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Schistosomiasis (mansoni) has a negative impact on serum levels of estradiol, progesterone and prolactin in the female baboon *(Papio cynocephalus anubis)*

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Summary
The baboon *(Papio cynocephalus anubis)* is a well-established discriminative model for human reproduction as well as for studies of human schistosomiasis (mansoni).
The present study examined the impact of experimental *Schistosoma mansoni* infection on ovarian hormones and prolactin in 28 female baboons (6-8 Kg).
Serum concentrations of estradiol, progesterone and prolactin were recorded 2 and 11 weeks post secondary infection challenge dose (1000 cercariae). Lower than normal levels (serum estradiol < 50 pg/ml, serum progesterone < 1 ng/ml and serum prolactin < 2ng/ml) were found in the circulation, particularly 11 weeks after the challenge, when 20% of the animals had low hormone levels, which may be indicative of an anovulatory ovary.
The results suggest that schistosomiasis may be a contributing factor to reduced fecundity among women in endemic regions.

Introduction
Detailed studies on exposure, intensity of infection and development of pathology in schistosomiasis as related to gender, age and prior exposure are very limited in experimental animals and have been only indirectly studied in humans. With regard to pathology, ultrasound examinations of human livers for fibrosis in children correlate with intensity of infection (*Mohamed-Ali et al., 1991*), but in adults are strongly associated with gender and duration of infection (*Mohamed-Ali et al., 1999*).
Typically, women in schistosomiasis endemic areas become infected during early childhood and usually maintain infection into middle age. Stress induced cessation of estrus cyclicity and prolonged periods of an anovulatory ovary is well described in the human; but the potential disruption of the estrus cycle, and subsequent reduction in fertility as the result of schistosoma infection in endemic areas has received very little attention.
Praziquantel is the drug of choice for treatment of schistosomiasis. In praziquantel-treated schistosoma infected mice serum concentrations of progesterone and 17 β-estradiol decreased significantly 30 days after treatment following an early increase (*Abdalla et al., 1994*). It appears likely that the estrus cycle in these animals will have been affected by these hormonal changes.
The aim of the present study was to examine the impact of experimental schistosomiasis on ovarian function in the baboon, *Papio cynocephalus anubis,* to test the hypothesis that schistosomiasis induced stress may severely impair fertility through the induction of anovulatory ovaries in young adult females. The Kenyan baboon has been utilized in schistosomiasis research because of a multiplicity of features that make them more appropriate models than rodents (*Damian et al., 1992; Nyindo & Farah, 1999, Farah et al., 2001*).
It is well established that the age-related changes

in the hormonal function of the adrenal and gonadal glands in the baboon significantly correlate with similar changes in humans (*Goncharova & Lapin, 2000*). The pathophysiological responses in baboon schistosomiasis are very similar to those of the human. Hence it is a most discriminative high fidelity animal model for human schistosomiasis (*Nyindo & Farah, 1999; Farah et al., 2001*).

Materials and Methods.

Animals

Twenty-eight female baboons (*Papio cynocephalus anubis)*, 6–8 Kg at the start of the study, whose main emphasis was not on reproduction, were used. The animals were screened for common bacterial, viral and parasitic infections and tuberculin tested, according to the standard operating procedures, at the Institute of Primate Research in Nairobi, Kenya. They were found negative for prior patent schistosomiasis infection by both the Kato technique (*Katz et al., 1972*) and serology by assaying for specific IgG against soluble schistosome worm antigens (*Farah et al., 2000*).

The animals were housed in single outdoor cages. They were fed on Monkey cubes (Unga feeds, Kenya) twice a day and water was provided ad libitum. In addition, fresh fruits and vegetables were provided three times a week.

Parasites and infection schedules

S. mansoni eggs obtained from an infected human in Machakos district, Kenya, were used to infect naive freshwater snails, *Biomphalaria pffeifeiri*, maintained at the Institute of Primate Research. Cercariae shed from the snails were quantified and used to infect the baboons. All infections were done percutaneously by the pouch method (*Sturrock et al., 1976*), and all baboons received a combined dose of 1,000 cercariae.

All the animals were treated with an oral dose of praziquantel (PZQ; 60mg/kg body weight) given on weeks 19, 27 and 30 post primary infection. The secondary challenge (1,000 cercariae) was done at four weeks after the last PZQ treatment.

Blood sampling and worm recovery

Peripheral venous blood was obtained every 2 to 3 weeks throughout the course of the experiment. The animals were perfused 16 weeks after the secondary infection to recover worms as described previously (*Smithers & Terry, 1965*).

Hormonal assays

Estradiol (estra-1,3,5(10)-triene-3,17β-diol), progesterone and prolactin were quantified in baboon serum samples by radioimmunoassay using commercial kits (Immunotect, Marseille, France) at weeks 2 and 11 post secondary infection. All samples were analyzed in duplicate according to the manufacturer's instruction manuals.

Ethical Review

The Institutional Ethical and Scientific Review Committee at the Institute of Primate Research, Kenya approved the study design. The maintenance and care of the baboons complied with the National Institutes of Health (Institute of Laboratory Animal Resources) guidelines for the humane use of laboratory animals.

Results

Serum concentrations of estradiol, progesterone and prolactin were measured in serum samples obtained 2 and 11 weeks post secondary infection and the results are presented in Table 1ab along with the numbers of worms recovered at the termination of the experiment.

Animals were defined as fertile with a likely undisturbed estrus cycle when they had estradiol serum concentrations > 100 pg/ml or progesterone levels > 2 ng/ml. In contrast, animals with serum estradiol concentrations < 50 pg/ml and progesterone concentrations < 1 ng/ml and prolactin concentrations < 2 ng/ml were categorized as anovulatory, probably acyclic and thus infertile. Using this definition, 9 out of 24 animals had a positive fertility score 2 weeks post infection and 3 out of 24 a negative score. The latter animals had consistent high numbers of worms recovered (> 250) after the experiment. By week 11, only 7 out of 25 animals had positive fertility scores and 5 of the 25 animals had

Table 1
Serum hormone concentrations in samples obtained from challenged infected animals as well as number of worms recovered after perfusion at the termination of the experiment. The criterion used for "disturbed cycle" was prolactin levels < 2 ng/ml and progesterone levels < 1 ng/ml and estradiol levels < 50 pg/ml. The criterion used for "normal cycle" was progesterone levels > 2 ng/ml or estradiol levels > 100 pg/ml.
v= classification of estrus cycle.
ND = Not Done.

Table 1a

2 weeks post challenge infection

Baboon Number	Prolactin ng/ml	Progesterone ng/ml	Estradiol pg/ml	Parasite Count	Disturbed Cycle	Normal Cycle
1541	2.8	11.59	49	673		v
1777	5.9	0.18	138	187		v
1804	1.8	0.25	53	292		
1826	3.2	5.00	50	55		v
1905	1.7	0.25	50	138		
1930	ND	4.70	54	163		v
1979	1.9	0.18	49	468	v	
2008	1.5	0.25	ND	302		
2010	0.9	0.22	49	258	v	
2069	ND	0.25	76	133		
2072	3.2	0.24	99	415		
2074	2.6	0.18	49	384		
2083	1.7	0.25	50	600		
2093	3.4	0.25	149	288		v
2111	1.7	0.25	50	263		
2127	3.2	0.25	52	218		
2129	3.5	5.90	49	169		v
2130	2.8	7.50	57	350		v
2138	2.3	0.25	83	120		
2147	1.4	0.19	49	523	v	
2148	ND	0.25	50	266		
2151	11.1	5.70	ND	652		v
2152	4.3	0.25	60	243		
2154	2.2	0.25	135	120		v

Table 1b

11 weeks post challenge infection

Baboon Number	Prolactin ng/ml	Progesterone ng/ml	Estradiol pg/ml	Parasite Count	Disturbed Cycle	Normal Cycle
1541	0.9	0.18	73	673		
1777	2.8	0.18	156	187		v
1804	0.6	0.18	49	292	v	
1826	4.3	6.60	64	55		v
1905	0.7	2.60	63	138		v
1930	1.4	5.20	122	163		v
1979	0.6	0.26	49	468	v	
1980	1.1	0.16	55	290		
2008	1.5	0.45	81	302		
2010	0.6	0.18	49	258	v	
2069	1.4	0.18	54	133		
2072	0.6	0.18	ND	415		
2074	1.0	ND	49	384		
2083	1.0	0.16	49	600	v	
2093	0.6	0.18	90	288		
2111	0.6	0.43	62	263		
2127	2.1	ND	78	218		
2129	3.5	0.18	82	169		
2130	3.8	6.20	68	350		v
2138	1.4	0.18	83	120		
2147	0.6	0.16	52	523		
2148	0.7	0.18	49	266	v	
2151	3.4	12.40	91	652		v
2152	2.8	0.18	73	243		
2154	3.0	3.40	84	120		v

negative scores. Prolactin serum levels were lower in 16 of 21 animals by week 11 compared with week 2 post secondary infection.

Discussion

There are few reports on the potential negative impact of schistosomiasis (mansoni) on human reproduction and fertility. It has been demonstrated, however, that the frequency of stillbirth or infant mortality was 16% among expectant mothers who had a previous record of schistosoma infection compared to 8% among those who had no history of schistosomal infection (*Patana et al., 1995*). The present study is the first in a series planned in mouse and baboon models to address the influence of this parasitic disease on fecundity.

Experimental infections have been demonstrated to have a negative impact on pregnant mice resulting in fetal loss in a significant proportion of the animals (*Bindseil et al., 1989; Bindseil et al., 1990; El-Nahal et al., 1998*). In infected mice with acute schistosomiasis the serum level of progesterone was significantly lower than in non-infected control animals, probably due to the atrophy of corpora lutea (*Tiboldi et al., 1979*).

In the present study challenge with a new dose of schistosoma cercariae in already infected baboons resulted in dramatic hormonal changes indicative of a disruption of the ovarian cycle in approximately 20% of the female baboons. Fertility or estrus cycle state cannot be accurately determined from hormone values obtained from single samples collected on selected time points after an infection. For this, the pattern of hormone secretion over time must be assessed. However, all of these animals had very low levels of estradiol, progesterone and prolactin. It is noteworthy that the worm counts of these animals were all high. However, the presence of two animals with very high worm count and normal hormone serum levels clearly demonstrated that a high infectious burden is not necessarily associated with a cessation of the estrus cycle. After the challenge dose a progressive negative impact on the hormonal status of the animals became apparent. Two weeks after the challenge, the fertility, as judged from the hormonal data, seemed unaffected in 9 out the 25 animals. Eleven weeks after the challenge, however, only 7 of the animals seemed to have normal hormone values. This deterioration during the course of the infections was confirmed by an overall reduction in serum concentration of prolactin from week 2 to week 11 post secondary infection in 16 out of the 25 females. The physiological role of prolactin in the baboon is not known in detail, but it has been reported to act as an anabolic and stress-modulating hormone (*Dorshkind & Horseman, 2000*); and both in the rhesus monkey (*Laudenslager et al., 1999*), and in the African green monkey (*Suleman et al., 2001*), stress results in a decrease in prolactin levels in the circulation. Interestingly, it has been reported that the uterus also synthesizes prolactin in the baboon during the estrus cycle as well as during pregnancy (*Frasor et al., 1999*).

The ultimate test for fertility would have been to mate the female baboons with normal males and establish which of the females conceived. Unfortunately the design of this study did not allow this.

Considering the close homology between the baboon and the human with respect to reproductive physiology and etiology and course of schistosoma infection (*Farah et al., 2001*), the results obtained in the present study may be of relevance to the increasing body of evidence pointing to *Schistosoma mansoni* as contributing to infertility problems in endemic regions.

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