



Emergence of infections with *Schistosoma mansoni* in the Dhofar Governorate, Oman

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Abstract

Infections with *Schistosoma mansoni* were identified in an area of Dhofar (Oman), where this parasite had been virtually absent during recent years and was reported only very sporadically before 1992. In the present survey, performed late in 2001, between 1 and 13% of children ($n = 519$) were found to excrete eggs (one Kato-Katz-smear) in four schools, from four different villages, but no infections were detected in additional five schools ($n = 281$). Infections were light (<72 eggs/g of faeces) in 19 of the 36 children found infected. Serologic examination of sera ($n = 511$) was done by ELISA (based on soluble worm antigen) and immunofluorescence tests (IFT, based on cryostat sections of adult *S. mansoni*). The prevalence according to serological tests was between 3 and 43% in the four schools with infected children. Positive test results were taken to reflect active infections, since false positive reactions could largely be excluded. According to ultrasound (US) examinations performed on 96 individuals (children and adults) from the four villages, livers were normal in all except three cases of mild pathology, which could be assigned to schistosomiasis mansoni (pattern C, ages 32–40 years). All data suggest that transmission of *S. mansoni* has been re-introduced only recently in Dhofar and that this emergence of schistosomiasis is limited to at most a few foci.

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1. Introduction

The potential transmission of schistosomiasis mansoni in Oman has been a concern for some two decades after it was first diagnosed in a farm close to Salalah, the capital of the Dhofar Governorate, South of Oman (Arfaa, 1982; Githaiga, 1983). However,

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the prevalence of schistosomiasis in Oman was extremely low. The total number of notified cases due to infections with both *Schistosoma haematobium* and *Schistosoma mansoni* detected by routine passive surveillance between 1991 and 2001 was 74 (internal documents of Ministry of Health, Oman). The predominant part of those infections occurred in expatriate workers from schistosomiasis endemic areas in Africa. In general, infections with *S. mansoni* were reported from several provinces, but *S. haematobium* was reported only from the North. Snails with a potential to transmit schistosomes are known to occur all over Oman (Brown and Gallagher, 1985). However, in the most Southern Governorate, Dhofar, which is separated by nearly 800 km of desert from the northern provinces, the potential vector of *S. haematobium* (*Bulinus wrighti*) was detected only in one isolated effluent of a spring (Idris et al., 1994). By contrast, the potential vector snail for *S. mansoni* (*Biomphalaria arabica*) is wide spread in Dhofar (Wright and Brown, 1980; Idris et al., 1994) and occurred in 15 out of 28 water bodies which we recently surveyed (Moné et al., 2003). Thus, in spite of a wide distribution of intermediate host snails, schistosomiasis had been well under control in Dhofar (Shaban, 1995) and the incidence of schistosomiasis had fallen to zero (Scrimgeour et al., 1999).

In Dhofar, the first case of infection with *S. mansoni* was detected in 1979 (Shaban, 1995). A few autochthonous infections were reported by Githaigap (1983) and very low numbers of cases, ranging only between 2 and 17 per year, were reported until 1991 (National Surveillance Organization, Ministry of Health, Oman). No autochthonous infections were found between 1991 and 1995, only 2 in 1996 and one each in 1998 and 1999 (Awaity and Shaban, 2002). With respect to the case of 1999, eggs of *S. mansoni* were diagnosed in the stools of an 8-year-old boy from Dhofar, who had been hospitalized at Sultan Qaboos University Hospital, Muscat, because of neurological disorders, and who had never left Oman (Scrimgeour et al., 2001). During follow-up examinations performed in 2000 by the Directorate of Health Affairs of Dhofar, eight additional cases of schistosomiasis *mansoni* were detected among his larger family and further stool surveys identified additional six cases. A cooperative study was then started with the aim to clarify the situation of *S. mansoni* transmission in

Dhofar. This led to the first detection in the field of snails shedding cercariae of *S. mansoni* (Moné et al., 2003). Here, we report on a parasitological and serological survey among the school children from those villages where autochthonous schistosomiasis cases were recently identified.

2. Materials and methods

2.1. Study population

Stool and serum samples were obtained from school children (6–17 years old) from selected localities in the area suspected for active transmission. Details are given in Table 1. Informed consent was obtained from the heads of the families and participation in this survey was voluntary. Most of the children agreed to give blood, but the majority did not provide stool samples. Obtaining repeated stool samples was not accepted by the population. Stools were examined at the Public Health Laboratory in Salalah using a single 41.6 mg Kato-Katz-smear. Eggs of *S. mansoni* were calculated per gram of feces (eggs observed \times 24 = eggs/g); eggs of other parasites were recorded, but not quantified.

2.2. Serological tests

Serum samples were maintained frozen and tested in Heidelberg. ELISA was performed as described (Idris et al., 1994). Soluble antigen (1.35 mg protein per ml) was prepared from adult *S. mansoni* (Puerto Rican strain maintained in Heidelberg). Microwell plates (Maxisorp Nunc-Immunoplates; Nunc, Wiesbaden, Germany) were coated with antigen (12.5 μ g/ml in 9.6 M sodium carbonate buffer, 0.1 ml per well, over night, 4 °C), washed with 0.9% NaCl, 0.05% Tween 20 (polyoxyethylenesorbitan monolaurate; Sigma, Deisenhofen, Germany) and blocked (200 μ g per well, at least 1 h, room temperature) with 5% newborn calf serum (NCS; Gibco, Karlsruhe, Germany), 0.05% Tween 20 in 0.01 M phosphate buffered saline (PBS, pH 7.6). After 3 washes, sera (1:200 in PBS, 2% NCS) were added (100 μ l per well) and plates incubated for 2 h at room temperature. After washing, peroxidase-conjugated rabbit antibodies against human IgG (Sigma) were added (100 μ l per well with PBS, 2% NCS, 0.05% Tween) and left for 1.5 h. Following further washing, the

Table 1
Stool survey using a single Kato-Katz-smear in selected schools close to Salalah (Dhofar, 2001)

Name of village of school	Number of students examined	Stools with eggs of <i>S. mansoni</i>		Other intestinal parasites (hookworms)	
		Number	%	Number	%
Schools with at least one schistosome infection					
Sheer	210	27	12.9	35 (12)	16.7 (5.7)
Zeek, secondary	96	7	7.3	13 (9)	13.5 (9.4)
Estah, preparatory	63	1	1.6	11 (6)	17.5 (9.5)
Qeiron Heiriti, boys	150	1	0.7	36 (32)	24.0 (21.3)
Total	519	36	6.9	95 (59)	18.3 ^a (11.4)
Schools without detected schistosome infection					
Wadi Aion	42	0	0.0	12	28.6
Qeiron Heiriti, girls	95	0	0.0	12	12.6
Hajif, secondary	94	0	0.0	12	12.8
Adebdo, boys	28	0	0.0	12	42.9
Ashenhib, boys	22	0	0.0	9	40.9
Total	281	0	0.0	57	20.3 ^b
Grand total	800	36	4.5	151	18.9

^a In addition to hookworms (11.4%) the parasites were: *Ascaris* 3.1%; *Hymenolepis* 2.9%; *Giardia* 0.8%, *Trichuris* 0.2%.

^b Intestinal parasites were not specified.

substrate ortho-phenylenediamine (OPD, tablets of 30 mg; Sigma) was added (final concentration of 0.25 mg/ml of OPD in 0.025 M citric acid, 0.05 M Na₂HPO₄; 100 µl per well) and the reaction left to proceed in the dark at room temperature. After 15–20 min, the reaction was stopped with 2 M H₂SO₄ (30 µl per well) and the optical density (OD) recorded in an ELISA reader (MR5000; Dynatech, Guernsey, Channel Islands) at 490 nm with a reference filter at 630 nm.

Immunofluorescence tests (IFT) were performed with cryostat sections of adult *S. mansoni* fixed on slides and processed essentially as described (Ruppel et al., 1985). Sera were serially diluted (1:20 to 1:1280) into PBS (0.1 M phosphate, 0.9% NaCl, pH 7.2), applied to the sections (15–20 µl per place) and left to react 30 min at 37 °C. After washing, FITC-labeled goat globulins against human immunoglobulins (Fluoline-“H”; bioMérieux; Marcy-l'Étoile, France) appropriately diluted in PBS containing 0.01% Evans blue were applied for 30 min at 37 °C. The slides were washed again, covered with glycerol and viewed in a fluorescent microscope (Axioscop; Zeiss, Göttingen, Germany). Fluorescent reactions were determined separately for the parasite gut and

parenchyma and titers expressed as reciprocal serum dilutions.

2.3. Ultrasound (US) examinations

US examinations were performed in the local health station of Qeiron Heiriti. A total of 96 individuals were examined with the following age distribution: ≤10 years: 33; 11–20 years: 41; 21–30 years: 4; ≥31 years: 18. The examinations were performed using a portable device equipped with a 2–5 MHz convex transducer (Sonosite 180 plus, Bothell, USA). Ultrasonographic results were assessed according to WHO-standards (Richter et al., 2000).

2.4. Snail surveys

Snails were scooped in water bodies close to Sheer at repeated occasions between 2000 and 2002 (Moné et al., 2003). Another survey was done in September 2002. For the geographical coordinates of the sites, see Moné et al. (2003). Each water place was visited for 1–2 h and scooping done with 30 cm × 30 cm nets. Snails were transported to the laboratory in Salalah, screened for shedding cercariae and these

were microscopically identified as being of human schistosomes or other trematodes.

3. Results

Children of nine schools were included in this survey. A total of 800 stool samples were obtained and examined by one Kato-Katz-smear in late 2001. Eggs of *S. mansoni* were detected in 36 children. These came from four schools (Table 1; closed circles in Fig. 1). Most of the infections with *S. mansoni* were detected in Sheer, some in Zeek, only one child was found infected each in Estah and Qeiron Heiriti (boys), but no eggs were detected in the stools from the five remaining schools (Table 1; open circles in Fig. 1). Geographically, the four schools with at least one parasitologically positive child clustered in the mountains North of Salalah, and are separated by at least one steep valley (all running North to South) from other schools without detected infections and which are located in the North–East or North–West from the city. There are no water courses running West to East or the reverse direction which would link these areas. The percentage of children yielding a “parasitologically positive” result varied greatly among the 4 schools, ranging from 0.7 to 12.9%. The distribution of in-

Table 2

Intensity of infection among children of the four schools (Sheer, Zeek, Estah, Qeiron—boys; see Table 1) with at least one infected child (Dhofar, 2001)

Eggs detected per slide	0	1–3	4–10	11–20	>20
Calculated eggs/g stool	0	24–72	96–240	264–480	>480
Number of cases	483	19	8	8	1

tensity of infection based on egg counts is shown in Table 2. Among the 36 parasitologically positive children, 19 excreted only 72 or less eggs/g of stools, i.e. only 1–3 eggs were detected per slide.

Antibodies (IgG) against schistosome adult worm antigen were determined by ELISA for a total of 511 sera from the four schools with at least one egg-excretor. Negative control sera were from healthy German blood donors. Positive control sera were German patients with heavy infection for a known duration of about 2 months (Ruppel et al., 1985). Reactivity in ELISA was considered positive, if the OD value was above the mean value plus 2 standard deviations of negative control sera ($n = 7$). The percentage of serologically positive reactions is shown in Table 3. It was considerably higher than the parasitologically positive results. Importantly, positive OD

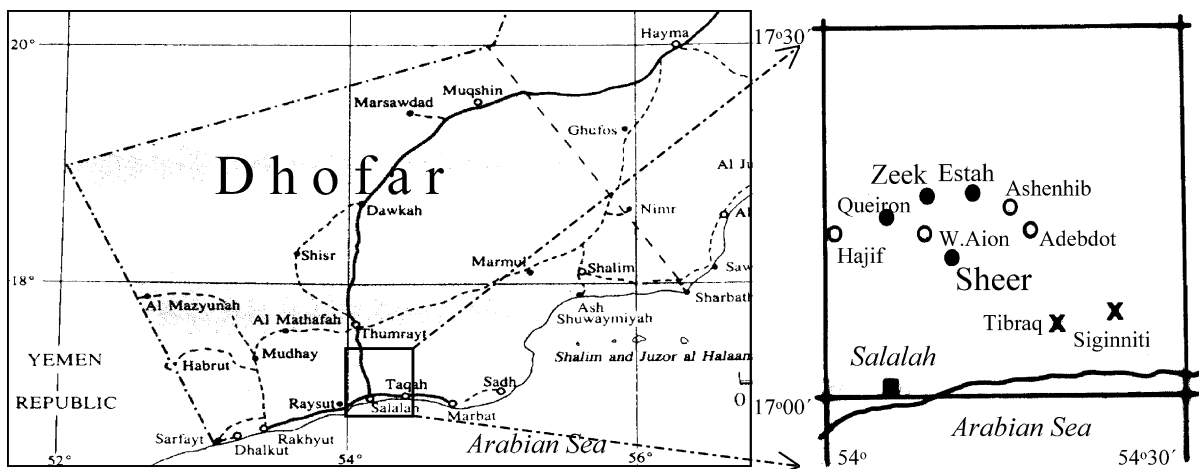


Fig. 1. Map (left) of the Dhofar Governorate (capital: Salalah), which is located in the South of the Sultanate of Oman at the border with the Yemen Republic. The rectangle indicates the study area, which is enlarged on the right. Locations are shown of schools where no *S. mansoni*-infected children (open circles) were detected, or where at least one infected child was found (closed circles; larger letters indicate higher prevalences; see Table 1). Two water bodies are shown (crosses), in which at least 1 (Siginniti) or a total of 43 (Tibraq) snails shedding cercariae of *S. mansoni* had been detected in previous surveys (Moné et al., 2003), but not in this study.

Table 3
Survey by ELISA of schools with at least one child excreting eggs (Dhofar, 2001)

Name of school	Number of students examined	Reaction of sera in ELISA			
		Negative (number)	Borderline (number)	Positive	
				Number	% ^a
Sheer	204	143	8	53	26.0
Zeek, secondary	144	76	6	62	43.1
Estah	63	56	0	7	11.1
Qeiron Heiriti	100	97	0	3	3.0
Total	511	372	14	125	24.5

^a The percent positive reactions were significantly ($P < 0.05$; Z-test) different between all schools.

values ($n = 125$) were well above the cut-off value ($OD = 0.14$) and borderline results with readings just above the cut-off value (OD up to 0.20) were obtained only in about 10% of all positive sera ($n = 14$). OD values obtained for individual sera with repeated determinations (100 sera tested) never varied more than about 10% and were never contradictory with respect to positivity or negativity.

Anti-schistosome antibodies (IgG plus IgM) were also tested by immunofluorescence tests (IFT) and titrated for gut- and parenchyma-associated reactions. A gut-associated reaction with a serum dilution of 1:40 or higher was considered positive. All ELISA-positive sera from Zeek were also positive in IFT. Among 20 randomly chosen ELISA-negative sera, only 1 was found positive in IFT. Thus, results with IFT confirmed those of ELISA. IFT reactivity was very pronounced with the parasite gut, but much weaker with the parenchyma. Titers of gut-associated IF were 1:1240 or more in 14 sera. Similarly high values are often obtained with German tourists presenting with a short but, heavy infection.

Ultrasound examinations were performed in the village of Qeiron Heirity on 96 individuals from the four villages from where infections were reported. Appearance of the majority of the livers was normal. Mild pathology, which could be undoubtedly assigned to schistosomiasis mansoni (image pattern C) was observed only in three men (age 32, 40, 40 years). Severe pathology was not observed. In particular, among the population studied, no case of advanced fibrosis, ascites or collateral circulation was observed.

Snails (*Biomphalaria arabica*) were collected from several water bodies (Zaikat, Wadi Ain, Aisham,

Jairah, Afilayiah, Sahanout, Aayyun, Hajeef; for geographical locations, see Moné et al., 2003) located close to the schools with at least one egg-excreting child (Zeek, Sheer, Estah, Qeiron). Upon screening in the laboratory in Salalah, none of the snails was found to shed cercariae of *S. mansoni*, neither in previous surveys (Moné et al., 2003) nor during an additional one in September 2002. On the other hand, two water bodies (see crosses in Fig. 1) in relative vicinity to the schools without detected egg-excreting children (Wadi Aion, Hajif, Adebdot, Ashenib) and which had been surveyed between November 2000 and February 2002 (Moné et al., 2003) yielded a few *S. mansoni*-infected snails: Tibraq (prevalence of infected snails decreasing from 7.9 to 0.4%) and Siginitti (0.2% in the first survey). However, among additional 1300 snails individually screened in September 2002, no infected *B. arabica* were detected from Tibraq ($n = 150$), Siginitti ($n = 268$) nor four additional water places (Sahanout, Birin, Arazat farm, Wadi Dharbat).

4. Discussion

This is the first evidence of emerging infections with *S. mansoni* in a community in Oman at large and specifically in Dhofar. Whereas in this Governorate the first case of schistosomiasis mansoni had been detected in 1979 (Shaban, 1995) and few additional cases in the years to follow (Githaiga, 1983), the number of egg-excreting individuals detected during the present survey by only one single stool examination surpasses in number the cumulated cases reported over the past 10 years in Dhofar. In addition,

the total number of individuals with clearly demonstrable anti-schistosome antibodies was at least three times more numerous. On the following grounds, these positive reactions in ELISA and IFT indicate that the respective children are infected.

First, the ELISA and IFT used here are highly specific for schistosomes. The absence of cross-reaction, at least with several other parasites, is shown by the low number of sero-positive children in schools with high percentages of other intestinal parasitoses (Estah, Qeiron Heiriti). Cross reactivity in IFT may occasionally occur in the schistosome parenchyma in IFT with malaria patients, but neither is malaria endemic in Dhofar, nor was parenchymal fluorescence observed in the absence of gut-associated fluorescence. Thus, false positive reactions can be excluded at least for the vast majority of the results.

Second, in the community studied here, schistosomiasis had not previously been detected and chemotherapy was never given, except possibly for very few cases. Consequently, antibody-reactivity cannot result from “immunological scars” following treatment and, thus, very likely represents active infection.

Third, testing for egg excretion has limited sensitivity. With low level infections, which predominate in this study, and when only one slide is screened, about one third of patients excreting 1 egg per slide (calculated as 24 eggs/g) and more than 10% of those with 2 eggs per slide are missed simply for statistic reasons (Lewert, 1984). In practice, also variation exists between stools obtained on consecutive days, and “much of this variation is undoubtedly contributed by persons with low egg counts, particularly those who had counts of zero” (Barreto et al., 1978). In a quantitative study to evaluate the Kato-Katz-technique, 45% of patients excreting 1–100 eggs/g (according to the Bell technique) were missed after examination of a single slide (Sleigh et al., 1982). For comparison, more than 50% of children in our study excreted less than 100 eggs/g. Also in a previous study in Sudan we obtained 33% negative results based on one Kato-Katz slide, but only 10% were negative in an antibody test (Ruppel et al., 1990).

Thus, we interpret our serologic data to indicate the “true prevalence” which ranged between 43 and 3%. Independent support for these values may be obtained from the correlation between prevalence as de-

termined by single stool examinations and the geometric mean of eggs counted per slide (de Vlas et al., 1997). The chart proposed by these authors to estimate the “true prevalence” of *S. mansoni* in a population can be applied, among the schools studied here, to Sheer: the prevalence based on the stool samples is about 13%, the geometric mean egg count is 3.516, and the “true prevalence” extrapolated from the chart is about 30%. This is compatible with our serology-based prevalence of 26%.

Epidemiologically it is most pertinent to evaluate, whether schistosomiasis mansoni had been present over the past years in Dhofar without having been recognized, or whether a recent introduction lead to this outbreak? Several arguments support the latter hypothesis.

First, eggs of *S. mansoni* were never observed in over 7000 stool samples examined during our previous surveys for hookworms (Idris et al., 1993, 1995, 2001) and schistosomiasis (Idris et al., 1994), nor from other stool tests performed at various health centres. The few cases of serologically positive individuals detected in the early 90s (Idris et al., 1994) did not pass detectable eggs and probably resulted from earlier infections in the eighties which may have been very mild or treated with praziquantel.

Second, snails shedding cercariae of schistosomes had never been reported, including our earlier survey of 16 water bodies, when several 1000 snails were screened (Idris et al., 1994). Only in late 2000 were snails shedding cercariae of *S. mansoni* detected for the first time in water bodies of Dhofar (Moné et al., 2003). Importantly, these sites (Tibraq and Siginitti; see Fig. 1) are located at some distance from the villages from where infections are reported here, whereas several waterbodies closer to these villages did not yield infected *Biomphalaria* in earlier surveys (Moné et al., 2003) nor in the present context. This apparent discrepancy does not suggest that the epidemiological cycle of schistosomiasis is now clarified. It requires further investigation.

Third, the immunofluorescence reaction of the sera showed generally high titers with the schistosome gut, but was low with the parasite parenchyma. This pattern of reactivity had been associated with relatively recent rather than long standing infections (van Helden et al., 1975; Kanamura et al., 1979). Also, schistosomiasis patients as well as experimentally infected

animals develop antibodies against the schistosome gut already 4–6 weeks after infection, when other parasite tissues have not yet induced a detectable antibody response (Ruppel *et al.*, 1985). It thus appears that the children in our study acquired their infections relatively recently.

Fourth, the ultrasonographic studies did not show more than mild liver pathology in few individuals and none in all of the other tested persons. Although, for logistic reasons, this group of patients was different from those of the serologic survey, they were from the same villages. Thus, the absence of serious liver pathology from all 96 persons examined and the normal appearance of liver in most of the individuals argues against long established focus or foci of transmission in those villages.

Fifth, a significant difference in prevalence was found, by parasitological as well as by immunological techniques, between individual schools: prevalence was relatively high in two, and low in two others. Therefore, children from the “high prevalence schools” may either have been infected at other water bodies than those frequented by the “low prevalence schools”, or the infections may have been acquired at the same locality, but at different times. In any way, the very different levels of prevalence including the apparent absence of infections in five other schools support the concept of a recent start of transmission in only one or a few water bodies, from where infections have not yet spread to other places.

The epidemiology of schistosomiasis in Dhofar appears to be dramatically different from well known areas of high schistosomiasis endemicity in Africa, e.g. along the rivers, lakes or irrigation schemes: (i) water bodies in Dhofar are comparatively very small, a few meters in diameter or stretches of a maximum of a few hundred meters along small rivers or discontinuous water courses; (ii) snails are subject to floods and dry periods according to the monsoon season, which contribute to strong variations in population size; (iii) concrete water reservoirs are increasingly built at all accessible water sources with a strong influence on snail population; (iv) importantly, human water contacts are not a necessity of daily life in Dhofar, but rather of recreational character. All of these conditions may contribute to restrict the spread of schistosomiasis.

In conclusion, the bulk of the data strongly suggests a recent and focal introduction of schistosomiasis *mansoni* in Dhofar. The origin remains obscure. One site in particular, Ain Sahanout, which is close to Sheer and Zeek, was suspected to have been the origin of this emergence of schistosomiasis and had been treated with the molluscicide Bayluscide already in August 2000 (Shaban, unpublished). However, the discrepancy remains to be clarified: close to the villages with infected children no infected snails were detected (this report), but water places (Tibraq and Siginitti) which are also close to schools without egg-excreting children (Adebhot and Ashenib) yielded infected snails (Moné *et al.*, 2003). Epidemiological work is under progress to identify possibly persisting transmission site(s) including a potentially seasonal transmission. Locally adapted control strategies are now being developed for Dhofar.

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