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HETEROLOGOUS IMMUNITY STUDIES IN CALVES IN IRAN:

Schistosoma haematobium versus *Schistosoma bovis* and *Ornithobilharzia turkestanicum*

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ABSTRACT

In a series of heterologous immunity studies of *S. haematobium*, *S. bovis* and *O. turkestanicum* in calves, a large number of *S. haematobium* cercariae (21,000 cercariae) were used repeatedly as the immunization agent. At autopsy, 22 weeks after initial exposure, immature *S. haematobium* were detected. Thirteen weeks after initial exposure a group of calves was challenged with *S. bovis* and another group with *O. turkestanicum* cercariae. The result of this investigation showed that the calves had developed limited *S. haematobium* infections. The number of worms recovered by perfusion varied from 250 to 640 (378 ± 99) and the mean percentage of worms recovered was 1.8%. Most of the worms were found in the portal veins, but in one calf some larger worms were detected in the lower mesenteric veins of the large intestine with some deformated eggs in th caecum substances. No worms or eggs were found in the vesical plexus or bladder tissue.

The immune response which developed in calves with *S. haematobium* infection was considerable. The protective effects of immunity was measured by detecting the effects on the expected worm burden and tissue egg counts of the challenge infection compared with the control group. It was found that the mean reduction of worm recovery and tissue egg counts was 42.3%

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and 62.2% in group *S. haematobium* versus *S. bovis,* and 31.4% and 81.0% in group *S. haematobium* versus *O. turkestanicum* infection. A part of this experiment was the reverse of those reported by Hsu *et al.* (1966), who repeatly immunized rhesus monkeys with Iranian *S. bovis* cercariae and then challenged with Iranian *S. haematobium* and produced a strong protection.

This suggests that the immune phenomenon will occur in endemic areas like Khuzestan, Iran, and that under natural conditions this might reduce the severity of the disease in livestock.

INTRODUCTION

There are no previous records of *S. haematobium* infection in cattle. Kuntz and Malakatis (1955) exposed goats to *S. haematobium* cercariae and only a very few small worms were recovered; Leiper (1915) and MacHttie (1933) failed to infect sheep with *S. haematobium.* In this study we tried to expose calves to a large number of *S. haematobium* cercariae in order to observe the degree of development of the worms, and also understand the degree of prophylaxis produced.

Material and Methods,

In the present experiments, 4 calves 7-9 weeks old from Khuzestan Iran, were exposed to a total of 21,000 cercariae of *S. haematobium,* each in 3 inoculations at 4 weeks intervals (in each inoculation 7,000 cercariae were used). For each inoculation, cercariae were pooled from 20-30 *Bulinus truncatus* which had been infected from a human source in the laboratory.

Thirteen weeks after initial exposure, the calves were challenged with *Schistosoma bovis* or *Ornithobilharzia turkestanicum* cercariae in order to stury cross-immunity against animal schistosomes.

For infection, the foot-immersion technique of Massoud (1973) was used. The worm recovery technique was similar to that used by Cheever (1968) on human cadavers. From each organ, 20 grams were digested in 10 volume of 4.5% Potassium hydroxide (KOH) and incubated 5 hours at 50C. After inculation, when all the tissues were digested, egg counts were performed.

RESULTS

Experiment 1 :

S. haematobium versus *S. bovis*

The calves were divided into an immunized Group I and a non-immunized Group II. In Group I, the calves were first immunized with cercariae of *S. haematobium* and then challenged with cercariae of *S. bovis.* The immunization of calves in Group I was by 7,000 *S. haematobium* cercariae for each exposure at 4-week intervals, giving a total of 21,000 cercariae per calf.

It was found that the mean worm recovery of*S. bovis* in immunized Group I was much lower than that in the control Group II, with a reduction of 42.3% (Table 1). Distinguishing *S. haematobium* from *S. bovis* was easy, since all of the *S. haematobium* worms were very small and immature with undeveloped internal organs.

S. bovis egg counts in the liver, small intestine and large intestine were very much reduced in Group I as compared with control Group II. The mean reduction in *O. turkestanicum* egg load was 62.2% (Table 2).

Experiment 2:

S. haematobium versus *Ornithobilharzia turkestanicum*

The procedure of this experiment was mainly the same as in Experiment 1. The mean recovery of adult *O. turkestanicum* was 31.4 % lower in challenge Group I than in control Group II. The corresponding reduction in tissue egg count per gram was 81.0% (Table 3 & 4).

At autopsy, 22 weeks after intial exposure to *S. haematobium* cercariae, it was found that all 4 calves had developed limited *S. haematobium* infections. The number of worms recovered by perfusion varied from 250-640 (378 ± 99) and the percentage of worms recovered was 3.0%, 0.9%, 1.9% and 1.2% with a mean of 1.8%.

In tissue digestion for egg counts, only calf No. 1 revealed a few deformed, rudimentary black eggs with no miracidia. Most of the worms were found in the portal veins, but in calf No. 1 some large worms with deformed intra-uterine eggs were recovered from the lower mesenteric veins of the large worms with deformed intra-uterine eggs were recovered from the lower mesenteric veins of the large intestin and som non-viabl eggs from the caecum substances, but no worms or eggs were found in the vascical plexus of the bladder wall.

DISCUSSION

Records of *S. haematobium* infection in animals are rare. Kuntz and Malakatis (1955) exposed 3 goats to about 200,000 cercariae of *S. haematobium* each, and succeeded in infecting 2 of them. However, the number of parasites recorded was extremely small. Leiper (1915) and MacHattie *et al.* (1933) were unable to infect sheep with *S. haematobium*. Saoud (1966) also failed to infect pigs with *S. haematobium* in the laboratory.

Our experiments with *S. haematobium, S. bovis* and *O. turkestanicum* show that success in experimentally infecting calves depends largely upon the technique used. With an efficient technique, calves were shown to take *S. haematobium* infection, but the worms recovered were immature. This may also happen to calves in nature in endemic areas, since they live in very close contact with infested natural waters.

The only previous reports of heterologous studies in calves were by Hussein *et al.* (1970), and Massoud *et al.* (1972), who reported a high degree of partial protection. In these experiments, *S. haematobium* in calves failed to develop to maturity. Nevertheless, immunization with 3 inoculation of *S. haematobium* cercariae produced very strong cross-protection against *S. bovis* and *O. turkestanicum*. A part of these experiments was the reverse of those reported by Hsu *et al.* (1966), who immunized rhesus monkeys repeatedly with Iranian *S. bovis* cercariae and then challenged them with Iranian *S. haematobium* and produced strong protection.

The conclusion is that natural heterologous immunity between human and bovine schistosomes could be of great importance in protecting animals from the severe effects of subsequent infections and reducing the dieases in livestock.

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TABLE 1

Experiment 1 - Immunization against S. bovis with cercariae of S. haematobium in calves: effect on adult worm counts

Group	Calf No.	Number of Cercariae			Autopsy (Weeks after Initial Exposure)	Worm Recovery						Average Number of S.bovis Adults	Reduction in S.bovis Adult Loads %
		Initial Exposure to S.haem.	Interval (weeks)	Challenge Exposure with S.bovis		Female		Male		Total			
						S.ha.	S.b.	S.ha.	S.b.	S.ha.	S.b.		
Group I													
S.haem. X	1	21,000 (7000 X3)	13	5,000	22	251	1104	167	934	418	2038	1819	42.3
S.bovis	2	"	13	5,000	22	111	794	139	806	250	1600		
Average						181	949	153	870	334	1819		
Group II	1	---	---	5,000	9	---	1250	---	1533	---	2783	3156	
S.bovis only	2	---	---	5,000	9	---	1661	---	1669	---	3530		
Average							1455		1701		3156		

TABLE 2

Experiment 1 - Immunization against S. bovis with cercariae of S. haematobium in calves: effect on tissue egg counts

Group	Calf No.	Number of Cercariae			Autopsy (Weeks after Initial Exposure)	Tissue Egg Counts per Gram						Average Number of S.bovis eggs/gm	Reduction in S.bovis eggload (mean)%
		Initial Exposure to S.haem.	Interval (weeks)	Challenge Exposure with S.bovis		Liver		Small Intestine		Large Intestine			
						S.ha.	S.b.	S.ha.	S.b.	S.ha.	S.b.		
Group I													
S.haem. X	1	21,000 (7000 X3)	13	5,000	22	0	2900	0	1600	0	380	1232	62.2
S.bovis	2	"	13	5,000	22	0	1400	0	750	0	480		
Average							2150		1175		430		
Group II	1	---	---	5,000	9	---	3597	---	1350	---	7312	3312	
S.bovis only	2	---	---	5,000	9	---	2556	---	2927	---	2130		
Average							3076		2138		4721		
Reduction of S. bovis eggs per gram of tissue (%)							30.1		45.0		90.8		

TABLE 3

Experiment 2 - Immunization against *O. turkestanicum* with cercariae of *S. haematobium* in calves: effect on adult worm counts

Group	Calf No.	Number of Cercariae			Autopsy (Weeks after Initial Exposure)	Worm Recovery						Average Number of *O.turk.* adults	Reduction in *O.turk.* adult loads %
		Initial Exposure to *S.haem.*	Interval (weeks)	Challenge Exposure with *O.turk.*		Female		Male		Total			
						S.ha.	*O.t.*	*S.ha.*	*O.t.*	*S.ha.*	*O.t.*		
Group I *S.haem.* X *O.turk.*	1	21,000 (7000 X3)	13	8,000	22	186	1199	454	1530	640	2729	2230	31.4
	2	"	13	8,000	22	43	828	161	903	204	1731		
Average						114	1013	308	1216	422	2230		
Group II *O.turk.* only	1	---	---	8,000	9	---	1022	---	1420	---	2442	3251	
	2	---	---	8,000	9	---	824	---	1028	---	1862		
	3	---	---	8,000	9	---	2377	---	3073	---	5450		
Average							1411		1840		3251		

TABLE 4

Experiment 2 - Immunization against O. turkestanicum with cercariae of S. haematobium in calves: effect on tissue egg counts

Group	Calf No.	Number of Cercariae: Initial Exposure to S.haem.	Number of Cercariae: Interval (weeks)	Number of Cercariae: Challenge Exposure with O.turk.	Autopsy (Weeks after Initial Exposure)	Tissue Egg Counts per Gram: Liver S.ha.	Liver O.t.	Small Intestine S.ha.	Small Intestine O.t.	Large Intestine S.ha.	Large Intestine O.t.	Average Number of O.turk. eggs/gm	Reduction in O.turk. eggload (mean)%
Group I S.haem. X O.turk.	1	21,000 (7000 X3)	13	8,000	22	16	453	33	3040	40	---	1706	81.0
	2	"	13	8,000	22	0	212	0	3118	0	---		
Average						8	333	16	3019	20			
Group II O.turk. only	1	---	---	8,000	9	---	280	---	22830	---	---	8985	
	2	---	---	8,000	9	---	116	---	7773	---	---		
	3	---	---	8,000	9	---	816	---	22100	---	---		
Average							404		17567				
Reduction of O. turk. eggs per gram of tissue (%)							17.5		82.4				