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The Specifity of Serous Antibodies in Coccidioses

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Abstract. We studied 4 species of coccidia—*Eimeria tenella* from chickens, *E. stiedai* and *E. magna* from rabbits and *E. pragensis* from mice—in order to obtain information on the specificity of antibody detected with the indirect fluorescence antibody reaction (IFAR). We tested the antisera of all these species against their own (homologous) antigen and against foreign (heterologous) antigens. The results of these cross-tests revealed a common component in the antigenic structure of the merozoites of the two rabbit species *E. stiedai* and *E. magna*. In cross-tests of the antisera *E. tenella* × *E. magna*, *E. stiedai*, *E. tenella*, and *E. magna*, and *E. stiedai* × *E. pragensis*, using heterologous antigens, the results showed either no reaction to the IFAR or a reaction in several instances only if the dilutions of the sera were very low.

In my previous papers (ČERNÁ 1966a, b, 1967) I drew attention to the use of the indirect fluorescent antibody reaction (IFAR) for revealing serous antibodies in various coccidia species of the genus *Eimeria*. In an attempt to detect whether a positive IFAR is always associated with the homologous antigen of the species under consideration or whether such reaction can be obtained also by using antigen from another coccidial species (heterologous antigen), I cross-tested with the IFAR a group of chicken antisera with antibodies against *E. tenella*, a group of rabbit antisera with antibodies against *E. magna* and *E. stiedai* and a group of mice with antibodies against *E. pragensis* using both homologous and heterologous antigens. The results of these tests are presented in this paper.

MATERIAL AND METHODS

For obtaining antisera, antigens and also sufficient numbers of oocysts for our experiments we infected a total of 45 rabbits aged 1—3 months. Of these, 20 rabbits were infected with the species *E. stiedai*, 25 rabbits with the species *E. magna*. The species *E. tenella* was used for infecting 60 chickens, the species *E. pragensis* for 80 white laboratory mice—strain H.

Before administering the oocysts, we examined repeatedly the faeces of the experimental animals with Faust's flotation method to make sure that these were not infected with any other coccidian

species. We evaluated only experiments in which antisera were obtained from a pure infection. The faeces of the experimental animals were examined also during the course of the experiment; whenever a contamination with another coccidian species was detected, the experiment was interrupted immediately and the sera from this experiment were discarded. Control sera were obtained from uninfected animals.

Antisera: These were obtained after artificial infection of the hosts with high doses of oocysts of the coccidian species under consideration. Antisera against *E. stiedai* obtained from rabbits aged 1—3 months fed orally with 4,000—5,000 oocysts. Antisera against *E. magna*—from rabbits of the same age (1—3 months) fed with 15,000—25,000 oocysts of *E. magna*. Antisera against *E. tenella*—from chickens aged 14—21 days, infected with 15,000—20,000 oocysts (per os). Antisera against *E. pragensis*—from white mice infected with 50,000 oocysts of this coccidian species.

The blood of the experimental animals was collected at regular intervals; at first (i.e. during the first 14 days after inoculation) at intervals of 3—4 days, later 10—14 days.

Antigens: The method of obtaining and elaborating antigens for the individual coccidian species has been described in previous papers. For our cross-tests we used always antigens obtained from fixed tissue containing asexual stages (schizonts, merozoites). Antigens of *E. pragensis* (EP_s) to be used for the IFAR were obtained from caeca and the large intestines of white mice on day 5 after inoculation. Antigens of *E. stiedai* (ES_s) were collected on day 13 and 14 after inoculation. A dose of 40—50,000 oocysts per rabbit had to be administered for obtaining large enough numbers of schizonts from the liver. Antigens of *E. magna* were collected from the small intestine of rabbits killed on day 4 after infection with the oocysts; antigens of *E. tenella* were obtained from the caeca of chickens on day 5 after infection.

Conjugates. A titred conjugate of horse- or goat serum with FITC-labelled rabbit gamma globulin was used for the IFAR with antisera of *E. stiedai* and *E. magna*; rabbit conjugate with labelled chicken anti-gamma globulin was used for antisera of *E. tenella*. Pig conjugate with labelled mouse anti-gamma globulin was used for antisera of *E. pragensis*. All conjugates were obtained commercially (ÚSOL, Prague). For IFAR techniques see ČERNÁ 1966a, b, 1967.

IFAR control. For this control we used mainly sera of uninfected hosts, which were coprologically negative. In addition, the reaction was controlled by leaving the conjugate only (without serum) to act on the antigen to exclude an eventual activity of antibody present already in the conjugate itself.

Reading of the IFAR. For reading the reactions, the Soviet produced fluorescent microscope ML-2 was used with filters FS-1, BS-8-2 and the barrier filter ŽS-18-2; the light source was a high-pressure mercury discharge lamp DRŠ 250.

RESULTS

1. Cross-reactions of chicken antisera of *E. tenella* with the homologous antigen ET_s and the heterologous antigens ES_s and EM_s.

The results of our cross-examinations of 26 antisera of *E. tenella* are given in Table 1. This table shows that when using for the IFAR antisera of *E. tenella* against the antigen of *E. magna*, the reaction with this heterologous antigen is completely negative. The heterologous antigen of *E. stiedai* gave a weak positive reaction with 3 sera of the examined group (Table 1), while all other reactions were negative.

2. Cross-reaction of rabbit antisera of *E. magna* with the homologous EM_s-antigen and with the heterologous ET_s and ES_s antigens.

We examined in the IFAR with the aid of the three mentioned antigens a total

Table 1. Antisera of *E. tenella* examined by IFAR with homologous and heterologous antigens

Serum no.	Titer of the antigens			Serum no.	Titer of the antigens		
	ET_s	ES_s	EM_s		ET_s	ES_s	EM_s
1	20	neg	neg	14	80	neg	neg
2	20	neg	neg	15	80	neg	neg
3	20	neg	neg	16	80	neg	neg
4	20	neg	neg	17	80	neg	neg
5	20	neg	neg	18	160	neg	neg
6	40	neg	neg	19	160	neg	neg
7	40	neg	neg	20	160	neg	neg
8	40	neg	neg	21	160	neg	neg
9	40	neg	neg	22	160	neg	neg
10	40	neg	neg	23	160	10	neg
11	40	neg	neg	24	320	neg	neg
12	40	neg	neg	25	320	20	neg
13	80	neg	neg	26	320	20	neg

Table 2. Antisera of *E. magna* examined with their own (EM_s) and with foreign (ET_s and ES_s) antigens

Serum no.	Titer of the antigens			Serum no.	Titer of the antigens		
	EM_s	ES_s	ET_s		EM_s	ES_s	ET_s
1	10	neg	neg	9	160	neg	neg
2	20	neg	neg	10	160	neg	neg
3	20	neg	neg	11	160	neg	neg
4	80	neg	neg	12	160	neg	neg
5	80	10	neg	13	160	10	neg
6	80	20	neg	14	320	10	neg
7	160	10	neg	15	640	20	neg
8	160	10	neg				

of 15 antisera of *E. magna*. The results are given in Table 2. This table shows that no positive reactions were obtained in the IFAR using antisera of *E. magna* with the heterologous chicken antigen of the species *E. tenella*; a weak positive reaction was obtained using antisera of *E. magna* of a higher antibody level with the heterologous rabbit antigen of *E. stiedai* (Table 2).

3. Cross-reactions of rabbit antisera of *E. stiedai* with the homologous ES_s-antigen and the heterologous antigens ET_s and EM_s.

Table 3. Cross-reaction of rabbit antisera *E. stiedai* with the homologous antigen ES_s and with the heterologous antigens ET_s and EM_s

Serum no.	Titer of the antigens		
	ES_s	EM_s	ET_s
1	80	40	10
2	80	40	neg
3	160	40	neg
4	320	80	40
5	320	40	10
6	320	40	20
7	640	80	10
8	640	80	20
9	1280	160	20

Table 3 shows that the antisera of *E. stiedai* reacted positively to the IFAR with the antigen of *E. tenella* only if the dilution of the sera was low. By contrast, sera with high titers reacted positively to the IFAR with the antigen of *E. magna* (coccidia from the same host) up to considerably high dilutions (1 : 80, 1 : 160).

Table 4. Antisera of *E. pragensis* examined by IFAR with the homologous antigen and with heterologous antigens

Serum no.	Titer of the antigens			
	EP_s	ET_s	ES_s	EM_s
1	20	neg	neg	neg
2	40	neg	neg	neg
3	40	neg	neg	neg
4	80	neg	neg	neg
5	80	neg	neg	neg
6	80	neg	neg	neg
7	80	neg	neg	neg
8	160	neg	neg	neg
9	160	neg	neg	neg
10	320	neg	neg	neg
11	320	neg	neg	neg
12	320	neg	neg	neg
13	640	neg	neg	neg
14	1280	neg	neg	neg
15	1280	10	neg	neg

1. Cross-reaction of antisera of *E. pragensis* with a homologous EP_s-antigen and with heterologous antigens ET_s, ES_s and EM_s.

We examined a total of 15 mice sera containing antibodies against *E. pragensis* of differently high titers using the heterologous antigens of the coccidia under consideration. The results are shown in Table 4. The IFAR of the antisera of

Table 5. IFAR with antigens of *E. tenella* and with the antigen of *E. pragensis*

Serum no.	Titer with the homologous antigen ET_s	Titer with the heterologous antigen EP_s
1	80	neg
2	80	neg
3	80	neg
4	160	neg
5	160	neg
6	160	neg
7	160	neg
8	320	10
9	320	10
10	320	neg

E. pragensis with various heterologous antigens (ET_s, EM_s, ES_s) were all negative with the exception of one serum with a high titer of antibody (1,280). In this case we obtained a weak positive reaction with the antigen of *E. tenella* only at the dilution of 1 : 10.

Table 6. IFAR with antisera *E. stiedai* and antigen *E. pragensis*

Serum no.	Titer with the homologous antigen ES_s	Titer with the heterologous antigen EP_s
1	80	neg
2	80	neg
3	160	neg
4	320	neg
5	320	neg
6	320	neg
7	640	neg
8	640	neg
9	1280	neg

5. Cross-reaction of antisera *E. tenella*, *E. stiedai* and *E. magna* with the heterologous antigen EP_s.

The results of these cross-reactions are recorded in Tables 5, 6 and 7 which reveal that the IFAR was nearly always negative using the antigen of *E. pragensis* with

Table 7. IFAR with antisera *E. magna* and antigen *E. pragensis*

Serum no.	Titer with the homologous antigen EM_s	Titer with the heterologous antigen EP_s
1	80	neg
2	80	neg
3	80	neg
4	160	neg
5	160	neg
6	160	neg
7	160	neg
8	320	neg
9	640	neg

heterologous antisera. Only twice was there a slight cross-reaction between *E. tenella* and *E. pragensis* (Table 5). This was a similar result to that previously observed (Table 4) where the test was weakly positive at 1 : 10.

CONCLUSIONS

In general, the IFAR was negative when used to examine the reaction between heterologous antisera and antigens the occasional positive results being obtained only at low dilutions (1 : 10 or 1 : 20); however, different results were obtained when observing the cross-reaction between material derived from the two rabbit species *E. stiedai* and *E. magna*. For example antisera of *E. stiedai* (homologous titers 320—1,280) reacted positively with the heterologous antigen of *E. magna* even at 80 and 160 (Table 3). This suggests the existence of a certain common component in the antigenic structure of the merozoites of the two rabbit coccidia *E. magna* and *E. stiedai* which would be in conformity with a similar observation of Diggs and Sadun (1965) when studying the IFAR in similar circumstances using the causal agents of malaria *Plasmodium falciparum* and *Plasmodium vivax*. The existence of a common antigenic component in coccidial species from the same host must be remembered especially when trying to apply the IFAR for the differential diagnosis of different species of coccidia.

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