moderately-infected calves. These two animals, however, could easily be detected as positives for cysticercosis by CIEP and ELISA procedures. Except for these two instances, IEP and CIEP techniques, in general, can be considered to yield similar sensitivities. On certain occasions, especially among the moderately-infected calves, the CIEP procedure showed better sensitivity. It was occasionally better than ELISA, in so far as the appearance of first positive serological reaction among the experimentally-infected calves is concerned. In the present investigations, the spectrophotometric extinction values of ELISA were considered positive only when these values were above the 95 per cent confidence limit. Because of this rigorous criterion, some of the samples, despite being considered negative by ELISA, could react in CIEP to show a qualitative positive reaction. However, with ELISA the antibodies among the heavily-infected calves could be detected as early as one week post-infection and the antibodies in a calf harbouring just two cysticerci were demonstrable. These two exclusive features prove that out of these three procedures ELISA is the most sensitive, at least under experimental conditions.

The sensitivities of IEP, CIEP and ELISA for the detection of *T. saginata*

TABLE I

The first detection of antibodies in the sera of calves harbouring experimental *T. saginata* cysticercosis by IEP, CIEP and ELISA

Lightly infected				Moderately infected				Heavily infected			
No. of metacestodes	Antibodies[1]			No. of metacestodes	Antibodies[1]			No. of metacestodes	Antibodies[1]		
	ELISA	CIEP	IEP		ELISA	CIEP	IEP		ELISA	CIEP	IEP
2[3]	12	—[2]	—[2]	106	10	10	15	1329[3]	5	5	6
3	—[2]	—[2]	—[2]	109[3]	10	7	6	1610	1	2	4
47[3]	6	6	6	158[3]	4.5	4.5	4.5	1730[3]	4.5	3.5	3.5
50[3]	14	15	14	185[3]	11	6	—[2]	1984[3]	3	3	3
87	14	16	16	232[3]	5.5	5.5	—[2]	2207[2]	4	3	3
				296[3]	10	7	7	2216[3]	4	5	4
				328[3]	4	5	4	2365[3]	3	5	5
				339[3]	4	3	4	2555	1	4	4
				356[3]	5	3.5	3.5	3053	4	3	3
				373	9	7	10	3150	3	2	2
				468[3]	3.5	4.5	4.5	3695	5	3	4
				739[3]	6	4	4	5285	3	2	2
				990	4	4	4	6510	1	2	2
								9455	2	3	3

[1] Weeks post infection.
[2] No antibodies.
[3] Mostly viable metacestodes.

cysticercosis in 24 naturally-infected conventionally raised field cattle are compared in Table II. It may be pointed out that while IEP and CIEP are purely qualitative methods yielding clear cut positive or negative results, ELISA is a quantitative method where certain statistical criteria are to be applied to designate a positive or negative serological reaction.

The lower percentages of sensitivities of different procedures given in Table II are to be interpreted with caution. In the first place these serum

TABLE II

Comparison of the sensitivity of IEP, CIEP and ELISA for the detection of *T. saginata* metacestodes in naturally infected cattle

No. of infected cattle	No. of animals revealed positive by		
	IEP	CIEP	ELISA
24	6 (25%)	13 (54.2%)	8 (37.5%)

samples from naturally-infected cattle originated from just one farm where the animals were exposed to identical ecological risks of infection. The meat inspection results had shown clearly that the intensity of infection was light; between one and five metacestodes, the majority of which were caseous or calcified, were recovered from the predilection sites. Under these situations the level of serum antibodies of these animals was probably lower than it would have been if the infection was active and more severe. The animals harbouring more recent and heavy infection, could have produced large amounts of serum antibodies resulting in higher percentages of sensitivities for the different tests. Another factor which could have enhanced the sensitivities of the tests, was the extraordinary care exercised by the abattoir authorities in carrying out the meat inspection in the light of their prior knowledge that this farm had a history of cysticercosis. It is possible that under the normal circumstances of meat inspection a few of the lightly infected animals might have escaped detection, resulting in at least a theoretical increase in the sensitivities of the different serological procedures.

Specificity

The specificities of the three sero-diagnostic methods were evaluated by incorporating the serum samples of cattle and sheep infected naturally or artificially with the heterologous helminths of cattle host. The results are summarised in Table III. Of the 35 serum samples originating from healthy parasite-free calves (serial preinfection samples of experimental calves), three showed false-positive reactions with ELISA while none did in IEP or CIEP. The serum samples of these three animals showed a progressively

TABLE III

Specificity of IEP, CIEP and ELISA

A.	Experimental infections	Host	Number of animals	Number of cross-reactions		
				IEP	CIEP	ELISA
	None (pre-infection samples)	Cattle	35	—	—	(3)[2]
	F. hepatica	Cattle	1	—	—	—
		Sheep	3	—	—	—
	D. viviparus	Cattle	4	—	2	—
	M. expansa	Sheep	2	—	—	—
	C. tenuicollis	Sheep	1	1	1	1
	E. granulosus hydatid cysts	Sheep	10	—	1	—
B.	Natural infections					
	F. hepatica[1]	Cattle	9	—	1	1
	Gastro-intestinal nematodes[1]	Cattle	69	1	2	3

[1] Free of *T. saginata* metacestodes in the heart.
[2] Probably originating from colostral antibodies.

decreasing trend in the spectrophotometric extinction values in ELISA. This trend suggests that the initial high preinfection extinction values were caused through transfer of colostrum antibodies which waned with increasing age of the calves. The authors did not examine the serum antibody response of the mothers of these calves. A similar phenomenon was shown by Van Knapen et al. (1979) in the offspring of cows known to be infected. On subsequent inoculation of these three calves with *T. saginata* eggs, a rise in their extinction values was observed.

The serum sample from a *C. tenuicollis*-infected sheep showed cross-reaction with all three methods. However, because *C. tenuicollis* is not a frequent parasite of cattle, this cross-reaction would not be a major drawback.

One of nine serum samples from cattle naturally-infected with *F. hepatica* cross-reacted both in CIEP and ELISA while the four samples from the experimentally-infected hosts which were harbouring massive *F. hepatica* infections did not react using any of the three methods. This cross-reaction wit the naturally-infected host harbouring mild infection of *F. hepatica* is unique since it does not appear to be related to the presence of antibodies against this parasite.

Of the 69 serum samples from animals with gastro-intestinal nematodes, but which were found free of *T. saginata* cysticercosis by the abattoir authorities, one showed positive reaction with IEP, two with CIEP and three with ELISA. It is difficult to tell whether or not these positive reactions are caused by gastro-intestinal parasitism. If these were real cross-reactions, a

larger proportion of these 69 animals could be expected to show positive results. Here, the reason for so few positive reactions cannot be known with any certainty.

One serum sample out of the ten sheep harbouring experimental hydatidosis showed cross-reaction with CIEP. Similarly, two of the four *D. viviparus*-infected cattle serum samples showed cross-reactions. These cross-reactions were discussed by Geerts et al. (1980a). Irrespective of all these considerations, a general conclusion can be drawn that IEP and ELISA are quite specific tests.

Abattoir studies

Of the 100 hearts obtained from slaughtered cattle, only 25 showed cyst-like lesions. However, after a detailed histological study of these lesions, only nine were assigned to *T. saginata* cysticercosis (Geerts et al., 1980b). The results of the serological examinations of the 100 slaughtered cattle and their relationship to the occurrence of cyst-like lesions in the hearts are given in Table IV. Only three of the nine animals showing lesions of cysticercosis were serologically positive by these methods. The majority of the serological positive animals did not have cysticercosis lesions in the heart. This may also imply that few of the latter animals might have been undetected cases of *T. saginata* cysticercosis.

This becomes especially relevant in view of the report of Mc Cool (1979) suggesting that up to 51 per cent of the weakly-infected animals may not

TABLE IV

Results of IEP, CIEP and ELISA with the sera of 100 slaughtered cattle, declared free of *T. saginata* cysticercosis by abattoir authorities

Animal no.	Positive reaction in			Presence of cysticercosis lesions[1]
	IEP	CIEP	ELISA	
2		+		
5	+	+	+	
13			+	
16		+	+	
17		+		
27			+	
67		+		+
73		+		
82		+		+
83	+	+	+	+
92			+	
96	+			

[1] Based on detailed examination of the heart.

harbour any cysticerci in the classical sites of predilection. Some of the positive sera in the present case may be false-positives since these procedures are not completely error-proof.

Only two serum samples (Nos. 5 and 83) were concurrently positive by the three serological methods and only one (No. 83) out of these had lesions of cysticercosis in the heart. It is quite probable that the abattoir authorities had missed the diagnosis of these two cases of mild cysticercosis.

CONCLUSION

It appears that under experimental conditions, IEP is quite a specific test for the detection of antibodies against *T. saginata* cysticercosis but on occasions it lacks the desired level of sensitivity. The IEP procedure is lengthy and demanding which makes it unsuitable for routine laboratory use. In suspicious cases, however, IEP can be a complementary or supplementary method to CIEP and ELISA in arriving at a definitive diagnosis. In addition, IEP is the only method to provide, based on the precipitation pattern, a stage-specific diagnosis of *T. saginata* cysticercosis (Geerts et al., 1979). Compared to IEP, the CIEP technique is a rapid, simple and more sensitive method but at times fails to provide the desired level of specificity. ELISA seems to be the test of choice as it yields the desired levels of sensitivity and specificity. It is useful for routine laboratory use and, when the procedure is mechanised and automated in conjunction with more objective spectrophotometric reading of the results, it lends itself more easily to large scale use (Ruitenberg et al., 1977).

It is evident from the results obtained that the three serological methods gave different results for the detection of cysticercosis in conventionally-raised cattle. When used in 24 proven cases of *T. saginata* cysticercosis in cattle raised conventionally these tests yielded high numbers of false-negative results. On the other hand, these methods showed a number of false-positive reactions among 100 slaughtered cattle declared free of cysticercosis by the abattoir authorities. Moreover, serological evidence is available that the abattoir authorities failed to detect at least two cases of cysticercosis out of these 100 cattle. It is proposed therefore that the schedule described by Ruitenberg et al. (1979) for the control of parasitic infections where the serologically negative animals may be released without direct examination at the abattoir is at present not effective for the control of *T. saginata* cysticercosis. ELISA or CIEP show limited possibilities for sero-epizootiological studies to provide basic data about the frequency of cysticercosis on a herd basis.

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