Trematode larval stages infecting *Radix natalensis* (Gastropoda: Lymnaeidae) in Qena Governorate, Egypt, with special reference to fasciolid cercariae

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Abstract: A faunistic field study of 2237 specimens of lymnaeids found in Qena Governorate, Upper Egypt, was carried out Malacological analyses showed that only the species *Radix natalensis* was included in the snail materials collected Snail population dynamics studies showed that lymnaeid numbers peak in spring and summer, and decrease in autumn and winter A total of 91 snail specimens (41%) was found infected by trematode larval stages, with the highest infection rate in autumn followed by spring, and the lowest in winter Fasciolid larval stages were detected in 35 individuals (156%), including very low rates of snail infection during July (048%) and September (063%), an appreciable rise in April (307%) followed by another increase in October (412%), and the highest percentage during November (1296%) No fasciolid infection could be found in lymnaeids collected during the rest of the year Three species of cercariae, other than *Fasciola*, were found among a total of 56 (25%) infected snails: xiphidiocercariae were found in 53 lymnaeids (236%), echinostome cercariae in 2 snails (009%), and bird schistosome cercariae in only 1 snail (005%)

Keywords: Lymnaeidae, *Radix natalensis*, trematode larval stages, *Fasciola*, Qena, Upper Egypt

Resumen: Se efectuó un estudio faunístico de campo de 2237 ejemplares de lymnaeidos encontrados en la región de Qena, en el Alto Egipto Los análisis malacológicos mostraron que los materiales de caracoles recolectados pertenecían a una única especie *Radix natalensis* Los estudios de la dinámica poblacional de los caracoles mostraron que el número de lymnaeidos resulta máximo en primavera y verano, para decrecer en otoño e invierno Un total de 91 especímenes de caracoles (41%) fue detectado presentando la infestación por estadios larvarios de trematodos, con una tasa de infestación más alta en otoño seguido de primavera, y una tasa menor en invierno Los estadios larvarios de fasciólidos fueron detectados en 35 individuos de moluscos (156%), incluyendo desde tasas de infestación de los moluscos muy bajas durante Julio (048%) y Septiembre (063%), hasta un apreciable incremento en Abril (307%) seguido de otro incremento en Octubre (412%), y un porcentaje máximo durante Noviembre (1296%) No se encontró infección alguna de los lymnaeidos por fasciolidos durante el resto del año Otras tres especies de cercarias, diferentes de *Fasciola*, pudieron también ser halladas en un total de 56 (25%) caracoles infestados: xiphidiocercarias en 53 lymnaeidos (236%), cercarias de echinostomátidos en 2 caracoles (009%), y cercarias de una especie de schistosomátido de ave en un sólo caracol (005%)

Palabras clave: Lymnaeidae, *Radix natalensis*, estadios larvarios de trematodos, *Fasciola*, Qena, Alto Egypt

1. Introduction

Trematodes are plathelminthes almost exclusively transmitted by molluscs . Among the latter, gastropods of the families Planorbidae and Lymnaeidae include most of the snail species of applied interest because of developing the role of first intermediate host harbouring cercariogenous sporocysts and rediae of digeneans of medical and veterinary importance (Maleck, 1962, 1985; Erasmus, 1972; Brown, 1978). Lymnaeids are of applied interest because of transmitting several trematode species of large medical and veterinary impact. The fasciolids *Fasciola hepatica* and *F gigantica* are the most important (Mas-Coma and Bargues, 1997). Therefore, many studies have focused on concrete lymnaeid species which are known to

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participate in the transmission of the fasciolid species *F .hepatica* and *F. gigantica* in the different endemic areas In recent years, this interest has markedly increased because of the detection of true human endemics, ranging from low to very high prevalences and intensities, indicating that human fascioliasis must no longer be considered merely as a secondary zoonotic disease, but be included in the list of important human parasitic diseases (Mas-Coma *et al*, 1999a, b, 2005; Mas-Coma, 2004a, b, 2005).

Moreover, several lymnaeid species are infected by schistosomatids of birds and mammals whose cercariae they shed cause dermatitis or swimmer's itch in humans (Degentile *et al*, 1996; Kolarova *et al*, 1997; Horak and Kolarova, 2001). Echinostomatidae are another group of trematodes including species developing in lymnaeids, of applied interest because of their capacity to infect humans (Yu and Mott, 1994; Graczyk and Fried, 1998). *Fascioloides magna* and *Paramphistomum daubneyi* are examples of other trematodes of great economic importance in veterinary medicine, which also use lymnaeids in their life cycles (Erhardova, 1961; Horak, 1971; Moukrim *et al*, 1993).

Examples of faunistic field studies in Europe, on fasciolids or on trematodes in general infecting lymnaeids, are those performed by Hovorka (1960) in Czechoslovakia, Manga-Gonzalez *et al* (1991) and Toledo *et al* (1998) in Spain, Dreyfuss *et al* (1994) in France, and Loy and Haas (2001) in Germany In Asia, the study of Ito and Blas (1978) in the Philippines, can be mentioned. In Africa, similar studies were made by Mc Cullough (1965) in Ghana, and Schillhorn van Veen (1980) in Nigeria

In Egypt, lymnaeids are also of great importance because of being involved in the transmission of fascioliasis, a large health problem in that country (Esteban *et al*, 2003). Faunistic cercarial studies on lymneids were performed in Abis village, Alexandria, Lower Egypt (Allam, 1992). In Upper Egypt, the only previous works did not mention any type of cercariae from lymnaeid snails (Omran, 1973). The present paper has the purpose to analyse the results obtained in the faunistic study of trematode larval stages found infecting a very large number of lymnaeid snails collected in the governorate of Qena, Upper Egypt, with special emphasis on fasciolid larval stages

2. Materials and methods

2.1. Snail collection and classification

Lymnaid snails were collected in water bodies of 14 different localities of the governorate of Qena, Upper Egypt, which is bordered on the north by the Sohag Governorate and on the south by Asswan. The localities surveyed were the following (from north to south): Nag Ahmed Bekhet, Nag El-Rebba, El-Nagma and Houmran (Abu-Tesht district), Nag Hammady city (Nag Hammady district), Dandara, El-Tramsah, El-Sale and Karm-Omran (Qena district), El-Keratia (Qus district), Armant city and Gezerat Armant El-Hate (Armant district), and Nag El-Maala and El-Shaghab (Isna district). Snail collections were performed following standard malacological methods (Maleck, 1962, 1985) to assess population densities. Lymnaeid snails were found in areas where water was stagnant or presented only a slow current, usually in places where livestock was present. A total of 2237 lymnaeid snails could be collected. Appropriate shell analyses allowed us to classify all specimens as belonging to the African species *Radix natalensis* Krauss, 1948 (Fig 1A), according to Frandsen (1983) and Brown (1994) This snail species appears to be distributed throughout the whole country, although it is usually called *Lymnaea callaudi* Bourguignat, 1883 in the Nile Delta region, Lower Egypt *Lymnaea callaudi* was synonymized with *Radix natalensis* by Hubendick (1951).

2.2. Collection and study of trematode larval stages

Cercariae were obtained after natural emergence from collected lymnaeid snail individuals placed in natural water in Petri dishes. Non-shedding snails were kept in the laboratory and re-examined weekly to verify that cercariae were not shed by snails which could have been infected only shortly before being collected. When snails proved to be negative after several examinations by the shedding method, they were gently crushed in a Petri dish containing a small amount of dechlorinated water. The fleshy part was removed and was dissected under a microscope. When intramolluscan trematode larval stages were detected in the dissected snails, they were transferred to a glass slide for detailed examination under the binocular microscope. The same process was followed for snails dying or recently died. The number of snails shedding cercariae and those presenting infection by intramolluscan larval stages were recorded.

Cercarial types were classified according to Combes *et al* (1980) and Frandsen and Christensen (1984).

3. Results

3.1. Snail population dynamics and general prevalences

Snail population dynamics studies showed monthly fluctuations in which the number of snails appeared highest in April (n = 390) and June (302), whereas the lowest numbers appeared in January (65) and December (12) The seasonal variation of the snail populations showed that lymnaeid numbers peak in spring (767) and summer (681), and decrease in autumn (437) and winter (352).

The general infection rate by trematodes was 41%, that is, only 91 snails appeared to be infected. The infection rates with trematodes were 308% in April (12

infected snails out of a total of 390 studied), 048% in July (1 out of 208), 063% in September (1 out of 159), 412% in October (7 out of 170), and 196% in November (14 out of 108) According to seasons, the highest infection rate was in autumn (66%) followed by spring (48%), and the lowest was in winter (006%).

3.2. Lymnaeid infection by fasciolid larval stages

Out of 2237 *R natalensis* snails, 35 individuals (156%) were shedding *Fasciola* cercariae. Fasciolid cercariae were found in Nag El-Rebba (26 infected snails out of a total of 616 snails analysed) (Abu-Tesht), El-Tramsah (1 out of 41) and El-Sale (1 out of 208) (Qena), and El-Shaghab (7 out of 170) (Isna).

A monthly analysis shows that *R natalensis* snail infection by fasciolid cercariae fluctuated between 0% and 1296%, including very low rates of snail infection during July (048%) and September (063%), an appreciable rise in April (307%) followed by another increase in October (412%), and the highest percentage of *Fasciola* infection being recorded during November (1296%). No fasciolid infection could be found in lymnaeids collected during December, January, February, March, May, June and August.

A seasonal analysis shows that all *R natalensis* snails collected during winter (December-February) were negative for infection with *Fasciola* spp. This is followed by a peak of infection during spring (March-May) (156%). A sharp decrease in infection rate was noticed during summer (June-August) (015%), while the highest rate of snail infection was found to occur during autumn months (September-November) (503%).

3.3. Lymnaeid infection by other trematode larval stages

During the present study three species of cercariae, other than *Fasciola*, were found among a total of 56 (25%) infected snails out of 2237 *R natalensis* individuals examined Xiphidiocercariae were found in 53 lymnaeids (236%) (Fig 1G), echinostome cercariae in 2 snails (009%) (Fig 1C), and bird schistosome cercariae in only 1 snail (005%) (Fig 1E). The sporocysts of both xiphidiocercariae (Fig 1F) and bird schistosome cercariae (Fig 1D), and rediae of *Fasciola* and echinostome cercariae (Fig 1B) were also found in the respective infected snails.

The infection with xiphidiocercariae was the most prevalent and varied from 118% in October to 2615% in January. It was detected in Nag El-Rebba (15 infected snails out of a total of 369 snails analysed) (Abu-Tesht), El-Tramsah (5 out of 41) and El-Sale (26 out of 423) (Qena), Armant city (1 out of 74) and Gezerat Armant El-Hate (3 out of 171) (Armant), and El-Shaghab (3 out of 235) (Isna). The 2 snails infected by echinostome cercariae were both collected in Nag El-Rebba (2 out of 108) (Abu-Tesht) in November, whereas the only 1 infected by bird schistosome cercariae was also collected in Nag El-Rebba (1 out of 390) (Abu-Tesht) but in April. No mixed infections were encountered.

4. Discussion

In the field studies carried out for the collection of lymnaeid snails in different localities of the southern Egyptian governorate of Qena, only specimens belonging to the species *R natalensis* could be found Although this freshwater snail species is the only lymnaeid species present in most of the countries of Africa (Brown, 1994), in Egypt other lymnaeid species are known to be present *Galba truncatula* have been recorded from many places in Lower Egypt, such as in the northern governorate of Behera of the Nile Delta region, as well as in Baharia, Dakhla and Kharga Oases, and Cairo (Frandsen, 1983; Brown, 1994). *Lymnaea stagnalis* was also reported inhabiting a marsh and ditch in Wadi El-Natroun (Nagaty *et al*, 1959; Frandsen, 1983). And finally *Pseudosuccinea columella* is also known from throughout the Nile Delta region (Brown, 1994). Our results suggest that these three lymnaeid species may perhaps not geographically expand southward in Egypt. In another southern governorate as Assiut, located between Cairo and Qena, studies on lymnaeid snails are unfortunately lacking (Omran, 1973) However, owing to the difficulties in detecting lymnaeid species of small size and the patchy distribution of freshwater snails which may present low population densities in southern Egyptian latitudes because of climatic conditions more extreme than in the mildered Nile Delta region, further studies should be performed to definitively assess which is the lymnaeid species faunistic composition in Qena Governorate.

Monthly fluctuation studies of *R natalensis* populations in Qena governorate showed higher densities during April, June and July and lowest densities in December, although the snails were collected throughout all months of the year. The more dense populations in spring may be related to climatic conditions favourable for snails as well as for aquatic plants, which offer shelter and food for the *R natalensis* snails, the resulting freshwater environment becoming appropriate for their breeding. Seasonal fluctuations of *R natalensis* populations have already been described in other countries. In Ghana, the snail populations begin to increase during the later part of the wet season, reach a peak during the early dry season, and decline during the late dry season and early-to-mid wet season (Mc Cullough, 1965). In the Samaru stream, Nigeria, which contains water throughout the yearly period, the lowest number of *R natalensis* was found when the water level was lowest (April to June), and the highest numbers occurred in the middle of the dry season. Results indicated that at least two, and probably three, generations of snails develop in one year (Schillhorn van Veen, 1980).

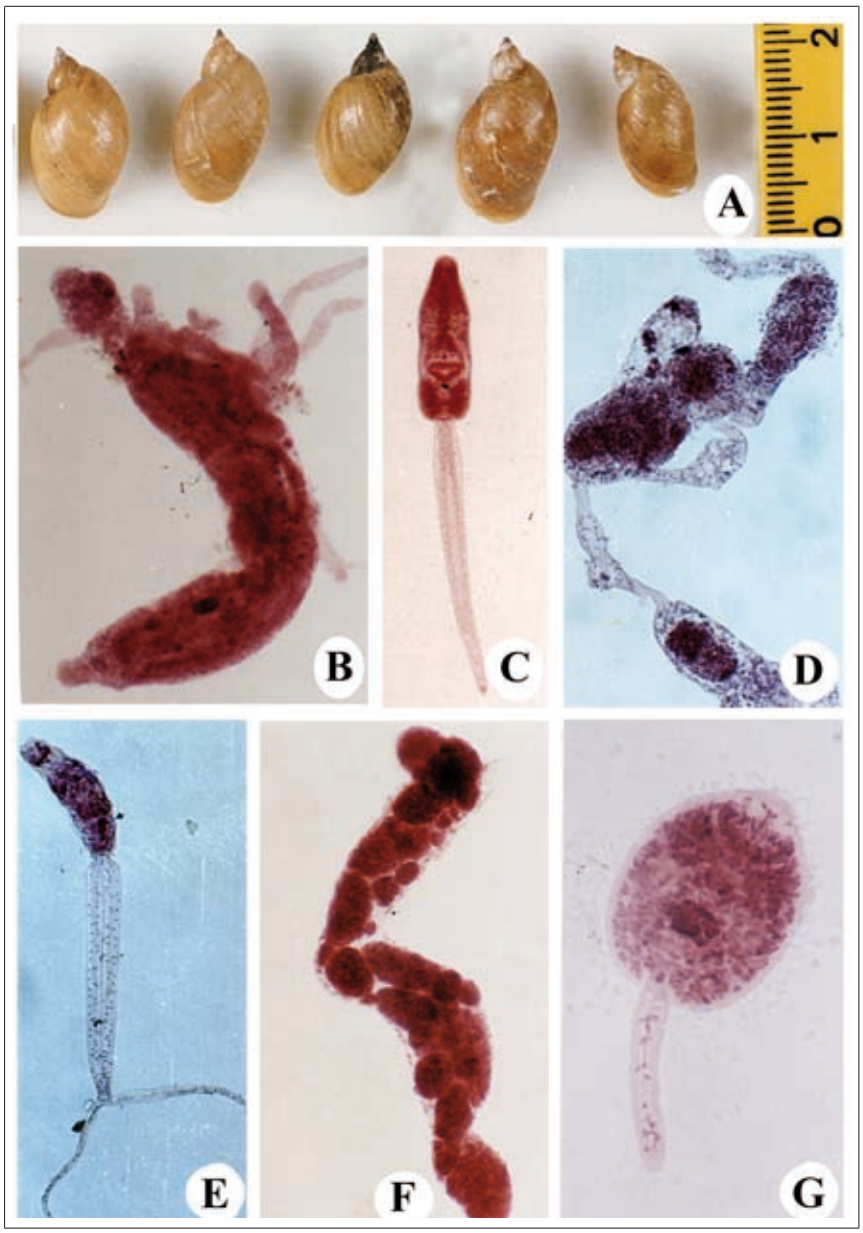

Fig 1 Trematode larval stages found in *Radix natalensis* in Qena Governorate, Egypt: A) shells of collected *R natalensis* snails; B) redia of echinostome cercaria (40x); C) echinostome cercaria (100x); D) sporocyst of bird schistosome cercaria (40x); E) bird schistosome cercaria (100x); F) sporocyst of xiphidiocercariae (40x); G) xiphidiocercaria (100x)

In Nigeria, the infection rate of *Fasciola*-infected *R natalensis* snails increased from the end of the wet season until December, after which it declined. Redial infections were most common during and directly after the rains (Schillhorn van Veen, 1980). In Egypt, different infection rates of lymnaeid snails with fasciolid cercariae have been reported: 1664% in Baharia Oasis (Nagaty *et al*, 1959), 35% in Edfina (Zaki, 1960), 10-40% in different Egyptian provinces (Zein El Din, 1971), 49% with a maximum of 103% in Alexandria (Abdel-Latif, 1985; Allam, 1992). Concerning monthly fluctuations of fasciolid infection rates, there appear to be marked differences according to the different areas. Thus, highest infection rates have been reported during summer months (Nagaty *et al*, 1959; Hiekal and El-Sokkary, 1987), in winter (Abdel-Ghani, 1962) and also in autumn (Nada, 1983; Abdel-Latif, 1985). In monthly surveys carried out in Abis village, Alexandria, from June 1990 to May 1991, fasciolid prevalence was highest in June (2894%), July (2804%) and November (2010%), and lowest in December (93%), April (00%) and May (167%), which represented maximums in summer (28%) and autumn

(16%), and minimums in winter (78%) and spring (27%). The consequent transmission potential was 351% in summer, 313% in autumn, 237% in winter, and 99% in spring (Allam, 1992). In Qena, the highest infection rate appears in autumn, which may be due to the very high temperatures of summer in this governorate.

In Egypt, faunistic studies on trematode larval stages infecting *R natalensis* are very few. In Lower Egypt, a xiphidiocercaria and an echinostome were described in Alexandria (Allam, 1992). In Upper Egypt, previous malacological studies did not mention any type of cercariae in *R natalensis* snails (Omran, 1973; Hassan, 1987). Comparing the general features of the xiphidiocercariae and echinostome cercaria found in the governorate of Qena, they seem to be completely different from those described in Alexandria (Allam, 1992). The detection of a bird schistosome cercaria appears to be the first report of such a schistosomatid infection in lymnaeids in Egypt.

The findings of xiphidiocercariae, an echinostome cercaria and a bird schistosome cercaria in Qena Governorate agree with results obtained in other countries. Lymnaeids participate in the life cycles of at least 71 trematode species belonging to 13 different families whose members use birds and both domestic and sylvatic mammals as definitive hosts (Brown, 1978), and this without counting digeneans of lower vertebrates such as amphibians. A compilation by Erasmus (1972) of the numbers of cercariae species recorded from freshwater molluscs in Europe showed the importance of several species of the genus *Lymnaea* Lamarck, 1799 *sensu lato*: 29 different species in *Lymnaea stagnalis*, 17 in *L palustris*, 3 in *G truncatula*, 7 in *R ovata*, 6 in *R auricularia*, and 20 in *R peregra.* Sometimes, the same lymnaeid species is even used by more than one digenean species simultaneously (Moukrim *et al*, 1993). Trematode species transmitted by a given lymnaeid species differ depending from the geographic region considered (eg, Adam and Lewis, 1993; Toledo *et al*, 1998a) and local ecological characteristics (Esch and Fernandez, 1993), but mainly depend on parasite-host specificity.

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