Human Urogenital Myiasis Caused by Psychoda Species Larvae: Report of Five Cases and Morphological Studies

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INTRODUCTION

Myiasis is the infestation of body tissues or organs by dipterous fly species and is often associated with poor hygiene. Although infestation by fly larvae is much more prevalent in animals, it occurs relatively frequent in humans in rural, tropical and subtropical regions of Africa and America. Urogenital myiasis is one of accidental myiasis that may be seen in humans. Urogenital myiasis is commonly associated with poor personal and environmental hygiene, low educational level and urogenital troubles. The current study presented five cases of urogenital myiasis. Patients were residing in Assuit and Qena Governorates (Upper Egypt). Some patients complained of intermittent passage of small, motile, greyish black wormiform objects in their urine and some were discovered accidentally. Larvae were collected and studied microscopically and detailed structures were described using scanning electron microscope (SEM). The larvae were morphologically identified as Psychoda spp. larvae. Special attention was given to cephalic region, vestiture, setae distribution and caudal extremity. It is worth mentioning that the sensillary necklace–like structure at the junction of the head with the first thoracic segment and the hollow appearance of setae were also clarified. It was concluded that despite the fact that urinary myiasis is very rare in humans; it should be considered in patients with urinary complaints.

ABSTRACT

Myiasis is the infestation of body tissues or organs by dipterous fly species and is often associated with poor hygiene. Although infestation by fly larvae is much more prevalent in animals, it occurs relatively frequent in humans in rural, tropical and subtropical regions of Africa and America. Urogenital myiasis is one of accidental myiasis that may be seen in humans. Urogenital myiasis is commonly associated with poor personal and environmental hygiene, low educational level and urogenital troubles. The current study presented five cases of urogenital myiasis. Patients were residing in Assuit and Qena Governorates (Upper Egypt). Some patients complained of intermittent passage of small, motile, greyish black wormiform objects in their urine and some were discovered accidentally. Larvae were collected and studied microscopically and detailed structures were described using scanning electron microscope (SEM). The larvae were morphologically identified as Psychoda spp. larvae. Special attention was given to cephalic region, vestiture, setae distribution and caudal extremity. It is worth mentioning that the sensillary necklace–like structure at the junction of the head with the first thoracic segment and the hollow appearance of setae were also clarified. It was concluded that despite the fact that urinary myiasis is very rare in humans; it should be considered in patients with urinary complaints.


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Until recently, the traditional treatment of myiasis of the body cavity is mechanical removal (Gopalakrishnan et al., 2008). Surgical intervention is sometimes needed, which is painful, costly and sometimes made impossible in smaller cavities (Wadhwa et al., 2006).

The aim of the present study was to report five cases with urogenital myiasis caused by larvae of a dipterous fly of the genus Psychoda. We used ordinary microscopy for identification of the larvae; in addition, scanning electron microscopy (SEM) of the maggots was carried out as a supportive measure to illustrate some important ultrastructures that may constitute useful criteria in the larval identification and species differentiation.

Patients and Methods

Clinical Data: Table (1)

Five cases of urogenital myiasis were referred from Urology Department, Assiut University Hospital to Parasitology Department, Faculty of Medicine, Assiut University for identification and proper diagnosis from February 2009 to June 2013.

History taking, clinical examination, and laboratory investigations in the form of complete urine examination, blood picture, stool examination using direct smear and formol ether concentration technique were conducted.

Imaging and interventional investigations including pelvic ultrasonography, x-ray, contrast radiography and CT scanning of the urinary system, cystoscopy and bladder wash were also done.

Medical treatment in the form of antihelmenthic (two oral doses of ivermectin 200 μg/Kg to the adult patients and 100 μg/Kg for the child patient) and antibiotic treatment were prescribed. The patients were followed up two months later.

Table 1: Clinical data and presentation of studied cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Occupation</th>
<th>Complaint</th>
<th>Duration</th>
<th>Urinary lesion</th>
<th>Urine analysis</th>
<th>Stool analysis</th>
<th>Blood exam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>44</td>
<td>male</td>
<td>employer</td>
<td>intermittent passage of living worms in urine</td>
<td>15 days</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Case 2</td>
<td>9</td>
<td>Female</td>
<td>student</td>
<td>Dysuria</td>
<td>2 months</td>
<td>No</td>
<td>Normal</td>
<td>Giardia lamblia</td>
<td>Normal</td>
</tr>
<tr>
<td>Case 3</td>
<td>23</td>
<td>male</td>
<td>Not work</td>
<td>intermittent passage of living worms in urine, urinary discharge, frequency, Dysuria</td>
<td>3 months</td>
<td>Previous operation for stone</td>
<td>Pus cells casts</td>
<td>Normal</td>
<td>Anaemia, mild eosinophilia</td>
</tr>
<tr>
<td>Case 4</td>
<td>18</td>
<td>male</td>
<td>Student</td>
<td>intermittent passage of living worms in urine</td>
<td>1 month</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Case 5</td>
<td>68</td>
<td>Male</td>
<td>Farmer</td>
<td>Haematuria and dysuria</td>
<td>12 months</td>
<td>Previous operation for cancer</td>
<td>Blood, pus. Living larvae</td>
<td>Entamoeba coli</td>
<td>Anaemia,</td>
</tr>
</tbody>
</table>

The data collection was conducted after written consent from the patients, and permission from the ethics committee of the Faculty of Medicine, Assiut University. The investigation was carried out in accordance with the declaration adopted by the 18th WMA (World Medical Association) General Assembly, Helsinki 1964, and as revised by 64th WMA General Assembly in Fortaleza, Brazil, October 2013.

Precautions took place to protect the privacy of research subjects and the confidentiality of their personal information, including limiting the amount of personal information to the absolute minimum, assigning an identification number to each subject and attaching the identification number to the actual research information, removing the subject names as soon as data were analyzed and maintaining any identifying information and lists of identification numbers in a safe and locked file.

Collection and processing of larvae for entomologic study:

Collected larvae were washed several times in saline. Larvae were fixed in 70% ethyl alcohol for examination and identification.

Preserved larvae were brought down to water in descending grades of alcohol 50 % and 30%, 5 minutes each. They were transferred to 10 % potassium hydroxide, after puncturing the specimens on the ventral side, overnight until soft parts were dissolved. The specimens were washed thoroughly in distilled water, dehydrated in ascending grades of ethyl alcohol 5 minutes each, cleared in clove oil for 10 minutes, mounted in Canada balsam and dried in an oven at 38°C for five days (Adams and Hall 2003).

For SEM, preserved larvae were washed thoroughly in distilled water and fixed for 2 hr in 4% glutaraldehyde and 5% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.2. They were rinsed overnight in cacodylate buffer, dehydrated in graded aqueous ethanol (30%, 50%, 70%, 90%) followed by critical-point drying according to Hayat (1981), sputter-coating them with gold in the sputter coating apparatus for 6 minutes. Specimens were processed, examined and photographed in the Scanning Electron Microscope Unit, Assiut University by JEOL-JSM–5400 LV.

Following taxonomic identification keys of Zumpt (1965), Greenberg (1971), Foote (1991), the description of Rutledge and Gupta (2002) and based on biogeography the wormlike organisms were identified as larvae belonging to genus Psychoda, subfamily Psychodinae (Nematocera: Psychodidae). The mounted larvae were illustrated by photomicrographs. The measurements were considered from the average measurements of five larvae to the nearest millimeters.
RESULTS
Four patients were adult males (age range 18–68), the 2\textsuperscript{nd} case was a young girl aged 9 years. All the patients were from Assiut Governorate except for third case was from Qena Governorate. All cases were outpatients except for the fifth case that was hospitalized.

Three of the patients complained of intermittent passage of 6 to 10 living worms in every urine void. They described the worms as being small, grayish black in color, motile and slender. In the second and fifth cases, worms were discovered during routine urine examination at clinical pathology laboratory.

The 3 patients who complained of passing worms had no history of abdominal pain; bowel irregularity or any other symptoms related to either gastrointestinal or genito-urinary system; while the second and fifth cases had intermittent abdominal pain and diarrhea. General and systematic examination revealed no findings except mild pallor and non-tender hepatomegaly of 2 cm size in fifth case.

The results of the laboratory tests are shown in table (1). The imaging and cystoscopy were within normal in all patients.

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**Figure 1:** Showing *Psychoda* sp. Larva has ventrally bended anterior end (arrow) and tapered posterior end (arrow head).

**Figure 2:** Larval triangular head, carrying two short hairy antennae (arrow head) and a double-toothed mandible (arrow).

**Figure 3:** showing body segments; covered with irregular rows of numerous sharp rose thorn shaped minute spinules, hypopigmented saddle shaped chitinous sclerites (arrow heads), its long filiform setae (arrow), irregularly distributed lateral brown setae(double headed arrow) and anterior spiracle(S).

**Figure 4:** Showing breathing tube extending along the length of the body of the larva (arrows) and long filiform setae (arrow heads).

**Figure 5:** Last abdominal segment showing a sharp edge separated it from the remaining segments (double headed arrow), posterior spiracles apically (arrow head) and anal papilla (arrow).

**Figure 6:** Showing higher magnification of the ventrally located large spinose anal papilla.

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Plate I: Light Microscopy

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Macrosopic examination of the larvae:
The larvae were dark brown to black in colour when freshly passed except the well-developed head, dorsal plates, and the last abdominal segment at the end of larva which were darker. Larvae were immotile when presented to the laboratory, measured from 8 – 12 mm in length. They had rounded and ventrally curved anterior end and tapered dorsally curved posterior end (Plate I, Figure 1).

Light microscopic findings are illustrated in plate I:
The body of the larvae was more or less cylindrical and segmented. The body length ranged from 8 to 12 mm and its width ranged from 0.25 to 0.75 mm (Plate I, 1). Each larva possessed a distinct triangular head, which carried two short hairy antennae and a double-toothed mandible. Mandibles were in a horizontal plane and opposed, so that their lips can be brought together (Plate I, 2). The body of the larvae consisted of 10 segments (3 thoracic and 7 abdominal). Hypopigmented saddle shaped chitinous sclerites were present on the dorso-lateral sides of each body segment, giving the larvae their banded appearance (secondary annulation). These chitinous sclerites varied in width. They were covered with long filiform setae arising from button-like basal plates on each sclerite (Plate I, 3). Body segments were covered with irregular rows of numerous minute sharp and rose thorn shaped spinules (Plate I, 3). Long filiform brown setae were irregularly distributed laterally and arising from nearly circular chitinous button-like basal plates (Plate I, 3).

Two breathing tubes were seen extending along the length of the body starting from a pair of anterior spiracles at the second subdivision of the prothorax and ending at the tip of terminal segment by a pair of posterior spiracles (Plate I, 4). The caudal end of the body was obliquely truncated while the last abdominal segment was cylindrical chitinous pigmented and separated from the remaining segments by a sharp edge (Plate I, 5). It was prolonged and tapering into two distinct respiratory tubes bearing the posterior spiracles apically (Plate I, 5). The posterior spiracles were guarded by dorsal and ventral brushes of hair tufts on each tubular projection. The anal papilla was large spinose and located ventrally (Plate I, 6).

SEM findings are illustrated in plates II – V.
In general, the body of the larvae was elongated, segmented with secondary annulations and slightly tapered at one end. The vestiture of thoracic and abdominal segments was scraggy coat of pointed hair-like elements; long and numerous on the dorsal surface and partially devoid from the ventral surface (plate II, 1). Randomly orientated long setae covered the whole body segments (plate II, 1). Dorso-lateral view of the head and thoracic region (Plate II, 2) revealed smooth, shiny egg-shaped head with few scattered medium sized setae. Head capsule slightly bent ventrally and the mouth filled with bunch of hair.

Figure 1: Showing elongated, segmented body, tapered at both ends: anterior end (arrow head), posterior end (arrow) and long, numerous setae on the dorsal surface (curved arrow).

Figure 2: Showing dorso-lateral view of the head and thoracic region revealed ventrally bended egg-shaped head with few scattered medium sized setae and mouth filled with bunch of hair.

Figure 3: Antero-posterior view of the cephalic region with central projection, two lateral small ear like structures (arrows), hemispherical necklace-like structure (arrow head) and a pair of anterior spiracles situated laterally (double headed arrow).

Figure 4: Magnification of the anterior spiracles appeared twice as long as wide and had a single pore in the apex with a skirt of very fine hairs at the base.
Antero-posterior view of the cephalic region (Plate II, 3) appeared like an elephant face illustrating central projection bent ventrally with two lateral openings filled with filament like network. Two lateral small ear like structures were located lateral to both openings. A sensillary hemispherical necklace-like structure situated at the junction of the head with the first thoracic segment reaching only to the mid-lateral cephalic region (Plate II, 3).

A pair of anterior spiracles was situated laterally at the anterior edge of the second thoracic segment. Although spiracles were small, they were placed at the top of a globular bulge (Plate II, 3). Magnification of this part (Plate II, 4), the anterior spiracles appeared twice as long as wide and had a single pore in the apex with a skirt of very fine hairs at the base.

Plate III, 1 & 2 illustrated the pattern of sensory spination of the ventral surface of thoracic and abdominal segments; they were condensed and gradually increased in size towards the posterior margin of the segments. Plate III, 3 & 4 showed spination on the dorsal surface of thoracic and abdominal segments, the middle of each segment is free from spines. The dorsal surface (Plate III, 5) revealed scattered sessile papillae in the center of the abdominal segments. Long setae covered the whole segments and were scattered in different directions.
dispersed in different directions. Under higher magnification (Plate IV, 1) the detailed characteristics of the spines on the ventral surface of the abdominal and thoracic segments showed irregular rows of minute sharp rose thorn shaped curved spinules at the anterior margin and long spine with sharp end towards the posterior margin of the segments. The long spines were almost covering the inter–segmental sutures. Dorsal spinations were nearly similar to those seen on the ventral surface but more dispersed at the middle of the segments (Plate IV, 2). Plate IV, 3 illustrated that setae were long, slender and easily overlooked in the vestiture. The distal end of the setae appeared either curly or somewhat like a rat’s tail. On magnification (Plate IV, 4 & 5) they were individually arising from ring like bases with raised edges (button like basal plates). The setae appeared like hollow tubes with central opening at their ends. They seemed to have a shroud of very fine filamentous hair skirting them. Plate V, 1 & 2 showed general appearance of the dorsal and ventral surface of the caudal extremity, the respiratory siphon looks like a cone with moderately broad base narrowing rapidly into slender tapering distal portion, few scattered setae covering its smooth surface.
On magnification (Plate V, 3), the posterior spiracles appeared at the end of tapering tubular siphon, with their opening (curved arrows) between the dorsal two pairs (arrows) and ventral one pair of processes (arrow heads).

DISCUSSION

Family Psychodidae includes six subfamilies. Only two of them have public health significance. Phlebotomineae (sand flies); are blood suckers and their larvae inhabit places where there is high organic matter; they are best known as vectors of leishmaniasis (Wagner, 1990; 1997). Psychodinae (moth flies); their mouthparts are short and not adapted for blood sucking. They breed in moist places and are often found around sewerage installations, hence are called ‘filter flies’ or they may originate in drain pipes and emerge from sink and bath tub drains.

Myiasis is endemic throughout the tropics and subtropics. It occurs more readily in warm and humid environments. In the tropics, cases present the year round, but in more temperate zones, myiasis is generally restricted to the summer months (Noutsis and Millikan, 1994). It is to be noted that in our patients, the presentation took place in summer. In our report, the cases were residing in rural areas and had low standards of living, which is the usual standard of living for urogenital myiasis patients as previously described (Markel et al., 1992). It is possible that some eggs may have been laid in or around the moist urogenital region when the patients were urinating; and after hatching, the larvae burrow in to the urogenital tract to feed giving rise to urogenital myiasis. This explanation was previously suggested by Salimi et al. (2010) and Lotfy (2011).

In a literature search by Oğuz et al. (2012), there were less than 30 case reports of urogenital myiasis. In general, it is uncommon to encounter dipterous fly larvae in human urine in Egypt. To the best of our knowledge, three cases of urinary myiasis with Psychoda spp. infestation were reported from Egypt (Sakla et al., 2003; Ezzat and Younis, 2009; Lotfy, 2011). More scattered cases of urogenital myiasis by larvae of the same genus have been reported before including; Kuwait (Hira et al., 1997), Turkey (Dinçer et al., 1995; Taylan–Ozkan et al., 2004), UK (Patton and Evans, 1929), China (Wang and Liu, 2006; Zhou et al., 2008), Argentina (Mariluis et al., 2007) and Spain (Fuentes et al., 2007).
Moreover, Psychoda spp. was reported to cause bronchial myiasis in Japan (Hirosi et al., 1963). Urogenital myiasis by larvae of other species of flies have been reported in the world including the United States of America by Dermatobia hominis (Massey and Rodriguez, 2002), India by Chryomyza bezziana (Wadhwa et al., 2006), Slovakia by Lucilia sericata (Nagy, 2012), Iran by Chryomyza bezziana (Jadlayer et al., 1978) and by Lucilia sericata and Wohlfahrtia magnifica. (Salimi et al., 2010) in Spain by Eristalis tenax (Gonzalez et al., 2009), in Nigeria by Eristalis species (Utsalo and Khalifa, 1983) and in Saudi Arabia by Musca domestica (Wakid, 2008).

Identification of the species of maggots prior to treatment is important since not all types of myiasis are benign (Singh and Rana, 1989). The present work focused on an ordinary microscopy for identification of the larvae. However, SEM of the maggots was carried out as a supportive measure to illustrate some important features that may constitute useful criteria in the larval identification and species differentiation. SEM has enabled us to visualize the ultrastructure of different parts of the maggots such as the anterior end, the respiratory spiracles, the pattern and density of sensory spination on ventral and dorsal surface of body segments and the setae distribution.

As reported by previous authors Whitten (1955) and Abonnenc (1972), the larvae of all Psychodidae are amphipneustic, having a pair of thoracic and abdominal spiracles. The oligopneustic (which comprises the amphipneustic type) and apneustic larvae, with 0–4 pairs of functional spiracles may be used for subsequent adaptation to a subemerged life in a fluid or semifluid medium (Fausto et al., 1998). Among Psychodidae, the aquatic larvae of Psychodinae have the post–abdominal spiracles opening at the end of a respiratory siphon as an adaptation to the aquatic mode of life in a good agreement with our description. In the current study, the anterior spiracles emerge on top of globular structures and project from the surface of the larval body. This localization probably favors the contact between the spiracles and the air.

The pattern of setae distribution is used as the main character that distinguishes the larvae of the subfamily Phlebotominae from other subfamilies of Psychodidae (Sther, 1991). According to Killick-Kendrick et al. (1989) the function of these setae is obscure. The distinctive back– and forth movement of the long setae of larvae and their fine hair suggest the presence of sensilla. In another study, Pessoa et al. (2001) proposed that the setae of all species examined bear several pores scattered along their structure, further suggesting that they may have a chemosensitive function. This suggestion is probably confirmed by the hollow structure of the setae in our description.

In this work much attention was given to the spination through which we could use them in differentiation. Spine structure of larval segment should be taken into account to determine specific patterns since the shape, density and orientation of spines varies along the length of body and also between dorsal and ventral surfaces (Colwell and O’Connor, 2000). In the current work, the spines appeared different in size and shape, almost all of them having single points, some with sharp pointed and curved tips and others with blunt terminations. The differences in spine pattern and size together with non–sucking mouthparts could explain the variability of the clinical manifestation of this parasitism (i.e. some cases presented with urogenital problems, others only passed larvae).

However, given the difficulty in identifying the larvae at specific level, they were reported in the present study as Psychoda sp., although only P. albipennis has been related with urogenital myiasis (Oğuz et al., 2012). Accurate identification of fly larvae which rarely cause myiasis is sometimes difficult and requires thorough study of unfamiliar morphological details of the larvae. The detected larvae in the present work were mostly fourth stage larvae because of their colour, size and the morphological characters of the respiratory system. Another more conservative explanation is that mature fly larvae leave the human body when they are ready to pupate; this is in agreement with the explanation given by Ezzat and Younis (2009) and Oğuz et al. (2012). Draber–Mońko et al. (2009) stated that: “It seems early to identify Psychoda spp. larvae on SEM bases only and we should resort to DNA findings to provide a thorough documentation of adult and larval stages”.

Ivermectin is a semisynthetic macrocyclic lactone that has been demonstrated to have activity against Strongyloides stercoralis, Ancylostoma braziliense, Cochliomyia hominivorax, Dermatobia hominis, Filaria bancrofti and Loa loa, as well as ectoparasites, such as Sarcoptes scabiei, Pediculus humanus and Demodex folliculorum. Ivermectin has, therefore, become an important alternative for the treatment of patients with different forms of ectoparasite infestations, such as scabies, head lice, demodicidosis, and myiasis (De Tarso et al., 2004; Osorio et al., 2006). In agreement to the previous studies; the patients responded to oral dose of Ivermectin without any recurrence in the followed patients in the present work.

According to the available literature, this study reported and described cases of urinary myiasis caused by Psychoda spp. Under SEM observation, it seems that the sensillary necklace–like structure at the junction of the head with the first thoracic segment, the scattered sessile papillae on the dorsal aspects of the body segments, the shape and pattern of spine distribution and the hollow structure of the setae have not been described before.

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CONFLICT OF INTEREST

No conflict of interest.

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