**Pseudechinoparyphium echinatum** (Digenea: Echinostomatidae): experimental observations on cercarial specificity toward second intermediate hosts

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**SUMMARY**

Infectivity of *Pseudechinoparyphium echinatum* cercariae to 11 species of gastropod was examined experimentally. Broad specificity and differential host–parasite compatibility were exhibited. Nine gastropod species functioned as second intermediate hosts. *Planorbarius corneus, Physa fontinalis, Lymnaea peregra* and * Biomphalaria alexandrina* showed high levels of compatibility with the parasite. In single-species exposures over 90% of cercariae encysted in each of these hosts. Low compatibility with the first intermediate host species *Lymnaea stagnalis* may be a mechanism preventing super-infection of emitting snails. Cercariae did not infect the prosobranchs *Bithynia tentaculata* and *Viviparus viviparus*. Experimental infection of a host community comprised of 8 European gastropod species revealed an order of host utilization similar to that shown in single-species exposures. However, cercarial transmission success in *P. fontinalis* and *L. peregra* (compared to that in *P. corneus*) was significantly reduced. This may have been due to the marked preference of cercariae for *P. corneus* compared to the other two highly suitable hosts for whom cercariae showed equal preference.

Key words: *Pseudechinoparyphium echinatum*, echinostome, cercariae, host specificity, second intermediate hosts, host utilization.

**INTRODUCTION**

*Pseudechinoparyphium echinatum* von Siebold 1837 (*Kanev & Vassilev, 1986* (= *Echinoparyphium aconiatum* Dietz 1909)) is an intestinal parasite of wildfowl occurring in Britain, Europe and Asia, which utilizes the freshwater pulmonate gastropod *Lymnaea stagnalis* as first intermediate host. The cercariae of this 37 collar-spined echinostome are known to exhibit broad specificity towards the second intermediate host. Organisms functioning in this role in the natural environment include tadpoles and adults of the amphibian *Rana ridibunda*, the freshwater terrapin *Emys orbicularis* and freshwater gastropods, (*Vassilev & Kambourov, 1972; Kanev, 1982*). At present no quantitative information is available on the degree of compatibility of the parasite with different species of gastropod second intermediate host, although investigations carried out in Bulgarian biotopes in the Danube Valley have suggested that a high degree of preference is shown for the planorbid *Planorbarius corneus* and the lymnaeid *Lymnaea peregra* while the prosobranch *Bithynia tentaculata* is rarely infected.

A study by Evans & Gordon (1983b) on the echinostome *Echinoparyphium recurvatum* discovered close parallels between the experimentally determined order of host utilization exhibited by cercariae among gastropod second intermediate hosts and that determined by an earlier detailed field study (Evans, Whitfield & Dobson, 1981). The former authors showed that differential susceptibility of gastropods to infection by the parasite was likely to be one of the most important factors in determining the pattern of host utilization in the natural environment. The present study set out to examine the pattern of second intermediate host snail utilization by *P. echinatum* with the aim of providing some information on the possible spectrum of second intermediate hosts in natural systems.

**MATERIALS AND METHODS**

Parasite and host material

*P. echinatum* cercariae were obtained from naturally infected specimens of the first intermediate host *Lymnaea stagnalis*. The snails were collected in June 1987 from freshwater channels close to the River Danube, 80 km north of Mikhaylovgrad, in Bulgaria. In total 11 species of freshwater gastropod were exposed to infection; the planorbid *Planorbarius corneus, Planorbiis planorbis, Biomphalaria alexandrina*, the bulinid *Bulinus truncatus*, the lymnaeids *Lymnaea peregra*, *Lymnaea stagnalis*, *Lymnaea palustris* and *Lymnaea truncatula*, the physid *Physa fontinalis* and the prosobranchs *Viviparus viviparus* and *Bithynia tentaculata*. The identities of European snail species used were checked by reference to guides such as Macan (1977). All snails, with the exception of *B. tentaculata* obtained from a natural site known to be free from echinostome infection,
were laboratory bred and infection-free. Snails used throughout this study were of similar size and in the range 3–5 mm, Evans & Gordon (1983a) having shown host size to be an important determinant of cercarial transmission success.

Infection of single host species

Cercarial infectivity towards the range of potential second intermediate hosts shown above was determined using the following method. Thirty specimens of each species were exposed (with the exceptions of B. tentaculata, V. viviparus and B. truncatus of which 10 specimens of each were used and B. alexandrina of which 12 specimens were used). Snails were exposed singly to freshly emitted cercariae (within 0.5 h post-emission) using a constant ratio of 20 cercariae/snail in 20 ml of dechlorinated water at pH 7 and 23 ± 2 °C. Exposures were conducted in shallow glass dishes. Snails were examined for infection 24 h post-exposure. Examination was carried out by crushing each snail between two glass microscope slides and recording the number of metacercarial cysts formed using a stereo-microscope. Specific organs of each snail were not examined separately for cysts. Cercarial transmission success was calculated as: (total number of metacercarial cysts established/total number of cercariae) × 100.

Infection of host communities

In order to investigate patterns of cercarial transmission in host communities two experiments were carried out.

Experiment 1. This experiment was designed to yield information on the pattern of cercarial transmission in a multi-species host community. Cercariae were provided with a choice of 8 potential gastropod second intermediate hosts known to occur commonly in European habitats from which P. echinatum has been recorded. Ten specimens each of P. corneus, P. planorbis, L. peregra, L. stagnalis, L. truncatula, P. fontinalis and B. tentaculata were placed in a cylindrical aquarium containing 1·6 litres of dechlorinated water. Exactly 1600 freshly emitted cercariae were then introduced, thus maintaining the density of 1 cercaria/ml, employed in single host species exposures. Snails were examined for infection 24 h post-exposure.

Experiment 2. In this experiment the degree of preference exhibited by cercariae for three species of pulmonate host P. corneus, P. fontinalis and L. peregra in mixed populations was examined. Exposures were conducted in glass dishes containing 40 ml of dechlorinated water at a host–parasite density equivalent to that employed in Exp. 1. Details of the experimental design are given in Table 3.

RESULTS

Infections of single host species

The infectivity of P. echinatum towards 11 species of potential second intermediate host, 9 European and 2 African, is shown in Fig. 1 and Table 1. It is obvious that 3 European pulmonates, Planorbarius corneus, Physa fontinalis and Lymnaea peregra displayed a similar degree of high compatibility with the parasite. Analysis of variance using cyst counts transformed by log(x + 1) showed that the numbers of cercariae establishing as cysts in each of these hosts were not significantly different, (P(F = 2.23) > 0.05, d.f. = 2 and 8). The African planorbid Biomphalaria alexandrina showed a similar high degree of compatibility whereas the African bulinid Bulimus truncatus showed intermediate compatibility comparable to that of the European lymnaeid Lymnaea palustris. The low degree of compatibility of the planorbid Planorbis planorbis is similar to that shown by Evans & Gordon (1983b) for this snail species with Echinoparyphium recurvatum cercariae. The first intermediate host of P. echinatum, Lymnaea stagnalis, also exhibited low compatibility with only 5 % of cercariae successfully establishing as cysts. The prosobranchs Bithynia tentaculata and Vivi-parus viviparus proved to be completely refractory to infection. It should be noted that in this experiment (and the others described in this study) no ectopic encystment of cercariae on snail faeces or mucus trails was observed.

Infection of multi-species host communities

The pattern of host utilization in a multi-species host community comprised of 8 species of potential second intermediate host is shown in Fig. 2 and Table 2. The general order of host utilization was very similar to that determined under conditions of single host species exposures, P. corneus, P. fontinalis and L. peregra remaining the most heavily utilized hosts. However, the pattern of cercarial transmission among these high compatibility hosts differed in one important respect from that shown under individual species exposure conditions. Under the latter conditions each of these hosts showed a similar high degree of compatibility with the parasite. Under multi-species exposure, analysis of variance on cyst counts transformed by log(x + 1) revealed significant differences between the numbers of cercariae establishing as cysts in each of these hosts (P(F = 7.05) < 0.05, d.f. = 2 and 27). Using a t-test on cyst counts transformed by log(x + 1) no significant difference was found between the number of cysts formed in P. fontinalis and L. peregra (P(t = 0.63) >
Table 1. Infectivity of *Pseudechinoparyphium echinatum* cercariae to a range of gastropods under conditions of single host species exposure

<table>
<thead>
<tr>
<th>Gastropod species</th>
<th>No. of snails exposed to infection</th>
<th>No. of cercariae/snail</th>
<th>Mean no (± s.e.) metacercariae recovered/snail</th>
<th>Percentage snails infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Planorbarius corneus</em></td>
<td>30</td>
<td>20</td>
<td>19-1 (±0-21)</td>
<td>100</td>
</tr>
<tr>
<td><em>Physa fontinalis</em></td>
<td>30</td>
<td>20</td>
<td>18-46 (±0-33)</td>
<td>100</td>
</tr>
<tr>
<td><em>Lymnaea peregra</em></td>
<td>30</td>
<td>20</td>
<td>18-11 (±0-43)</td>
<td>100</td>
</tr>
<tr>
<td><em>Lymnaea palustris</em></td>
<td>30</td>
<td>20</td>
<td>11-33 (±1-26)</td>
<td>83</td>
</tr>
<tr>
<td><em>Lymnaea truncatula</em></td>
<td>30</td>
<td>20</td>
<td>3-7 (±0-85)</td>
<td>47</td>
</tr>
<tr>
<td><em>Lymnaea stagnalis</em></td>
<td>30</td>
<td>20</td>
<td>1-03 (±0-43)</td>
<td>20</td>
</tr>
<tr>
<td><em>Planorbis planorbis</em></td>
<td>30</td>
<td>20</td>
<td>0-3 (±0-17)</td>
<td>10</td>
</tr>
<tr>
<td><em>Bithynia tentaculata</em></td>
<td>10</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Viviparus viviparvs</em></td>
<td>10</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Biomphalaria alexandrina</em></td>
<td>12</td>
<td>20</td>
<td>18-92 (±0-22)</td>
<td>100</td>
</tr>
<tr>
<td><em>Bulinus truncatus</em></td>
<td>10</td>
<td>20</td>
<td>8-4 (±2-38)</td>
<td>70</td>
</tr>
</tbody>
</table>

Fig. 1. Transmission success of *Pseudechinoparyphium echinatum* cercariae in a range of gastropods under conditions of single host species exposure.

<table>
<thead>
<tr>
<th>Gastropod species</th>
<th>No. of snails exposed to infection</th>
<th>Percentage snails infected</th>
<th>Mean no (± s.e.) metacercariae recovered/snail</th>
<th>Percentage of total cyst population in species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Planorbarius corneus</em></td>
<td>100</td>
<td>48-2 (±11-23)</td>
<td>43-7</td>
<td></td>
</tr>
<tr>
<td><em>Physa fontinalis</em></td>
<td>100</td>
<td>25-3 (±5-0)</td>
<td>22-9</td>
<td></td>
</tr>
<tr>
<td><em>Lymnaea peregra</em></td>
<td>100</td>
<td>21-9 (±4-37)</td>
<td>19-9</td>
<td></td>
</tr>
<tr>
<td><em>Lymnaea palustris</em></td>
<td>60</td>
<td>7-9 (±2-66)</td>
<td>7-2</td>
<td></td>
</tr>
<tr>
<td><em>Lymnaea truncatula</em></td>
<td>60</td>
<td>4-6 (±1-8)</td>
<td>4-1</td>
<td></td>
</tr>
<tr>
<td><em>Lymnaea stagnalis</em></td>
<td>20</td>
<td>2-0 (±1-41)</td>
<td>1-8</td>
<td></td>
</tr>
<tr>
<td><em>Planorbis planorbis</em></td>
<td>10</td>
<td>0-4 (±0-4)</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td><em>Bithynia tentaculata</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

0-05, D.F. = 18), and therefore these hosts maintained the same degree of compatibility with the parasite compared to each other as they did under individual species exposures. However, a *t*-test on cyst counts transformed by \(\log(x+1)\) indicated that the number of cysts established in *P. corneus* was significantly greater than that in both *P. fontinalis* (*t* = 3-5 < 0-05, D.F. = 18) and *L. peregra* (*t* = 3-7 < 0-05, D.F. = 18). The transmission success of cercariae in *P. corneus* was in fact approximately double that of
which also exhibits broad 
paryphium recurvatum,
decline in any single mollusc host species. Such
dependence effects of local population 
a habitat. It may also help to protect the parasite 
function as a ‘decoy’ conferring a significant 
degree of protection from infection by E. recurvatum 
cercariae on the high suitability hosts L. peregra and

The marked preference shown by cercariae for 
P. corneus was confirmed by the results shown in 
Table 3. Again, t-tests on cyst counts transformed 
by log(x + 1) indicated that the number of cercariae 
establishing as cysts in P. corneus was significantly 
greater than in either P. fontinalis (P(t = 9.1) < 0.05, 
D.F. = 38, or L. peregra (P(t = 8.0) < 0.05, D.F. = 38), under conditions where a choice of host was made available. The results also confirm that although P. echinatum cercariae show a preference for 
P. corneus they do not discriminate between 
P. fontinalis and L. peregra. Cyst counts from the 
latter hosts transformed by log(x + 1) were not found 
to be significantly different, (P(t = 1.2) > 0.05, 
D.F. = 38).


discussion

The order of second intermediate host utilization by 
P. echinatum cercariae determined experimentally shows close parallels with that suggested by occasional field studies carried out in north Bulgarian 
biotopes along the River Danube in recent years, which indicate that the parasite shows a preference for the pulmonates P. corneus and L. peregra, but rarely encysts in prosobranchs such as B. tentaculata. 
It is probable that differential compatibility of the parasite with different second intermediate host snails is an important factor in determining the order of host utilization exhibited by P. echinatum cercariae in the natural environment.

The broad host specificity exhibited by 
P. echinatum cercariae toward second intermediate host gastropods probably confers a similar set of eco-
logical advantages on the parasite as those suggested by Evans et al. (1981) for the cercariae of Echinophysnum recurvatum, which also exhibits broad specificity towards second intermediate hosts. Broad specificity increases the density of potential hosts in a habitat. It may also help to protect the parasite from the deleterious effects of local population 
decay in any single mollusc host species. Such 
ecological advantages may suggest the reason why 
the trait of broad specificity toward second inter-
mediate hosts has evolved in several echinostome 
species.

Although differential compatibility clearly 
operates in the case of P. echinatum cercariae among 
gastropod second intermediate hosts, the degree of 
host–parasite compatibility seems, to a certain ex-
tent, to be independent of the phylectic position of the 
host. For example, cercariae show significantly 
different degrees of compatibility with the con-
generic lymnaeid species Lymnaea peregra, L. palus-
tris, and L. stagnalis. The parasite also shows 
markedly different compatibility with the planorbid 
species P. corneus and P. planorbis. There is, 
however, no significant difference between its com-
patibility with the lymnaeid L. peregra, and the 
planorbid P. corneus. A similar type of finding was 
made by Evans & Gordon (1983b) for cercariae of the echinostome E. recurvatum. It was discovered 
that the prosobranch snail Valvata piscinalis and the 
lymnaeid pulmonate L. peregra were second in-
termediate hosts of similar high compatibility, whereas the prosobranch B. tentaculata and the 
lymnaeid L. stagnalis were both hosts of very low 
suitability.

The first intermediate host of P. echinatum, 
L. stagnalis, showed a consistently low degree of 
compatibility with cercariae in this study. This situation is the opposite of that shown by Evans & 
Gordon (1983b) for E. recurvatum cercariae which show a high degree of preference for the first 
intermediate host of this species L. peregra. The functional significance of low compatibility of 
P. echinatum cercariae with L. stagnalis may be that 
it is a mechanism preventing metacercarial super-
infection of emitting hosts and also preventing 
parasite pressure on the first intermediate host 
population in general. Low compatibility of cercariae with the first intermediate host may therefore be 
likely to be of advantage to P. echinatum.

The ability of the low suitability host L. stagnalis 
to function as a ‘decoy’ conferring a significant 
degree of protection from infection by E. recurvatum 
cercariae on the high suitability hosts L. peregra and

<table>
<thead>
<tr>
<th>Community composition</th>
<th>No. of cercariae</th>
<th>No. of replicate experiments</th>
<th>Mean no. (± s.e.) of cysts/snail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 P. corneus + 1 P. fontinalis</td>
<td>40</td>
<td>20</td>
<td>23.1 (±0.79)</td>
</tr>
<tr>
<td>1 P. corneus + 1 L. peregra</td>
<td>40</td>
<td>20</td>
<td>24.0 (±1.18)</td>
</tr>
<tr>
<td>1 P. fontinalis + 1 L. peregra</td>
<td>40</td>
<td>20</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3. Transmission of Pseudechinoparyphium echinatum cercariae in communities of high compatibility 
second intermediate hosts.
P. fontinalis has been shown by Evans & Gordon
(1983b). The role of low suitability host decoy snails in reducing transmission levels of schistosome
miracidia is also well documented (Christensen,
1980; Combes & Moné, 1987). In the present study
we have shown that the high suitability host
P. corneus effectively has the ability to confer a
significant level of protection on two other high
suitability hosts, L. peregra and P. fontinalis. When
exposed individually to infection P. corneus,
L. peregra and P. fontinalis showed an insignificantly
different degree of compatibility with the parasite.
However, in situations where a choice of these hosts
was made available, P. corneus was preferred to both
of the other two high suitability hosts. It is probable
that the apparently strong attractiveness of P. corneus to
the parasite was largely responsible for the fact that
both L. peregra and P. fontinalis showed lower
degrees of infection with the parasite compared to
P. corneus in the community infection experiment
than they did under conditions of individual host
species exposure. This illustrates the point that the
transmission success of P. echinatum cercariae into a
given second intermediate host is not a constant but
a variable, an important determinant of which is the
species composition of the surrounding host com-

At present it is not known how the strong
attractivity of P. corneus for P. echinatum cercariae is
generated. However, it is well documented that
attraction to chemical stimuli produced by the host
plays a role in attraction and attachment to in-

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and Biomphalaria glabrata (Gastropoda) with
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