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STUDIES ON SCHISTOSOMIASIS. 3.* DETECTION OF ANTIBODIES AGAINST *SCHISTOSOMA MATTHEEI* BY THE INDIRECT IMMUNO-FLUORESCENT METHOD

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ABSTRACT

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The indirect fluorescent antibody test was employed to determine levels of serum antibodies in cattle and sheep infested with *Schistosoma mattheei*. Using smears of schistosome cercariae as antigen, a high degree of specificity and sensitivity was obtained. High titres were obtained in sera from all the animals infested with schistosomiasis, while those from animals infested with various other helminths gave negative results. The antigen-antibody complex appeared to be localised in the cuticle of the cercaria.

INTRODUCTION

The antigen used in the indirect immuno-fluorescent technique to detect serum antibodies against *Schistosoma mansoni* has been prepared in various ways. Niel, Pinon & Gentilini (1970), employing frozen sections of adult *S. mansoni* in the ventricular cavity of a guinea pig heart, recorded titres up to 1/2560 and found cross-reactions with hydatidosis and fascioliasis. With *S. mansoni*, Sadun, Williams & Anderson (1960) and Sadun, Anderson & Williams (1962) obtained greatest uniformity with fresh carcariae in direct fluorescent antibody tests and formalised cercariae in indirect fluorescent antibody (IFA) tests. They recorded extensive cross-reactions with sera obtained from trichinosis patients as well as with those from patients infested with *Schistosoma haematobium* and *Schistosoma japonicum*. Using formalised cercariae, Kagan, Sulzer & Carver (1965) also obtained cross-reactions with trichinosis.

This report describes a different method of preparing the antigen to determine antibodies against *S. mattheei* infestation in cattle and sheep by means of the IFA technique.

MATERIALS AND METHODS

Sera

Fifty-eight serum samples from the following animals were used:

1. S. mattheei *group*

Twelve cattle and 15 sheep experimentally infested with varying numbers of *S. mattheei* cercariae and 6 cattle harbouring natural infestations.

2. *Control group*

Twenty sheep and 5 cattle experimentally infested with pure strains of the following helminths: *Paramphistomum microbothrium* (7 sheep), *Fasciola hepatica* (4 sheep), *F. gigantica* (3 sheep), *Oesophagostomum columbianum* (1 sheep), *O. radiatum* (1 calf), *Gaigeria pachyscelis* (1 sheep), *Haemonchus contortus* (1 sheep), *H. placei* (1 calf), *Chabertia ovina* (1 sheep), *Nematodirus spathiger* (1 sheep), *Trichostrongylus* spp. (1 sheep), *Ostertagia circumcincta* (1 calf), *Bunostomum phlebotomum* (1 calf) and *Cooperia* spp. (1 calf).

Antigen

Cercariae of *S. mattheei* were collected from *Bulinus* (*Physopsis*) *globosus* and *B.* (*P.*) *africanus* as described by Heitmann, 1969. The cercariae were concentrated by sedimentation at 4°C until the suspension contained approximately 10^4 cercariae per ml. A droplet of cercarial suspension was placed in a 1 cm diameter circle on a clean glass slide. The fluid part of the suspension was allowed to evaporate for ½ to 1 minute and the droplet subsequently spread over the entire surface of the circle by means of a platinum loop.

Test procedure

The slides containing the antigen were fixed in acetone at room temperature for 10 minutes. A few drops of three-fold serum dilutions in buffered physiological saline were allowed to react with the antigen in a moist chamber at room temperature for 20 minutes. After two washings in buffered saline, a few drops of fluorescein-conjugated rabbit anti-sheep or anti-bovine globulin (Pasteur Institute, Paris) were placed in contact with the antigen for 20 minutes. After two further washings in buffered saline, the preparations were mounted in buffered glycerine and examined under a Zeiss binocular miscroscope equipped with a 200 watt Wild mercury burner. A BG12 exciter filter and a dark field condensor were used.

The absence of non-specific fluorescence of the antigen was tested by allowing the conjugated anti-species globulin to react with the antigen without prior contact with serum.

The specificity of the test was controlled by testing sera obtained from sheep and calves (listed above) infested with other helminths.

RESULTS

The results of the IFA test in sheep and cattle infested with *S. mattheei* are summarized in Table 1. The antibody titres obtained varied considerably, and there was no correlation between the titres and the number of cercariae used for infestation on the one hand and the number of exposures on the other. In both sheep and cattle levels of antibodies demonstrable by means of the IFA test remained elevated for long periods after infestation.

In positive cases the strongest fluorescence was observed consistently in the cuticle of the cercaria, while the rest of the cercariae showed less fluorescence. At the endpoint of the reaction the fluorescence was confined to the cuticle and the dilution at this point was taken as the highest titre.

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TABLE 1 IFA titres of sera against *S. mattheei*

Animal No.		Infestation			Worm Count	IFA Titre
		No. of exposures	No. of cercariae	Interval*		
Cattle Exp. cases	BE1	1	17 278	365	6 946	1/90
	BE2	1	21 010	348	3 757	1/2430
	BE3	1	48 721	350	9 208	1/810
	BE4	1	19 864	340	6 604	1/7290
	BE5	1	36 719	349	—	1/2430
	BE6	1	161 260	53	121 317	1/810
	BE7	2	25 007 & 143 287	349(50)	32 306	1/2430
	BE8	2	41 298 & 110 314	337(43)	—	1/7290
	BE9	2	33 501 & 110 028	337(43)	65 420	1/810
	BE10	2	7 926 & 149 794	217(48)	25 728	1/270
	BE11	2	12 645 & 165 397	224(55)	46 568	1/270
	BE12	2	5 711 & 143 320	222(39)	39 542	1/810
Cattle Nat. cases	BN1	***–	—	—	63 930	1/7290
	BN2	–	—	—	56 833	1/2430
	**BN3	–	—	—	—	1/810
	**BN4	–	—	—	—	1/810
	**BN5	–	—	—	—	1/21870
	BN6	–	—	—	54 603	1/810
Sheep	01	1	3 600	294	—	1/270
	02	1	5 800	964	—	1/270
	03	1	3 850	1142	—	1/270
	04	1	4 500	1143	—	1/30
	05	1	2 000	1246	—	1/90
	06	1	2 000	1246	—	1/90
	07	1	1 092	254	—	1/7290
	08	1	2 422	289	—	1/7290
	09	1	1 559	110	—	1/2430
	010	1	1 559	110	—	1/7290
	011	2	2 700 & 11 454	582(523)	—	1/2430
	012	2	2 600 & 2 900	586(328)	1 223	1/810
	013	2	3 160 & 2 900	583(328)	—	1/810
	014	2	5 000 & 4 750	574(310)	2 217	1/810
	015	2	2 030 & 3 960	583(310)	—	1/90

*Interval in days between first infestation and collection of serum. In parentheses, interval in days between last infestation and collection of serum
**Diagnosis confirmed by demonstration of schistosome eggs in the faeces of the animals
***– = Unknown or not determined

In positive sera strong fluorescence was observed at the oral end of cercariae and at breaks in the cuticle. This fluorescence, which was also observed to a lesser extent in negative control sera and in preparations of antigen plus anti-species globulin, was considered non-specific and ignored.

The sera from the control animals were all negative at a dilution of 1/30.

DISCUSSION

Marked fluorescence of the cercarial cuticles in positive sera was a distinctive feature in these tests. Both the specificity and sensitivity of the IFA test appeared to be related to the affinity of serum antibodies for the cuticular antigens. This phenomenon was also observed by Du Plessis, Pienaar & Basson, 1969, in antigens prepared from larvae of *Strongyloides papillosus* and was recorded by Sadun *et al.* (1962), who used formalised cercariae as antigen.

These results support the findings of other workers, as reported by Sadun (1967), in that we found no correlation between the IFA titre and the worm burdens of the various animals infested with schistosomes. Our results differed from those of other workers (Niel *et al.* 1970), however, as there were no cross-reactions with other helminths and, moreover, no false negative results were obtained. Nevertheless, it must be pointed out that in our case the parasites and definitive hosts differed mostly from those used by the latter workers.

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