

Klossiella Muris Infecting Laboratory and Wild Mice in Egypt

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Abstract: This investigation is considered the first report about klossiellosis in Egypt. It studies the parasitic infection of mice kidney with various developmental stages of *Klossiella muris* as a cause of renal coccidiosis in mice. This study describes the sporocysts excreted in mice urine and the different developmental stages of *klossiella muris* diagnosed by histology of the renal tissues collected during mice necropsy. The schizont was detected in the glomeruli, while the sporonts, sporoblasts and macrogametes were identified in the epithelial cells of kidney tubules. The prevalence of renal coccidiosis caused by *K.muris* among examined mice was estimated at 28.18% of laboratory mice (Swiss albino mice) and 37.14 % of wild mice (*Mus musculus*). The infection of mice with *Klossiella muris* was associated with mild interstitial nephritis which is likely to be of minimal pathogenic effect and they were not suffering from any renal disorders.

Key words: Klossiella • Mice • Pathology • Kidney • Sporocysts

INTRODUCTION

Klossiellidae and *Klossiella* are erected for *Klossiella muris* a renal coccidian parasite of mice. *Klossiella* spp. parasitize vertebrates and usually infect kidneys [1]. *Klossiella muris* is a polysporous coccidium which parasitizes the kidney of both wild and laboratory mice; it was first reported in 1889 [2]. Various developmental stages of the organism had been observed in the invaded kidneys which are slightly enlarged with uneven surface. The most characteristic feature is the very delicate mottling of the kidneys whole surface with minute, barely visible, grayish spots. This appearance of the kidneys may be considered almost diagnostic of the infection [3]. The previously described pathogenicity of *K. muris* involved lowering of the metabolic capability [4]. The infection occurs through ingestion of sporocysts and spreads throughout the body to the kidney [5]. While, the endurance time of the examined feral mice and interstitial nephritis in the spiny mouse (*Acomys cahirinus*) were described by Meshorer [6]. Other species of *Klossiella* are *Klossiella hydromys* recorded in Water Rat (*Hydromys Chrysogaste*) [7]. Bennett *et al.* [1], Barker *et al.* [8] and Pearce [9] identified *Klossiella quimrensis* in Australian marsupials. Recorded *Klossiella sp.* of Guinea pigs. Austin

and Dies [10] described *Klossiella equi* in the Kidneys of a horse and they added that *Klossiella equi* induced clinical signs in horses in the form of persistent long hair, intermittent bouts of sweating and increasing weakness. Arrington [11] and Fleck and Moody [12] reported the morphology, pathology and prevalence of *K. muris* in Swiss albino mice and they concluded that such infection may affect their performance as well as affecting obtained results when using laboratory animals in Biomedical Research.

The current research was carried out to investigate *K. muris* infection in the regional wild mouse population (*Mus musculus*). Whereas such population may be a potential reservoir of *K.muris* to domesticated laboratory animals in Egypt.

MATERIALS AND METHODS

A total of 110 laboratory mice of both sexes (Swiss albino mice), 2-12 months old, obtained from a single housed colony at the Laboratory Animal House, Faculty of Veterinary Medicine, Benha University, Moshtohor, Egypt was used in the present experimental work. They were kept in open cages (170 X290X135 mm) and had free access to water and a standard diet. Also, 175 wild mice of

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both sexes (*Mus musculus*) were collected by rat trap from different localities, housed in Stainless steel cages and supplied with standard ration and water till examination. Each examined mouse was kept in a separate box for clinical examination. The urine of each animal was collected, centrifuged at 2000 [g] for 5 minutes and the sediment was screened microscopically for the evidence of urinary spookiest [6]. The recovered sporocysts were thoroughly examined under microscope X40 and then kept in 2.5% Potassium dichromate at 4°C. The infected mice were anaesthetized by inhalation, necropsied and their kidneys were examined grossly and representative samples of kidney tissues were collected into 10% neutral buffered formalin. Once fixed, these tissues were embedded in paraffin, sectioned at 4 µm and stained routinely with Hematoxylin and Eosin (H and E). Sections of renal tissue were examined for evidence of klossiellosis and any associated renal pathology using a Leica light microscope. All alterations from the normal structure were registered. All measurements were made using an ocular micrometer. The following criteria were used for scoring the intensity of parasite per microscopic field, +++, for a severe infection, of, +++ for, a moderate infection and ++, for low infection.

All procedures dealing live animals were approved by the Local Ethics Committee for animal experiments in Egypt.

RESULTS

The result of urine examination revealed the presence of already sporulated sporocysts which are oval in shape, measuring 5-5.5 X 8-9.2µm in diameter and each has about 12 sporozoites. The infected animals did not show any signs of kidney malfunction. The percentage of infection among examined mice was listed in table 1.

Gross and Microscopic Examination of Kidneys: The parasitized kidneys of mice with *klossiella muris* appeared either normal, or contained white foci, without gross changes in their size. Microscopic examination of

the fresh sample of the kidney revealed that the typical oocyst did not formed, but a number of sporocysts developed within a membrane and pass already sporulated in urine, each measured 5-5.5 X 8-9.2µm in diameter with up to 12 sporozoites (Fig1).

Histopathological Examination: Moderate numbers of protozoan parasites in various stages of development are present in infected kidneys. These are identified as *K. muris* based on morphological appearance, sporocysts and their location. The gametogony, Schizogony and sporogony occur in different location of the kidney the schizogonic cycle occurs in the endothelial cells of the glomeruli, the young gametocytes then enter the tubule. Their subsequent development occurs in the epithelial cells of the convoluted tubules whereas the sporonts develop to mother sporoblasts, daughter sporoblasts and finally sporocysts containing sporozoites.

For the nomenclature of *Klossiella* spp. Schizonts were mainly recorded in the glomeration [Fig. 5]. Sporonts are round to ovoid, approximately 8.12-22X6.6-24.2 µm and are observed within the cytoplasm of the collecting tubules and distal convoluted tubular epithelial cells surrounded with a halo with nuclear displacement to eccentric position [Figs 2 and 6]. A daughter sporoblasts are marked by a rosette arrangement of 9 or more spheres [15.4-33.5X 19.8-25.4µm] which budded from large central mass of the cytoplasm [Fig. 3]. Numerous free mature sporoblasts are detected within the delicate wall and they are measured 4.18-7.26X 3.74-8.8µm [Fig.7]. Large thin wall oocysts [22-41.8X26.4-37.4] containing two to fourteen sporoblasts are located within the epithelial cells of the kidney [Fig. 6]. The gamonts appear as a small eosinophilic rounded masses within the vacuolated epithelium of the convoluted tubule. [Fig.4]. The macrogametocytes are demarked by their single rounded nucleus and were measured 3.74-5.5X 3.3-4.84µm.

Kidneys containing *klossiella muris* stages were affected with interstitial nephritis, congestion of the blood vessels, vacuolation in the cytoplasm of the tubules with atrophy in the epithelial cells, pyknosis of the nucleus and nuclear displacement.

Table 1: Prevalence of infection with *Klossiella muris* among examined mice

Mice	No. of examined mice	No. of infected mice	%	No. of examined Females	No. of infectedFemales	%	No. of examined males	No. of infected males	%
Lab. Mice	110	31	28.18	60	14	23.33	50	17	34
Wild mice	175	65	37.14	75	20	26.66	100	45	45
Total	285	96	33.68	135	34	25.18	150	45	30



Fig. 1: *K. muris* sporocyst in a urine sample. (Bar= 10 μ m)

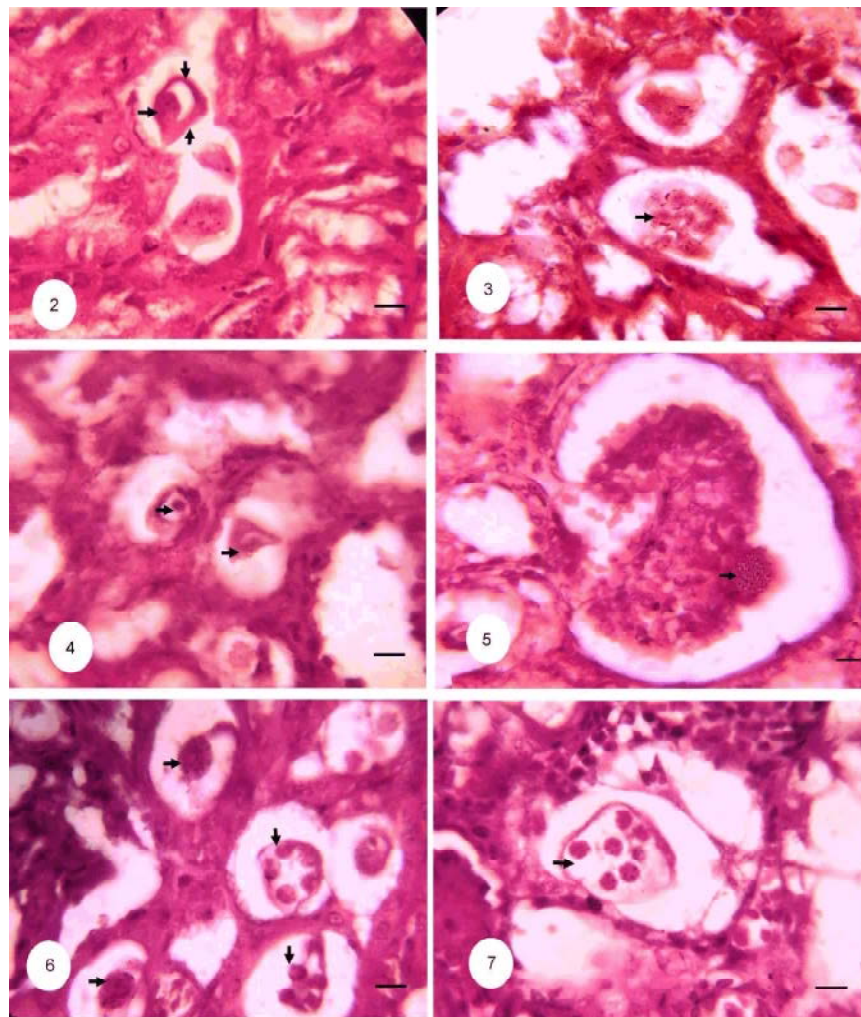


Fig. 2-7: Photomicrographs of *K. muris* life cycle stages in renal tissues from Swiss albino mice and *Mus musculus* in Egypt. [2] Sporont within the tubule with atrophy of cytoplasm and nuclear displacement. [3] Budding sporoblast. [4] Gamont appears within a vacuole in epithelial cell of the tubule. [5] Schizont within the glomeruli. [6] An early sporont and sporoblast. [7] Free mature sporoblast.

DISCUSSION

Reppas and Collins [13] showed that there is no reliable ante mortem test available to diagnose renal coccidiosis in mice. Sporocysts of *Klossiella* spp. are detected in urine infrequently and may be refractory to traditional concentration methods. Therefore microscopic examination of urine cannot be relied upon to diagnose *klossiellosis*, nor can mild to moderate renal coccidiosis be detected by macroscopic inspection of kidneys. Taylor [14] stated that histology of multiple sections of kidney tissue remains the gold standard for definitive diagnosis of *Klossiellosis*. Smith and Johnson [7] and Bogovsky [15] added that this parasite exhibited different developmental stages of the sexual and asexual cycles and followed the description of Gardiner *et al.* [16]. The presence of schizogonic stage in the glomerulus was firstly reported by Smith and Johnson [2]. It has the same morphological character of schizogonic phase of *Klossiella muris* and the round small cell with a single nucleus represent the gametocytes Wilson [18]. The presence of fully mature sporulated sporocysts containing up to 12 sporozoites passing in the urine and in histological sections of renal tissues supports the view of Yang and Grice [17] and Levine [9] that the mode of transmission is through ingestion of already sporulated sporocysts in urine and ensures that the sporulation occur in the kidney. Other species of *Klossiella* produce nonsporulated oocysts in urine as *K. queerness* [1]. We did not encounter clinical cases of *klossiellosis* in mice, all *Klossiella muris* affected kidneys demonstrated moderate parasite burden that would not by themselves affect renal function. In this respect, Bogovsky [15] and Meshorer [4] reported pathological changes associated with intracellular life cycle stages including mild interstitial nephritis. The prevalence of *klossiella muris* was estimated as 28.18% in laboratory mice (Swiss albino mice) and 37.14 % in wild mice (*Mus musculus*). Also, *Klossiella muris* had been recorded in spiny mice [18]. Moreover, this parasite was detected in a single *Mus musculus* [18], 40% of mice [15] and 60% of Australian water rats were infected by *Klossiella hydromys* [6]. The prevalence of infection was higher among wild mice than the laboratory mice, this may be due to unhygienic nature of wild mice which helps in parasite transmission as the disease appeared to be related to unsanitary housing conditions and disappeared when hygiene is improved [4]. The absence of any clinical signs relevant to kidney malfunction on *K. muris* infected animals in the present study coincide with the finding of many authors

such as Wilson *et al.* [18] and Gardiner *et al.* [16] who concluded that many cases of *klossiella* are not perceived except upon histological examination or in experimental work.

In conclusion, Experimental laboratory animals must be reared under hygienic condition and obtained from standard source free from *klossiella* infection to avoid its possible drawbacks.

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