Trematode infection among freshwater gastropods in the Gharb area, Morocco

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Abstract
In the summer of 2012 and the spring of 2013, a snail survey aimed at determining relationships between the distribution of molluscan fauna, various environmental factors and trematode infection was carried out in the Gharb area, Morocco. The molluscan fauna consisted of eleven species belonging to 6 families, (including 9 gastropods and 2 bivalves). Cercariae belonging to five families were collected. Only Melanopsis praemorsa, Lymnea peregra and Lymnaea truncatula issued the cercariae. No case of mixed-species infections was found.

The present study, first of its kind at the Gharb region, paved the way for other studies to know information about the distribution and ecology of freshwater molluscs in the region.

Index Keywords: Cercariae, freshwater gastropods, Trematode infection, The Garb area, Morocco.
1. INTRODUCTION
The majority of studies on trematode infections in snails in Morocco have focused on those of medical and veterinary importance, while little information on other trematode species is available.

Several studies suggest that the distribution and prevalence of trematodes of medical and veterinary significance will be altered and in some cases enhanced by global climate change (Patz et al. 2000, Dobson et al. 2003, Mas-Coma et al. 2009).

Environmental changes following irrigation development are likely to modify the pattern of distribution of snails and trematodes. Consequently the cercariae shed by snails can be used to assess the environment impact (Lafferty, 1997). Information on snail distribution and infection, ecology and behavior could strengthen the efficiency of control measures against snails of medical and veterinary importance. The relevance of such studies was particularly emphasized in the control of Schistosomiasis transmission (Madsen 1992; Sturrock 1993). A combination of different methods of control, when possible, is recommended as well (World Health Organization 1993). Possible competitive interactions between larval stages of native trematodes have been suggested as candidates for biological control of schistosomiasis (Combes 1982). Several field experiments on the use of sterilizing trematodes to control snail intermediate hosts have been conducted and reviewed by Combes (1983).

The present investigation aimed at providing information on distribution patterns of molluscan species in relation to habitat parameters with emphasis on the importance of trematodes. The purpose of the present note is to list cercariae shed by different snail species collected.

2. MATERIALS & METHODS
2-1 Study Area (Figure 1)
The Gharb area is situated in north-western Morocco, covers an area of 8805 km² and has a population of 1,859,540 (2004 census).

He is the second most important agricultural region of Morocco after Tadla-Azilal. In 2011, the total agricultural area is estimated at 324.551 ha. The agricultural area is estimated at 200.168 ha with 93.590 ha irrigated, rainfed 106578 ha, 19916 ha of forests, 35.506 ha of rangeland and 11500 uncultivated. (ORMVAG, 2011)

Figure 1 : Map showing location of the Tessaout Amont

2-2 Sampling method
Between May 2012 and May 2013, A total of 200 sites spread over 5 towns were surveyed. Sampling sites were selected so as to cover the entire region, the widest possible range of habitats, including
swamps, canals and rivers, their epidemiological history in Schistosomiasis and their favorable biotope conducive to the development of freshwater molluscs. At selected secondary canals, tertiary canals were randomly sampled. Nearby swamps were always inspected for the presence of water and if possible, sampled. A standardized semi-quantitative snail sampling technique was used. At first, visible species to the naked eye are harvested by hand. This easy and inexpensive method requires no special equipment and has been used by several authors (Cameron and Down, 1980; Magin and Tatoni, 1995; Gosteli, 1996). We also used two types of drags by area prospected: for irrigation canals, a rectangular drag (20cm x 12,5cm x 1,70m) provided with a cutting frame covered with a 1mm mesh net.

The dredge is used to scrape the bottom and shoreline vegetation serving as support for freshwater molluscs. This technique was also used by (Khallaayoune and Laamrani, 1992) and (Maqboul, 1996).

For other habitats (rivers and swamp), we used a rectangular drag with a diameter of 20cm and a length of 1.70 m. The drag is pushed through the vegetation or on the bedrock surface and the contents are stirred carefully to detach molluscs to the vegetation.

The content are sorted in a tray using a gripper, all specimens are then packaged in a wooden box with holes in order for ventilation to keep them alive.

The samples were transported to laboratory of Biology and Health in the Sciences Faculty of Kenitra where snails were identified according to Brown (1980) and Kristensen (1985). All snails were placed individually in small troughs containing 12 ml of canal water and exposed to artificial light for at least four hours to induce cercarial emergence.

Detailed morphology of the cercariae was observed on a slide under a trinocular microscope. Cercariae were photographed using a camera fitted to the microscope, and they were identified by a combination of staining methods and chaetotaxy, following Combes (1980), Frandsen & Christensen (1984), Nasir (1984) and Schell (1985).

3. RESULTS

3-1 Habitats sampled

In total, 200 sites representing six categories of habitats were sampled. The habitats consisted of canals of different sizes and their structures, traditional canals, drains; swamps and the Tessaout River. In the irrigation system, a diverse aquatic flora, including algae and macrophytes was found. Representatives of Characeae, Diatomiacae, Chlorophyceae, Cyanophyceae, Herbaceae and Graminae. Rooted macrophytes were found mostly in shallow habitats with intermittent and/or permanently slow-flowing water and thick substratum, such as drains, traditional canals, river banks and a few canal structures.

3-2 Molluscan fauna

A total of 11 Molluscan species belonging to familie Physidae, Lymnaeidae, Thiariidae, Hydrobiidae and Unionidae were collected. Thiariidae snails were the most frequent, found at 63% of the sampled sites, whereas Melanoïde tuberculata was collected at 10% of the sites.

Table 1 shows that Melanopsis praemorsa was present in all kind habitats even canals with high water velocity. With Melanopsis Cariusis, It was the most frequent and abundant snail species in rivers and canals with moderate velocities.

Physa acuta is more abundant in the irrigation canals and swamp. The Other species were present at very low density.
### Table 1: Mean density of mollusc species (individual/m²) in different habitats in Gharb area

<table>
<thead>
<tr>
<th>Species</th>
<th>N° Of Sites</th>
<th>River</th>
<th>Swamps</th>
<th>lakes</th>
<th>canals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physa Acuta</td>
<td>10</td>
<td>Mda</td>
<td>179</td>
<td>31,9</td>
<td>34,5</td>
</tr>
<tr>
<td>Melanopsis Praemorsa</td>
<td>10</td>
<td>Drader</td>
<td>13,5</td>
<td>21,6</td>
<td>18,7</td>
</tr>
<tr>
<td>Melanopsis Cariousis</td>
<td>10</td>
<td>Fouarat</td>
<td>0,0</td>
<td>61,3</td>
<td>14,7</td>
</tr>
<tr>
<td>Melanopsis Scalaris</td>
<td>34,4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Peregra</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Truncatula</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Stagnalis</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U. Duriei</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Cygnae</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Confusa</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Tuberculata</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3-3 Parasite fauna

We examined a total of 2400 individual freshwater snails. 12% of species collected emitted Cercariae but only three species harboured larval stages of trematodes, it is melanopsis praemorsa, Lymnae truncatula and Lymnea peregra. (Table 2)

Altogether, five morphologically distinguishable cercarial types were recovered (Figure 2):
- Furcocercous Longifurcate pharyngeate, brevifurcate lophocercous and Xiphidiocercaire parasitizing melanopsis praemorsa.
- Echinostome parasitizing Lymnea peregra.
- Cercariae of fasciola hepatica parasitizing Lymnae truncatula.

No mixed infections were found, and no infections were recorded in Lymnaea stagnalis, Melanopsis cariousis, Melanopsis Scalaris, Lymnea Stagnalis, Union duriei, Mercuria confusa, Melanoide tuberculata or in Physa acuta, which was very frequent and abundant in the area.

The highest prevalence of trematode cercaria was recorded from snails collected in rivers (31% in Fouarat river) followed by irrigations canals (9%), grazing lakes (8%) and swamp (6%).

### Table 2: Types of cercariae and prevalences of infection among gastropods in Gharb area

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of cercaria</th>
<th>Famille</th>
<th>Positive Case</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanopsis Praemorsa</td>
<td>Furcocercous Longifurcate pharyngeate</td>
<td>Prohemistomatidae</td>
<td>150</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>brevifurcate lophocercous</td>
<td>Sangunicolidae</td>
<td>73</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Xiphidiocercaire</td>
<td>Lecithodendriidae</td>
<td>57</td>
<td>6%</td>
</tr>
<tr>
<td>Lymnea truncatula</td>
<td>cercariae of fasciola hepatica</td>
<td>Fasciolidae</td>
<td>2</td>
<td>5%</td>
</tr>
<tr>
<td>Lymnea peregra</td>
<td>Echinostome</td>
<td>Echinostomatidae</td>
<td>2</td>
<td>2%</td>
</tr>
</tbody>
</table>
Figure 2: Trematode cercariae recovered from freshwater snails in the Gharb region, Morocco

4. DISCUSSION
The present report represents the first study on the diversity of larval trematode parasites (cercariae) of the freshwater snails in the Gharb area (Morocco).

Five morphologically distinguishable types of trematode cercariae were observed in three freshwater snail species with 12% the average prevalence rate in snail hosts. Melanopsis praemorsa was found to shed cercariae belonging to three families with high prevalence of infection (6% - 17%).

In comparison with other region of Morocco, in Tessaout Amont, Lamrani and al (2004) collected cercariae belonging to nine families. Melanopsis praemorsa are shedding cercariae belonging to four families, Ancylus fluviatilis and Mercuria confusa showed the highest prevalence of infection (1% and 2%, respectively), and he found no case of mixed-species infections.

On the Haouz plain, (Ghamizi, 1994) found six families to cercariae shed by Melanopsis praemorsa with high prevalence of infection (4 - 28%). (Idaghdour 1991) reported the cases of mixed infection with
Philophthalmidae/Sanguinicolidae, Monorchiidae/ Lecithodendriidae or Prohemistomidae/ Lecithodendriidae. Mouahid (1989) noticed that Melanopsis praemorsa collected in the region of Marrakech carried natural infections with Szidatia joyeuxi belonging to Cyathocotylidae. Dollfus (1952) reported the occurrence of Szidatia nemethi (Cyathocotylidae) in Oued Cherrat in central Morocco.

In the north western region of Morocco, Saud (1995) described seven types of cercaria of trematodes may parasitize freshwater molluscs of this region.

In eastern Morocco, Kharboua (1994) reported a prevalence of infection of 1.5, 5 and 1.2 % for the following host parasite combinations: Melanopsis praemorsa/leucochloridiid cercariaeum, Melanopsis costellata/Armatiae cercariae and Melanopsis costellata/brevifurcate aphyryngeate cercariae, respectively.

In Algeria, Kechemir (1988) found that Bulinus truncatus carried natural infections, with larval stages of nine trematodes, including Schistosoma haematobium and Paramphistomum microbothrium with cases of mixed infections. Pojmanska & Combes (1990) reported that six types of cercariae were shed by Melanopsis praemorsa in northwestern Algeria, including the furcocercaria identified as Szidatia joyeuxi that is common in North Africa. Furthermore, they found cotylicercous cercariae that were identified as Nicolla gallica.

Lymnaea truncatula is the intermediate host of Fasciola hepatica in different regions of Morocco. In the Gharb region, several author reported the presence of this species (Laamrani 1994, Saoud, 1995, Fadli, 2003, Maqboul, 1996).

Natural infections have been reported from different regions, including the Middle Atlas (Khallaayoune et al. 1991), the northwestern region of Morocco (Saoud 1995) and Souss Massa (Moukrim 1991). In Tessaout Amont, Khallaayoune & El Hari (1991) found Lymnae truncatula naturally infected with Fasciola hepatica.

No cercariae were shed by Physa acuta, even if she was very frequent and abundant in the area.

5. Conclusion
This study showed the diversity of snail fauna and larval digenes in the Gharb region. Freshwater snails distributed in different habitats in this region were infected with five forms of trematode cercariae. One trematodes of medical and veterinary importance were found. The low prevalence of infection indicates that snail infection would play a minor role as a factor regulating snail density. Among the three infected gastropod species, Melanopsis praemorsa was less specific and showed the widest spectrum of susceptibility to trematode infections.

The diversity of the digenean fauna is dependent on whether conditions are conducive for transmission, i.e. presence of intermediate hosts and sufficient final host contact with the habitats to allow transmission. However, the digenean fauna seems reasonably diverse in these highly artificial habitats.

We hope this study will serve to stimulate further work on patent and pre-patent infections of snail hosts; larval behavior and morphology, geographical distribution of trematodes; infections in human beings, livestock and wildlife; and clinical manifestations of trematode infection in the Gharb area.

6. References


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