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THE ORAL ROUTE AS A POTENTIAL WAY OF TRANSMISSION OF *SCHISTOSOMA BOVIS* IN GOATS

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ABSTRACT: The infectivity of *Schistosoma bovis* cercariae administered orally was evaluated in Sahelian goats. Compared to the percutaneous route, a single massive oral dose resulted in a worm burden and in fecal egg excretion reduced by one-half. Surprisingly, tissue egg counts were increased by more than 4-fold. Fecundity of individual female schistosomes was, therefore, markedly increased. When infective doses were administered weekly for 20 wk, both worm and egg burdens were doubled without modification of the individual worm pair fecundity. Repeated oral infections seem to have induced an acquired tolerance toward parasite antigens. These results confirm the epidemiologic relevance of the oral route in a host species inclined to become infected through drinking water rather than percutaneous exposures.

Unlike most parasitic species, schistosomes affecting humans and livestock penetrate into their definitive host by actively crossing the skin (Sturrock, 1993). The possible contribution of the oral route to schistosome transmission has been poorly explored. However, a good example of its potential is provided by the model of small ruminants infected with *Schistosoma bovis*, the agent of the main bilharziosis affecting livestock in Africa.

Epidemiologic surveys in West Africa devoted to *S. bovis* have shown that, in bovines, prevalences could locally reach 80% (Rollinson et al., 1990). In contrast, screenings performed on small ruminants found only limited numbers of animals harboring active *S. bovis* infections (Fritsche et al., 1993). In Niger, the prevalence recorded in the slaughterhouse of Niamey peaked at 3.3% in sheep and at 3% in goats (Mouchet et al., 1989). However, small ruminants, especially goats (Saad et al., 1984), display a sensitivity to the parasite pathogenic impact comparable to what is observed in bovines (Saad et al., 1980). Yet, in contrast to cattle, small ruminants are reluctant to enter water and usually contact contaminated water only when drinking. Using radiolabeled cercariae, an oral route has been demonstrated as an actual mechanism of transmission in goats (Kassuku et al., 1985). A smaller propensity for contact with drinking water as a source of contamination could explain the low prevalence of *S. bovis* in small ruminants. To examine this hypothesis, we have compared, in caprines, the development of an experimental infection performed with a single massive dose of cercariae applied either on the skin or in drinking water. In order to parallel more closely the natural conditions of infection encountered in the field, 20 light doses of larvae were also administered per os every week for 5 mo. The results of this comparison are shown in the present paper.

MATERIALS AND METHODS

Animals

Eighteen Sahelian goats (mean weight ± SD at the start of the experiment = 17.2 ± 4.6 kg) were purchased in Niamey (Niger) city market. Animals were kept for 1 mo in quarantine and treated for intestinal nematodes (Pyrantel tartrate, Exhelm®, Pfizer, Orsay, France) and coccidia (sulfadimidine + diaveridine, Darvisul®, Mallinckrodt, Meaux, France). The parasitic status of the goats was confirmed by repeated negative fecal examinations. The goats were maintained in open-air pens with free access to a permanent shelter. The diet of the goats was composed of dried bean leaves and of millet bran.

Infection procedure

The schedule of infection is shown in Table I. The same local strain (Mada, central Niger) of *S. bovis* maintained in *Bulinus truncatus* snails was used for the 3 experiments. The required number of cercariae was manually counted. For the percutaneous infection, goats were anesthetized with xylazine (Rompun®, Bayer, Leverkusen, Germany), then infected for 45 min, using the ring method (Hussein, 1971). The mean (± SD) percentage of nonpenetrating cercariae, determined by binocular examination of the ring washing fluid, was 2.0 ± 1.9. For the oral infection, cercariae were suspended in about 1 L of H_2O kept in a glass container. Preventing the animals from accessing water for 12 hr before infection resulted in a rapid completion of drinking in all cases. Binocular examination of the container surface allowed the calculation of a mean percentage of cercarial absorption, which ranged from 94.1 to 98.7% (mean ± SD = 96.0 ± 1.3).

Clinical and parasitologic follow-up

Body weight and packed cell volume (PCV) values from heparinized blood were measured weekly. To obtain comparable data, fecal eggs were monitored for 19 wk after the single infection (groups 1 and 2) or last-trickled dose (group 3) by a modification of Bell's method (1963) from samples weighing approximately 2 g collected once weekly from the rectum. On each day of collection, all the animals were isolated in individual stalls for 24 consecutive hours. The stools excreted during this period were weighted, permitting an estimate of the daily egg excretion. After goats were killed (via heparinized pentobarbital), major vessels were clamped and the abdominal organs except the liver were removed. Worms were recovered by dissection from the mesenteric vessels by careful examination of the entire gut. Separate liver perfusion was performed by injection of citrated saline under pressure through the aorta and recovery from the portal vein. Then, worms were counted and sexed. Less than 5% of total worm recovery was found in the portal system and these were mainly single male worms. Results are expressed as worm pairs, that is, number of females, and as total number of worms (number of pairs + number of single males). Tissue egg counts were performed by KOH digestion from an approximately 25% tissue sample according to a previously described method (Bushara et al., 1980). Records of the organ weights allow the calculation of total tissue eggs per organ. Because the follow-ups were performed at a late stage of infection, parasitologic data from animals that died before the end of the experiment were included in the means calculations.

Data comparison

Because the data distribution was not normal, statistical comparisons were made by the Mann–Whitney *U*-test for nonpaired values and by the Spearman rank correlation coefficient for correlations analysis.

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TABLE I. Schedules of infection of goats with *Schistosoma bovis*.

	Group 1	Group 2	Group 3
Number of animals	6	6	6
Mode of infection	Percutaneous	Oral	Oral
Number of cercariae/dose	2,000	2,000	100
Number of doses	1	1	20
Duration of infection (wk)	19	19	37

RESULTS

Fecal egg excretion

Daily egg excretion is shown in Figure 1. Eggs first appeared 6 wk after the single massive infection (groups 1 and 2) followed by 2 peaks on weeks 8 and 12. Peak intensities were higher in the percutaneous group. Eggs were not detected before 9 wk after the first trickle infection in group 3. Afterwards, the egg output increased slowly, but then dramatically increased 4 wk after the last trickle dose. Compared to the percutaneously infected group, the overall mean daily excretion of eggs in the feces was significantly lower (−53%, $P < 0.01$) in group 2 (1,423 eggs/day vs. 2,999 eggs/day in group 1), but almost twice as high in group 3 (5,549 eggs/day, $P < 0.01$).

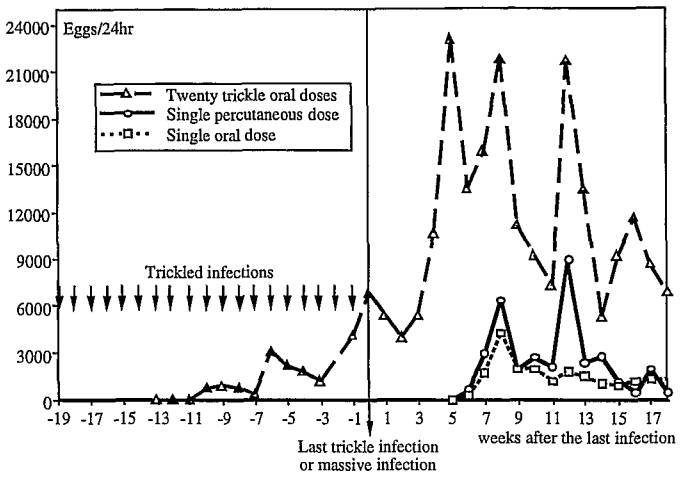

FIGURE 1. Compared kinetics of the daily fecal eggs in the 3 groups of goats experimentally infected with *Schistosoma bovis*.

Clinical data

Overall, PCV values were significantly ($P < 0.05$) and negatively correlated with the intensities of fecal egg excretion. Group 2 experienced only a limited PCV decline, whereas groups 1 and 3 were more seriously affected (data not presented). Body weight losses were also observed, but with smaller and not significant differences between groups (data not presented). In group 1, 2 animals of 6 died 14–16 wk after the percutaneous exposure. Massive oral infection (group 2) did not result in any death. In contrast, 1 goat died in week 20 and a second in week 33 when the oral infective dose was trickled (group 3). All deaths were attributed to acute schistosomiasis.

Perfusion data

Perfusion data are summarized in Table II. Compared to group 1, worm and tissue egg burdens were increased in group 3, but only the number of female worms was significantly higher. Massive single oral infection (group 2) elicited not only a significantly ($P < 0.05$) lower worm burden, but also a much higher number of tissue eggs compared to the percutaneous group ($P < 0.01$). Females were reduced by more than one half, but tissue eggs were multiplied by more than 3. As a consequence, the number of tissue eggs per female was considerably higher in group 2 compared to the other 2 groups ($P < 0.01$). Tissue egg distributions were somewhat erratic. However, the highest proportion of eggs was found in the small intestine in groups 1 and 3, whereas egg deposition was more equally distributed in group 2.

Synthesis

Figure 2 shows a comparison between both schedules of oral infection and the percutaneous route. Results are expressed as the ratio orally infected group mean : percutaneously infected group mean. To use the oral rather the percutaneous route to infect goats, a massive single dose of *S. bovis* (group 2) yielded about one half of the number of female worms and fecal eggs, but 4 times more tissue eggs than in group 1. As a consequence,

TABLE II. Adult worm recoveries and tissue egg counts in goats infected with *Schistosoma bovis* according to the schedules shown in Table I. Results are expressed as mean ± SD.

	Group 1	Group 2	Group 3
Number of animals	6	6	6
Adult worm recoveries			
Worm pairs	102.0 ± 38.2	42.3 ± 11.2*	224.3 ± 113.7*
Total	318.5 ± 214.3	89.7 ± 35.2*	448.0 ± 237.4
Tissue egg counts			
Liver (%)	18 ± 17	38 ± 11	30 ± 10
Small intestine (%)	50 ± 6	27 ± 14	51 ± 15
Large intestine (%)	32 ± 23	35 ± 3	19 ± 19
Total (×1,000)	2,011 ± 962	8,820 ± 927†	3,496 ± 3,840
Total/female (×1,000)	23.1 ± 18.1	218.4 ± 64.5†	31.0 ± 46.1

* $P < 0.05$ compared to group 1.
† $P < 0.01$ compared to group 1.

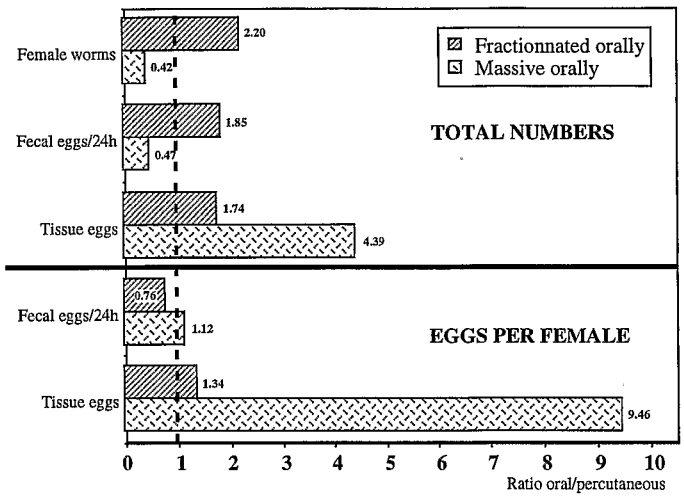

FIGURE 2. Parasitologic parameters of the 2 groups of goats orally infected with *Schistosoma bovis* compared to the percutaneously infected group (vertical dotted line: ratio = 1.00).

the fecal egg output per female was not modified (ratio = 1.12), whereas the mean number of tissue eggs per female was increased by more than 9-fold. On the other side, infection with an equivalent total dose of cercariae (2,000), but weekly and orally administered (group 3), resulted in a parallel increase of worm, fecal, and tissue egg burden compared to group 1, suggesting that the fecundity of each female schistosome was similar in both groups.

DISCUSSION

Although it resulted in one-half fewer worms and fecal eggs compared to the percutaneous route, single oral infection has confirmed an epidemiologic potential already suggested in the same model (Kassuku et al., 1985; Kassuku et al., 1986). The observed lesser efficacy has several possible explanations, for example, mechanical barrier due to corneous epitheliums impassable on some parts of the proximal digestive tract (buccal cavity, rumen, and so on), nonspecific inactivation of cercarial metabolism by mucous secretions, and induction at the site of penetration of a specific immune response preventing some of the larvae from completing their internal migration (Long et al., 1980). Indeed, one major limit in the experimental study of oral infection is the doubt about the number of cercariae that actually reach the main bloodstream.

The most original observation concerns the tissue egg counts, which were dramatically increased. In baboons infected with *Schistosoma mansoni,* eggs trapped in the tissues are believed to account for 75% in the overall female schistosome egg-laying productivity (Damian and Chapman, 1983). By extrapolation to our model, it seems that individual worm fecundity, as well as the total number of laid eggs, have been exacerbated by the oral route of infection, discounting the crowding effect hypothesis (Woolhouse et al., 1994). Alleviation of a physiological inhibition of worm-pair fecundity also is a possible explanation. The control by the immunocompetent host of schistosome vitality, or fertility, or both, is a common observation made in laboratory rodents (Rashed et al., 1996), in primates (Damian et al., 1986), in ruminants (Bushara et al., 1983), and also suspected in humans (Agnew et al., 1996; Karanja et al., 1997). The alternative explanation would be reduced tissue-egg destruction, based on immune tolerance to egg antigens (Doenhoff et al., 1986). In both cases, the mechanisms by which the method of infection could influence the immune sensitization to schistosome antigens remain unclear.

Numerous publications have shown that mucosal administration, especially per os, of defined antigens elicits immune responses quite distinct from what is observed after parenteral injection (McGhee et al., 1992). Crossing by a schistosome larva of a mucous epithelium in the orodigestive area, which is rich in specialized lymphoid tissue (bronchus-associated lymphoid tissue [BALT] and gut-associated lymphoid tissue [GALT]), is indeed propitious in the recruitment of cell populations different from those located in the skin (Croitoru and Bienenstock, 1994). Consequently, specific responses to the parasite can be preferentially oriented toward tolerance instead of defence pathways.

In an effort to more closely reproduce field conditions, the second aim of the present study was to attempt, for the first time, a trickled infection procedure via the oral route. Considering the 3 parasitologic parameters that were assessed (worm burden, fecal egg excretion, and tissue egg counts), this method of challenge was twice as successful when compared to the classical single massive cercarial dose administered percutaneously. However, the fecundity of the established worm pairs was not modified. The potential of light-trickled doses has already been evaluated in the context of schistosomiasis, but only by using the percutaneous route. Results differed according to the model. For example, compared to singly-infected controls, baboons trickle-infected with *Schistosoma haematobium* (Reid et al., 1995) or by *S. mansoni* (Damian et al., 1976; Farah et al., 1997) displayed lowered parasitologic criteria, whereas goats infected with *S. bovis* (our model) underwent more severe pathogenic effects after repeated infections (Monrad et al., 1995). A similar acquisition of resistance has been noted in baboons repeatedly infected with *S. mansoni* (Damian et al., 1974; Sturrock et al., 1984). Thus, our data suggest that goats do not protect themselves against small, trickled numbers of larvae by the oral route of infection, whereas they develop an acquired immunity when exposed to repeated percutaneous exposures (Lindberg et al., 1995). The route of cercarial penetration can explain these differences. As previously noted, the induction of tolerance to antigens is a classical phenomenon when they are administered repeatedly per os (Chen et al., 1995). Local or general clonal depletion and the development of suppressive T-cell responses are the most frequently proposed mechanisms. Indeed, repetition of an antigenic stimulus in a common lymphatic drainage is known to favor the production of immunosuppressive cells, including in the murine model of schistosomiasis mansoni (Pemberton and Wilson, 1995).

Taken as a whole, our results exclude a weak infective yield of the oral route as a hypothesis to explain the differences of prevalence observed between small and large ruminants. Therefore, it is likely that the number of cercariae actually swallowed with drinking water is much lower than the number of larvae able to cross the skin of bovines when they stand in contaminated water.

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