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New recording of *Kalicephalus* sp (nematode) and its histological effect on *Eryx jaculus* snake in Diwaniyah province / Iraq.

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Abstract

During the period between March 2017 and March 2018, 28 *Eryx jaculus* snakes were collected by snake-hunting enthusiasts, some by farmers in villages in Diwaniyah, Afak, Hamzah al-Sharqi, Dagara, Nafer and Al-Badair districts of Diwaniyah province. Diagnosis of snake samples in the Museum of Natural History at the University of Baghdad.

The results of the current study on the examination 28 snakes revealed that 21 of them were infected with nematode *Kalicephalus* sp. with a rate of infection reached %75 , and through study sites where the presence of worm nematode it was noted that most of the infections concentrated in the small intestine compared to the stomach and large intestine.

The results of the reading of the histological sections prepared from the small ,large intestines and stomach of the snakes in the study showed the presence of tissue changes caused by the parasites in their specimens represented by infiltration of inflammatory cell , degeneration of villi,atrophy , necrosis and local death of some affected tissues,and all results of the current study are new and recorded for the first time in Iraq.

Keywords: Snake , *Eryx jaculus* , *Kalicephalus* sp.,Nematode, Iraq.

1.Introduction

Eryx jaculus belongs to the family of Boidae and is locally known as the sand-spotted or Arabian sand snake . It isn't endangered species due to its widespread in North and South-Eastern Europe, southern Russia, Morocco, Egypt, Saudi Arabia, Iraq, Iran , the Caucasus Mountains in Armenia and Azerbaijan(Ananjeva *et al*.,2006;Cox *et al*.,2006) , In Iraq, it was recorded in Basra, Baghdad, Amarah, Nasiriyah, Halabja, Sulaymaniyah, Najaf, Arbil, al-Kut,

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Baquba, Mandali, Diwaniyah and Mosul Habeeb and Pouyani (2016).

This snake able to bear a wide range of temperatures and long periods of drought but are inactive during March and October, where they are hibernation in fragile sand or in burrows rodents or cracks under the rocks, and catch their prey by ambush where the body is hidden under the sand, except the area of the eye and nose to detect the passage of the prey and when close to it, they attack at an amazing speed and by nature squeeze her prey to death and swallow, it also can swallow alive small prey such as rodents (Lanza & Nistri, 2005).

Reptiles, including snakes, are exposed to a variety of pathogens, which may be bacterial, fungal, viral, parasitic agents, There are many studies have indicated that snakes are the intermediate or definitive hosts for many of the internal parasites (Klingenberg,2000; Parc , 2008; Rataj *et al*.,2011 ; Al-Mayali &Anah ,2018)as well as being an intermediate host of *Toxoplasma* sp. (Anah & Al-Mayali,2018), as well as blood parasites (Telford,2009; Jacobson , 2007), It is also affected by external parasites (Rataj *et al*.,2011;Pietzsch *et al*.,2006).According to our literatue review ,despite of wide distribution of these snake in Iraq,there is not comprehensive and adequate publisher data about parasite of this snake in Iraq,so this study was conducted.

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2.Materials and methods

Area of study

Al-Diwaniyah province(180 KM south of Baghdad) is one of the Middle Euphrates provinces and its territory is part of the plain sedimentary Iraqi, which is characterized by the simple decline from north-west to the south and south-east also show minor differences and other local in the surface of the province because of several factors, most important process of wind sedimentation and can be explained nature by dividing the province to ,the first part consists of the flood plain, which includes most areas of the province and the area of the shallow and semi-shallow depressions,which the second part represents, the third part which is located in the sand dunes area,Such as the districts of Afak and Al-Badir, and the fourth part, which is represented by the sandy area and covers the southwestern part Of the province in the area between the west of the Euphrates River and the Western administrative boundaries of the province. (Al-Janabi&Ghaleb,1992).

2.1.Examine snakes and collect parasites

The dead snakes are kept in the refrigerator at 7 ° C and dissected within seven days while living species, they are anesthetized with ether (Fontenot &Font,1996),Then

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process of snakes dissection is carried out according to the method of the reptile anatomy **(**Jacobson,1978), the snakes are placed on a large piece of cork designed for this purpose. Then opened from abdominal side ,starting from anus slot towards forefront (Fig.1,2). The digestive tract and its components,and then each part is longitudinalized by a sharp scissors in a sterile petri dish to look for intestinal worms. It also examines the yellow sac near the pancreas adjacent to the stomach as well as the liver and lungs. In the case of isolation of intestinal worms, it is washed with water and kept in containers of %70 ethyl alcohol, glycerin For the purpose of clarification and confirmation. Nematodes are placed in lactophenol solution and then carried on a clean glass slide using Canada's balsam (Chaiyabutr & Chanhome, 2002) .all nematode examined under lower and higher magnification and necessary measurements were taken using ocular and stage micrometer.Samples used for histopathology were fixed in %10 formalin ,embedded in wax paraffin sectioned at 5 µm.Sections were stained with Hematoxylin and Eosin ,mounted on glass slides .

Fig.1:Morphology of *Eryx jaculus*

Fig. 2:*Eryx jaculus* after dissection

3.Results

This nematod worm *Kalicephalus* sp.(Rudolphi,1819) was recorded highest percentage from *Eryx jaculus*,it was found in 21 samples %75 and found in both the large and small intestines as well as stomach(Table.1), This nematoda female describe short, strong worms with a white color that does not exceed 6 mm long. The

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mouth is equipped with four pyramid cuticular structures,it has Oesophagus 280µM long by 75µM wide ,funnel shape(Fig.4) may contain three small teeth and with strong kaitinine lining ,excretory pore near to posterior end ,uterine branches opposed or parallel and full of eggs(Fig.5) ,posterior end conical form .(Fig.6).

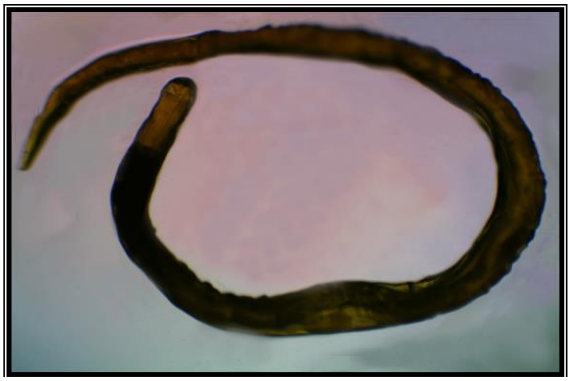

Fig.3:Morpholoy of *Kalcephalus* sp.(40x)

Fig.4:Anterior end of *Kalicephalus* sp.female(100x)

Fig.5:Eggs of *Kalicephalus* sp.female(100x)

Fig.6:Posterior end of *Kalicephalus* sp.female(100x)

Table 1: Number &percentage of snake (*Eryx jaculus*)infection with *Kalicephalus* sp.

Number of snake examined	Number infection	%	Number of parasites	Site of infection					
				stomach		Small intestine		Large intestine	
				n	%	n	%	n	%
28	21	75	44	9	20.45	23	52.27	14	31.82
LSD(P≤0.05)			To compare the infection rate according to site of infection =33.98						
			F.calculated:20.91 F.table:4.45 (to site infection)						

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When microscopic histological sections were examined, some microbiological changes were observed in the gastrointestinal tract infected with *Kalicephalus* sp. compared to the normal intestine. microscopic histological changes included infiltration of inflammatory cell , degeneration of villi ,atrophy , necrosis and local death of some affected tissues .

Fig.7:Small intestine of the snake is in normal state (H&E.100X)

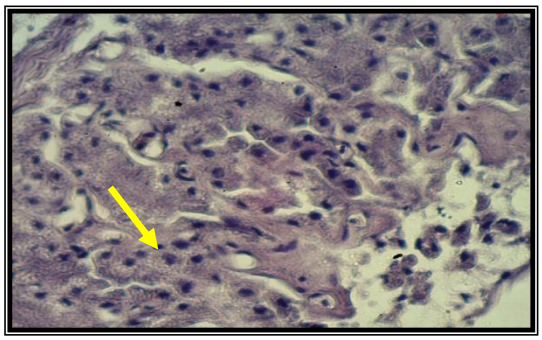

Fig.8: Infiltration of inflammatory cell(H&E.400x)

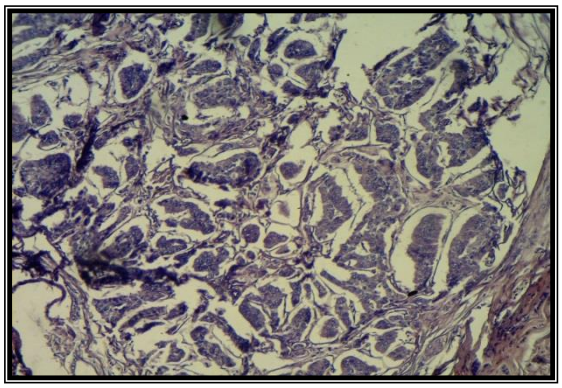

Fig.9: Nicrosis and disappearance of the features of the villi completely (H&E.100x)

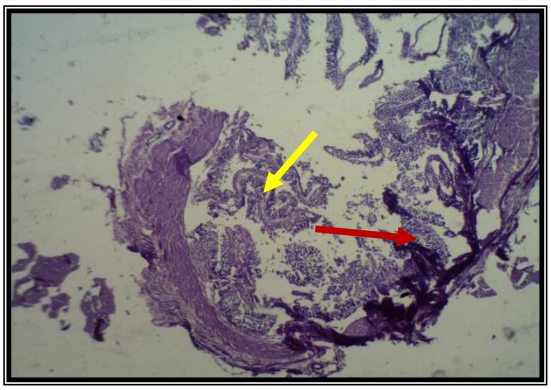

Fig.10:Degeneration(←) and atrophy of villi (→) (H&E.100x)

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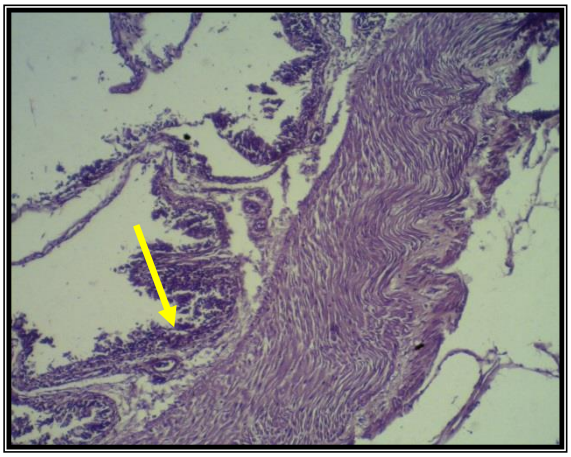

Fig.11: Extensive degeneration of the villi (H&E.100x)

4.Discussion

Eryx jaculus had a %75 nematode infection,The isolation of *Kalicephalus sp*. from the wild snake was considered the first recorded in Iraq. Iraqi studies did not indicate that it was previously recorded. Globally, Fontenot & Font (1996) in South-East Louisiana recorded in the *Nerodia fasciata* %13 and *Agkistrodon piscirorus* %30, Chaiyabutr & Chanhome (2002) in Thailand (%30.5) of five poisonous snakes Three species of nonvenomous snakes , Santos *et al*. (2006) in the Iberian Peninsula of two species of *vipera latastei* and *V.aspis* %5.3 , Dusen *et al*. (2010) in north-western Turkey and %18 of the *Coronella austriaca* , Ribas *et al* (2010) in the north - east of Spain, noting that the snake *Malpolon monspessulanus* recorded the lowest rate among the snakes studied as it reached 5.9% , Rataj *et al.* (2011) in Scandinavia from A group of snakes %20.4 , Santoro *et al*. (2013) in southern Italy and %12 of the snake *Hierophis viridiflavus carbonarius*, The description of *Kalicephalus sp*. accorded with mentioned Punshyam & Mahendra (2016) as the mouth is equipped with four pyramid cuticle structures, a strong muscular esophagus with strong kaitinine lining, and an aperture position.

Sprint (1978) points out that this parasite has an indirect life cycle and uses rodents as an intermediate host, while Baker (2008) indicated that it has a direct life cycle and the infection is obtained by ingestion of larvae by mouth or by penetrating the skin, (Santoro *et al*., 2013) reported that the life cycle of this parasite is unknown until now, and the snake acts as final host. According to Anderson (2000), the snakes are infected by larval suspension, indicating that there is no clear evidence of the skin penetrates , and it is possible that the animals with soft bodies such as shells, mollusks and

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amphibian are associated with snakes in living as a vector host.

The examination of the tissue sections prepared from the small and large intestine obtained from the infected snakes with *Kalicephalus* sp. revealed the presence of a large number of chronic inflammatory cells along the mucous layer, especially the lymphocytes, and that inflammatory cell sprays are only the expression of the inflammatory response resulting from the necrosis and crash in the intestinal villi peaks resulting from the parasites on host cells. The case was caused by the infection of the nematode *Kalicephalus* sp. Similar cases of gastroenteritis and inflammatory cell infiltration have been reported by schistenaar *et al*. (2000) in lizard intestines infected with nematode , Brewer & Cranfield (2001) in snakes infected with *Cryptosporidium* sp. (2007) in his study of pathological changes caused by intestinal parasites in reptiles as well as Kuroki *et al*. (2008) in snakes infected with *Cryptosporidium* sp.

It was noted that the atrophy of the villi, some of which appeared short , wide and form of abnormal .This state may be due to the feeding of parasites on the villi, leading to its disappearance and the lack of surface area of absorption and thus low efficiency of absorption of nutrients and mineral salts.

Another disease has also emerged which death and necrosis of some cell, which were clearly destroyed by the infection *of Kalicephalus* sp. Nasiri *et al*. (2014) pointed to a clear necrosis of snake intestines infected with *Centrorhynchus corvi*.

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