

Die invloed van blootstelling aan mirasidiums van *Schistosoma mansoni* op mortaliteitskoerse van kohorte van vier geografiese stamme van *Biomphalaria pfeifferi*

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UITTREKSEL

Eerstegenerasiekohorte van 25 eksplare elk van parentaalbevolkings van *Biomphalaria pfeifferi* afkomstig uit vier verskillende lokaliteite, is elk blootgestel aan drie *Schistosoma mansoni*-mirasidiums per slak op 'n ouderdom van vier weke. Die invloed op oorlewing is geëvalueer deur die weeklikse per capita-mortaliteitskoerse en gemiddelde lewensverwachting van die blootgestelde slakke te bereken. Geen betekenisvolle verskille kon aangetoon word tussen die per capita-mortaliteitskoerse van F₁-kohorte van die twee parentaalstamme afkomstig uit endemiese bilharziagebiede nie. Desgelyks was daar ook geen betekenisvolle verskille in hierdie opsig betreffende F₁-kohorte van die twee parentaalstamme afkomstig uit nie-endemiese bilharziagebiede nie. Per capita-mortaliteitskoerse van F₁-kohorte van die twee stamme uit endemiese bilharziagebiede was egter betekenisvol hoër en hul gemiddelde lewensverwachting derhalwe veel korter as die ooreenstemmende waardes wat vir nakomelinge van die parentaalslakke uit die nie-endemiese bilharziagebiede bereken is. Die gevolgtrekking word gemaak dat per capita-mortaliteitskoerse as sodanig, vir 'n bepaalde varswaterslakspesie betekenisvol deur die herkoms van die parentaalstam beïnvloed kan word. Gevolglik moet dit deeglik in berekening gebring word in die beplanning van eksperimentele ondersoeke van hierdie aard en by interpretasie van die resultate.

ABSTRACT

The effect of exposure to miracidia of *Schistosoma mansoni* on mortality rates of cohorts of four geographical strains of *Biomphalaria pfeifferi*

First generation cohorts of 25 specimens each of parental populations of *Biomphalaria pfeifferi* from four different localities, were each exposed to three *Schistosoma mansoni* miracidia per snail at an age of four weeks. The effect on survival was evaluated by calculating the weekly per capita mortality rates and the mean life-expectancy of the exposed snails. No significant differences could be demonstrated between the per capita mortality rates of F₁ cohorts of the two parental strains originating from localities in endemic bilharzia areas. Likewise there were no significant differences in this respect as far as F₁ cohorts of the two strains originating from localities in non-endemic bilharzia areas were concerned. However, per capita mortality rates of cohorts of the two parental strains from endemic bilharzia areas were significantly higher and their mean life-expectancy therefore much shorter than the corresponding values calculated for offspring of the parental snails from non-endemic bilharzia areas. It is concluded that per capita mortality rates of a particular freshwater snail species can be significantly influenced by origin of the parental strain and should be accounted for in the planning of experimental investigations and in the interpretation of the results.

INLEIDING

Ontplooiing van *Schistosoma* en varswaterslakke wat as geskikte tussengashere kon dien, het in Gondwanaland plaasgevind nog voordat Pangaea begin opbreek het.¹ Ten spyte van hierdie lang tydperk van assosiasie en koevolusie is daar afdoende eksperimentele getuieis vir die bestaan van graduele vatbaarheid van slaktussengasheerstamme onderling vir hulle verenigbare skistosoomspesies, wat kan wissel van algehele weerstandbiedendheid tot absolute verenigbaarheid.^{2,3,4} Die "uiteindelike doel" van digeniese trematoodparasiete is nie net om slaktussengashere te besmet nie, maar eerder om die

parasietlewensiklus voort te sit,⁵ derhalwe is dit tot voordeel van die parasiet om die tussengasheer so min moontlik te benadeel. Dat besmetting met hulle verenigbare skistosoomparasiete die lewensverwachting van tussengasheerslakke soms drasties kan inkort, is reeds wyd gerapporteer.^{5,6,7,8}

Voldoende en voorspelbare hoeveelhede parasietmateriaal is noodsaaklik om die wisselwerking tussen die helmintparasiet en tussengasheerslak sinvol te kan bestudeer. Dit is ook bekend dat stamme van dieselfde varswaterslakspesie afkomstig uit verskillende geografiese gebiede onder eksperimentele toestande betekenisvol kan verskil in hulle reaksie op eksterne faktore.^{9,10,11}

Hierdie ondersoek is gedoen om die per capita-mortaliteitskoerse¹² van kohorte van stamme van *Biomphalaria pfeifferi* afkomstig uit vier verskillende lokaliteite, na blootstelling aan mirasidiums van *Schistosoma mansoni*, te bepaal en met mekaar te vergelyk in 'n poging om onderlinge verskille in die verband te demonstreer, en 'n slaktussengasheerstam te identifiseer waarvan die lewensduur so min as moontlik deur besmetting met sy verenigbare helmintparasiet beïnvloed sal word.

MATERIAAL EN METODEDES

Vier parentaalbevolkings (± 100 eksemplare elk) van *Biomphalaria pfeifferi* (Krauss) is in natuurlike habitats in verskillende geografiese gebiede in Suid-Afrika versamel. Twee van hierdie habitats is uit endemiese bilharziagebiede gekies (Nelspruitdistrik, ruitverwysing 25° 28' S; 31° 06' O en Durbandistrik, ruitverwysing 29° 52' S; 30° 55' O) en die ander twee uit nie-endemiese gebiede (Lichtenburgdorpgebied, ruitverwysing 26° 09' S; 26° 10' O en Molopo Oog, ruitverwysing 25° 53' S; 26° 04' O). Hierdie parentaal-slakke is in die laboratorium in 'n eksperimentele opstelling gehuisves wat uit drie vlekvrystaalakwariums bestaan het waarin die kultuurwater voortdurend gesirkuleer is en die temperatuur op 25 °C konstant gehou is deur sentrale prosesseerders.^{13,14}

Slakke wat vir die ondersoek nodig was, is geteel van eiers wat gedurende 'n 24-uurperiode deur elk van die vier groepe parentaal-slakke gelê is. Pasuitgebroeide slakkies is versorg soos beskryf deur Joubert en De Kock.¹⁵ Jeugdige en volwasse slakke is gevoer met 'n 1:1 mengsel van TetraMin "Staple Diet" en Tetra "Conditioning Food" (Tetra Werke, Duitsland).¹⁶

Drie replikaatkohorte van 25 eksemplare elk is uit F₁-nakomelinge van elk van die vier stamme op 'n ouderdom van vier weke geselekteer om aan mirasidiums van die verenigbare ingewandsbilharziaparasië, *Schistosoma mansoni*, bloot te stel. 'n Parasietstam wat reeds vir dekades in die laboratorium van die Nasionale Instituut vir Siektes in 'n Tropiese Omgewing (NISTO) in stand gehou is, is geselekteer omdat dit ook elders, insluitend die buiteland, vir eksperimentele doeleindes beskikbaar gestel is. Vir elk van die vier stamme is 'n onbesmette kontrolekohort van 25 slakke aangehou.

Al die lede van die kohorte wat vir blootstelling geselekteer is, is individueel vir 'n periode van drie uur aan drie mirasidiums per slak in 2 ml water in multiselhouers blootgestel. Lede van die kontrolekohorte is op presies dieselfde wyse behandel, behalwe dat geen mirasidiums in die selle van die multiselhouers geplaas is nie. Na die blootstellingsperiode is die slakke verwyder en elke sel mikroskopies vir die teenwoordigheid van mirasidiums ondersoek. Volgens Christie en Prentice¹⁷ en Christensen¹⁸ is dit nie moontlik om die getal mirasidiums wat 'n slak gepenetreer het, te kan voorspel sonder om die getal mirasidiums wat na blootstelling oorgebly het, te tel nie, omdat tot 30% lewensvatbare mirasidiums daarin mag faal om 'n verenigbare tussengasheer binne te dring. In die huidige ondersoek is slakke waarvan die selle steeds mirasidiums bevat het na afloop van die blootstellingsperiode weer aan die ooreenstemmende getal pasuitge-

broeide mirasidiums blootgestel en die werkwyse herhaal.

Al die eksperimente is by 25 \pm 0,2 °C uitgevoer. Mortaliteit is daagliks aangeteken en die gemiddelde per capita-mortaliteit per week bereken volgens die vergelyking $N_{t+1} = N_t \cdot e^{-d}$, waar N_t die getal slakke is wat leef teen tyd t en d die mortaliteitskoers is.¹² Gebrek aan enige respons op meganiese prikkels onder 'n stereomikroskoop, is as kriterium vir dood van 'n slak geneem.¹⁹ Alle slakke wat tydens die ontwikkelingsperiode van die parasiet dood is, is tussen twee mikroskoopvoorwerpglasies platgedruk en vir die teenwoordigheid van onvolwasse parasietstadiums ondersoek.^{4,20,21}

Lede van elke blootgestelde kohort is vanaf dag 24 na blootstelling elke derde dag gesamentlik vir die afskeiding van serkariëë ondersoek. Die kontrolekohorte is op presies dieselfde wyse behandel. Nadat die eerste afskeiding van serkariëë waargeneem is, is slakke individueel vir drie opeenvolgende drie-dagperiodes vir afskeiding ondersoek, waarna eksemplare wat nog nie met afskeiding begin het nie, uit die eksperiment onttrek is. Die eksperiment is volgehou totdat alle lede van die blootgestelde kohorte dood was.

RESULTATE

Afskeiding van serkariëë het by al die blootgestelde kohorte tussen dag 33 en dag 36 'n aanvang geneem. Geeneen van die slakke het later as dag 36 met afskeiding van serkariëë begin nie en slakke wat nie afgeskei het nie, is nie in ag geneem in berekening van per capita-mortaliteit nie. Die gemiddelde besmettingspersentasie het gewissel tussen 92 en 96% (tabel 1). Geen getuienis vir spontane self-genesing van skistosoombesmette slakke,^{6,8,22} is by enige van die F₁-kohorte van enige van die vier parentaalstamme gevind nie. Soos gerapporteer deur Theron²³ is staking in die afskeiding van serkariëë in geen geval voor die dood van die slak waargeneem nie.

Omdat geen mortaliteite by een van die kontrolekohorte voorgekom het nie en by die ander drie onbeduidend was (tabel 1), is sterftes wat tydens die ontwikkelingsperiode by die blootgestelde kohorte waargeneem is, in navolging van Sturrock,⁶ aan ontwikkeling en vermenigvuldiging van onvolwasse parasietstadiums toegeskryf. Die vroegste sterftes is op dag 18 by die F₁-kohorte van die stamme afkomstig van Nelspruit en Durban waargeneem, terwyl dit eers op dag 27 by die F₁-kohorte van die stam afkomstig van Molopo Oog plaasgevind het.

Die gemiddelde per capita-mortaliteit het vir die F₁-kohorte van die Lichtenburg- en Durbanstam onderskeidelik van 0,136 tot 0,341 gestrek (tabel 2). Tukey se studentereekstoets vir veranderlike mortaliteit dui 'n getal van 0,05612 aan as die minimum betekenisvolle verskil vir hierdie stel data. Hiervolgens het die gemiddelde per capita-mortaliteitskoerse van die F₁-kohorte van die Nelspruit- en Durbanstam nie betekenisvol van mekaar verskil nie, so ook die waardes van die F₁-kohorte van die Molopo Oog- en Lichtenburgstam. 'n Betekenisvolle verskil is egter wel aangetoon tussen die eersgenoemde twee stamme aan die een kant en die laasgenoemde twee stamme aan die ander kant (tabel 2).

Uit die per capita-mortaliteitskoerswaardes is die ge-

TABEL1 Gemiddelde oorlewing van F ₁ -kohorte van parentaalbevolkings van <i>Biomphalaria pfeifferi</i> afkomstig uit vier verskillende lokaliteite								
Dae*	Getal lewende slakke							
	Lokaliteite							
	Nelspruit Eks** K***		Durban Eks K		Molopo Oog Eks K		Lichtenburg Eks K	
0	25	25	25	25	25	25	25	25
3	25	25	25	25	25	25	25	25
6	25	25	25	25	25	25	25	25
9	25	25	25	25	25	25	25	25
12	25	25	25	25	25	25	25	25
15	25	25	25	25	25	25	25	25
18	24	25	24	25	25	25	25	25
21	22	25	21	25	25	25	24	25
24	20	25	16	25	25	25	24	25
27	17	25	11	25	24	25	23	25
30	14	25	10	25	23	25	20	24
33	9	25	7	25	22	25	17	24
36	5	25	3	24	19	25	14	24
36	1+	25	2+		2+	25	2+	
	96%++		92%++		92%++		92%++	
39	4	25	3	24	18	25	13	24
42	3	25	2	24	17	25	13	24
45	2	25	1	24	16	25	12	24
48	2	25	1	23	15	25	11	24
51	1	24	1	23	14	25	10	24
54	1	24	0	23	13	25	9	24
57	1	24			12	25	9	24
60	1	24			11	25	8	24
63	1	24			11	25	7	24
66	0	24			9	25	6	24
69					9	25	6	24
72					8	25	6	24
75					8	25	5	24
78					7	25	3	24
81					6	25	2	24
84					3	25	1	24
87					2	25	1	24
90					2	25	0	24
93					1	25		
96					1	25		
99					1	25		
102					0	25		

- * Getal dae na blootstelling
 ** Gemiddelde oorlewingsyfer van drie replikaatkohorte van 25 slakke elk
 *** Kontrole van 25 nie-blootgestelde slakke
 + Getal slakke wat nie serkarië afgeskei het nie
 ++ Besmettingspersentasie

middelde slakmortaliteit en gemiddelde lewensverwagting vir elk van die F₁-kohorte van die vier stamme bereken. Tabel 2 toon dat die berekende gemiddelde lewensverwagting van drie tot ongeveer agt weke vir die F₁-kohorte van die Nelspruit- en Molopo Oogstam onderskeidelik, gestrek het.

BESPREKING

'n Oorsig van die resultate van vorige laboratorium-ondersoeke wat die oorlewing van skistosoombesmette slakke met onbesmette slakke vergelyk, dui op 'n toename in per capita-mortaliteitskoerse, alhoewel hoogs veranderlik, in die orde van 0,100.⁷ Woolhouse¹² rapporteer dat ryp besmettings met *S.mansoni*, per capita-mortaliteitskoerse van *B. pfeifferi* met 0,122 laat toeneem het. Waardes wat tydens die huidige ondersoek vir die per capita-mortaliteitskoerse bereken is, was heelwat hoër, dog het ook sterftes ingesluit wat tydens die ontwikkelingsperiode van die parasiet voorgekom het. In ooreenstemming met die resultate van Sturrock⁶ is groot getalle slakke tydens die ontwikkelingsperiode dood, terwyl mortaliteite by die kontrolekohorte negeerbaar klein was (tabel 1).

Die gemiddelde sterftes per week vir besmette slakke was ongeveer twee keer hoër vir nakomelinge van die twee stamme uit endemiese bilharziagebiede (Durban en Nelspruit) gevolglik was hul berekende totale lewensduur meer as twee keer korter as by die twee stamme uit nie-endemiese bilharziagebiede (tabel 2).

Tabel 2 Per capita-mortaliteitskoerse en lewensverwagting van F₁-kohorte van parentaalbevolkings van *Biomphalaria pfeifferi* afkomstig uit vier verskillende lokaliteite

Lokaliteit	Replikaatnommer	Per capita-mortaliteitskoers/week	Gem.	Tukey* groepering	Gem. slakmortaliteit/week	Gem. lewensverwagting (weke)
N+	1	0,339				
N	2	0,327	0,333	A	8,32	3,00
N	3	0,333				
D++	1	0,373				
D	2	0,307	0,341	A	8,53	2,93
D	3	0,344				
M+++	1	0,100				
M	2	0,161	0,127	B	3,19	7,84
M	3	0,122				
L++++	1	0,124				
L	2	0,159	0,136	B	3,41	7,34

* Tukey se studentereekstoets vir veranderlike mortaliteit
 Minimum betekenisvolle verskil = 0,05612
 Gemiddeldes met dieselfde letter verskil nie betekenisvol nie

- + Nelspruitdistrik (ruitverwysing, 25° 28' S ; 31° 06' O)
 ++ Durbandistrik (ruitverwysing, 29° 52' S ; 30° 55' O)
 +++ Molopo Oog (ruitverwysing, 25° 53' S ; 26° 04' O)
 ++++ Lichtenburgdorpsgebied (ruitverwysing, 26° 09' S ; 26° 10' O)

Hierdie resultate was teen die verwagting in. Dit sou verwag kon word dat slakbevolkings wat reeds vir dekades in die endemiese gebiede aan parasitisme deur 'n verenigbare skistosoom-parasietstam onderhewig was die nadelige gevolge van die parasiet beter sou kon verdra. Hierdie resultate is egter in ooreenstemming met die slotsom van Frandsen² dat dit nie altyd die reël is dat lokale kombinasies van *S.mansoni* en *Biomphalaria* spp. die mees verenigbare blyk te wees nie.

Hierdie ondersoek toon dat per capita-mortaliteit van kohorte van *B.pfeifferi* as gevolg van besmetting met 'n verenigbare skistosoomparasiet net so betekenisvol deur die herkoms van die slakstam beïnvloed kan word, as deur die getal mirasidiums waaraan 'n slak blootgestel word,²⁴ of die ouderdom van die slak tydens blootstelling.²⁵ Dit bevestig dus die bestaan van groot onderlinge variasie tussen sommige stamme van dieselfde slakspesie soos reeds vroeër deur verskeie outeurs gerapporteer.^{9,10,11} Daarbenewens bied dit 'n aanvaarbare verklaring vir die teenstrydige bevindinge met betrekking tot slaksterftes tydens die ontwikkelingsfase van die parasiet wat in die literatuur gerapporteer is.^{5,6,26}

Die resultate van die huidige ondersoek maak dit moontlik om die invloed van besmetting met *S.mansoni* op oorlewing van bepaalde stamme van *B.pfeifferi* te kan kwantifiseer en te kan voorspel, wat 'n groot bate kan wees in die beplanning van navorsing wat rondom parasiet-tussengasheerwisselwerking sentreer. Dat verskillende parasietstamme 'n bepaalde slaktussengasheer verskillend kan beïnvloed, is reeds elders gerapporteer^{2,4,27} en is 'n aspek wat ook vir die Suid-Afrikaanse spesies nadere ondersoek regverdig.

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SUMMARY

Coevolution of *Schistosoma* and Mesozoic snails began in Gondwanaland prior to the breakup of Pangaea. In spite of this long period of association and coevolution, there is decisive experimental evidence to prove the existence of gradual susceptibility between strains of snail intermediate hosts to their compatible schistosome parasites which may range from total insusceptibility to absolute compatibility. It is also known that strains of a given freshwater snail species originating from different localities may differ significantly in their reactions on external factors under experimental conditions.

This study was conducted to compare the per capita mortality rates of four geographical strains of *Biomphalaria pfeifferi* after exposure to *Schistosoma mansoni* miracidia (a compatible helminth parasite) in order to demonstrate possible differences between the strains in this respect and to identify a strain of which the lifespan would be less adversely affected.

Four parental populations of *B. pfeifferi* were collected from natural habitats of which two were situated in

nonendemic bilharzia areas (Lichtenburg township, grid reference 26° 52'S; 26° 10'E and Molopo Oog, grid reference 25° 53'S; 26° 04'E) and two in endemic bilharzia areas (Nelspruit district, grid reference 25° 28'S; 31° 06'E and Durban district, grid reference 29° 52'S; 30° 55'E). These parental snails were kept in the laboratory in an experimental set-up consisting of three stainless steel aquaria through which water was continuously circulated and the temperature kept constant at 25° C by central processing units.

Three replicate cohorts of 25 specimens each were selected at random from four-week old F₁ offspring of each of the four parental populations and exposed to three *S. mansoni* miracidiums per snail. A control cohort of 25 unexposed snails was kept for each of the four strains. All experiments were conducted at 25 ± 0,2° C. Mortalities were recorded daily and the mean per capita mortality rates per week calculated from the equation $N_{t+1} = N_t e^{-d}$ where N_t is the number of snails alive at time t and d is the mortality rate.

Members of each exposed cohort were screened together every third day for shedding of cercariae from day 24 post-exposure onwards. After initial shedding was observed, snails were individually screened for three consecutive three-day periods and non-shedding specimens were discarded. The experiment was continued until all members of the exposed cohorts were dead.

Shedding of cercariae commenced between day 33 and 36 in all the exposed cohorts. Non-shedding snails were not considered as part of the infected cohorts in calculating the per capita mortality rates. However, as no mortalities at all occurred in the control cohorts at corresponding times, mortalities during the prepatent period were ascribed to effects of developing parasites and therefore incorporated in the calculations.

Percentage infection ranged from 92 tot 96% and a stop in cercarial production was never observed before the death of a particular snail. The earliest mortalities were recorded on day 18 for the F₁ cohorts of the strains from Nelspruit and Durban, but only took place on day 27 at the F₁ cohorts of the Molopo Oog strain.

Tukey's studentised range test for variable mortality indicates 0,056 as the minimum significant difference for the set of per capita mortality rate values calculated from these results. According to this there was no significant difference between the mean per capita mortality rates of 0,333 and 0,341 calculated respectively for the F₁ cohorts of the Nelspruit strain and the Durban strain. Likewise, the values for the F₁ cohorts of the Molopo Oog strain (0,127) and the Lichtenburg strain (0,136) did not differ significantly. However, there was a significant difference between the cohorts of the former two strains and those of the latter two strains, in this respect.

The mean mortalities per week for infected snails were approximately twice as high for the offspring of the two strains from endemic bilharzia areas and their mean life expectancies accordingly more than two times shorter than the values calculated for the two strains from non-endemic bilharzia areas. These findings were contrary to expectations. It could be reasoned that snail populations subjected to parasitism by compatible schistosomes in the endemic

areas for decades would be more tolerant to the adverse effects of these parasites. The results of this investigation, however, are in agreement with similar findings reported elsewhere for other schistosome and snail species combinations.

The present study revealed that per capita mortality rates of cohorts of *B. pfeifferi* due to infection with a compatible schistosome parasite could be influenced just as significantly by the origin of the snail strain as by the number of miracidia to which individual snails are exposed, or by the age of the snails at the time of exposure, as reported elsewhere in literature. It also supports the evidence for the existence of great variation amongst certain strains of the same snail species, reported earlier by several other authors. It also supplies a plausible explanation for the contradictory results reported in literature, in respect of mortalities recorded during the prepatent period in groups of exposed snails.

The results of this investigation make it possible to quantify and to predict the effect of infection with *S. mansoni* on survival of given strains of *B. pfeifferi*. This could be of great benefit in the planning of research involving schistosome parasite-snail intermediate host interactions in the laboratory.

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