

# GENETIC ANALYSIS OF PRAZIQUANTEL RESISTANCE IN *SCHISTOSOMA MANSONI*

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**Abstract.** To determine the genetic basis of the resistance of *Schistosoma mansoni* to praziquantel (PZQ) and to understand whether the resistance is dominant or recessive trait, a schistosome cross was undertaken between a PZQ-susceptible and a PZQ-resistant isolate using infections of the single-sex cercariae which were identified by a direct W1-specific PCR technique. The resistances of F<sub>1</sub> and F<sub>2</sub> generation to PZQ were evaluated using *in vitro* egg, miracidial and cercarial tests. The F<sub>1</sub> hybrid progeny from crosses between the susceptible and resistant isolates were resistant to PZQ. The resistant phenotype reappeared in the F<sub>2</sub> progeny. It can thus be considered that the PZQ resistance behaves like a dominant trait.

## INTRODUCTION

Schistosomiasis still remains a major public-health concern in tropical and sub-tropical areas. Praziquantel (PZQ) is currently the drug of choice for treatment of human schistosomiasis (World Health Organization, 1993). However, in recent years the resistance of *Schistosoma* to PZQ has been found in laboratory studies (Fallon and Doenhoff, 1994) and in a number of foci in Africa (Gryseels *et al*, 1994; Fallon *et al*, 1995; Stelma *et al*, 1995; Ismail *et al*, 1996; Guisse *et al*, 1997). Determining the genetic basis of schistosome resistance to PZQ and understanding whether resistance is a dominant or recessive trait is important in predicting the spread of resistance. Although these are time-consuming experiments, the results from this type of work may reveal the genetic basis of the transmission of the PZQ resistant phenotype. If resistance segregates in a strict Mendelian pattern as a recessive or a dominant trait then these result should reveal a predictable pattern. Obviously, if the allele is not

constant in its expression (*ie* reduced penetrance) then it may be difficult to determine the genetic nature of the phenotype. This factor, coupled with the possibility that the phenotype may be the result of the expression of more than one gene, could produce results that are difficult to interpret. On the other hand, if a clear Mendelian pattern is detected then this information would be relevant for the development a molecular-based assay to detect PZQ resistance. Finally, the notion that resistance to PZQ is due to a dominant or a recessive gene has important implications for the interpretation of both the mechanism of drug resistance and the mechanism of drug action. In the present study, a schistosome cross was undertaken between a PZQ-susceptible and a PZQ-resistant isolate of *Schistosoma mansoni*.

## MATERIALS AND METHODS

### Parasite isolate

Two isolates of *S. mansoni* were used: one was PZQ-susceptible isolate Keneyan-field isolate which was collected from an infected snail in 1994 and had not previously been subjected in PZQ treatment; the other was PZQ-resistant Senegalese-3 isolate which was obtained from an individual infected with *S. mansoni*, who persisted

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in passing eggs in feces after 3 treatments with 40 mg/kg PZQ. Details for these isolates have been described previously (Liang *et al*, 2001a). The drug-sensitivity/resistance profile of both has been tested by treating mice with mature parasite infections with 3 x 200 mg/kg PZQ and the reduction in worm burden of the Kenyan-field and Senegalese-3 was found to be 99% and 67% respectively following treatment (Liang *et al*, 2001a).

### **Infecting snails with a single miracidium of the two parental isolates**

One hundred *Biomphalaria glabrata* snails for each isolate were placed in 24-well flat bottom microplates and a single miracidium was added to each well in three ml of 28°C tap water. Infection was undertaken under a tungsten light for two hours. On day 40 post-infection, the single sex cercariae obtained by individually placing snails in three ml of tap water in each well of the microplates for cercarial shedding, and single cercariae were isolated by successive dilution steps in water drops using a micropipette with an elongated tip.

### **Identifying the sex of *S. mansoni* cercariae from a monomiracidial mollusc infection snail using PCR**

A single cercaria was transferred into a PCR reaction tube, and DNA amplification was performed using a PCR technique with W1-specific primers without DNA extraction (direct PCR protocol) (Grevelding *et al*, 1997) with Taq PCR Master Mix Kit. To determine the gender of cercariae, a primer (Gasser, 1992) was used that is directed against the repetitive W1 sequence of *S. mansoni* (Webster *et al*, 1989) and synthesized by Sigma-Genosys Ltd (Cambridgeshire, UK). The W1 repeated sequence which was isolated and characterized from female W chromosome (Webster *et al*, 1989) was shown to be female-specific in each stage of the life cycle of the Puerto Rican strain (Gasser, 1992). This allows the determination of sex since the gender of larvae cannot be distinguished by phenotype. Amplification of one W1 repeat unit results in a 473-bp product. Due to the repetitive nature of the target DNA, bands were expected that represented multiples

of the repeat unit. The details of the primer and PCR procedure have been described by Grevelding *et al* (1997).

### **Schistosome crosses**

Genetic crosses were performed by infecting mice with the female and male cercariae of the resistant and susceptible isolate of *S. mansoni*. Each mouse was infected by Standen's padding method (Standen, 1949) as modified by Christensen *et al* (1979) and Frandsen (1981) with a mixture of 100 female cercariae of Senegalese-3 isolate and 100 male cercariae of Kenyan-field isolate. Twenty mice were used in this study. On day 57 post-infection, eggs were isolated from the feces of the infected mice and used to produce miracidia for snail infection (Liang *et al*, 2001a). The eggs, miracidia and the cercariae shed from these snails represented the F<sub>1</sub> generation to be tested for drug sensitivity. The cercariae of F<sub>1</sub> were employed to infect mice and to obtain the F<sub>2</sub> generation.

### **Assessment of PZQ resistance**

The phenotype of *Schistosoma* of F<sub>1</sub> and F<sub>2</sub> generations with respect to PZQ sensitivity/resistance was defined *in vitro* using the egg, miracidial and cercarial tests described previously (Liang *et al*, 2000; 2001a;b). Briefly, about 1,000-2,000 eggs obtained from each isolate were incubated in 0.9% saline solution containing four concentrations of PZQ ( $5 \times 10^{-6}$ ,  $10^{-6}$ ,  $5 \times 10^{-7}$  and  $10^{-7}$  M) at 28°C in the dark for 24 hours. The eggs were transferred to fresh tap water under tungsten light at 28°C for miracidia hatching, and the numbers of miracidia were counted by collecting the water containing the miracidia every half hour and adding several drops of Lugol's iodine. The miracidial tests were made in 24-well flat bottom microplates (Iwaki Glass, Japan) using freshly hatched miracidia of each isolate. In each well, 20-40 miracidia were observed and morphological changes checked by adding a drops of Lugol's iodine. Miracidia were examined under the microscope at 0, 1 and 5 minutes at 26°C using four concentrations of PZQ ( $5 \times 10^{-6}$ ,  $10^{-6}$ ,  $5 \times 10^{-7}$  and  $10^{-7}$  M). Batches of 100-200 freshly shed cercariae from snails infected with each isolate were transferred to a glass Petri dish held at a constant tem-

perature by the circulation of 28°C water under the dish. PZQ dissolved in dimethyl sulfoxide (DMSO) was added to cercariae to give a maximum concentration of 1% DMSO. This concentration of DMSO has no effect on cercarial behavior. Observations of tail shedding of cercariae were made under a dissecting microscope at 20, 40, 60, 80 and 100 minutes after administering PZQ at four concentrations ( $10^{-4}$ ,  $10^{-5}$ ,  $6 \times 10^{-7}$  and  $4 \times 10^{-7}$  M). The PZQ susceptibilities of the two parental isolates were compared with those of  $F_1$  and  $F_2$  generations.

### Statistical analysis

The statistical significance between groups evaluated using  $\chi^2$  tests. Analysis of variance and Dunnett's *t*-test were used to test the significance of difference between groups. P-value of <0.005 was considered significant.

## RESULTS

### Identifying the sex of *S. mansoni* cercariae and schistosome cross

At 6 weeks post-infection, a cercaria from each infected snail was isolated to perform a W1-PCR reaction and 200 cercariae were used to infect a mouse. Two of the 10 cercariae from 10 snails infected with Senegalese-3 isolate showed a DNA band (data not shown), and infection and perfusion of mice showed that these snail infections were female. Eight of the 10 cercariae left showed no band, and infection and perfusion of mice showed that all the snail infections were male. None of the 12 cercariae from 12 snails infected with Kenyan-field isolate showed any DNA band, and infection and perfusion of mice also showed that all the snail infections were male. By crossing the female cercariae of Senegalese-3 isolate with the male cercariae of Kenyan-field isolate  $F_1$  generation was obtained. Similarly, by crossing  $F_1$  cercariae the  $F_2$  generation was obtained as well.

### Assessment of PZQ resistance of the $F_1$ and $F_2$ generation

**Egg hatch test.** The hatch rates of the eggs of  $F_1$  generation were significantly higher ( $10^{-6}$  M PZQ:

$\chi^2=411.7$   $p<0.0001$ ;  $5 \times 10^{-7}$  M PZQ:  $\chi^2=659.79$   $p<0.0001$ ) than those of the parental susceptible (Kenyan-field) isolate, but were as same as those of the parental resistant (Senegalese-3) isolate following the incubation in  $5 \times 10^{-6}$ ,  $10^{-6}$  and  $5 \times 10^{-7}$  M PZQ solutions for 24 hours (Table 1). The hatch rates of the eggs from  $F_2$  generation were significantly higher ( $10^{-6}$  M PZQ:  $\chi^2=1777.34$   $p<0.0001$ ;  $5 \times 10^{-7}$  M PZQ:  $\chi^2=168.66$   $p<0.0001$ ) than that of Kenyan-field isolate, but significantly lower ( $10^{-6}$  M PZQ:  $\chi^2_s=7.279$   $p=0.007$  and  $\chi^2_{F_1}=60.239$   $p<0.0001$ ;  $5 \times 10^{-7}$  M PZQ:  $\chi^2_s=93.496$   $p<0.0001$  and  $\chi^2_{F_1}=149.43$   $p<0.0001$ ) than those of Senegalese-3 isolate and  $F_1$  generation following the incubation in  $10^{-6}$  and  $5 \times 10^{-7}$  M PZQ for 24 hours (Table 1). There were no differences among the parental isolates,  $F_1$  and  $F_2$  generations following the incubation in  $10^{-7}$  M PZQ for 24 hours (Table 1).

**Miracidial test.** Miracidia (14.4%) from the  $F_1$  generation exposed to  $5 \times 10^{-6}$  M PZQ immediately changed their shapes. This was lower than that of the parental PZQ-susceptible (Kenyan-field) isolate, but the same as the parental PZQ-resistant (Senegalese-3) isolate. The percent change in shape of miracidia of  $F_2$  generation was lower than that of the Kenyan-field isolate, but was significantly higher ( $t_{F_1}=7.555$   $p<0.0001$ ;  $t_s=4.15$   $p=0.0032$ ) than those of the Senegalese-3 isolate and  $F_1$  generation. When exposed to  $10^{-6}$  M PZQ no miracidia from Senegalese-3 isolate,  $F_1$  and  $F_2$  generation immediately changed their shapes, while 27.4% of miracidia from the Kenyan-field isolate changed their shapes. After 1 minute of drug exposure only 6.5% of miracidia from  $F_1$  generation changed their shapes which was significantly lower ( $t=8.673$   $p=0.001$ ) than that of the parental susceptible isolate, but the same ( $t=1.642$   $p=0.3214$ ) as that of the parental resistant isolate. The percent change in shape of miracidia of  $F_2$  generation was significantly higher ( $t_s=4.938$   $p=0.0078$ ;  $t_{F_1}=5.181$   $p=0.0035$ ) than those of the Senegalese-3 isolate and  $F_1$  generation, but significantly lower ( $t=3.78$   $p=0.054$ ) than that of the Kenyan-field isolate. After five minutes of drug exposure, similar results were achieved (Table 2).

**Cercarial test.** When exposed to  $10^{-4}$  M PZQ for

Table 1

The percent hatch rates of eggs of parental, F<sub>1</sub> and F<sub>2</sub> generations of *Schistosoma mansoni* following incubation in different concentrations of PZQ for 24 hours. One thousand to 2,000 eggs were used in each PZQ concentration group.

Parasite isolates	Praziquantel concentration (M)			
	5x10 <sup>-6</sup>	10 <sup>-6</sup>	5x10 <sup>-7</sup>	10 <sup>-7</sup>
Kenyan-field	0	3.1	27.9	92.7
Senegalese-3	0.3	22.7	69.3	91.3
F <sub>1</sub> (Kenyan-field ♂ x Senegalese-3♀)	5.4	30.7	72.9	93.6
F <sub>2</sub> (F <sub>1</sub> ♂ x F <sub>1</sub> ♀)	7.7	18.6	51.8	89.4

Table 2

The percent change in shape of miracidia of parental, F<sub>1</sub> and F<sub>2</sub> generations of *Schistosoma mansoni* following incubation in PZQ. The results are means (±SD) of five tests using 30-50 miracidia per test.

Parasite isolate	Incubation time (minute)	Praziquantel concentrations (M)			
		5x10 <sup>-6</sup>	10 <sup>-6</sup>	5x10 <sup>-7</sup>	10 <sup>-7</sup>
Kenyan-field	0	100	27.4 ± 9.2	0	0
	1	100	64.2±14.3	0	0
	5	100	88.3±21.5	14.8 ± 5.4	0
Senegalese-3	0	15.5 ± 2.1	0	0	0
	1	100	8.4 ± 3.2	0	0
	5	100	13.9 ± 9.9	0	0
F <sub>1</sub> (Kenyan-field ♂ x Senegalese-3♀)	0	14.4 ± 1.2	0	0	0
	1	100	6.5 ± 4.1	0	0
	5	100	13.6 ± 8.1	0	0
F <sub>2</sub> (F <sub>1</sub> ♂ x F <sub>1</sub> ♀)	0	19.9 ± 1.1	0	0	0
	1	88.0±17.2	33.7±11.0	5.8 ± 2.7	0
	5	100	41.4±14.1	7.8 ± 3.2	0

40, 60, 80 and 100 minutes, the cercariae of F<sub>1</sub> generation gave significantly lower ( $t_{40}=3.866$   $p=0.0181$ ;  $t_{60}=4.227$   $p=0.0083$ ;  $t_{80}=8.709$   $p<0.0001$ ;  $t_{100}=11.762$   $p<0.0001$ ) tail shedding rates than those of the parental PZQ-susceptible (Kenyan-field) isolate, but there were no differences of tail shedding rates between the F<sub>1</sub> generation and the parental PZQ-resistant (Senegalese-3) isolate ( $t_{40}=1.127$   $p=0.2925$ ;  $t_{80}=1.227$   $p=0.2547$ ;  $t_{100}=1.402$   $p=0.1986$ ) except for the 60 minute exposure ( $t=4.535$   $p=0.0019$ ) (Table 3). When exposed to 10<sup>-5</sup> M PZQ for 40, 60, 80 and 100 minutes, the cercariae of F<sub>1</sub> gen-

eration also gave significantly lower ( $t_{40}=4.417$   $p=0.0022$ ;  $t_{60}=14.329$   $p<0.0001$ ;  $t_{80}=9.571$   $p<0.0001$ ;  $t_{100}=8.799$   $p<0.0001$ ) tail shedding rates than those of the parental susceptible (Kenyan-field) isolate, but there were no differences of tail shedding rates between the F<sub>1</sub> generation and the parental resistant (Senegalese-3) isolate ( $t_{40}=1.586$   $p=0.1515$ ;  $t_{80}=0.9745$   $p=0.3584$ ;  $t_{100}=1.498$   $p=0.1726$ ) except for 60 minutes ( $t=3.023$   $p=0.0165$ ) (Table 3). When exposed to 6x10<sup>-7</sup> M PZQ for 20-100 minutes, none of the cercariae from the F<sub>1</sub> generation had shed their tails (Table 3).

## DISCUSSION

The results from egg, miracidial and cercarial tests showed that the  $F_1$  generation was as resistant to PZQ as the parental PZQ-resistant Senegalese-3 isolate. The  $F_2$  generation was also resistant, but the level of resistance was somewhat lower than those of  $F_1$  generation and Senegalese-3 isolate with a proportion of the offspring from the phenotypically resistant hybrid parents behaving as susceptible individuals.

These results were in agreement with the pattern of Mendelian genetics. It also suggested that starting populations, *ie* the parental susceptible and resistant isolates, being 'stabilized' with respect to PZQ sensitivity or resistance, were homozygous. The present results show that the  $F_1$  hybrids obtained by crossing resistant schistosome with susceptible ones displayed a resistant phenotype, and this resistant phenotype also reappeared in  $F_2$  generation. The simplest way to interpret these data is to assume that PZQ resistance in *Schistosoma mansoni* is determined by a single or a group dominant gene(s). The results also imply that resistance will spread fast in a setting such as Senegal. This is a preliminary ex-

periment and the results and hypothesis require confirmation by further experiments on crosses of the four possible sexual combinations, as well as backcrosses and mouse chemotherapies.

The mode of action of PZQ is unknown. Understanding the genetics of PZQ resistance may provide important insights into the biochemical mechanisms of resistance to the drug and into the mode of action of PZQ itself as well. In some biological systems, drug resistance results from the acquisition of a new activity in resistant organisms (*eg* the ability to degrade or inactivate the drug), while in other systems resistance results from the loss of a pre-existing activity (*eg*, the loss of drug-activating mechanisms)(Cioli *et al*, 1993). The data from the recent research work suggested that in *Schistosoma* a possible target for PZQ action is the interaction between the  $Ca^{2+}$ -channel pore-forming  $\alpha_1$  subunits and  $\beta$  subunits which were cloned and expressed in *Schistosoma* and can confer PZQ sensitivity to an otherwise PZQ-insensitive mammalian  $Ca^{2+}$ -channel. (Kohn *et al*, 2001). Coexpression of the PZQ-insensitive  $\alpha_1$  subunits with  $\beta$  subunits resulted in an expressed channel that is sensitive to PZQ, indicating that  $\beta$  subunits, in combination with par-

Table 3

The percent tail shedding of cercariae of the parental and  $F_1$  generations of *Schistosoma mansoni* following incubation in PZQ. The results are the means ( $\pm$ SD) of five tests using 100-200 cercariae.

Praziquantel concentration (M)	Parasite isolate	Incubation time (minutes)				
		20	40	60	80	100
$10^{-4}$	Kenyan-field	0	12.9 $\pm$ 5.8	30.3 $\pm$ 6.2	67.2 $\pm$ 9.8	84.8 $\pm$ 4.9
	Senegalese-3	0	3.5 $\pm$ 1.2	23.6 $\pm$ 1.7	30.2 $\pm$ 8.8	48.3 $\pm$ 6.9
	$F_1$ (Kenyan-field $\sigma$ x Senegalese-3 $\phi$ )	0	2.8 $\pm$ 0.7	17.8 $\pm$ 2.3	24.7 $\pm$ 4.8	42.4 $\pm$ 6.4
$10^{-5}$	Kenyan-field	4.8 $\pm$ 1.1	41.1 $\pm$ 9.2	71.1 $\pm$ 4.8	82.3 $\pm$ 8.4	88.2 $\pm$ 8.7
	Senegalese-3	0.6 $\pm$ 0.2	24.5 $\pm$ 6.4	36.9 $\pm$ 3.7	46.7 $\pm$ 2.7	53.6 $\pm$ 4.7
	$F_1$ (Kenyan-field $\sigma$ x Senegalese-3 $\phi$ )	0	17.4 $\pm$ 7.7	27.6 $\pm$ 5.8	41.1 $\pm$ 4.7	49.1 $\pm$ 4.8
$6 \times 10^{-7}$	Kenyan-field	0	0.8 $\pm$ 0.3	14.1 $\pm$ 7.9	22.4 $\pm$ 2.4	37.5 $\pm$ 1.7
	Senegalese-3	0	0	1.4 $\pm$ 0.3	6.7 $\pm$ 1.9	11.1 $\pm$ 2.9
	$F_1$ (Kenyan-field $\sigma$ x Senegalese-3 $\phi$ )	0	0	0	0	0

ticular  $\alpha_1$  subunits, may be important molecular target of PZQ action (Kohn *et al*, 2001). If the demonstration that homozygous schistosomes are phenotypically PZQ-susceptible is confirmed, it indicates that the sensitive allele need not be completely absent for resistance to appear. The simplest model to account for such a behavior is to assume that the sensitive gene codes for a biological function (*eg*,  $\beta$  subunits or  $\alpha_1$  subunits) which is necessary for PZQ activity. The concept that resistance is associated with the lack of some activity can also be easily reconciled with the well known intrinsic resistance of immature schistosomes (Jansma *et al*, 1977; Cioli and Knopf, 1980), since juvenile stages of the parasite would have not yet switched on those biotransforming activities which are present in the adult PAQ-sensitive stage.

The classical technique previously employed to perform genetic crosses of schistosomes (Jansma *et al*, 1977) consists of the following series of operations: infecting snails with a single miracidium of the two parental strains; obtaining snails shedding single-sex cercariae (usually a small percentage of the infected snails); determining through mouse infections the sex of cercariae shed by each individual snail; infecting mice with cercariae of opposite sex from the two parental populations, and finally, obtaining F<sub>1</sub> hybrid eggs from the mature infections. It is tedious and lengthy procedure. In genetic analysis of hycanthone resistance in *Schistosoma mansoni* Cioli and Pica-Mattoccia (1984) and Cioli *et al* (1992, 1993) developed a worm transfer technique (one parental 25- to 27- day-old male worm and one parental female worm of the same age were transferred into the mesenteric veins of mice) which considerably reduced the length of time and complexity of the operations generally involved in performing schistosome genetic crosses. In the present study, a simple PCR technique that allows rapid determination of the sex of individual cercaria (Gasser, 1992) was used, although the effectiveness of this PCR method for identifying female cercariae, expressed in percentage of positive female cercariae, is 81.9% (Boissier *et al*, 2001). However, in our experiment 2 cercaria from separate monomiracidial mollusc infection showed a DNA band, and infection and perfu-

sion of mice confirmed that both of the snail infections were female; 12 cercariae showed no band, and infection and perfusion of mice confirmed all the snail infection were males. This PCR protocol permitted a rapid method for sex determination and allowed crosses shortly after snails started shedding cercariae.

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## REFERENCES

- Boissier J, Durand P, Mone H. PCR effectiveness for sexing *Schistosoma mansoni* cercariae: application for sexing clonal cercarial population. *Mol Biochem Parasitol* 2001; 112: 139-41.
- Christensen NØ, Frandsen F, Nansen P. The effect of some environmental conditions and final host and parasite-related factors on the penetration of *Schistosoma mansoni* cercariae into mice. *Z Parasitenk* 1979; 59: 267-75.
- Cioli D, Knopf PM. A study of the mode of action of hycanthone against *Schistosoma mansoni* *in vivo* and *in vitro*. *Am J Trop Med Hyg* 1980; 29: 220-6.
- Cioli D, Pica-Mattoccia L. Genetic analysis of hycanthone resistance in *Schistosoma mansoni*. *Am J Trop Med Hyg* 1984; 33: 80-8.
- Cioli D, Pica-Mattoccia L, Archer S. Drug resistance in schistosomes. *Parasitol Today* 1993; 9: 162-6.
- Cioli D, Pica-Mattoccia L, Moroni R. *Schistosoma mansoni*: hycanthone/oxamniquine resistance is controlled by a single autosomal recessive gene. *Exp Parasitol* 1992; 75: 425-32.
- Fallon PG, Doenhoff MJ. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. *Am J Trop Med Hyg* 1994; 51: 83-8.
- Fallon PG, Sturrock RF, Capron A, Niang M, Doenhoff MJ. Diminished susceptibility of praziquantel in

- a Senegal isolates of *Schistosoma mansoni*. *Am J Trop Med Hyg* 1995; 53: 61-2.
- Frandsen F. Cultivation of schistosomes for chemotherapeutic studies. *Acta Pharmacol Toxicol* 1981; 49 (suppl V): 118-22.
- Gasser RB. Sexing schistosome larvae. *Parasitol Today* 1992; 8: 286-8.
- Grevelding CG, Kampkotter A, Kunz W. *Schistosoma mansoni*: sexing cercariae by PCR without DNA extraction. *Exp Parasitol* 1997; 85: 99-100.
- Gryseels B, Stelma FF, Talla I, et al. Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. *Trop Geogr Med* 1994; 46: 209-19.
- Guisse F, Polman K, Stelma FF, et al. Therapeutic evaluation of two different dose regimens of praziquantel in a recent *Schistosoma mansoni* focus in northern Senegal. *Am J Trop Med Hyg* 1997; 56: 511-4.
- Ismail MM, Farghaly AM, Bruce J, Tao LF, Bennett JL. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerated high doses of praziquantel. *Am J Trop Med Hyg* 1996; 55: 214-8.
- Jansma WB, Rogers SH, Liu CL, Bueding E. Experimentally produced resistance of *Schistosoma mansoni* to hycanthone. *Am J Trop Med Hyg* 1997; 26: 926-36.
- Kohn AB, Anderson AV, Roberts-Misterly JM, Greenberg RM. Schistosome calcium channel (subunits: unusual modulatory effects and potential role in the action of the anti *Schistosoma* drug praziquantel. *J Biol Chem* 2001 (in press).
- Liang YS, Coles GC, Doenhoff. Detection of praziquantel resistance in schistosomes. *Trop Med Int Health* 2000; 5: 72.
- Liang YS, Coles GC, Doenhoff MJ, Southgate VR. *In vitro* responses of praziquantel-resistant and -susceptible *Schistosoma mansoni* to praziquantel. *Int J Parasitol* 2001a; 31: 1227-35.
- Liang YS, Dai JR, Ning A, et al. Susceptibility of *Schistosoma japonicum* to praziquantel in China. *Trop Med Int Health* 2001b; 6: 707-14.
- Standen OD. Experimental schistosomiasis. II. Maintenance of *Schistosoma mansoni* in the laboratory, with some notes on experiment infection with *Schistosoma haematobium*. *Ann Trop Med Parasitol* 1949; 43: 268-83.
- Stelma FF, Talla I, Sow S et al. Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. *Am J Trop Med Hyg* 1995; 53: 167-70.
- Webster P, Mansour TE, Bieber D. Isolation of a female-specific, highly repeated *Schistosoma mansoni* DNA probe and its use in an assay of cercarial sex. *Mol Biochem Parasitol* 1989; 34: 1132-6.
- World Health Organization. The Control of Schistosomiasis. Second report of the WHO Expert Committee. *WHO Tech Rep Ser* 1993; 830.