

Pathogenicity of Two Nematodes Tetramere sp. and Microtetramere spiralis in Proventriculus of Some Aquatic Birds from Al-Sanaf Marshes in Thi-Qar Province, South of Iraq

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Abstract: The present study was carried out to investigate the prevalence and mean of intensity of two nematodes *Tetramere* sp. and *Microtetramere spiralis* isolated from some of aquatic birds collected from Al-Sanaf marshes, Thi-Qar province. The pathological effects of these parasites on the tissues of the infected aquatic birds were recorded. The *Tetramere* sp. females were embedded in glands of proventriculus of the some aquatic birds including: *Ardea ralloides*, *Egretta garazeta*, *Nycticorax nycticorax*, *Himantopus himantopus*, *Larus genei*, *Bubulicus ibis*, *Ardea purpurea* and *Anas clypeata* with prevalence 12.57% and mean of intensity 3.00, while the *Microtetramere spiralis* embedded in gastric glands of proventriculus of *Bubulicus ibis* with prevalence 10% and mean of intensity 25.00. Histopathological examination of infected tissues showed dilation of the proventricular gland lumen, atrophy, necrosis and degeneration of the glandular cells of proventriculus.

Keywords: *Tetramere* sp., *Microtetramere spiralis*, Aquatic birds, Proventriculus, Pathogenicity, Iraq.

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I. Introduction

Tetrameres (Creplin, 1846) and *Microtetrameres* Nematodes are widely spread parasites of water birds (Ellis, 1967; Anderson, 2000). Those parasites are characterised by strong sex dimorphism and they are limited to the glandular stomach. The sexually mature form of the females is relatively large, strongly truncated and had a swollen egg-filled uterus, with conical anterior and posterior body ends. Males are small with filiform body residing in the

superficial layer of the mucous membrane (Borgarenko, 1990), and usually free in the lumen of the proventriculus or they may be present in the gastric glands in association with the females for copulation (Permin and Hansen, 1998).

The proventriculus worm *Tetrameres* used intermediate hosts such as arthropods which were in turn eaten by the birds. The signs of infection include weight loss, diarrhea, inactivity, and these parasites caused considerable inflammation in the proventriculus (Clark, 2000).

The pathogenicity of the parasite depended on parasite species, host resistance and parasite load, these nematodes fed on blood and caused anemia and may caused the death of some infected birds due to the migration of the larval stages to the proventriculus glands which caused irritation and inflammation (Soulsby, 1982).

Al-Mayah et al., (1991) reported that the *M. egret* caused severe pathological effects in *B. ibis*, it caused destruction in infected tissues, hemorrhage, intensive inflammatory response and dysfunction in the proventriculus of infected birds.

The present study aimed to show the prevalence and mean of intensity for these parasites in aquatic birds and to describe the pathological effects on the proventriculus of some aquatic birds infected with these two nematodes.

II. Materials and Methods

A total of 358 aquatic birds belonged to eight species: *Ardea ralloides* (121), *Egretta garazeta* (85), *Nycticorax nycticorax* (56), *Himantopus himantopus* (46), *Larus genei* (30), *Bubulicus ibis* (10), *Ardea purpurea* (5) and *Anas clypeata* (5) were collected from Al-Sanaf marshes in Thi-Qar province.

Females of *Tetrameres* sp. and *Microtetrameres spiralis* isolated from proventriculus by fine forceps used for directing light pressure around the both sides of gland to remove the worms. Nematodes are usually stored in 5% glycerine in 70% ethanol. The lactophenol and glycerine used to clear the internal features, the nematodes were identified according to (Yamaguti, 1961).

Tissue samples with attached parasites were isolated from infected birds and:

washed with normal saline and fixated in Bowen's fluid for 24 hours according to (Humason, 1972):

Washed many times with ethanol 50% to remove the fixative and then stored in ethanol 70%. Dehydrated by bathing them successively in a graded series of ethanol 80%, 90% each for one hour, then ethanol 100% for four hours and xylene was used to clear the tissue for 20 minutes. The tissue fragments were placed in melted paraffin in an oven at 58 °C for two hours. Transferred to clean paraffin and appropriate blocks were used, rotary microtome was used for sectioning the specimens at 6-7 micrometers thick. The sections were floated on water bath (40 – 45 °C), then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

The sections were stained with Hematoxylin - Eosin stain according to (Pantin, 1946) then mounted with Canada balsam, examined by compound microscope and photographed by digital camera.

III. Results and discussion:

The current study revealed that the *Tetrameres* sp. were embedded in gastric glands of proventriculus of seven aquatic birds: *Ardea ralloides*, *Egretta garazeta*, *Nycticorax nycticorax*, *Larus genei*, *Bubulicus ibis*, *Ardea purpurea* and *Anas clypeata* with prevalence 12.57% and mean of intensity 3.00, Table 1.

Nematodes	Birds				Worm No.	Mean
	Name	Examined	Infected	%		
1 <i>Tetrameres</i> sp.	<i>A. ralloides</i>	121	18	14.88	47	2.61
	<i>E. garzeta</i>	85	14	16.47	29	2.07
	<i>N. nycticorax</i>	56	3	3.36	18	6.00
	<i>H. himantopus</i>	46	5	10.87	32	6.40
	<i>L. genei</i>	30	1	3.33	1	1.00
	<i>B. ibis</i>	10	1	10.00	1	1.00
	<i>A. purpurea</i>	5	1	20.00	1	1.00
	<i>A. clypeata</i>	5	2	40.00	6	3.00
	Total	358	45	12.57	135	3.00
2 <i>M. spiralis</i>	<i>B. ibis</i>	10	1	10.00	25	25.00
	Total	10	1	10.00	25	25.00

Only female nematodes of *Tetrameres* sp. were recovered from infected birds in current study. The identification was limited to the genus level because the

identification to the species depend on some measurements of male structures.

The first record of *Tetrameres* sp. in Iraq was by (Abdullah, 1988) from *Porphyrio poliocephalus*, *Fulica atra* and *Gallinula chloropus* in Basrah. It was also isolated from *P. ruficollis*, *Anas strepera* and *Anas querquedula* in Basrah by (Al-Mayah, 1990; Awad et al., 1994) isolated *Tetrameres* sp. from the proventriculus of *E. garazeta* and *Phalacrocorax pygmaeus*.

Mizhir in (2002) reported *Tetrameres* sp. from *Aythya ferina* from Bahr Al-Najaf depression. (Al-Awadi et al., 2010) isolated *Tetrameres* sp. from the proventriculus of *F. atra* with an incidence of 18.5% and an intensity of 1.1.

Al-Tameemi (2013) isolated *Tetrameres* sp. from proventricular glands of five aquatic bird species: *L. genei*, *H. himantopus*, *Chettusia leucura*, *Hoplopterus indicus*, *A. ralloides* as new final host in Iraq and also from proventriculus glands of *E. garazeta*, *A. purpurea*, *A. cerca*, *G. chloropus*, *F. atra* in Basrah province.

The high prevalence 40.00% occurred in *A. clypeata* and the high mean of intensity 6.40 in *H. himantopus*, while the low prevalence 3.33% was occurred in *L. genei* and the low mean of intensities were recorded in three aquatic birds *L. genei*, *B. ibis* and *A. purpurea*, while the *Microtetrameres spiralis* embedded in gastric glands of proventriculus of *B. ibis* with prevalence 10% and mean of intensity 25.00.

The *Tetrameres* genus, characterized by different prevalence and intensity of *Results and Discussion* (2004).

The incidence of *Tetrameres* sp. was differed by season, the infections ranged from 89% in winter to 100% in spring and fall (Robel et al., 2003). *Tetrameres americana* infected 56.67% of the chickens of a rural district in Zimbabwe (Percy et al., 2012). Table 2. showed that the prevalence of *Tetrameres* sp. was 13.64% of females more than prevalence of males 11.54%, while the mean of intensity 3.33 of males more than mean of intensity (2.7) of females

Nematodes	Birds	♂					♀				
		Ex.	In.	%	Nem. No.	M.	Ex.	In.	%	Nem. No.	M.
1 <i>Tetrameres</i> sp.	<i>A. ralloides</i>	68	10	14.71	24	2.40	53	8	15.09	23	2.88
	<i>E. garzeta</i>	39	4	10.26	8	4.00	46	10	21.74	21	2.1
	<i>N. nycticorax</i>	31	2	6.45	16	8.00	25	1	4.00	2	2.00
	<i>H. himantopus</i>	19	2	10.53	17	8.50	27	3	12.00	15	5.00
	<i>L. genei</i>	12	-	-	-	-	18	1	5.56	1	1.00
	<i>B. ibis</i>	7	1	14.29	1	1.00	3	-	-	-	-
	<i>A. purpurea</i>	3	1	33.33	1	1.00	2	-	-	-	-
	<i>A. clypeata</i>	3	1	33.33	3	3.00	2	1	50.00	3	3.00
	Total	182	21	11.54	70	3.33	176	24	13.64	65	2.71
2 <i>M. spiralis</i>	<i>B. ibis</i>	7	1	14.29	25	25.00	3	-	-	-	-
	Total	7	1	14.29	25	25.00	3	-	-	-	-

Abdullah (1988) noted that the male and female of *Porphyrio poliocephalus* were infected with *Tetrameres* sp. with out any significant differences because both sexes of the birds were consume the same mount and quality of foods.

Tetrameres sp. infected lesser prairie-chickens *Tympanuchus pallidicinctus* in southwestern Kansas with

prevalence 92.00% and the male prevalence 100.00% more than female 70.00% (Robel et al., 2003).

The prevalence of duck females were more than males because the females consume in their food large amounts of invertebrates (Thul et al., 1985).

Histopathological examination showed that the *Tetrameres* sp. embedded in the lumen of gastric glands of the proventriculus of *A. ralloides*, *E. garazeta*, *N. nycticorax*, *H. himantopus*, *A. purpurea* and *A. clypeata*, causing severe damage and dilation on the gland lumen, Photo. (1, 2, 3, 4, 5, 6).

Embryonated eggs were noted in the uterus of *Tetrameres* sp. section with different stages of developments, Photo. (7, 8).

Kamil et al., (2011) showed that the *Tetrameres* infection in the proventriculus of the ducks caused dilation of the gland lumen, atrophy, degeneration and desquamation of the glandular cells, they also observed embryonated eggs of the nematode inside glandular lumen. *Tetrameres* sp. caused atrophy and degeneration of lining epithelial tissues of proventriculus of *H. himantopus*, *purpurea*, *A. ralloides*, *E. garazeta*, , Photo.(9,10) in compared with control, Photo. (11,12). Glandular atrophy and inflammation changes in proventriculus were induced by *Tetrameres* infection. Under stressful condition this nematode may caused mortality in poultry (Fakae and Paul-Abiade, 2003; Biu and Haddabi, 2005)

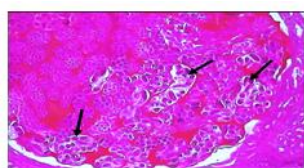


Photo. 7. C. S. through the uterus of *Tetrameres* sp. infected *H. himantopus*. Note embryonated eggs (→). 100X.

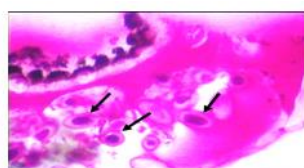


Photo. 8. C. S. through the uterus of the *Tetrameres* sp. infected *A. purpurea*. Note the embryonated eggs (→). 400X.

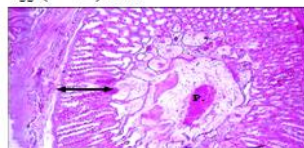


Photo. 9. C. S. of the gizzard of the *A. ralloides*. Note the atrophy (↔) of the glandular epithelial cells adjacent to the *Tetrameres* sp. 100X.

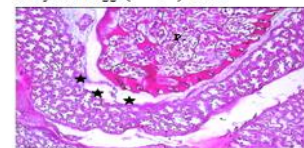


Photo. 10. C. S. of the gizzard of the *E. garazeta*. Note the atrophy (★). 100X.

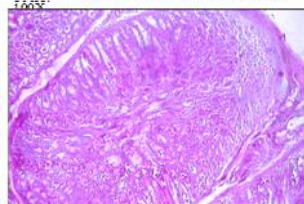


Photo. 11. C. S. Normal gastric gland of gizzard in *E. garazeta*. 100X.

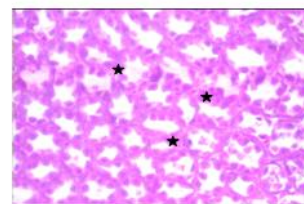


Photo. 12. C. S. Normal gastric epithelial tissue (★) of the gizzard in *E. garazeta*. 400X.

Necrosis and desquamation of the adjacent epithelial glandular cells caused by *Tetrameres* sp. growth were noted in the proventriculus glands of *A. ralloides*, *N. nycticorax*, *E. garazeta*, *A. clypeata*. Photo. (13,14).

(LaPage, 1956) reported greatest damage on the proventricular wall of avian host caused by *Tetrameres* juveniles migration which caused marked irritation and inflammation.

Tetrameres sp. caused pressure effect on the lining epithelial tissues of proventriculus glands of *A. clypeata*, Photo. (15). The *Tetrameres* infection in chickens produced vast structural and functional changes lead to organ

dysfunction and glandular necrosis which caused the sudden massive death on the infected birds (Kamani et al., 2008).

Abdullah and Al-Hadithi(1992) noted that the pathological effects of *Tetrameres* sp. in *Porphyrio poliocephalus* proventriculus glands were due to the large size of female and their pressure effect, and they recorded dilation, thickness in the infection area with swelling in the mucus layer and atrophy in the glandular cells.

T. americana has been reported both from wild (Ewing, 1967) and laboratory-raised pigeons (Flatt and Nelson, 1969) where heavy infections resulted in diarrhea, emaciation, and possibly death.

Tsvetaeva(1960) reported that the *T. fissispina* which commonly a parasite of wild or domestic ducks and geese caused considerable tissue reaction occurs like degeneration of the glandular tissue, edema, and extensive leucocyte infiltration.

In Brazil *Tetrameres confusa* was reported from the lumen of the proventriculus of the blue and gold macaw *Ara ararauna*. *Fistulae nodules* was observed in the proventriculus wall (Silva et al., 2005). *Microtetrameres spiralis*, Photo. (16). caused huge damage in the gastric glands of *B. ibis*, embryonated eggs were noted in the uterus section of the worms, Photo. (17,18).



Photo. 13. C. S. of the gastric gland of the gizzard of the *E. garazeta*. Note the necrosis (★) of the superficial cells adjacent to the *Tetrameres* sp. 400X.

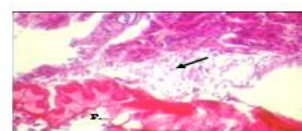


Photo. 14. Higher magnification showing necrosis (→) in the lining epithelial of gastric gland of *A. clypeata* caused by *Tetrameres* sp. 400X.

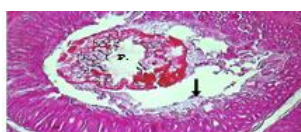


Photo. 15. *Tetrameres* sp. occupied the lumen of the gastric gland with pressure effect (→) in lining epithelial tissue of *A. clypeata*.

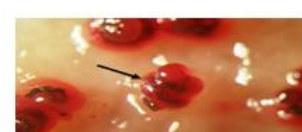


Photo. 16. *Microtetrameres spiralis* (→) attached to the gizzard of *Subulterus ibis*.



Photo. 17. T. S. through the gastric gland of the *B. ibis*. Note the huge damage to the gastric glands caused by *Microtetrameres spiralis*. 400X.

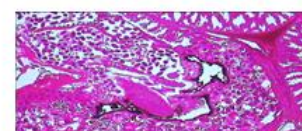


Photo. 18. T. S. in the gastric gland of the *B. ibis*. Note the uterus of *Microtetrameres spiralis* filled with embryonic eggs. 400X.

Microtetrameres centuri caused mechanical effect on host tissue which lead to the cystic dilation on the gland lobule and mild pressure atrophy on epithelial tissue of infected gland (Ellis, 1970).

Microtetrameres nestoris found in the ducts of the proventricular glands of a parrot *Nestor meridionalis* causes destruction of secretory cells and thickening and some necrosis of the epithelium (Clark et al., 1979).

IV. Conclusion:

The two nematodes *Tetrameres* sp., and *Microtetrameres spiralis* caused mechanical and histopathological effects in the tissues of infected birds differ according to number of the parasites and species of the bird, the sever cases could lead to bird death.

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