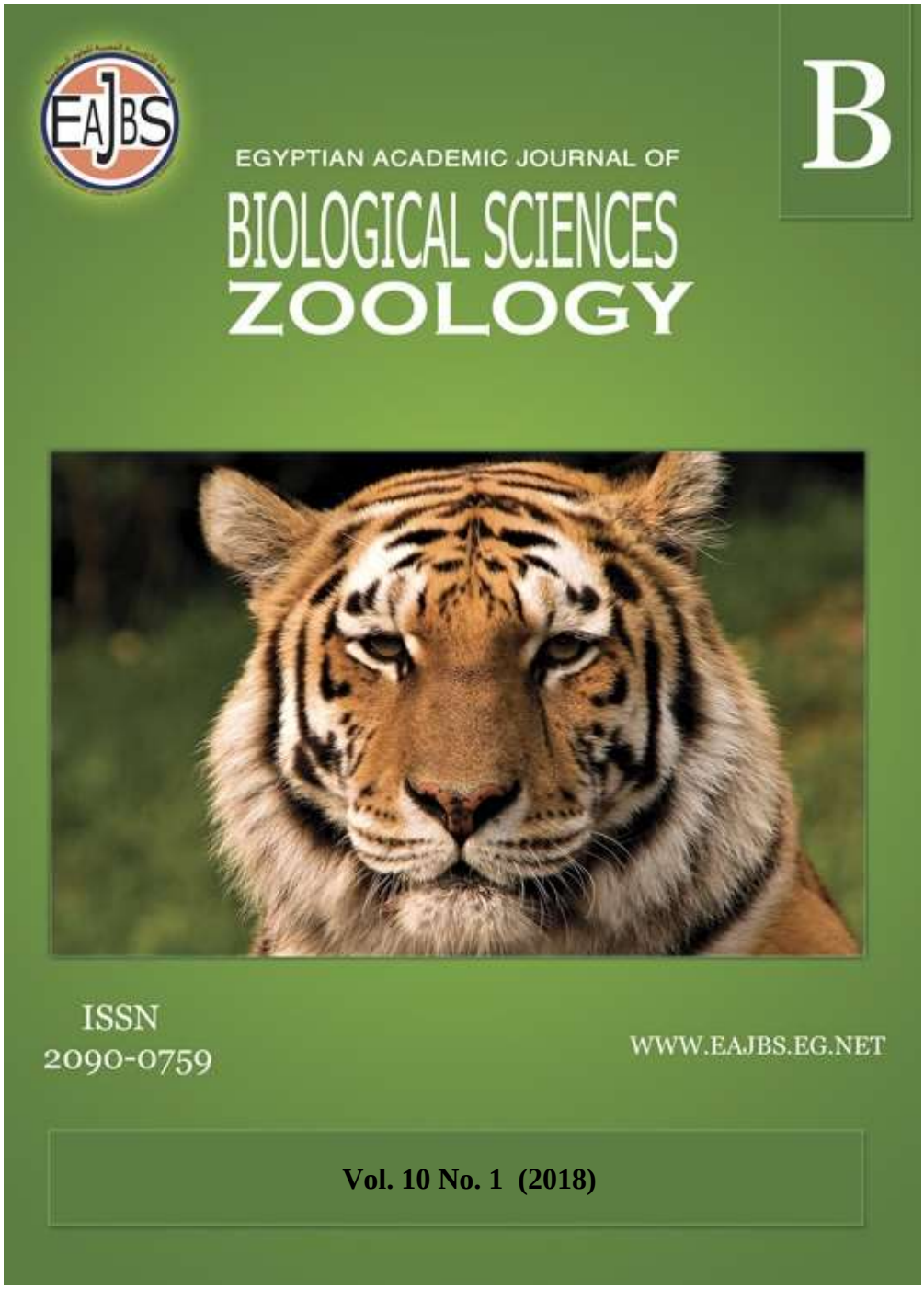

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Case Study*: Dioctophyma renale* Infection in Mice, Incidental Finding During Experimental Studies

Sara S. Abdel-Hakeem[1], and Mahmoud A. Abdel-Samiee [2].
1- Department of Zoology, Parasitology, Faculty of Science, Assiut University, Egypt (71516)
2 -Department of Pathology and Clinical Pathology, Faculty of Veterinary, Assiut University, Egypt
E.Mail:: sara_assiut86@yahoo.com

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ABSTRACT
Background: Accidental diagnosed of *Dioctophyma renale* parasite in experimental mice as the case in our report. **Methods:** *Dioctophyma renale* larvae had been reported in the renal pelvis of mice used for experimental studies. The parasite had two locations an intra-renal location (renal pelvis) and an extra-renal one (renal fat). **Results:** The larvae in the first location causing obvious pathomorphological changes in the renal tissues, consists of atrophy of the renal tubular epithelium dilation of the nephron. The glomerular tuft was also atrophied and it's Bowmen's were dilated. The parasite in the external location was confined by encystment were not associated with any pathological lesions. **Conclusion:** It had been concluded that *Dioctophyma renale* had been reported for the first time in mice and were associated with acquired polycystic kidney.

INTRODUCTION

The giant kidney worm *Dioctophyma renale* is an uncommon parasite which a debilitating and potentially lethal parasite of dogs, domestic and wild animals, and humans (Nahili *et al*., 2017). It is highly pathogenic in domestic animals it is most common in dogs which are considered an abnormal host (Mesquita *et al*., 2014). Mink, the most commonly affected animals, are believed to be the normal definitive host (Daniela *et al*., 2017). The adult worms are the largest of the nematodes and reside in the renal pelvis, although they may also be encountered in the peritoneal cavity (Mehlhorn , 2001). Eggs laid by adult worms pass in the urine and undergo a prolonged development of one to several months in water; they are then ingested by the intermediate host, a free-living annelid, *lumbriculus variegates*. Here they hatch, undergo further development and encyst. Frogs and fish may serve as paratenic hosts. Dogs, mink and other susceptible species become infected by ingesting the intermediate, or paratenic hosts. Larvae released in the intestinal tract penetrate the wall of the intestine and enter the peritoneal cavity. Subsequently, it penetrates the kidney to reside in the pelvis. This entire process requires a minimum time of about three months, but may take several years (Woodhead, 1950).

In dogs, the right kidney is more frequently affected than the left, but because dogs are not a usual host the parasite is more often found restricted to the peritoneal cavity, where it obviously cannot complete its life cycle (Osborne *et al*., 1969). The presence of the worms in the renal pelvis leads to slow destruction of the renal parenchyma, ultimately leading to a fluid-filled sac hydronephrosis (Jubb *et al*., 2016). In the peritoneal cavity, the worms and their eggs incite a chronic peritonitis with adhesions (Carter and Collely, 1979). They often are in close proximity to the liver where they may cause strangulation of a lobe of the intestine.

MATERIALS AND METHODS

One hundred of BALB- mice purchased from the Animal house in the Faculty of Medicine, Assiut University, Egypt. They were brought to the lab and sacrificed for screening of internal parasites. Different organs (liver, kidney, intestine and stomach) were collected for histological studies. The material of this study consists of kidneys and renal fat from three mice showing signs of renal enlargement. Samples from enlarged kidneys were obtained for histopathological studies.

Histopathological study

Kidney specimens were fixed in formol-alcohol fixative for 24-48h. Fixed specimens were dehydrated in ascending grades of alcohol, and then cleared in methyle benzoate three changes, infiltrated with parafine at 56℃ and subsequently embedded in paraffin blocks. The blocks were sectioned on microtome at 4-6 µm, and stained with hematoxylin and eosin (H&E) (Bancroft and Steven, 1982). Stained sections were examined on Optica microscope and a digital colored video camera (Optica 4083.B9 digital camera, Italy).

RESULTS

According to the location of the parasite, infected mice were divided into two groups: The first group with an intra-renal location of the parasite (renal pelvis).
Microscopical examination of sections from the kidney of this revealed that the parasite was located in the renal pelvis (Fig. 1A).The epithelium of the renal pelvis showed compressed and atrophied due to the pressure of the parasite (pressure atrophy) (Fig. 1B). The larvae in this location were exposed to the action of irritant products in the urine (urea, uric acid, ammonia and creatinine) hence most of the structure of the larvae was blared. Blood vessels in the vicinity of the renal pelvis were dilated and thrombophlebitis (Fig. 1C), the arteries in this area having a thickened wall with hypertrophy of its endothelium and perivascular edema with few mononuclear cells infiltration. The perivascular spaces at the cortico-medullary junction showed edema and few leucocytic infiltrations (Fig. 1C).

The renal collecting tubules in the vicinity of the renal pelvis were prominently dilated and showed severely atrophied epithelium which were sometimes disappeared and the tubules were bordered only by the basement membrane .Fig (1D and E). From 60-70% of the proximal and distal convoluted tubules of the renal cortex were prominently dilated (Fig. 1F).Fig (1D and E) showed extremely atrophic epithelium, sometimes the renal tubular epithelium of the proximal and distal tubules were completely sloughed and the tubules were only bordered by stretched basement membrane. Most of the glomeruli showed atrophic changes with prominent dilatation of its capillaries and increased density of the mesangial matrix (Fig. 2A). The Bowman's spaces of the most of the glomeruli were dilated and the capsular epithelium sometimes showed evidence of proliferation and epithelial crescent.

The second group of infected mice with an extra-renal location of the larvae (encysted in the renal fat). In these group, the larvae were located extra-renal (Fig. 2C and D) in the adipose tissue surrounding the kidney. In such location, the larvae were surrounded by connective tissue capsule composed of collagen fibers and fibroblast cells (Fig. 2E) with prominent space between the larvae and connective capsule. The parasitic larvae consist of closely packed tubules lined with cuboidal epithelium. The lumen of each tubule having a long axis and short axis, the diameter of each were (1477.9-907.9 µm) and (813.9-210.9 µm) respectively Fig. (3D-F).

In this situation, the somatic cells of the parasitic larvae were of healthy appearance. Both the nucleus and cytoplasm showed no evidence of degeneration or necrosis (Fig. 3A-C)

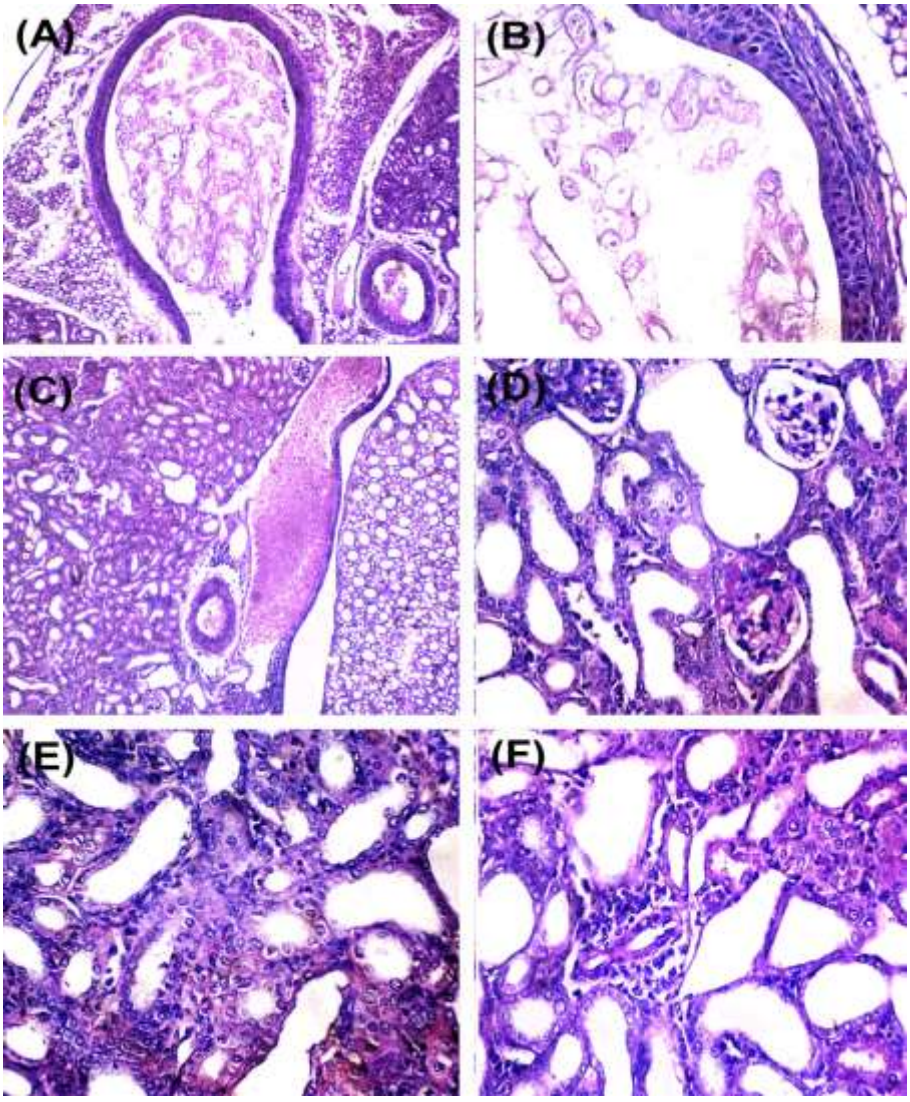

Fig (1): Transverse section of the kidney of mice infected with *Dioctophyma renale*: (A) the structure of the larvae of *D. renale* in the renal pelvis appear as closely packed tubules (10X); (B) high power showing the compressed transitional epithelium of the renal pelvis; (C) severe dilation of the vein and artery at the corticomedullary junction with evidence of thrombosis; (D & E) prominent and severe dilation of proximal and distal convoluted tubules where its renal epithelium was disappeared or atrophied, other tubules showed atrophy of renal tubular epithelium; (F) about 70% of the proximal and distal tubules showed prominent severe dilation and loss of the epithelium. H&E staining, (40X).

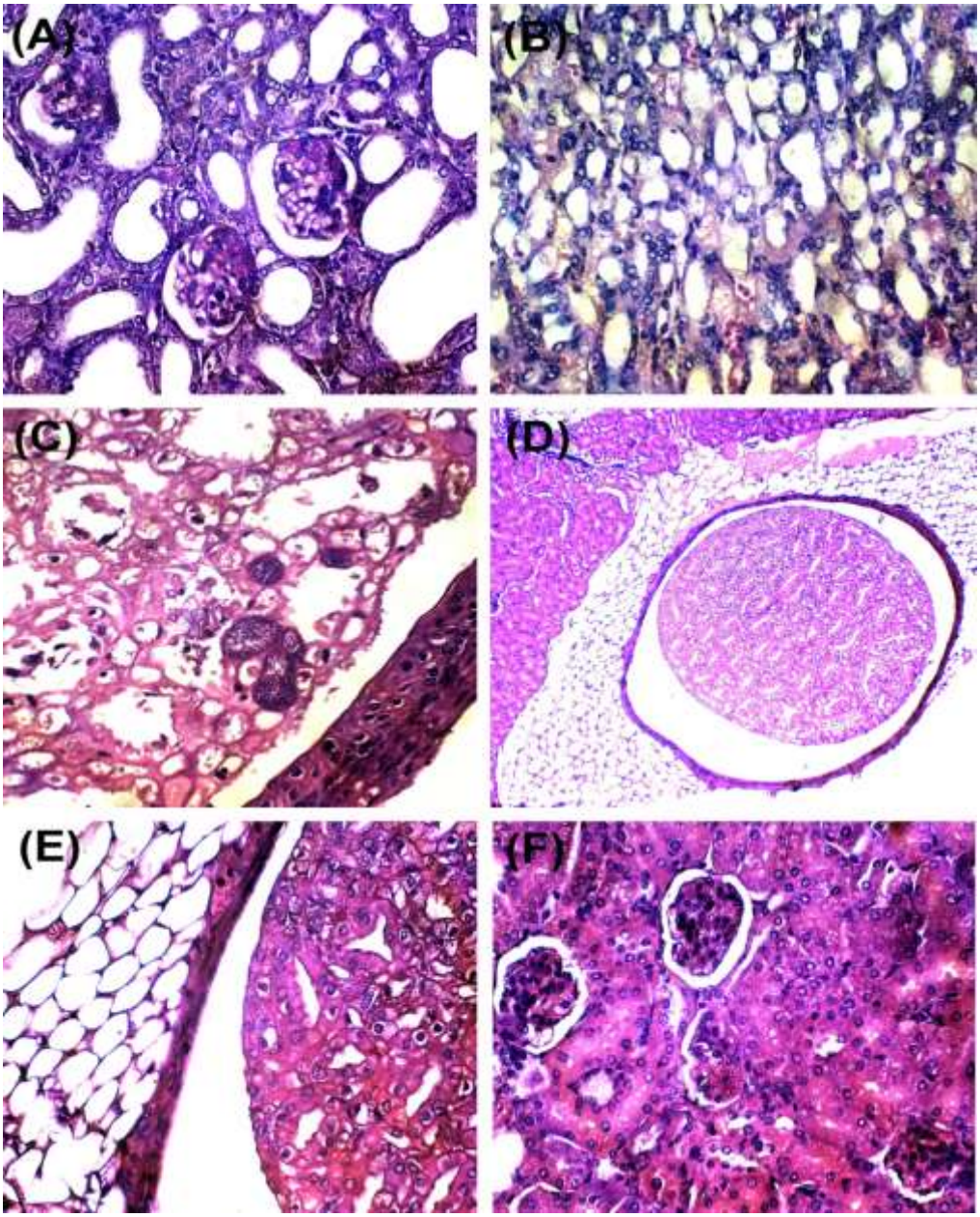

Fig (2): Transverse section of the kidney of mice infected with *Dioctophyma renale*: (A) prominent atrophy of glomerular tuft with prominent decreased cellularity, dilation of the capillaries and thickening of the glomerular membrane; (B) dilation of some collecting tubules of the medulla; (C) cross section showing aggregation of internal structures of the parasite; (D) cross section of the parasite surrounded by connective tissue capsule (extra-renal location of the worm); (E) fragment of the parasite appear as a healthy closely packed tubules lining with cuboidal epithelium surrounded by connective tissue capsule; (F) normal appearance of the kidney glomeruli and tubules associated with extra-renal location of the parasite. H&E staining, (40X)

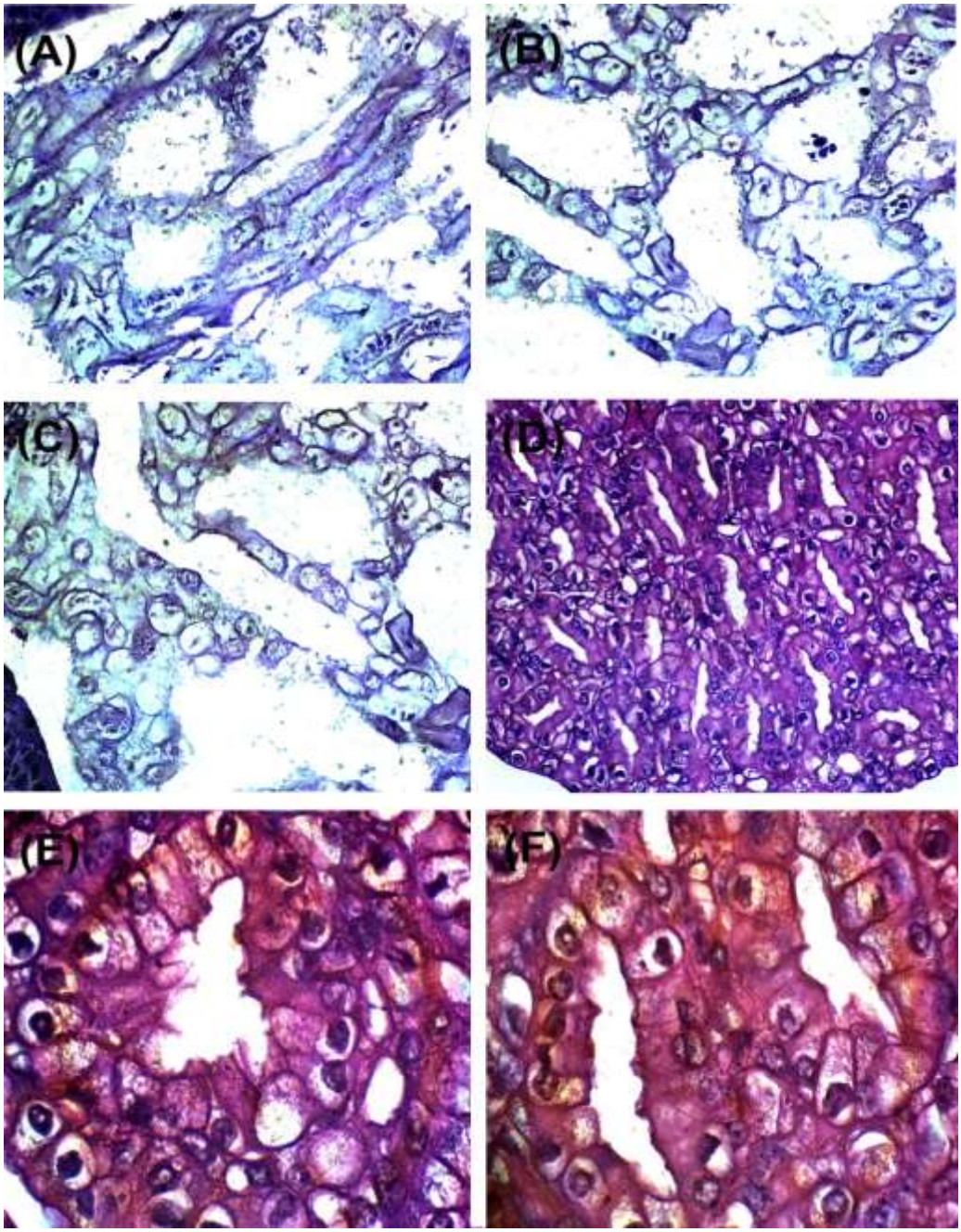

Fig (3): Transverse section of the kidney of mice infected with *Dioctophyma renale*: (A-C) parasitic larvae in the renal pelvis with seriously damaged structure 40X, (D-F) healthy appearance of the parasitic larvae in the extra-renal location showing closely packed tubules lined with a cuboidal epithelium (D , 40X; E and F, 100X) H&E

DISCUSSION

Pathology is considered a powerful and essential tool to diagnose different parasitic infection according to the morphological changes in target tissues. Information about the prevalence of the dictophymosis infection is difficult because most reports deal with isolated findings or clinical cases (Macpherson *et al*., 2000).

Structure greatly resemble the morphology of the larvae of *Dioctophyma renale* had been reported in the renal pelvis of two mice, in the third one, cross section of the parasitic larvae were reported to be encysted in the renal fat close from the renal pelvis. Analysis of all available literature revealed that parasite was always accidentally diagnosed as the case in our report (Schmitt *et al*., 2012; Katafigiotis *et al*., 2013). According to many animal/ human cases, the infection can be explained by indiscriminate eating habits of these animals (Kommers *et al*., 1999) that occurred by eating infected worms or paratenic hosts as fish, crayfish or frogs in which the larvae become encapsulated in the tissue. As well as it can result from direct contact with contaminated water (Sapin *et al*., 2016). This agreement with Carolina *et al* (2016) who discussed that probability of infection through drinking water infested with infected annelid intermediate host. Gutierrez *et al* (1989) suggested that such larvae eventually migrate to the kidney and grow to adult state these will take a long time that extends to about three months. In our cases no worm or egg was detected, the cases were diagnosed as a presence of closely packed tubules which were lined by cuboidal epithelium. The clinical expression of the parasitosis by *D. renale* is unspecific or may be absent (Kommers *et al*., 1999; Kano *et al*., 2003; Monteiro *et al*., 2003).

In the present case, the structures of the parasitic larvae encountered in our material were greatly confused with the Liesegang rings phenomenon (LRs). This LRs phenomenon is physic-chemical process which commonly occurs in vitro. It was first documented by German Chemist in 1896. LRs were initially believed to represent parasite *D. renale* (Tuur *et al*., 1987). Islam *et al* (2012) reported the LRs in the breast, but the author did not describe any histopathological lesions associated with it. The structure reported in our material was closely related to the parasite *D. renale* as some points of differential diagnosis must be considered. Firstly the structure in our material consists of closely packed regular tubules lined by cuboidal epithelium with histoid architecture some of the tubules were differentiated to cystic space filled with basophilic coarse granules. These granules could be considered remains of the internal structure of the parasite. But LRs structure showed great irregularly in shape and arrangement. Secondly, this structure was associated with severe histopathological lesions in the kidney (Acquired polycystic kidney and chronic sclerosing glomerular nephritis) which often lead to uremia and death of the affected host. As well as presence of a fibrous thick wall cyst which is filled with ring-like structure that is the characteristic pathological criteria of the *D. renale* eggs which harmonized with Sapin *et al* (2016). Finally, there are not record or documented larva migrate nematodes associated with polycystic kidney appearance except Ascaris which totally showed different in pathological criteria.

Instead of the above mentioned facts used for differential diagnosis, the confusion of parasitic structure and LRs is not accepted because of biological, histoid regular appearance of the structure in our material and the deleterous renal micromorphological changes associated with it .The organism reported in the renal pelvis in the extra renal location having identical biological micromorphology and exhibit a regular histoid architecture, these indicating its parasitic nature.

The intra pelvic location parasite caused a prominent lesion in the kidney. These lesions involve both cortex and medulla. It also involves the parenchymal tissue of the kidney (nephron) and the vasculature. The blood vessels in the vicinity of the renal pelvis were severely dilated and thrombosed, perivascular edema and mononuclear cells infiltration were commonly seen. In the cortex 70% of the proximal and distal convoluted tubules were severely dilated and its epithelium were stretched and atrophied. Most of the dilated tubule loss its epithelium and were only bordered by connective tissue basement membrane. About 80% of the glomeruli showed atrophic changes with prominent decrease in size of the glomerular tuft, and decrease in its cellular density. The capillaries were dilated and bloodless with thickening of their basement membrane. Sometimes the podocytes were proliferating and adhere to the parietal epithelium forming what is known as epithelial crescent. The bowman's spaces in most of the glomeruli were dilated and the Bowman's capsules were thickened .The epithelium of some renal tubules suffering necrotic changes of coagulative type. The collecting tubules in the renal medullary showed a moderate degree of dilatation. This condition was diagnosed as acquired polycystic kidney which apparently due to blockage of urine in renal pelvis where the parasite was located. Contrary to our finding, Mace (1975) demonstrated that severe hydronephrosis and renal cortical and medullary degeneration and necrosis are commonly encountered as a pathological finding. Lesions in the renal parenchyma consist of connective tissue proliferation in the interstitial tissue, tubular atrophy and fibrosis and periglomerular fibrosis. The luminal surface of the renal pelvis was formed of papillae covered with transitional epithelium (Mace, 1975; Measures and Anderson, 1985). These results were agreement with Mcneil (1948) who demonstrated that the pelvic mucosa had extreme papilliferous metaplasia and the margins of these polyps were composed of stratified transitional epithelium and this thickening of renal capsule was reported by Leite, *et al* (2005). The difference between the nature of lesion reported in our material and those described in the literature could be discussed on an etiological base, reported lesions reported in our material were associated with the immature worm, while those described in the literature were associated with mature kidney worm *D.renale*. The degree of blockage of renal pelvis by the parasites must be also considered.

Conclusion

We can conclude that acquired polycystic kidney and chronic glomerulo-nephritis were reported in two mice. These lesions were due to blockage of urine flow in the renal pelvis induced by larvae of *D. renale*. Moreover the toxic metabolic products of the parasite caused severe angiopathic changes in the renal vasculature. The encysted parasite in the extra-renal location (in the renal fat) caused no histopathological changes in the kidney.

Ethical considerations

All experiments were carried out in accordance with Egyptian laws and University guidelines for the care of experimental animals. The research will be approved by the committee of the Faculty of Veterinary medicine of Assiut University, Egypt.

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ARABIC SUMMERY

دراسة حالة عن وجود طفيل *Dioctophyma renale* كحالة عارضة أثناء التجارب العملية

سارة صلاح عبد الحكيم [١]، محمود عبد الظاهر عبد السميع [٢]
[١] قسم علم الحيوان والحشرات، كلية العلوم، جامعة أسيوط
[٢] قسم الباثولوجيا والباثولوجيا الأكلينيكية، كلية الطب البيطرى، جامعة أسيوط

يقدم التقرير تشخيص عرضى لطفيل *Dioctophyma renale* فى الفئران التجريبية. وقد تم تشخيص وجود الطفيل فى موقعين أحدهما داخلى (فى الحوض الكلوى) والأخر خارجى (فى الدهون الكلوية). وقد أظهرت النتائج وجود يرقات من طفيل *Dioctophyma renale* فى الموقع الأول الداخلى مما أدى الى العديد من التغيرات الباثولوجية المميزه المصاحبة والتى تشمل ضمور واضح فى طلائية الأنيبيبات الكلوية وتمدد للنفرون وكذلك ضمور فى كتلة الكُبة وتوسيعها. بينما لم يقترن تحوصل الطفيل فى الموقع الخارجى حدوث أى من هذة التغيرات الباثولوجية. وقد لُخصت النتائج بتسجيل وجود يرقات لطفيل *Dioctophyma renale* للمرة الأولى فى الفئران وما يصاحب ذلك من تغيرات باثولوجية.