A retrospective analysis of mortalities in Greater Red Musk Shrews (Crocidura flavescens)

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ABSTRACT—The Greater Red Musk Shrew (Crocidura flavescens) is found throughout Africa, but several species of insectivorous African shrew are in decline, mainly due to the adverse effects of human activities and pollution. There is a lack of literature on the etiology and pathology of mortalities occurring in shrews in general, and this species in particular. We therefore undertook a detailed and systematic histopathological, parasitological, and ultrastructural examination of thirty-five Greater Red Musk Shrews that had died mainly of natural causes in Egypt between March 2008 and April 2014. Parasitic enteritis caused by Hilmylepis spp. was observed most frequently (n = 20), followed by parasitic pneumonia (n = 5), gastric giardiasis (n = 5), intestinal coccidiosis (n = 4), testicular degeneration (n = 4), intestinal amebiasis (n = 3), mycotic pneumonia (n = 3), Streptococcus pneumonia (n = 3), and blood protozoal infection (n = 2). There was a causative agent in 20/32 (63%) cases and several etiological agents in 12/32 (37%) cases. Although a number of helminthic and parasitic gastrointestinal infections and lung infections are described in shrews, this is the first description of parasitic pneumonia, mycotic pneumonia, and testicular degeneration and in shrews. Awareness of these diseases can help in the management of shrews in captivity and prevent their population decline in the wild.

Keywords: Greater Red Musk Shrew; parasitology; histopathology; Giardia lamblia; mycotic pneumonia; testicular degeneration; parasitic pneumonia.

INTRODUCTION

Greater Red Musk Shrews (Crocidura flavescens) are a mammalian species belonging to the family Soricidae. They are reddish-brown or grey, about 16 cm long with 5-6 cm long tail, and weight between 22 and 27 g. Although particularly prevalent in sub-Saharan Africa, particularly South Africa, they are widely distributed throughout the continent and are found in Sudan, Ethiopia, and Egypt (Osborn and Helmy 1980). Greater red musk shrews prefer moist habitats, although they are also found in dry areas, and are often found in dense vegetation. Although mainly nocturnal, they also have active spells throughout the day (Osborn and Helmy 1980). Shrews are
considered to be one of the most important mammals in Egypt (Hoath, 2009) since they play a role in controlling rodent and reptile populations by feeding on small rodents, lizards, and snakes, in addition to their predominantly insectivorous diet. They have also contributed to the eradication of the predatory and invasive freshwater crayfish (*Procambarus clarkii*) along the shores of the river Nile (personal communication). Historically, shrews are likely to have played an important role in the religious practices of the ancient Egyptians, particularly during the late dynasties, and mummified shrews have been found in the ceremonial animal tombs of Akhmim near Thebes, dating at the 27th Dynasty of Egypt (around 500 BC) (Hutterer, 1994).

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However, several species of insectivorous African shrew are in decline, mainly due to the adverse effects of human activities and pollution. For example, Egyptian shrews have declined from six species to only one, the Greater Red Musk Shrew (*Nicoll and Rathbun* 1990). However, there is a paucity of literature on the pathology and causes of death in shrews. Although it has been suggested that shrews do not harbor large numbers of diseases, and might therefore be useful experimental animals, there are few pathological and parasitological studies to support this claim (Stunkard et al., 1975). We therefore aimed to systematically study the pathology occurring in Greater Red Musk Shrews in our practice in Egypt between 2008 and 2014 using light microscopy, electron microscopy, and parasitological examination.

**MATERIALS AND METHODS**

**Ethics statement**

Since the study did not use laboratory animals and was performed as part of the investigation of natural disease, no ethical approval from an animal ethics committee was required.

**Pathological examination**

In total, thirty-five shrews were examined during the period between March 2008 and April 2014. Thirty shrews were found dead in the wild (accidentally in rat traps) and five cases died in captivity within one month of capture as breed as pet animals. Tissues samples were fixed in 10% formalin, dehydrated in a graded alcohol series, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin stain according to (Godwin, 2011, Kiernan, 2008 and Culling et al., 1985), Periodic acid–Schiff (PAS) according to (Kiernan, J.A. 1999), and Gram stain (Black, J, 2012). Blood films were stained with Giemsa stain and Leishman stain according to (Godwin, 2011).

**Parasitological examination**

Intestinal Nematodes founded during examination s were mounted in glycerine jelly for further examination. Cestodes were prepared by flattening the worms between two slides and keeping in a refrigerator for two hours. They were then transferred to 2% formalin for 60 minutes, 5% formalin-acetic
acid (5% formalin plus 2% glacial acetic acid) for 60 minutes, and washed in water for 30 minutes before being stained overnight in Delafield’s hematoxylin plus 1% aceticarmine. The next day, they were washed in water, dehydrated in a series of alcohols (15%, 30%, and 60%), and destained in 10% acid alcohol (3% hydrochloric acid) until a pale pink color was obtained. The worms were then immersed in 80% alkaline alcohol until a pale blue color was obtained. Worms were placed between two glass slides bound with a rubber band and dehydrated in 80%, 90%, 95%, and absolute alcohol for 60 minutes at each grade. Finally, specimens were cleaned in xylene-alcohol for 30 minutes, pure xylene for 60 minutes, and then mounted in balsam. Identification of organisms was made according to the criteria in Table 1.

Scanning electron microscopy (SEM)

Tissue specimen were fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.3) for four hours, followed by post fixation in osmium tetroxide for two hours. Samples were rinsed three times in sodium cacodylate buffer, dehydrated through a graded ethanol series from 10 to 100% for ten minutes in each, except for 100% for 30 minutes with three changes (each one for ten minutes). Liquid carbon dioxide was used to achieve critical-point drying, the specimens were mounted on copper stubs with double-sided adhesive tape, and coated with gold using the Edwards S150A Sputter Coater. The specimens were viewed using the JXA-840A Electron Probe Microanalyser (JEOL, Japan) scanning electron microscope.

Transmission electron microscopy (TEM)

Tissue specimen (0.5-1mm in diameter) were fixed in modified Karnovsky solution, post-fixed in 2% osmium tetroxide solution, immersed in 2% tannic acid solution for one hour at room temperature, and dehydrated in an increasing series of ethanol and propylene oxide and embedded in Spurr resin. Thin sections were prepared using an ultra-microtome (Ultra-Cut; Reichert) with a diamond blade, mounted on 200 and 300 mesh grids, counterstained with uranyl acetate and lead citrate, and examined using the Joel JSM1010 transmission electron microscope (JEOL, Japan) at 100 kV.

RESULTS

A total of 35 Greater Red Musk Shrews (Crocidura flavescens) were examined. A summary of the pathological results including the occurrence of different pathological alterations were shown in Figure 1. Nine disease categories explained 32/35 (91%) of cases, with predation accounting for the other three deaths. Parasitic enteritis caused by Hilmylepis spp. was the commonest disease (n = 20), followed by parasitic pneumonia (n = 5), gastric giardiasis (n = 5), intestinal coccidiosis (n = 4), testicular degeneration (n = 4), intestinal amoebiasis (n = 3), mycotic pneumonia (n = 3), Streptococcus pneumonia (n = 3), and blood protozoal infection (n = 2). There was a single etiology in 20/32 (63%) cases and more than one etiology in 12/32 (37%) cases. The predation was due to mismanagement of the animals during transportation, since multiple captured animals were transported in a single bag (n=3) rather than in individual bags.

Parasitic enteritis

Hilmylepis sp. was identified in 20 cases by using histological and parasitological examination of the intestines. The disease was the only infection in eight cases, but the other twelve cases co-existed with other etiological agents, as shown in Figure 1. The animals were lethargic and emaciated with humped backs and ruffled hair. There was severe congestion of the internal organs and diffuse filling of the small intestine with sanguineous fluid (Fig. 2A).
Microscopic examination revealed the presence of large numbers of cestodal segments attached to the lining epithelium of the intestine, resulting in necrosis and desquamation of the epithelium (Fig. 2B-C). There was a pronounced inflammatory cellular reaction around the invaded cestodes, consisting mainly of lymphocytes and eosinophils, the eosinophils were degranulated around the scolex of the cestodes (Fig. 2D).

Parasitic and mycotic pneumonia

Parasitic pneumonia was recorded in five cases, together with two cases showed multiple pathological findings: one with co-existent parasitic enteritis by Hilmylepis spp., and one with parasitic enteritis and Streptococcus pneumoniae. The lungs had a “tiger spot” appearance, with areas of emphysema and atelectasis. Emphysematous alveoli were associated with spiruroid nematode larvae (Fig. 3A). There was a pronounced inflammatory cellular reaction, consisted mainly of lymphocytes and eosinophils, in the interstitium. Several eggs were also observed in the peribronchial areas, surrounded by thin fibrous tissue layers. An accumulation of brown-dark pigment in the alveolar septa and inside alveolar macrophages was also observed. In one case, mites were observed in the interstitium and surrounded by thickened alveolar septa and a dense inflammatory infiltrate mainly eosinophiles and lymphocytes with blood cells (Fig. 3B).

In three cases, the lungs were invaded with thick, branched, PAS-positive hyphae of Aspergillus fumigatus, and surrounded by massive tissue damage and neutrophils (Fig. 3C). In addition, Gram staining revealed the presence of Gram-positive cocci in one case of parasitic pneumonia and two cases of mycotic pneumonia, and presumed to be Streptococcus pneumonia (Fig. 4).

Gastric giardiasis

Gastric giardiasis was observed in five cases from the total cases, three of which were in association with other disease: one case of parasitic enteritis, other case with parasitic infection of the blood, and the third case with testicular degeneration. Macroscopically, black spots could be seen inside and the outside of the stomach, and, on opening, the gastric mucosa contained blood clots measuring between 0.1 and 1 mm in diameter (Fig. 2A). Microscopically, flagellated fusiform Giardia trophozoites were seen (Figure 5) invading the gastric mucosa, resulting in damage and distortion of the epithelium. In association, there was a well-developed inflammatory reaction consisted of lymphocytes and neutrophils associated with hemorrhage around the invaded trophozoites (Fig. 6). Transmission electron microscopy (TEM) examination of the stomach tissue confirmed trophozoite and cyst forms of Giardia (Fig. 7).

Intestinal amoebiasis

Intestinal infection with Entamoeba histolytica was observed in three cases. One case had co-existent infection with parasitic enteritis. Macroscopically, the intestinal wall had a dark red color externally, and on opening the intestinal lumen was filled with muco-sanguineous material. Microscopically, the mucosa was completely denuded due to the presence of numerous trophozoites associated with mucosal microulcerations (Fig. 8A,B).

Testicular degeneration

Testicular degeneration developed in four cases suffered from severe cestodal parasitic enteritis. The testes were pale and swollen (Fig. 2A). Microscopically, there was irregularity of the basement membrane of the seminiferous tubules and vacuolation of the seminiferous tubular epithelium and
only small numbers of sperm were seen (Fig. 8 C, D).

**Intestinal coccidiosis**

Four animals had evidence of intestinal coccidiosis in the absence of any other pathological agent (Fig. 9). At necropsy, affected animals had enlarged and distended intestinal tracts and filled with blood with petechial hemorrhages in parts of the lower intestine. Microscopic examination of the affected intestine showed severe tissue damage due to clusters of large schizonts causing necrosis and disintegration of glandular epithelial cells and hemorrhage in the sub-mucosa. Merozoites and oocysts were present in the mucosa and submucosa (Fig. 9 A,B). Sporulated oocysts were subspheroidal and measured about 17x15 μm with a length: width ratio of 1:1. Most of the mucosal epithelial cells contain schizonts, gamonts, or developing oocysts (Fig.9 C,D). Many intestinal villi are distorted or have been destroyed as a result of coccidial infection. Erosion and ulceration of the mucosa have occurred.

**Blood protozoa**

In two cases there were evidence of infection of Plasmodium sp infection in the red blood cells, as evidenced by multinucleated schizonts (Fig. 10 A), and direct visualization of trophozoites in red blood cells by TEM (Fig. 10 B) and SEM (Fig. 10 C). Liver was invaded by plasmodium showed hepatocellular lysis and nuclear damage(Fig. 11) ,kidneys showed marked obliteration of Bowman’s space and periglomerular accumulation of invaded plasmodium, and degenerated proximal convoluted tubules.( Fig.12).
**Table 1.** Criteria used for the identification of the main parasites seen in the Greater Red Musk Shrew (*Crocidura flavescens*)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Disease</th>
<th>Features used to identify the parasite</th>
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<tr>
<td><em>Hilmylepis</em></td>
<td>Parasitic enteritis</td>
<td>The major reliable diagnostic characters of <em>Hilmylepis</em> are the number and length of the rostellar hooks, the shape of the rostellum, and the host-range of <em>Hilmylepis</em> spp. (Khalil et al., 1994 and Schmidt GD., 1986).</td>
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<tr>
<td><em>Angiostrongylid</em> (Nematoda) spp.</td>
<td>Parasitic pneumonia</td>
<td><em>Angiostrongylus</em> sp. (Nematoda: Angiostrongylidae) is described from the pulmonary arteries of <em>Crocidura flavescens</em> in Egypt. It is distinguished by the morphology of the dorsal ray and spicules according to identification of parasitic metazoa in tissue sections (Chitwood M, Lichtenfels JR., 1972).</td>
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<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Intestinal amoebiasis</td>
<td><em>E. histolytica</em> is the only pathogenic <em>Entamoeba</em> species. It belongs to the subphylum Sarcodina, class Lobosea, and family Entamoebidae (Fritsche T, Smith J, Henry J, 1996).</td>
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<tr>
<td><em>Giardia lamblia</em></td>
<td>Gastric giardiasis</td>
<td><em>Giardia lamblia</em> has a characteristic tear-drop shape and measures 10-15 µm in length. It has twin nuclei and an adhesive disk which is a rigid structure reinforced by supelicular microtubules. Trophozoites start to encyst <em>in vivo</em> when they migrate to the lower part of the small intestine. During encystation, the adhesive disc disassembles into four crescent-shaped structures that are kept in the cytoplasm.</td>
</tr>
<tr>
<td><em>Plasmodium</em> spp.</td>
<td></td>
<td>Intracellular parasites from the genus Plasmodium reside and multiply in a variety of cells during their development. After invasion of animal erythrocytes, their presence were recorded in H&amp;E stained liver and lung tissues. Identification according to (Kreier JP., 1993).</td>
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**Figure 1.** A Venn diagram showing the distribution of the organisms causing death of Greater Red Musk Shrews in Egypt. The co-occurrence of two or more organisms in a single animal is not uncommon.

**Figure 2.** Humped lethargic shrew with Parasitic enteritis. (A) Shrew necropsy. Focal areas of hemorrhages are seen externally on the stomach (red arrows), as well as in the stomach (blue circle). Swollen pale testes. (B,C) Cestode segments surrounded by a mixed inflammatory infiltrate (H&E x100). Severe inflammatory cellular reaction (arrow) in the lamina propria surrounding the cestodes causing necrosis (H&E x100) (D)
Figure 3. Lung diseases. (A) Lungs of Greater Red Musk Shrew infested with spiruroid nematodes. Note accumulation of the eggs in the lung tissues (H&E x40). (B) Lung invaded with mites and causing thickening of the alveolar septa with inflammatory cells (arrow) (H&E x10). (C) Lung invaded with Aspergillus fumigatus and appearing as thick, branching hyphae surrounded by neutrophils and tissue debris (H&E x400). (D) Lung invaded with Aspergillus fumigatus and appearing as thick, branching hyphae (PAS stain; red arrow) and associated with Gram-positive bacteria (blue arrow) (background H&E x10).

Figure 4. Lung diseases. Lung invaded with Aspergillus fumigatus and appearing as thick, branching hyphae (PAS stain; red arrow) and associated with Gram-positive bacteria (blue arrow) (background H&E x10).
Figure 5. Giardia trophozoites in gastric mucosa (H&E x400).

Figure 6. Gastric giardiasis. Red blood cells around invaded trophozoites in the stomach (H&E x400).
**Figure 7.** TEM image of stomach showing crescent-shaped Giardia trophozoites invading the epithelium (arrow). Lead citrate and uranyl acetate x2000).

**Figure 8.** Intestinal amoebiasis. (A) Entamoeba histolytica invading the intestine and appearing elongated and rounded in shape (H&E x400). (B) Entamoeba H. invading the intestine and appearing in cyst form (arrow) (H&E x40). Testicular degeneration. Seminiferous tubules showing reduced sperm production (C, H&E x10 and D, H&E x40).
Figure 9. Intestinal coccidiosis. Many intestinal villi are distorted or have been destroyed with erosion and ulceration of the mucosa. A lymphoplasmacytic inflammatory reaction is present in the lamina propria. There is also segmental enterocyte necrosis with villous blunting in several sections of small intestine, in some sections coccidian oocytes are seen in the lumen of the small intestine among sloughed epithelial cells and necrotic debris. (6. Axs100). Most of the mucosal epithelial cells contain schizonts, microgamonts, macrogamonts and developing oocysts (6 B, C, DX1000).

Figure 10. Blood Plasmodium infection. (A) Erythrocytic schizont, multinucleated stage, in an RBC resulting from asexual multiplication of trophozoite. Each schizont contains merozoites. (Leishmann stain x100). (B) TEM showing the presence of Plasmodium inside the RBCs (colored arrow; uranyl acetate and lead citrate, x4000). (C) Distorted RBCs as a result of Plasmodium infection (SEM).
Figure 11. Livers showed invaded plasmodium (arrow) and hepatocellular lysis (arrow) and nuclear changes, H.&E. X1000

Figure 12. Kidneys showed marked obliteration of Bowman’s space and periglomerular accumulation of invaded plasmodium (arrow), and degenerate proximal convoluted tubules. H.&E. X1000.
DISCUSSION

Although it has been suggested that shrews do not harbor large numbers of diseases, there are few pathological and parasitological studies to support this claim (Stunkard et al., 1975). We were therefore prompted to undertake a detailed and systematic histopathological, parasitological, and ultrastructural examination of thirty-five Greater Red Musk Shrews that had died mainly of natural causes in Egypt. Overall, infectious causes were the commonest and most important causes of mortality, in line with other pathological studies of small mammals and shrews.

Inflammatory pathology of the gastrointestinal tract is well described in shrews in captivity and in the wild, including from helminthic infections. For instance, in a study of 218 white-toothed shrews in Spain, four species of cestodes were present at a prevalence of over 4% (Portoles et al., 2004), compare to 57% in our study, and a necropsy study of elephant shrews in the United States revealed gastrointestinal pathology in 13% of cases, although no causative organisms were found in this retrospective review (Clancy et al., 2013). Giardia is probably more prevalent in small mammals than suggested by the published literature. There are no reports of giardia occurring in land-dwelling shrews; however, 65% of fecal samples collected from small rodents in the mountainous North America contained Giardia spp (Pacha et al., 1987).

Likewise, respiratory diseases are common in small mammals, including shrews. In one study, respiratory disease accounted for 13% of elephant shrew deaths in captivity, which were mainly bacterial in nature with no parasitic cases identified (although no causative organism was found in 9/14 cases) (Clancy et al., 2013). The main organism usually identified in shrews is Pneumocystis carini, which has been reported in shrews in the United States and Europe (Laakkonen et al., 2001; Peters et al., 1994). However, although spiruroid nematodes are well known to infect insectivores when infected insects, which are intermediate hosts, are eaten (Fisher, 1941), to the best of our knowledge this is the first description of parasitic pneumonia occurring in shrews. Likewise, Aspergillus spp in our study, have not previously been described in shrews and are generally uncommon in mammals (Tell, 2005).

Some of the other observations are also first descriptions of etiologies affecting Greater Red Musk Shrews. This is the first time that amoebiasis has been described in shrews, with humans generally regarded as the definitive host. Plasmodium has been described in the African Elephant Shrew (Hoogstraal, 1950) but not the Greater Red Musk Shrew. Finally, testicular degeneration has been recorded in captured animals, and may represent a systemic manifestation of severe disease.

In conclusion, this comprehensive analysis of Greater Red Musk Shrews dying in the wild suggests that a range of parasitic organisms affects shrews, and multiple infections are common at the time of death. Awareness of these diseases can help to manage shrews in captivity and prevent their population decline in the wild.
REFERENCES


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