Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. IV, 243-247, 1992

IDENTIFICATION OF SCHISTOSOME-INFECTED SNAILS BY DETECTING SCHISTOSOMAL ANTIGENS AND DNA SEQUENCES

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Cercarial shedding tests do not provide species identification of the schistosomes concerned and cannot detect prepatent schistosomal infections. We have demonstrated that both immunodetection by ELISA of schistosomal antigens in snail hemolymph, and dot hybridization of snail extracts by a DNA probe representing highly repeated sequences, proved suitable for detecting infected snails during prepatency as well as patency. A group-specific monoclonal antibody was found to be suitable for detecting Schistosoma mansoni *infection in* Biomphalaria sp., *but not for positive identification of* S. haematobium *in* Bulinus sp. *Comparative evaluation of the diagnostic qualities, and technical aspects and cost of these tests, point to the superiority of the immunodetection approach for large scale detection of snails prepatently infected with* S. mansoni. *This approach is potentially useful for providing extended information on schistosome-snail epidemiology that may facilitate rapid evaluation of the danger of post-control reinfection, and help make decisions on the time and place of supplementary control measures. In this context the potential usefulness of the immunodetection or DNA probing approach for facilitating catalytic model representation of schistosome-snail epidemiology warrants further evaluation. Specific identification of* S. haematobium *in* Bulinus *by either of these approaches may be possible depending on the development of suitable antibodies or DNA probes.*

Key words: *Schistosoma mansoni* – snail infection – immunodetection – DNA probing

Snail infection-rate data have been traditionally collected as part of the overall epidemiological data required for evaluating transmission of schistosomes, particularly for evaluating the effect of community-based control measures on transmission (Sturrock, 1986). The cercarial shedding test served in these cases as the only available routine test for identifying infected snails. However, the fact that the shedding test does not detect prepatent infection results not only in sometimes highly inaccurate snail infection-rate values (Sturrock, 1973), but also makes impossible the detection of early post-control changes in snail infection rates.

The detection of prepatent schistosomal infection in snails can be accomplished by serial shedding tests in snails collected in the field and maintained in the laboratory (Sturrock et al., 1979), or by microscopical identification of larval stages in crushed snails (Chu & Dawood, 1970). Serial shedding requires prolonged maintenance of snails and is hampered by snail death. Microscopical detection of prepatent infections is cumbersome, time consuming, highly inaccurate, and requires high parasitological expersite. None of these approaches, however, nor the traditional shedding test can provide schistosome species identification which is particularly important for differential identification of *Schistosoma haematobium* from a number of animal schistosomes which can develop in *Bulinus* spp. We therefore concentrated our efforts on developing simple tests for detecting schistosomal antigens and DNA sequences in infected snails aiming at identifying prepatent infections and at eventual species identification. The abundant presence in snail hemolymph of specific parasite antigens early in infection (Hamburger et al., 1989a) made possible the identification of infected snails by one stage ELISA employing monoclonal antibodies (Hamburger

This study was supported by AID/PSTC grant DP-E-5542-G-SS-40433-00.

et al., 1989b). In addition, the cloning of a probe representing highly-repeated and species-specific schistosomal DNA sequences (Hamburger et al., 1987, 1991), made possible the application of dot hybridization for identifying infected snails.

In the present publication we present further information on the specificity of the monoclonal antibody employed by immunodetection of infected snails and discuss the performance and prospects of this approach vis a vis dot hybridization with cloned highly-repeated DNA sequence.

MATERIALS AND METHODS

The immunodetection of *Biomphalaria sp.* infected with *S. mansoni* in the laboratory and in transmission sites in Kenya was carried out by employing one stage ELISA for detecting parasite antigens in the snail's hemolymph. The test involved adsorption of hemolymph onto microtiter plate wells, blocking of non-specific binding sites, and probing with enzyme-linked 2nd antibody. The details of the test were previously described (Hamburger et al., 1989a, b). A similar procedure was applied for testing hemolymph collected in Kenya from laboratory-reared *Bulinus* (Africanus group) and from field snails collected from sites in Kisumu and Tiengre (both in the Lake Province). Similar tests were also carried out with hemolymph collected from laboratory-reared *B. truncatus* infected with *S. haematobium,* and from laboratory-reared *B. wrighti* infected with *S. intercalatum* (both kindly provided by Dr A. Agnew, Imperial College, London).

The details on the cloning of *S. mansoni* highly repeated sequences and on the identification of infected snails by probing with pSm1-7, a recombinant pUC18 containing a 0.64 Kb stretch of these tandemly repeated sequences, were recorded elsewhere (Hamburger et al., 1991).

RESULTS AND DISCUSSION

Immunodetection of snails infected with *S. mansoni* has been accomplished by one stage ELISA employing MoAb K. C. Smeg 22-3 and K. C. Smeg 22-4 directed to schistosome-specific antigens (Hamburger et al., 1989a, b). While the MoAb-employed could differentiate field collected *B. pfeifferi* infected with *S. mansoni* from snails infected with other trematodes with 100% specificity, the data presented here indicate that this MoAb is largely group-specific rather than species-specific. Thus, although it could detect schistosomes in *Bulinus* spp., its reactivity was highly variable, spanning from no reactivity to high reactivity, with infected snails collected at a location where *S. haematobium* as well as animal schistosomes are known to be present in *Bulinus sp.* (Table I). Further studies on this line involved testing of hemolymph collected from 8 laboratory-reared *B. truncatus* infected with *S. haematobium* and from 8 laboratory-reared *B. wreighti* infected with *S. intercalatum* (kindly supplied by Dr A. Agnew, Imperial College, London). ELISA results with both these schistosomes were negative (unpublished). In view of these results the MoAb employed is not suitable for positive identification of *S. haematobium*-in-

TABLE I

Detection of schistosomes in *Bulinus* (Africanus group)

Origin of snails (Special features)	Shedding test Results	No. snails tested		Positives[a]	ELISA results/No. snails tested Average O.D. High	Medium	Low
Laboratory reared (Known uninfected)	Non-shedders	15/15	→	0/15	–	–	–
Kisumu (Unidentified infection)	Non-shedders	378/400	→	0/20	–	–	–
	Schistosomes	20/400	→			Not tested	
	Other	2/400	→	0/2	–	–	–
Tiengre (known high transmission of both *S. haematobium* and animal schistosomes)	Non-shedders	20/100	→	3/20		3/20	
	Schistosomes	80/100	→	30/36	9/30 (1.215)	9/30 (0.607)	12/30 (0.327)
	Other	0/100			(6/36 were negative)		

a: ELISA O.D. $>$ 0.25, constituting twice the control value, was considered positive.

TABLE II

Comparison of diagnostic qualities of immunodetection and DNA probing

Diagnostic qualities	Immunodetection	DNA probing
Earliest detection	2nd week	1st week
Detection target	Antigens secreted by live larvae (probably daughter sporocysts)	Schistosomal DNA
Indicator of prospective prepatency to patency conversion	Very accurate	Presumed inaccurate early in prepatency
Quantitative aspects	Antigen quantity presumed to suggest reproductive capacity rather than actual parasite load	Quantitation of actual parasite load is possible
Specificity	Group specific. Subgroup specificity unclear with current MoAb	Presumed species specific (*S. mansoni*) with the pSm1-7 probe

fected snails. The question of whether ELISA negativity of a patent infected *Bulinus* can be taken as an indication of *S. haematobium* infection (in areas where *S. intercalatum* does not exist) would require further investigations. Detection of prepatent *S. haematobium* would require further investigations. Detection of prepatent *S. haematobium* would require development of MoAb capable of positive differential identification of this species. It thus emerges that the immunodetection test in its current form is suitable for detecting *S. mansoni* in areas where *S. rhodhaini,* the only other schistosome species developing in *Biomphalaria sp.,* is not present.

Of some interest is the potential usefulness of the test for large scale identification of snails carrying prepatent infection. The availability of such a test to supplement the routine determination of the prevalence of patent infection by shedding tests, may provide more accurate counts of infected snails, and an early measure of the post-control parasite flux from man to snail. Furthermore, successive determination of the prevalence of prepatent and patent infections following prevention of parasite flux from man to snail (e.g., by fencing of a site or by effective community-based treatment) may provide information suitable for constructing infection-rate-reduction/time curves. Theoretically such curves may facilitate assessments of the length of prepatent period, prepatency to patency conversion rate, and the combined rate of mortality and loss of infection of patent infected snails. All the above parameters are important for catalytic model representation of schistosome-snail epidemiology and are so far unobtainable by direct field studies (Woolhouse & Chandiwana, 1990).

When comparing the diagnostic qualities of immunodetection with DNA probing (Table II), it emerges that both are 100% sensitive for detecting patent infections (Hamburger et al., 1989a, b), but the DNA probing can detect prepatent infection during the first week after miracidial penetration (Hamburger et al., 1991), whereas ELISA positivity emerges during the 2nd week of infection. Very early detection of schistosomal DNA in the snail, however, does not necessarily indicate that the early larvae are viable or reproducing. By contrast, immunodetection which is indicative of the presence of antigens secreted by live larvae (Hamburger et al., 1989a), is more likely to represent reproductive capacity actual parasite load. in fact, preliminary studies in our laboratory (results not shown) indicate that the quantity of schistosomal antigens in the hemolymph does not correlate with the number of cercariae shed immediately prior to testing for antigens. It has been shown by Ward et al. (1988) that two populations of *B. glabrata* differing by about 20% in their susceptibility rate present a dramatic difference in cercarial output per snail when exposed to five miracidia per snail, but not when exposed to one miracidium per snail. Morphometric analyses led these authors to conclude that, once established, all primary sporocysts possess a similar reproductive potential. In search of a simple correlate of host-parasite compatibility it would be interesting to find out whether the quantity of schistosomal antigens in the hemolymph correlates with the capacity of miracidia to

TABLE III

Comparison of technical aspects of immunodetection and DNA probing

Basic features	Immunodetection		DNA probing	
	Current	Prospective improvement	Current	Prospective improvement
Shipment	Live snails	HL on plate	Crushed snails in NaOH	–
Sample	HL droplet	–	Supernatant of the above	–
Sample pretreatment	None	–	Boil and centrifuge	–
Sample applied on:	Microtiter plate	Recycled plate	Hyb membrane	–
Test employed	1 stage ELISA	–	Dot Hyb	–
Test steps	HL adsorption	Adsorption/shipment	Spotting	–
	Blocking by BSA	By powdered milk	Denaturation and neutralization	–
	ELAb binding	ELAb self production	Pre-Hyb	–
	Substrate Rxn	–	Hyb Rad probe	Hyb non-Rad probe
	Reading	–	Autoradiography	Autoradiography
Total test time (after sample application)	4-5 h	–	2 x O/N + 2 h	1-2 x O/N
Essential additions	None	–	Probe preparation:	Non-Rad probe preparation
			Rad labelling:	
Major equipment and facilities	Microscope	Magnifying glass	Microfuge	–
	Incubator	RT (Different Time)	Dot blotter	–
	Refrigerator	–	Vacuum pump	–
	(ELISA reader not required)	–	Oven, Water bath	–
			β-Counter	β counter not needed
			Dark room	–
			Water dionizer	–
			Sterilization facility	–

ELAb – Enzyme Linked Antibody; HL – Hemolymph; Hyb – Hybridization; O/N – Overnight; Rad – Radioactive; RT – Room Temperature; Rxn – Reaction.

establish and develop into reproducing mother sporocysts in a variety of schistosome/snail strain combinations. Unlike the quantity of schistosomal antigens in the hemolymph, DNA hybridization signals appear to be directly proportional to actual parasite load (Hamburger et al., 1987) and may therefore provide quantitation of the number of parasites in the snails at the time of testing. The epidemiological value of such information, however, is unclear. As for the specificity of the tests concerned, it has been shown that immunodetection with the available K. C. Smeg 22-3 or K. C. Smeg 22-4 monoclonal antibodies, can differentiate between schistosomes and a variety of other trematodes (Hamburger et al., 1989b), but not between *S. harmatobium* and other schistosomes developing in *Bulinus* spp., as described above. We assume that the antibodies employed are group-specific. The available DNA probe clearly differentiates between *S. mansoni* and two other schistosome species (*S. haematobium* and *S. magrebowei*). We assume that the cloned repeat or synthetic oligonucleotides based on its sequence should provide a highly species-specific probe suitable for identification of *S. mansoni* in a variety of biological materials.

Comparative evaluation of the technical aspects of the immunodetection and DNA probing tests described by us (Table III) points out the immunodetection approach as quicker, simpler, and requiring less of expensive facilities, equipment and materials. It is also more amenable to further improvements in cost efficiency and test simplicity, and therefore appears more suitable for large scale application in developing countries.

Further development of the immunodetection and/or DNA probing for extended studies on schistosome-snail epidemiology seems to be warranted considering that the widely applied morbidity control strategy through community-based chemotherapy, appears to have little effect on transmission (Kloetzel, 1989; Wilkins, 1989; Gryseels, 1990). In fact, any study on the effect of com-