Description and classification of abnormal cercariae of *Schistosoma mattheei* and a method of concentration and collection

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ABSTRACT

VAN WYK, J.A. & VAN RENSBURG, L.J. 2003. Description and classification of abnormal cercariae of *Schistosoma mattheei* and a method of concentration and collection. *Onderstepoort Journal of Veterinary Research*, 70:121–129

Over a period of about 12 years, 30 abnormal *Schistosoma mattheei* cercariae were found among a total of approximately 2.8 million examined. Initially seven were recovered from about 1.02 million (0.0007 %), which were examined individually while being counted with the aid of a stereoscopic microscope. Subsequently, on the strength of relatively high percentages of abnormal individuals recovered when counting cercariae that failed to penetrate into oxen, it appeared that the morphologically abnormal cercariae were unable to swim and would mostly sediment out of a suspension while most of the normal cercariae would remain swimming. This surmise is supported by recovery of 23 morphologically abnormal cercariae (0.001 %) from about 1.8 million, by examining the sediment after the cercarial suspension had been left standing undisturbed in glass measuring cylinders.

The abnormalities ranged from aberrant tails only (e.g. an underdeveloped tail, or different degrees of schism) or aberrant heads only, to abnormalities of both the heads and tails. A suggested schematic classification of abnormal cercariae is presented.

A young, adult hamster was exposed to eight *S. mattheei* cercariae with complete schism of the shaft of the tail, by pipetting the cercariae onto the shaved abdominal skin of the anaesthetised animal. Two underdeveloped females were subsequently encountered in squash preparations of the liver when the hamster was killed for worm recovery 10 weeks after infection, thus showing that some of the abnormal cercariae were viable.

A method is also described for killing and fixing cercariae while retaining some of the shining brilliance of live cercariae, without them becoming shrivelled, granular and semi-opaque, as occurs when cercariae die spontaneously or are killed with heat.

This is apparently the first report of abnormal cercariae of *S. mattheei*. In addition, a method of concentrating abnormal cercariae after emergence from a snail, a schematic classification of abnormal cercariae and a method for killing and fixing cercariae while retaining much of the shiny brilliance of live cercariae are also reported for the first time as far as is known.

Keywords: Abnormal cercariae, concentration of abnormal cercariae, fixing cercariae, *Schistosoma mattheei*

INTRODUCTION

A number of anomalies in cercariae of schistosomes and other digenetic trematodes have been described previously (Kuntz 1948; Hussey & Stahl 1961; Nasir 1973; Cheng & Harris 1974), but there is apparently no previous report on abnormal cercariae of *Schistosoma mattheei*.

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Accepted for publication 3 March 2003—Editor

Some abnormal cercariae were encountered while methods for estimating numbers of live cercariae in suspension were being tested (Van Wyk & Groeneveld 1973), and thereafter also in relatively high concentration among cercariae that had failed to penetrate into the skin of the tails of oxen exposed to cercarial suspensions.

We subsequently became interested in determining whether such cercariae could infect the primary host, and a method of concentration was developed for collecting them when required.

MATERIALS AND METHODS

A population of *S. mattheei* was isolated from the field towards the end of the 1960s and maintained in the laboratory in sheep and *Bulinus (Physopsis)* spp. snails. Infected snails were placed in tap water in glass vials of about 60 mℓ capacity and exposed to fluorescent light for a period of about 30 min after which the contents of the vials were pooled. After thorough mixing of the cercarial pool, the suspension was left standing for a few minutes until the snail faeces had sedimented, before the supernatant was decanted for use in various trials.

While examining aliquots of cercarial suspension in the process of developing methods for accurate estimation of infective doses (Van Wyk & Groeneveld 1973), seven abnormal cercariae were found among a total of 1.02 million. Five more abnormal larvae were encountered while estimating how many of 595 000 cercariae to which an ox had been exposed by dangling its shaved tail in a 2 ℓ measuring cylinder of suspension, had failed to penetrate. For this estimate an aliquot comprising only half of the suspension was examined after infection of the ox; it was thus assumed that the five abnormal individuals remained out of half of the 595 000 cercariae to which the partially immune ox had been exposed. An attempt was then made to obtain more easily an estimate of the percentage of abnormal cercariae in each batch of cercariae. This was done by estimating the total number of cercariae per batch using the method of Van Wyk & Groeneveld (1973), after which the cercarial suspension was left standing until currents in the fluid (visualised by watching the cercariae in suspension) had ceased. In order to obtain the sediment containing abnormal cercariae, if any, in about 20–30 mℓ of water, the supernatant fluid was removed by suction. The cercariae in the sediment were examined within a period of about 3 h with the aid of a stereoscopic dissecting microscope at a magnification of 250 x.

Abnormal cercariae that were alive when found and not required for testing for viability were killed by adding a few drops of 10 % formalin to approximately 20 mℓ of cercarial suspension. A few hours later more formalin was added, the better to fix the cercariae and prolong their preservation. This had the effect that the cercariae retained some of the shiny brilliance of live cercariae; they did not contract as much or become as shrivelled, semi-opaque and granular in appearance, as those that died spontaneously or were killed by heating the suspension. As an indication of the size of normal cercariae of *S. mattheei*, 30 were measured after they had been killed and fixed as described above.

The cercariae were photographed (generally at 1000 x magnification) after having been placed in fluid in the well of a hollowed glass slide which was covered with a coverslip. The microscope was sturdily supported to prevent movement of the cercariae while the photographs were being taken.

One test was conducted to ascertain whether abnormal cercariae were able to penetrate and develop in a primary host. A young adult hamster was anaesthetised with pentobarbital sodium and its abdomen shaved. It was positioned on its back, as illustrated in the article by Olivier & Stirewalt (1952). Eight cercariae, each having two tails instead of one furcous tail (similar to those depicted in Fig. 2, no. 6–9) were carefully and individually pipetted onto the shaved abdominal skin of the hamster which was kept immobilized for about 30 min. During this period it was ensured that the infection site remained moist. The faeces of the hamster so treated were examined twice for the presence of schistosome eggs by a sedimentation method (Reinecke 1983) during the period of 50–70 days after infection, whereupon, no ova having been seen, the animal was killed for worm recovery. After the hamster had been perfused by the method of Duvall & De Witt (1967), its liver was squashed between two glass plates and examined for worms with the aid of a stereoscopic dissection microscope.

On the strength of the range of abnormalities encountered, a schematic classification of such cercariae is suggested.

RESULTS

Table 1 contains a summary of the measurements of 30 normal cercariae of *S. mattheei* that were killed and later fixed in the same way as the abnormal cercariae that are described and/or depicted in this paper, and were alive when found.

Tables 2 and 3 contain, respectively, the suggested classification system for abnormal cercariae and its application to those recorded in this paper and/or previously, by others. In Fig. 1 a variety of deviations from normality are shown schematically, and micrographs of a range of affected cercariae are contained in Fig. 2. Some cercariae, e.g. one with what appeared to be an abnormally short tail that otherwise appeared to be normal (Fig. 1, no. II), were lost during handling and could therefore not be photographed or measured.

Thirty morphologically abnormal cercariae were detected among approximately 2.8 million (0.0011 %). Initially, seven (0.0007 %) of them were found when all of ± 1.02 million cercariae in different batches were examined microscopically (Van Wyk & Groeneveld 1973). Thereafter five abnormal cercariae were found among 3 430 in an aliquot comprising 50 % of a suspension of cercariae that had failed to penetrate into the skin of the tail of a partially immune ox exposed for 25 min to a suspension containing 590 000 cercariae in a 2 ℓ measuring cylinder. While the abnormal cercariae in the suspension remaining after exposure of the ox constituted 0.0017 % of 295 000 (half of the initial total), this comprised 0.15 % of the 1.2 % that remained in the suspension after having failed to penetrate the ox. Considering the similarity in the proportions of abnormal cercariae in the initial 1.02 million (0.0007 %) and in the remnant of the suspension to which the ox had been exposed (0.0017 %), it was assumed (for the purpose of calculating the overall prevalence of abnormal forms) that the latter cercariae were concentrated by inability to reach and/or penetrate into the host. Furthermore, inability to swim would cause such cercariae to sink to the bottom of the suspension, while most of the normal cercariae were still swimming.

When this hypothesis was tested, 16 abnormal cercariae were recovered from two batches of cercariae totalling 1.2 million. These comprised therefore a percentage of 0.0013. In this case only those

TABLE 1 Measurements of normal cercariae of *S. mattheei* ($n = 30$), killed and later fixed as described in the text

Anatomical feature	Measurements (µm)			
	Mean	Standard deviation	Minimum	Maximum
Tail furcae	88.2	8.6	73.5	102.9
Tail shaft	273.4	17.1	235.2	294.0
Head length	191.1	17.3	161.7	220.5
Head breadth	79.4	9.9	58.8	102.9
Head and tail shaft	464.5	nd	411.6	514.5

nd: Not done

TABLE 2 Classification system for topographical morphology of cercariae (normality is either not listed or, if preferred, is indicated by "n"—see text)[a]

Anatomical feature	No. per feature[b]	Size[c]	Shape
A: Tail appendage	0: Absent	a: Longer	i: Oval
B: Tail	1: Single	b: Shorter	ii: Triangular
C: Head	2: Twin	c: Broader	iii: Tripod-shaped
	3: Triplet	d: Narrower	iv. Quadrangular
	4: Quadruplet	e: Thicker	v: V-shaped
	5: Partially split	f: Thinner	vi: Kincked
			vii: Lopsided
			viii: Amorphous
			ix: Other

[a] If, for instance, only the tail of a cercaria is abnormal, the head is either not included in the classification, or is indicated by "n" (Cn)

[b] In case of a schism, use (1), (2), etc., for indicating the situation in each branch concerned (see Table 3)

[c] In relation to normality

TABLE 3 Various abnormal cercariae previously encountered

Theoretical illustration in Fig.1[a]	Species	Reference[b]	Micrograph or drawing of cercaria found[c]	Code[d]
No. II	*S. mattheei*	This paper	Not drawn	B_b
No. IV	*S. mattheei*	This paper	Fig. 2, no. 1	B_{vi}
No. V	*S. mattheei*	This paper	Fig. 2, no. 2	$A_{(1)1(2)1}B_{2,5}$
No. V	*C. cuman.*[f]	Nasir	Fig. 2 (6)	$A_{(1)1(2)1}B_{2,5}$
No. V	Unidentified	Mathias	Fig. 1 (II)	$A_{(1)2(2)2}B_{2,5}$
No. VI	*S. mattheei*	This paper	Fig. 2, no. 3	$A_{(1)1(2)1}B_{2,5}$
No. VII	*S. mattheei*	This paper	Fig. 2, no. 4	$A_{(1)1(2)2}B2_{(1)b}$
No. VIII	*S. mattheei*	This paper	Fig. 2, no. 5	$A_{(1)1(2)1}B_{2,5}$
No. IX	*S. mattheei*	This paper	Fig. 2, no. 6	$A_{(1)1(2)1}B_2C_{2,5}$
No. IX	*S. mattheei*	This paper	Fig. 2, no. 7	$A_{(1)2(2)?}B_{2?}C_{cvii}$
No. IX	Unidentified	H & S	Fig. 1 (2)	$A_{(1)1(2)1}B_{2?}C_{2,5}$
No. IX	*S. mansoni*	C & H	Fig. 1	$A_{(1)1(2)1}B_2C_{2,5c?}$
No. IX	*S. mansoni*	Kuntz	Fig. 4	$A_{(1)1(2)1}B_2C_{2,5}$
No. X	*S. mattheei*	This paper	Fig. 2, no. 8	$A_{(1)1(2)1}B_2C_{2,5ii}$
No. X	*S. mattheei*	This paper	Fig. 2, no. 9	$A_{(1)1(2)1}B_2C_{2,5ii}$
No. X	Unidentified	H & S	Fig. 1 (6)	$A_{(1)1(2)1}B_2C_{2,5vii}$
No. X	*S. mansoni*	Kuntz	Fig. 5	$A_{(1)2(2)2}B_2C_{2,5ii}$
No. X	*E. donosoi*[e]	Nasir	Fig. 1 (8)	$A_{(1)1(2)1}B_2C_{2,5ii,vii}$
No. XI	*S. mattheei*	This paper	Fig. 2, no. 10	$A_{(1)1(2)1}B_2C_{2,5iii}$
No. XI	*S. mattheei*	This paper	Fig. 2, no. 11	$A_{(1)1(2)1}B_2C_{2,5iii}$
No. XI	*S. mattheei*	This paper	Fig. 2, no. 12	$A_{(1)1(2)?}B_2C_{2,5iii}$
No. XII	*S. mattheei*	This paper	Fig. 2, no. 13	$A_{(1)1(2)1}B_2C_{2,5iii,v}$
No. XIII	*S. mattheei*	This paper	Fig. 2, no. 14	$A_{(1)1(2)1}B_2C_{2,5iii,v}$
No. XIII	Unidentified	H & S	Fig. 1 (4)	$A_{(1)1(2)1}B_2C_{2,5v}$
No. XIII	*C. cuman.*[f]	Nasir	Fig. 2 (7)	$A_{(1)1(2)1}B_2C_{2,5v}$
No. XIV	*S. mattheei*	This paper	Fig. 2, no. 15	$C_{2,5vii}$
No. XIV	*E. donosoi*[e]	Nasir	Fig. 1 (7)	$A_{(1)1(2)1}B_2C_{vii}$
No. XV	*E. donosoi*[e]	Nasir	Fig. 1 (6)	$A_{(1)1(2)1}B_2C_{2ii}$
Not incl.	Unidentified	H & S	Fig. 1 (7)	$A_?B_3C_{3?,5ii}$
Not incl.	*C. setifera*[g]	Mathias	Fig. 2 (II)	$A_?B_3C_{3?,5ii}$
No. XVI	Unidentified	H & S	Fig. 1 (5)	B_2C_{2iv}
Not incl.	*C. cuman.*[f]	Nasir	Fig. 2 (5)	$A_0B_0C_{2,5viii}$

[a] See Fig. 1 in this paper; Not incl.: not included in Fig. 1

[b] Nasir: Nasir 1973; Mathias: Mathias (1930); H & S: Hussey & Stahl 1961; C & H: Cheng & Harris (1974); Kuntz: Kuntz (1948)

[c] Depiction of cercaria

[d] See text for coding suggested for classifying cercariae showing aberrations. Note that normality is not included in the code listed in the table, and that "?" is used for any details that are unclear in illustrations or descriptions

[e] *Echinostoma donosoi*

[f] *Cercaria cumanensis*

[g] *Cercaria setifera*

fractions of the cercarial suspension in which the abnormal cercariae were expected to occur (in the sediment, constituting about 2.5 % of the total volume), were examined after the total in suspension had been estimated, and not the total suspension as in the initial trials. These 16 cercariae, all having complete schism of the tail (Fig. 2, no. 4–14), were alive when found. When they were observed, they did not appear to be able to swim, but only crawled along the bottom of the container, while twitching their tails, as also observed by Kuntz (1948) and Cheng & Harris (1974).

A large variety of forms and types of abnormalities were obtained (Fig. 2). These ranged from abnormalities of appendages of the tail, the tail itself, or the head, to abnormalities of all three structures and were either symmetrical or lopsided.

A suggested system for classifying abnormal cercariae is presented in Table 2. This classification

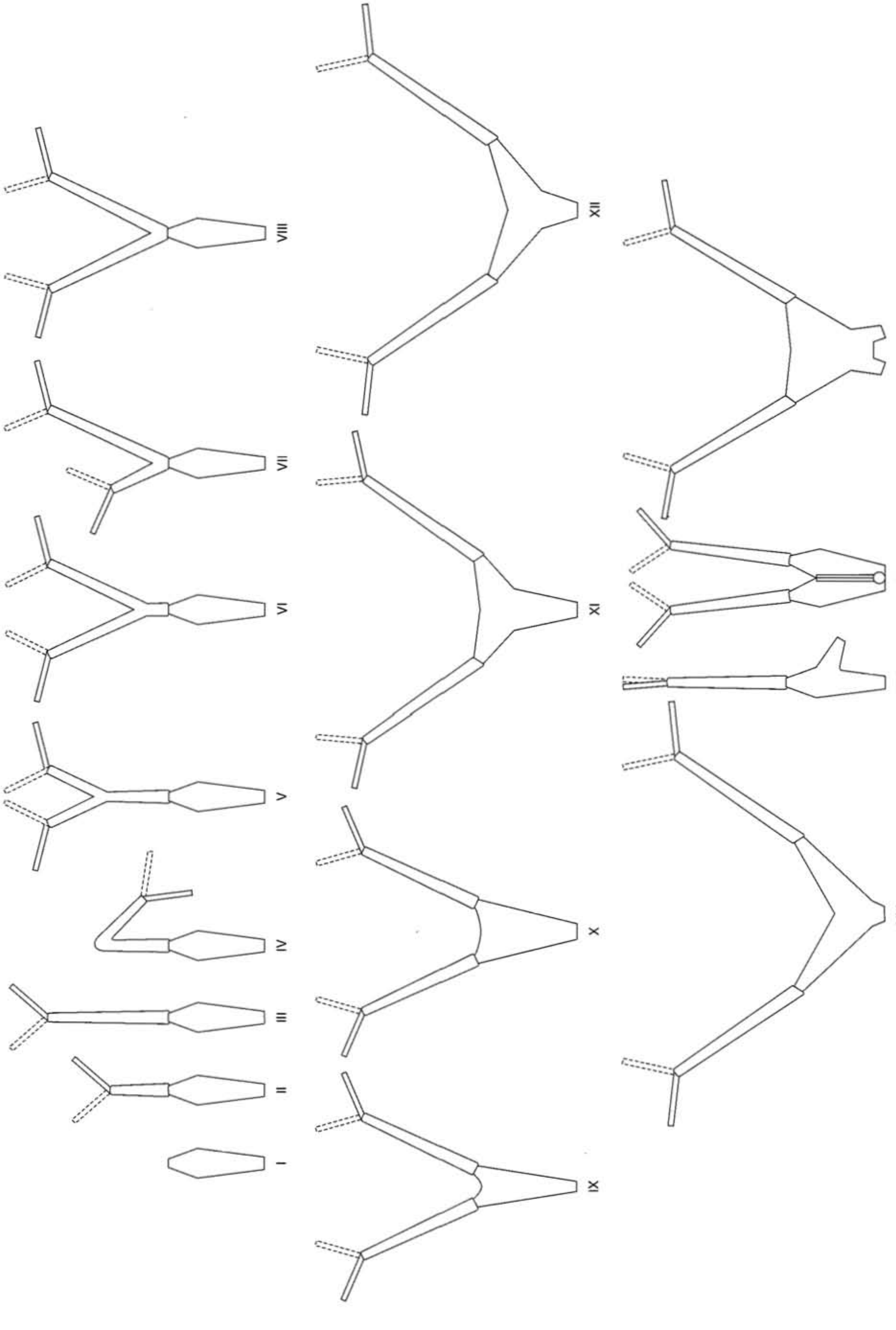

FIG. 1 Schematic illustration of abnormalities theoretically to be expected in furcocercous cercariae

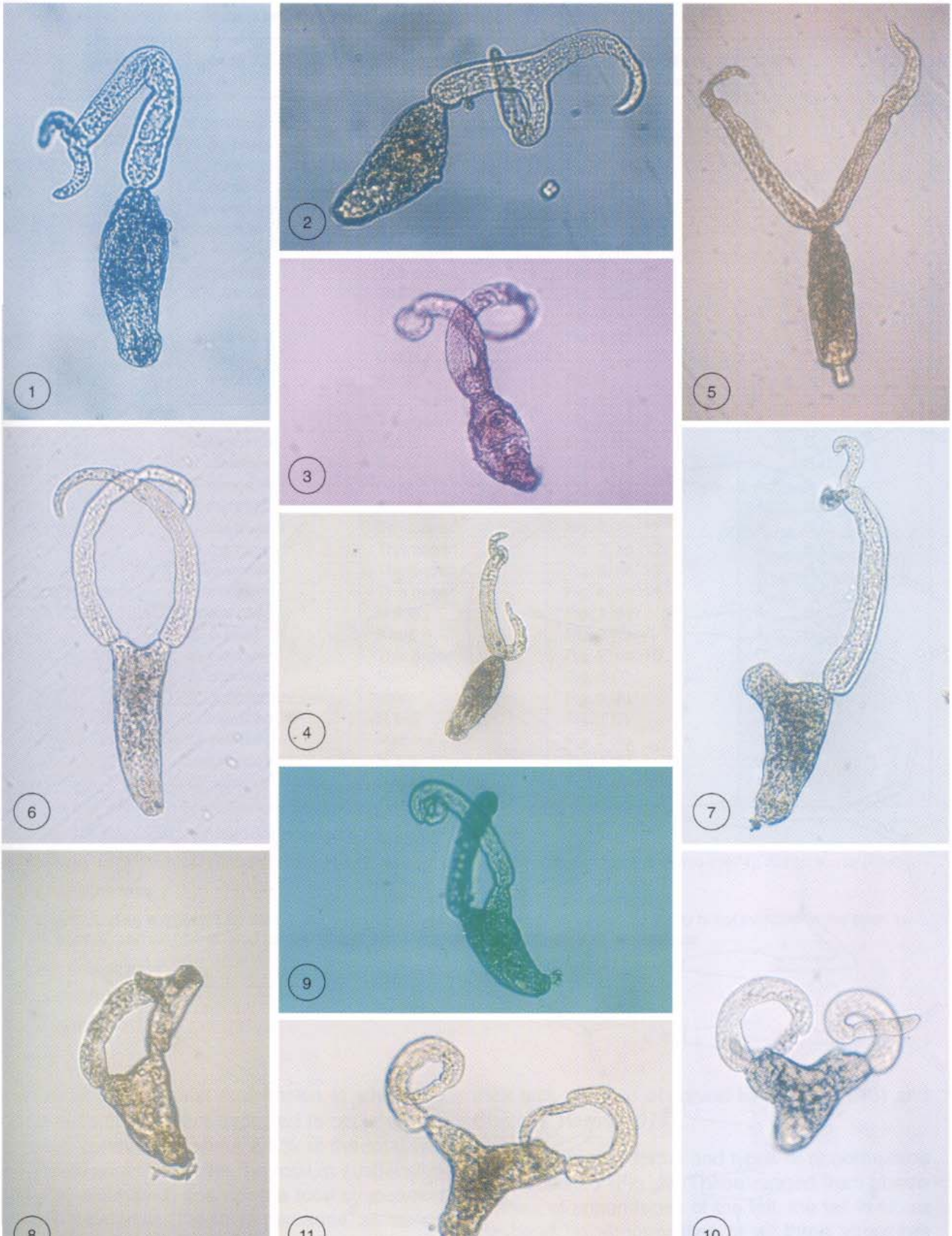

FIG. 2 Photomicrographs of 15 *Schistosoma mattheei* cercariae, illustrating some of the abnormalities encountered over a period of about 15 years, commencing when this population had been cycled for about 5 years in the laboratory

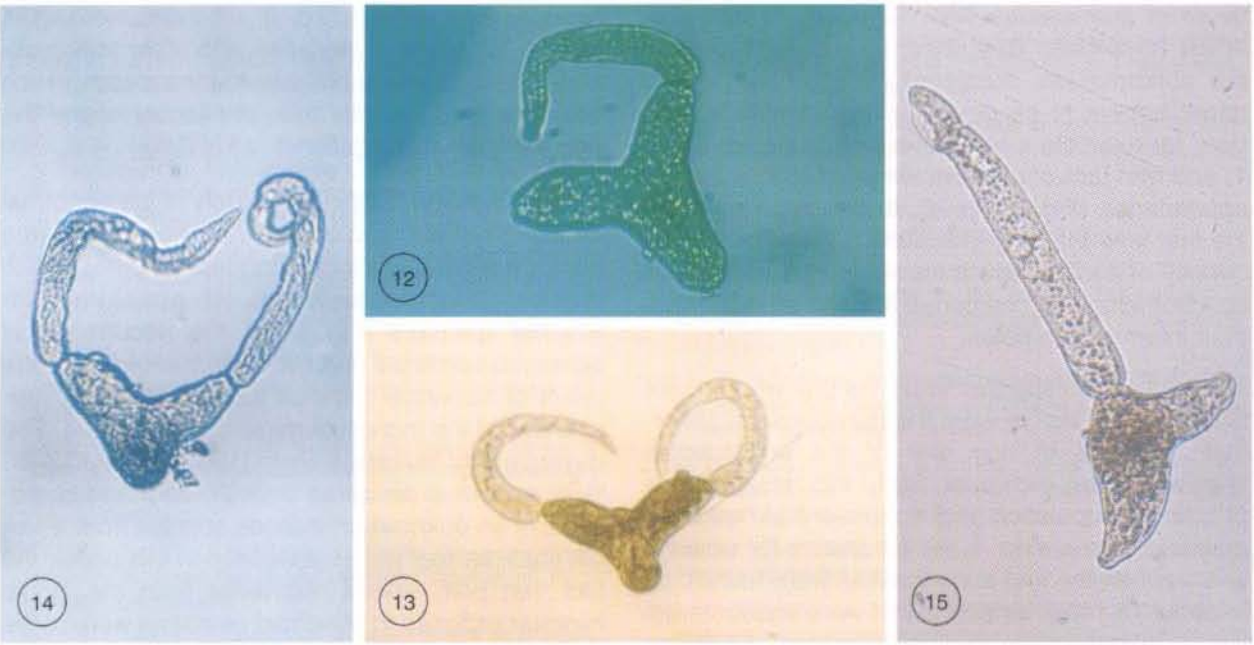

FIG. 2 (*cont.*) Photomicrographs of 15 *Schistosoma mattheei* cercariae, illustrating some of the abnormalities encountered over a period of about 15 years, commencing when this population had been cycled for about 5 years in the laboratory

includes abnormalities that are theoretically considered likely to occur (Fig. 1).

For each of the tail appendage, tail and head of the cercaria (respectively A, B and C in Table 2) abnormality can be coded by the contents of the other three columns, while normality is either excluded, or is indicated by "n". For instance, for a cercaria with two heads that are partially separated, and a normal tail and tail appendages (Fig. 2, no. 15) the formula will be $C_{2.5}$ or $A_nB_nC_{2.5}$. If there is some or other form of twinning and the different "individuals" differ from one another (e.g. a cercaria with two tails, of which one has two appendages and the other one, as in Fig. 2, no. 4), then each part is numbered and the number is enclosed in brackets, e.g. $A_{(1)2(2)1}$.

Infectivity of abnormal cercariae for the primary host

When the hamster that was exposed to eight abnormal cercariae (resembling those in Fig. 2, no. 6–9) was killed 10 weeks after infection, two female adult worms were detected in squash preparations of the liver but no worms were recovered from the perfusate (Duvall & De Witt 1967). While these females appeared to be anatomically normal (other than being underdeveloped as commonly occurs in schistosomes in the absence of males [Le Roux 1949; 1951b and Moore, Yolles & Meleney 1949)], they were unfortunately damaged during examination which precluded the taking of measurements for comparison with normal worms.

DISCUSSION

Only the topographical morphology of abnormal cercariae is considered, despite the fact that internal morphological abnormalities are likely to have been present in some cercariae depicted in this paper. Examples of internal anomalies are illustrated in Fig. II of Mathias (1930) and in Fig. 3–5 of Kuntz (1948).

Numerous reports have appeared on the occurrence of abnormal cercariae of schistosomes and other trematodes, as reviewed by Kuntz (1948); Hussey & Stahl (1961); Nasir (1973) and Cheng & Harris (1974). DiConza & Basch (1974) found branched daughter sporocysts of *Schistosoma mansoni*. While most cases were obtained while routinely examining wild-caught snails, we, together with Nasir (1973) are apparently the only ones who have reported on abnormal schistosome larvae after specifically searching for them by examining large amounts of material.

Apparently no previous reports have appeared of abnormal cercariae of *S. mattheei*, although abnormal adults (Le Roux 1951a; 1951b) and an abnormal ovum of this species (Van Rensburg & Van Wyk 2003) have been described. In addition, some of the abnormalities illustrated or described in this paper appear to be novel for any trematode species, for example a tail with a kink in it (Fig. 2, no. 1) and twin tails of different lengths and numbers of appendages (Fig. 2, no. 4). It also appears to be the first time that any indication is given of the frequency of such aberrant forms, or of a method for concentrating such cercariae after emergence from their intermediate hosts.

This paper also appears to be the first where there is some indication of what the prevalence of abnormal cercariae of any one of the schistosome species can be. However, firstly, this description is of a single population of *S. mattheei* that had been maintained in sheep in the laboratory for about 5 years (when the first abnormalities were found), up to about 15 years, when the last were encountered. Hence, extrapolation to other schistosome species or even to other field populations of the same species is not valid. Secondly, part of the estimate of abnormalities is from observation of abnormal cercariae in the remnant of a suspension to which an ox had been exposed, with the assumption that abnormal cercariae are not infective. The recovery of the two female *S. mattheei* from the liver of a hamster exposed to grossly abnormal cercariae deposited on the skin of its abdomen apparently belies this assumption. It seems unlikely that such cercariae are able to swim, with the result that they would hardly be able to infect animals in nature, or even an ox with its tail immersed in a measuring cylinder containing a cercarial suspension, especially since the animal in this case was apparently irritated and tended to move its tail continuously when, it is thought, many cercariae had started penetrating its skin.

The suggested system of classification (Table 2) is designed in such a way that it can make provision both for worm species with furcocercous cercariae and for those with single or no appendages to the tail, either by leaving any feature out of the code if it is normal for the species concerned, or else listing "n" for normality. The system will be of use mainly if a large number of abnormal cercariae are encountered, e.g. as described in this paper and those by Hussey & Stahl (1961) and Nasir (1973), or in cases where photography is impossible because of damage to or loss of them during the process of preparation.

The ability of two of the eight abnormal cercariae manifesting complete schism of the tail to infect a hamster was unexpected. It will be interesting to test the infectivity of cercariae with other abnormalities. The system reported here for detecting such cercariae more readily than previously opens the way for such investigations.

It is not known whether the majority of the abnormal forms described and/or depicted here originated from a few miracidia with a tendency to give rise to relatively high percentages of abnormals, or whether (perhaps less likely) the occurrence of abnormal cercariae is a random phenomenon, the result of abnormal genesis during formation, irrespective of the individual miracidia concerned. The description of Hussey & Stahl (1961) of from 13–80 % of abnormal cercariae from 20 daughter sporocysts of an unknown trematode species from a single snail, as well as, as described in this paper, the fact that both worms recovered from the single hamster exposed to abnormal cercariae were of the same sex, seem to lend some support to the hypothesis that a low proportion of miracidia give rise to relatively high percentages of abnormal cercariae. This is merely a surmise, but if the hypothesis is correct, then it is probable that the development of such cercariae is genetically based. On the other hand, Kuntz (1948) suggested that, in addition to genetic causes, injury or physiological changes in the host at critical points in the development of a trematode may cause abnormalities, and Cort, Hussey & Ameel (1960a, b) ascribe abnormalities of cercariae to hyperinfection of snails with microsporidian hyperparasites. Cheng & Harris (1974) thought it unlikely that an abnormal cercaria of *S. mansoni* (corresponding morphologically to our Fig. 2, no. 6) was genetic in origin.

The present results seem to invalidate the surmise of Hussey & Stahl (1961) that "Although these abnormal cercariae were alive and showed a certain amount of movement, they would probably never have emerged from the snail." Some grossly abnormal cercariae of *S. mattheei* did manage to emerge from their intermediate hosts, although it does seem unlikely that some of the forms illustrated by Hussey & Stahl (1961), for example no. 8–11 in their Fig. 1, could emerge unless considerable further development was to take place.

ACKNOWLEDGEMENTS

We are very grateful to Mr Tonie du Bruyn for his patience and his striving for perfection when the cercariae were photographed.

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