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CONTRIBUTION TO THE SEROLOGICAL DIAGNOSIS OF SARCOCYSTOSIS

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Abstract. The importance of the IFA-test (Indirect Fluorescent Antibody Test) in the diagnosis of sarcocystosis in the intermediate host has been confirmed in the present paper. We assessed the time of the first appearance of serum antibodies in the intermediate host in mice inoculated experimentally with the species *Sarcocystis dispersa*. By means of the IFA-test, the first antibodies were found on day 20 p.i. Cross-reactions among antisera of *S. dispersa* and a heterologous antigen of *S. cernae* disclosed that the reaction was not species-specific, but genus-specific. In addition, we confirmed serologically that the antigenic structure of the genus *Frenkelia* was identical to that of the genus *Sarcocystis*, because the results of cross reactions obtained with the IFA-test were identical.

Tadros et al. (1974), Aryeetey and Piekarski (1976) and Černá and Kolářová (1977) pointed out that the indirect fluorescent antibody test (IFA-test) was a satisfactory method for the detection of antibody in sarcocystosis. The present study was made mainly for the purpose of confirming the suitableness of the IFA-test on the infected intermediate host which constitutes a source of infection for carnivorous hosts.

We used *Sarcocystis dispersa* Černá, Kolářová et Šulc, 1978 as a model for our experiments; its life cycle in the mouse (*Mus musculus*) and in owls (*Tyto alba* and *Asio otus*) has been described earlier (Černá et al. 1978). Particular attention has been given to the specificity of the IFA-test when using different antigens, and to serological relationships between members of the genus *Sarcocystis* and related coccidians of the genus *Frenkelia*. Another point of interest was the time of appearance of serum antibodies in the infected intermediate host, i.e., at which time of the life cycle of the parasite these become detectable in the intermediate host by the IFA-test.

MATERIALS AND METHODS

Sera for the IFA-test were obtained from laboratory white mice (*Mus musculus*) infected perorally with a dose of 100 to 200 thousand sporocysts of *Sarcocystis dispersa* per mouse. Blood from the infected mice was drawn in different intervals p.i. (Table 1). Antigen of *S. dispersa* was prepared from the muscles of infected mice three months after the inoculation when muscle "cysts" could already be seen macroscopically. Part of the material was fixed and antigen was prepared in the mode suggested by Černá (1966) for one-host coccidians of the genus *Eimeria*; the remaining material was crushed in saline and the antigen was prepared from zoites released from the cysts and fixed with 1% neutral formalin.

The antigen used in cross-examinations was prepared from "cystic" stages in a common vole (*Microtus arvalis*) inoculated with the species *Sarcocystis cernae* Levine, 1977. The life cycle of this species in *Microtus arvalis and Falco tinnunculus* has been described in an earlier paper (Černá and Loučková 1976). The antigen of a *Frenkelia* species was prepared from "cystic" stages in the brain of a common vole (*Microtus arvalis*) with a spontaneous infection. The method used for the preparation of both antigens (*S. cernae* and *Frenkelia*) was essentially similar to that suggested earlier by Černá (1966). Also a *Toxoplasma*-antigen from free zoites was used for a comparison of the IFA-test results. The antigen was prepared by Dr. Chalupský, from the peritoneal exudate of mice inoculated with *Toxoplasma gondii* in a method suggested by Kramář (1964).

The IFA-test was essentially similar to that used for coccidians of the genus *Eimeria* (Černá 1966). It was read on the Soviet fluorescent microscope ML_2.

RESULTS

1. DETECTION OF ANTIBODY IN EXPERIMENTALLY INFECTED MICE INOCULATED WITH THE SPECIES *SARCOCYSTIS DISPERSA*

The results of the serological response obtained with the IFA-test are shown in Table 1. In this case, we used for the IFA-test the antigen of *S. dispersa* prepared histologically (see above).

As evident from Table 1, the first antibodies appeared in intermediate murine hosts at a time, at which the asexual development of the parasite was no longer occurring in the liver and was confined to the muscles only (see Discussion and conclusions).

We compared 2 types of antigens of *S. dispersa* in a set of 4 antisera collected 165 days after the administration of 10^5 sporocysts per mouse. One antigen type was prepared histologically from "cystic" stages, the other from free zoites. As indicated by Table 2, differences in the IFA-tests were negligible and independent of the mode of antigen preparation (histologically or from free muscle zoites).

Table 1. The indirect immunofluorescence reaction in mice infected experimentally with *S. dispersa*

Serum No.	Days after inoculation	Antibody titre
1	7	neg
2	7	neg
3	10	neg
4	20	10
5	28	40
6	70	80
7	70	20
8	70	40
9	70	80
10	85	80
11	120	80
12	165	40
13	165	10

Table 2. Comparison of two types of *S. dispersa* antigens

Serum No.	Antigen titre in tissue section	Antigen titre in free zoites
14	40	20
15	20	20
16	10	10
17	5	5

2. COMPARISON OF IFA-TESTS USING ANTIGENS OF *S. DISPERSA* AND *S. CERNAE*, AND *S. DISPERSA*, ANTISERA

The problem of a species- or genus specificity of the IFA-test was approached in that antigens of two different species of the genus *Sarcocystis* were used against antisera of one of these. Table 3 shows the results of cross-reactions for 7 antisera.

The results of the IFA-test (Table 3) were practically identical when using either a homologous (*S. dispersa*) or a heterologous (*S. cernae*) antigen.

3. THE IFA-TEST WITH *SARCOCYSTIS* ANTISERA AGAINST ANTIGENS OF OTHER GENERA: *FRENKELIA* AND *TOXOPLASMA*

Using 4 antisera with antibodies against *S. dispersa*, the IFA-test was performed also on an antigen from "cystic" stages of the genus *Frenkelia* in the brain and from fixed zoites of *Toxoplasma gondii*.

When using a heterologous antigen of *Toxoplasma gondii* (Table 4, sera 1-3,) the result of the IFA-test with *Sarcocystis* antisera was negative. The results obtained for a *Frenkelia* antigen were identical to those with a homologous antigen.

Table 3. Cross-reactions of *S. dispersa* and a homologous (*S. dispersa*) and a heterologous (*S. cernae*) antigen

Serum No.	Homologous antigen titre	Heterologous antigen titre
1	80	80
2	20	10
3	40	40
4	80	80
5	40	40
6	80	40
7	40	40

Table 4. Results of the indirect immunofluorescence reaction with *S. dispersa*-antisera and heterologous antigens of *Frenkelia* and *Toxoplasma*

Serum No.	Homologous antigen titre	Titre on a *Frenkelia* antigen	Titre on a *Toxoplasma* antigen
1	20	20	neg
2	40	40	neg
3	80	80	neg
4	80	80	—

DISCUSSION AND CONCLUSIONS

As pointed out in the introduction, also Tadros et al. (1974) and Aryeetey and Piekarski (1976) recommended the IFA-test for the detection of antibodies in sarcocystosis. Our examination of serum antibodies in mice infected experimentally with *Sarcocystis dispersa* indicated that the appearance of circulating antibodies coincided with the time of "cyst" formation if an antigen from cystic stages was used. It emerged from our study on the life cycle of *S. dispersa* in the intermediate host (Černá 1977) that asexual stages of the parasite multiplied first in the liver (up to day 8–9 p.i.); and then the zoites were transported by macrophages to the muscles and started to produce cystic formations. Muscle "cysts" could be demonstrated histologically on day 20 p.i.; at that time, antibody titres shown by the IFA-test were low (10), but several days later, the titres attained values ranging from 20–80 (Table 1).

Our results indicated that it was possible to use as an antigen both histologically treated cystic formations from the muscles and an antigen obtained from free zoites as recommended by the earlier mentioned authors (Table 2). Confirmation was obtained from a cross-examination with a homologous and a heterologous antigen of the genus *Sarcocystis* that the reaction was genus- and not species-specific (Table 4). By contrast,

a species-specific reaction was obtained for one-host coccidians of the genus *Eimeria* in serological cross-examinations (Černá 1970).

We have also confirmed that although a *Toxoplasma gondii*-antigen did not react with *Sarcocystis*-antisera, an antigen from another heteroxenous coccidian of the genus *Frenkelia* gave identicial results with the IFA-test to those of a homologous *Sarcocystis*-antigen. Close relationships of *Frenkelia* and *Sarcocystis* suggested by studies on the life cycle of members of these genera will be the subject of another paper.

It emerged from our results that the IFA-test can be used in the diagnosis of sarcocystosis of the intermediate host at the time of production of "cystic" stages in the muscles of the infested animals.

ДАННЫЕ О СЕРОЛОГИЧЕСКОЙ ДИАГНОСТИКЕ САРКОЦИСТОЗА

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Резюме. Доказано значение непрямой реакции флуоресцирующих антител (НРФА) для диагностики саркоцистоза у промежуточных хозяев. Первое появление сывороточных антител в промежуточном хозяине обнаруживали у экспериментально зараженных *Sarcocystis dispersa* мышей. Первые антитела появились на 20 день после заражения. С помощью перекрестной реакции антисывороток *S. dispersa* с гетерологичным антигеном *S. cernae* установлено, что реакция обладает не видовой, а родовой специфичностью. Кроме того с помощью серологических методов доказано, что антигенные структуры родов *Frenkelia* и *Sarcocystis* идентичны, так как перекрестные реакции дали идентичные результаты при НРФА.

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