DIAGNOSIS OF *MYXOBOLUS BRAMAE* (MYXOSPOREA: MYXOBOLIDAE) IN THE KIDNEYS TISSUE OF *CARASOBARBUS LUTEUS* AND HISTOPATHOLOGICAL CHANGES ASSOCIATED WITH INFECTION

N. T. Mansoor [1] **I. B. Falih [2]** **F. S. Al-Nasiri [3]**
Researcher **Assist. Prof.** **Prof.**
[1] Animal and Fish Res. Center, Agric. Res. Direc. Min. Sci. and Techn. Iraq.
[2] Dept. Patho. Coll. Veter. Med. University of Baghdad, Iraq.
[3] Dept. Biol. Coll. Sci. University of Tikrit, Iraq.
fshnasiri@yahoo.com. e.mail: nahlataleb1999@yahoo.com

ABSTRACT

This study was conducted during the period from March till the end of October 2018, to study the histopathological changes of *Myxobolus bramae* in kidney tissue of *Carasobarbus luteus* caught from Tigris River passing through Baghdad city. During the period of this study, a total of 60 fishes belonging to *Carasobarbus luteus* species from the family Cyprinidae were collected. The prevalence of infection with these protozoa was determinate (5.00%). Histopathological study due to *M. bramae* in the kidney tissue of *C. luteus* was done by using three types of stain: Hemotoxylin and eosin, giemsa and acid fast stain to observe plasmodia cyst and structures of spores. These changes characterized by tubular degeneration, necrosis, hyalinization of glomerular tuft, mild distension of Bowman's space with reduction in haemopoitic tissue together with inflammatory response, and accumulation of melanomacrophages at the site of infection. The results of this study revealed that *Carasobarbus luteus* from Tigris River at Baghdad city, it infected with *Myxobolus bramae* and this parasite cause severe histopathological changes in the infected kidneys tissue.

Key words: myxosbolidae, cyprinidae, Bowman's space, tigris river.

مجلة العلوم الزراعية العراقية -2020 :51 (3):874-884 منصور وآخرون

تشخيص البوغيات المخاطية *Myxobolus bramae* (Myxosporea: Myxobolidae) في نسيج كلية الحمري والتغيرات النسيجية المرضية المرافقة للإصابة

نهلة طالب منصور [1] انعام بدر فالح [2] فاطمة شهاب الناصري [3]
باحث أستاذ مساعد أستاذ
[1] مركز بحوث الثروه الحيوانية والسمكية، دائرة البحوث الزراعية، وزارة العلوم والتكنولوجيا، العراق
[2] فرع الأمراض، كلية الطب البيطري، جامعة بغداد، العراق [3] قسم علوم الحياة، كلية العلوم،جامعة تكريت، العراق

المستخلص

أجريت هذة الدراسة خلال المدة من شهر اذار الى نهاية شهر تشرين الأول 2018، لدراسة التغيرات المرضية النسجية التي تسببها أبواغ ال ***Myxobolus bramae*** في أسماك الحمري المصطادة من نهر دجلة خلال مروره بمدينة بغداد. جمعت 60 سمكة حمري من عائلة الشبوطيات خلال مدة الدراسة. حددت النسبة المئوية للأصابة (5.00%) . شملت الدراسة المرضية النسجية دراسة التغيرات المرضية النسيجية في نسيج كلية أسماك الحمري المصابة بالبوغ ***M. bramae*** وتضمنت إستعمال ثلاثة أنواع من الصبغات وهي : Hemotoxylin and eosin, giemsa and acid fast stain الملاحظة كيس البلازموديا وتركيب الابواغ وهذه التغيرا ت تميزت بالتنكس والتنخر، تزجج اللمه الكبيبية، توسع بسيط في حيز بومان، إختزال النسيج المكون للدم مع حدوث إستجابة التهابية مصحوبة بتجمع البلاعم الحاملة للميلانين في مكان الإصابة. أثبت نتائج الدراسة إصابة أسماك الحمري من نهر دجلة عند مدينة بغداد بالطفيلي ***Myxobolus bramae*** ويسبب هذا الطفيلي تغيرات مرضية شديدة في نسيج الكلية المصابة.

كلمات مفتاحية: عائلة الشبوطيات، علم التشريح المرضي، أسماك الحمري، حيز بومان.

*Received:12/7/2019, Accepted:2/10/2019

INTRODUCTION

Fishes are considered as a basis of protein, fat, phosphate, iron, calcium, amino acids and vitamins which are soluble in water and soluble in fat (41). So that, fishes are consider as a drug against many diseases for example Psoriasis that is treatment by fish oil (omega-3) as well as treatment of heart diseases. Therefore, fish meats are necessary for human health (39). All living organisms can in certain circumstances become subject to disease and fishes make no exception (13). All types of diseases are of common occurrence in fishes like bacterial, viral, fungal, and parasitic diseases (37). Relatively often mixed infections (bacterial and parasitic diseases) may occur in the aquarium (6, 21). Generally, fishes are found a large number of important commercial in natural and cultured environment, and are exposed to several invasions (23). Fish parasites divided into external and internal parasites (5). Phylum myxozoa are protozoan internal parasites, and are very common in freshwater and marine fishes, divided into two class including: class Myxosporea which parasitize of vertebrates particularly fishes, often toxic imports for their host (8), and class Actinosporea which parasitize of invertebrates specially Oligochaetes and Polychaetes (27). Some of fish parasites cause deformation in the skeleton like whirling disease cause by *Myxosoma cerebralis*, which cause loss of balance and death of infected fish (20). Myxosporidia infections can cause all categories of regressive and progressive pathological changes in the host, including atrophy, dystrophy, hypertrophy, hyperplasia, necrosis, infiltration Inflammatory leukocyte cells (white blood cells). These histopathological changes mainly depended on type, phase (adult or larval phase), and number of parasites and type of infect tissue (10, 1, 29). Nevertheless, most *Myxobolus* species cause tissue damage. Relatively, few species are now to cause serious or fatal infections. Phagocytosis by macrophages of small trophozoites or of mature spores released into tissue spaces is important for host control of Myxosporidia infections (16). According to above data, the current study was done for focusing on the following objectives.

1- Estimate histopathological changes that occur due to *Myxobolus bramae* in the kidneys tissue of infected *Carasobarbus luteus* fishes from Tigris River

2- Using of original types stains in the histological sections (Giemsa, and Acid fast stain) to determine the efficiency of these stains for the study of histopathological changes and appearance of parasite inside the infected kidneys tissue.

MATERIALS AND METHODS

During the present study a total 60 fish sample *Carassbarbus luteus* were collected from Tigris River at Baghdad city, during the period from March to the end of October 2018. The scientific names of fish samples were determined (15, 19). These fishes were belonged to family Cyprinidae. Fishes were examined as soon as possible after killing them by pithing method or by a beating to the head. Total and stander length were taken and fishes were weighted. The range and mean of total length (15-35) 23.45 cm. Also, the range and mean of weight (100-300) 200.83 gm. of all fish species were taken. Fish samples were dissected according to the method of (6, 22). *Myxobolus* species were identification according to the following references (11, 38). The parasites were photographed by compound microscope with digital camera. The *C. luteus* were subjected to complete parasitological examination that included studying *M. bramae* infection in the kidney tissue. The prevalence of infected fish with *M. bramae* was calculated as demonstrated by (30) as following:

Prevalence (%) =

$$\frac{\textbf{Number of infected host}}{\textbf{Total number of host examined}} \times \mathbf{100}$$

For histological examination, a piece of infected organ (kidney tissue) from infected fishes were taken and fixed in 10% formalin solution for 24-28 hours to prepare of histological sections. Samples of infected kidney were embedded in the wax blocks, which were sectioned by the Rotary microtome with a thickness of 4-6 µm and the sections were stained with haematoxylin and eosin according to (6), Giemsa stain according to (7) and acid fast stain (18). The histological study was done according to (9, 14, 36). The

histological sections were done in the department of pathology, college of veterinary medicine, university of Baghdad. The slides of histological study were photographed by a compound microscope with Digital camera.

RESULTS AND DISCUSSION

The results of the internal examination of these fishes demonstrated that 3 fishes (out of 60 examined fishes were infected with *Myxobolus bramae*) Figure 1. Therefore, the overall prevalence of infection was 5.00%.

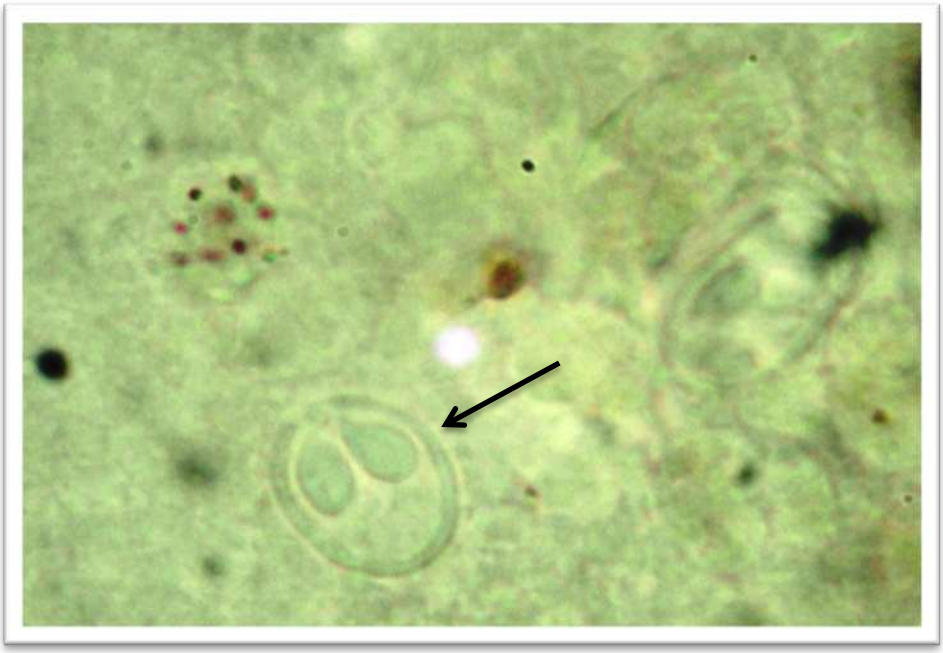

Figure 1. Spore of *M. bramae* (arrow) in the kidneys of *C. luteus* (100 x).

The histological analysis of kidney tissues from *C. luteus* infected with *M. bramae* showed severe tubular epithelial necrosis was recorded in majority of kidney sections that containing sloughed tubular epithelium in their lumen Figure 2. Also, hyalinization of glomerular tuft with mild distension of Bowman's space noticed in Figure 3. Also, renal manifestation showed mild capsulation of some necrotic tubules with fibrous zone accompanied with great reduction in adjacent hemopoitic tissue Figure 4. Also, the renal observation showed cystic distention of most collecting tubules with necrotic debris Figure 5. Another samples of histopathological finding of kidneys section from *C. luteus* that infected with *M. bramae* showed number of mature spores seen with hemopoitic tissue with prominent two polar capsules together with prominence of melanomacrophages adjacent to intact glomeruli Figure 6. Also, various structural forms of spores were identified in kidney tissue, some of them appeared mature spherical with prominent two polar capsules that stain dark blue seen in the interstitial tissue with number of melanomacrophages Figure 7. The histopathological finding of kidneys tissue from *C. luteus* infected with *M. bramae* showed both mature and immature spores visible together in renal tissue with minimal inflammatory response Figure 8, while other mature spores observed within lumen of degenerated tubules with scatter of melanomacrophages Figure 9 while large accumulation of melanomacrophages seen within immature unstained spores. As well as evidence of large plasmodia stage filled with immature spore and polar filaments noticed in Figure 10 as well as several of immature spores seen with minimal response. While other section showed large plasmodia cyst with number of mature spores stained positive with acid fast stain Figure 11.

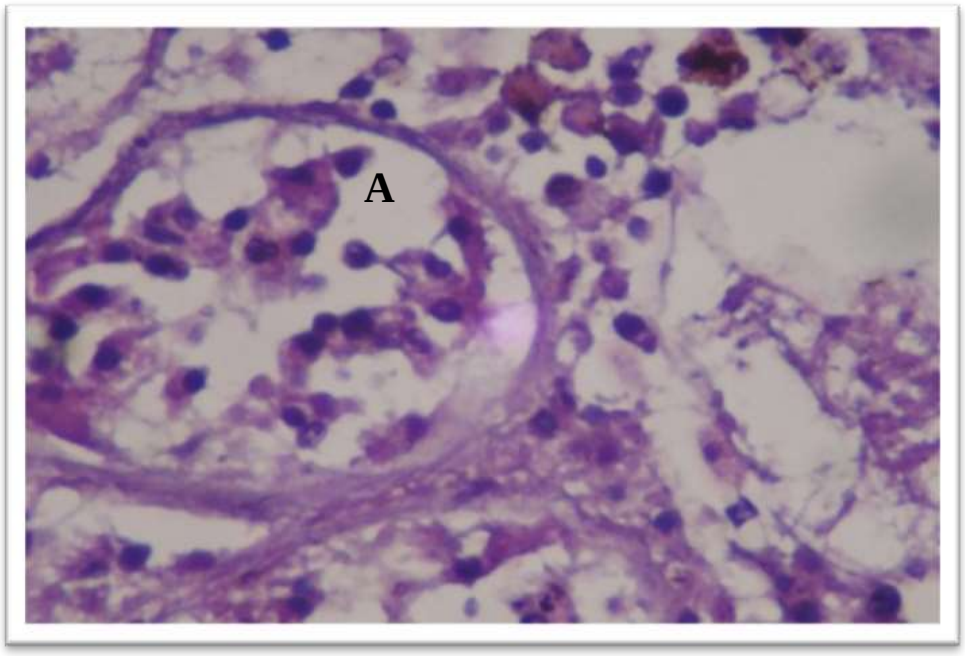

Figure 2. Histopathological section in the kidney tissue from *C. luteus* infected with *M. bramae* showed severe tubular epithelial necrosis that containing sloughed tubular epithelium in theirlumen (A) (H& E x40).

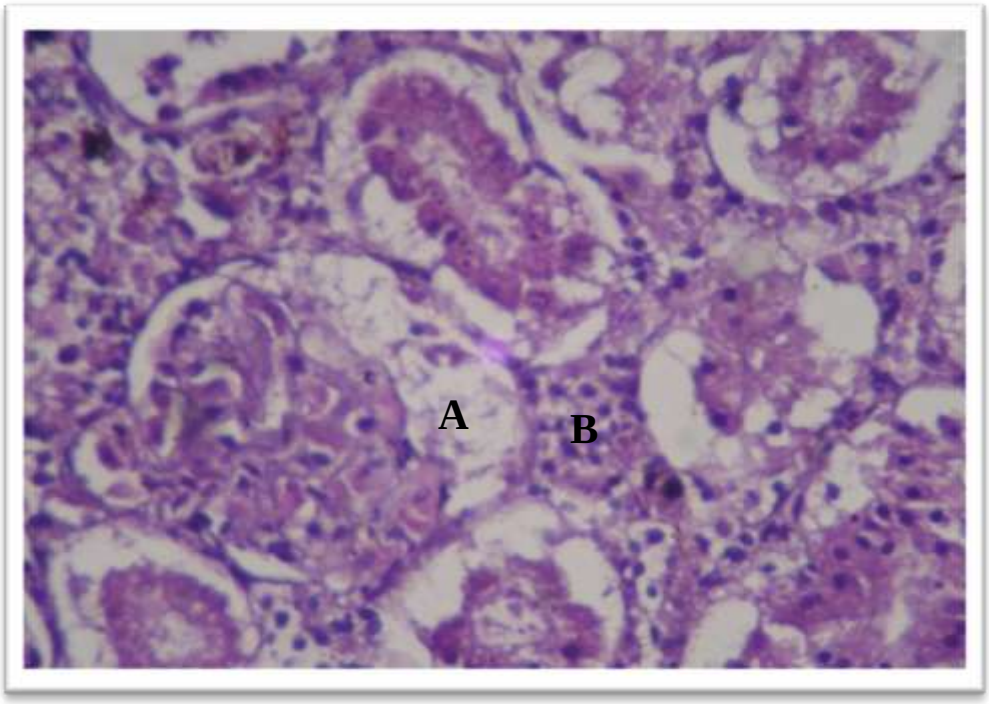

Figure 3. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed hyalinization of glomerulartuft, mild distension of Bowman's space (A) with reduction in haemopoitic tissue (B) (H&E stain 40x).

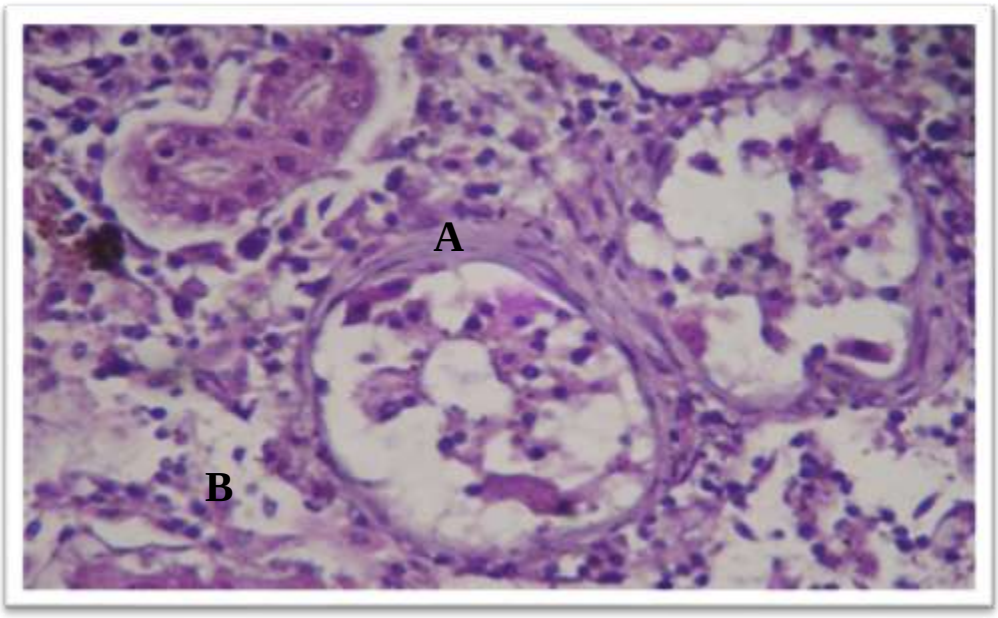

Figure 4. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed mild capsulation of some necrotic tubules with fibrous zone (A) with great reduction of hemopoitic tissue (B) (H&E stain 40x).

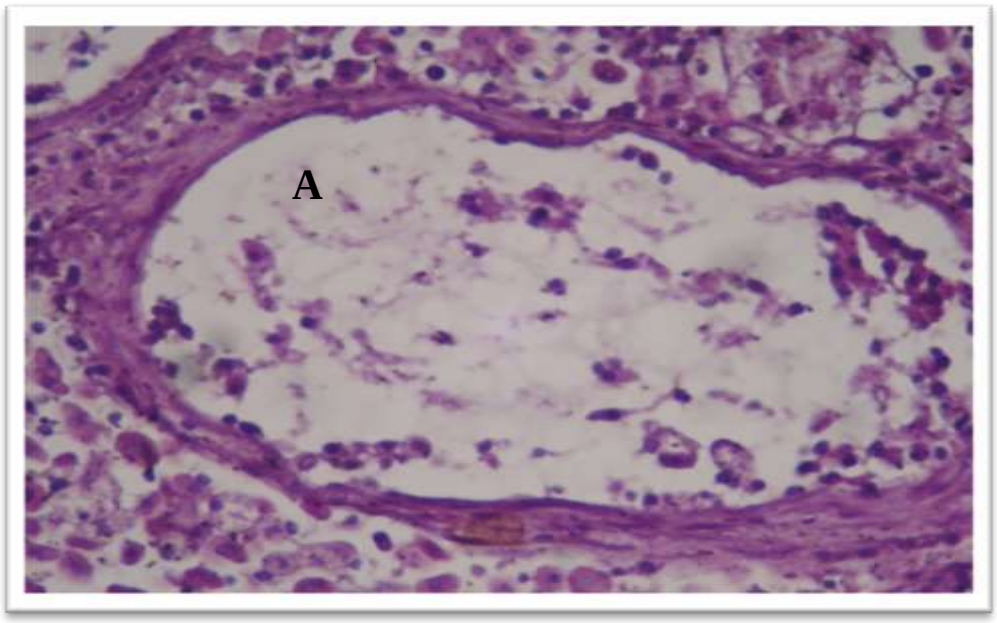

Figure 5. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed great cystic distention of collecting tubules with necrotic debris (A) (H&E stain 40x).

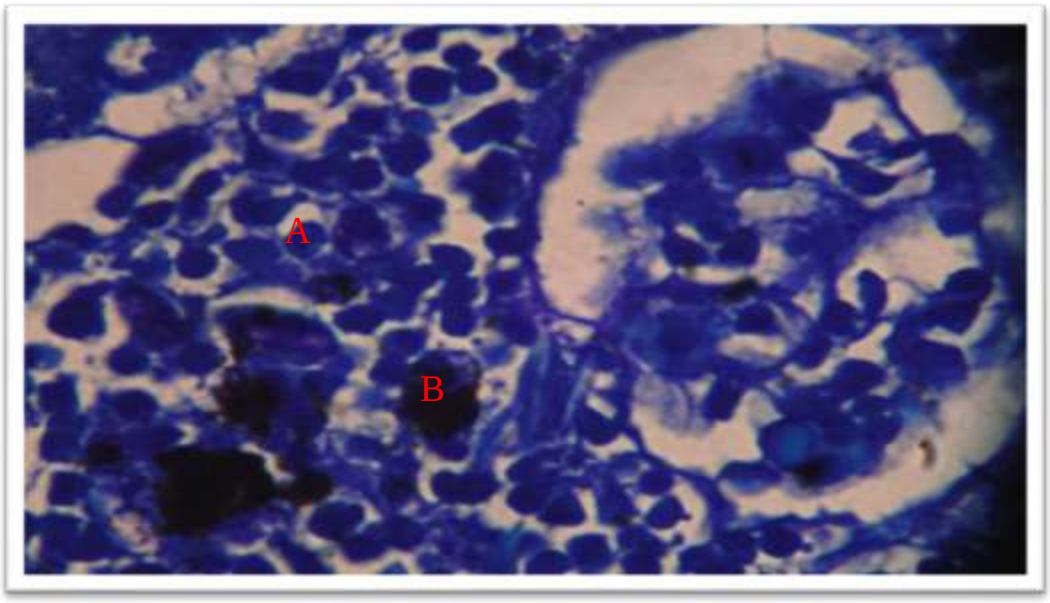

Figure 6. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed number of mature spores (A) with hemopoitic tissue and melanomacrophages (B) adjacent to intact glomeruli (Giemsa stain 100x)

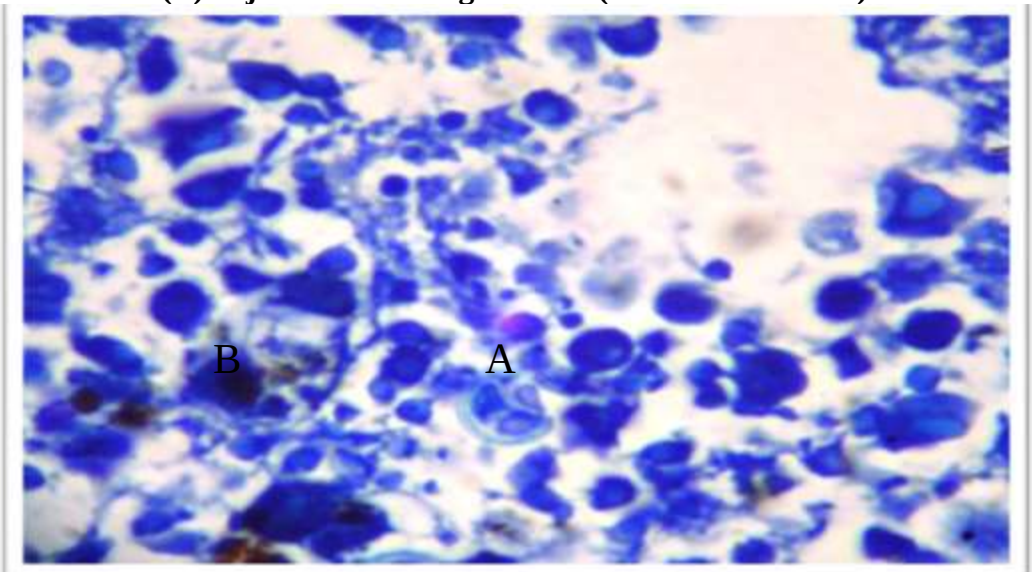

Figure 7. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed mature spherical spores with two polar capsules that stain dark blue (A) seen in the intestinal tissue with number of melanomacromhages (B) (Giemsa stain 100x).

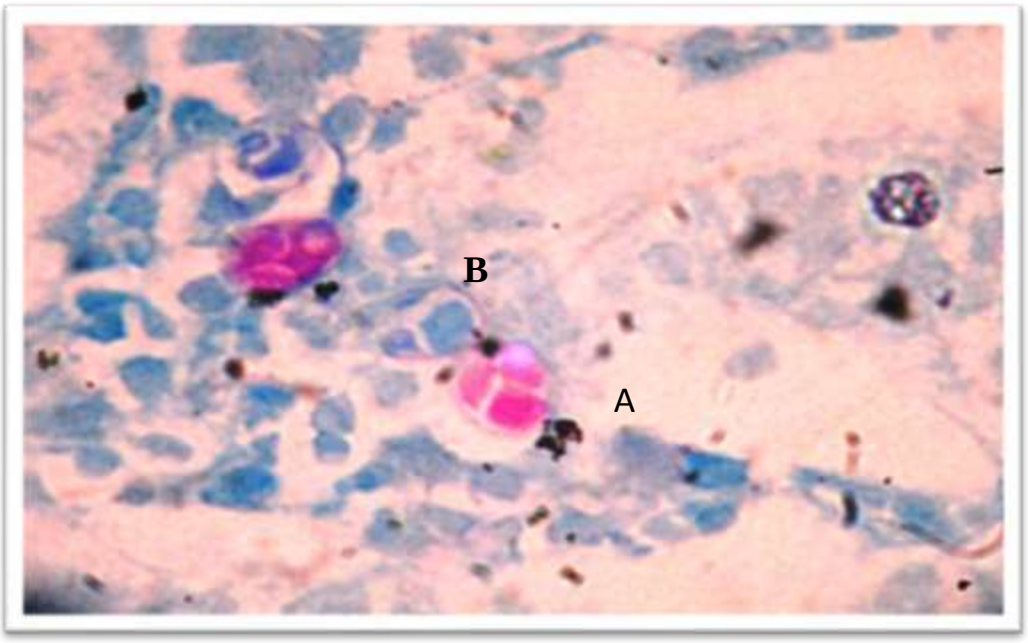

Figure 8. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed both mature (A) and immature spores (B) together in renal tissue and minimal inflammatory response (Acid fast stain 100x).

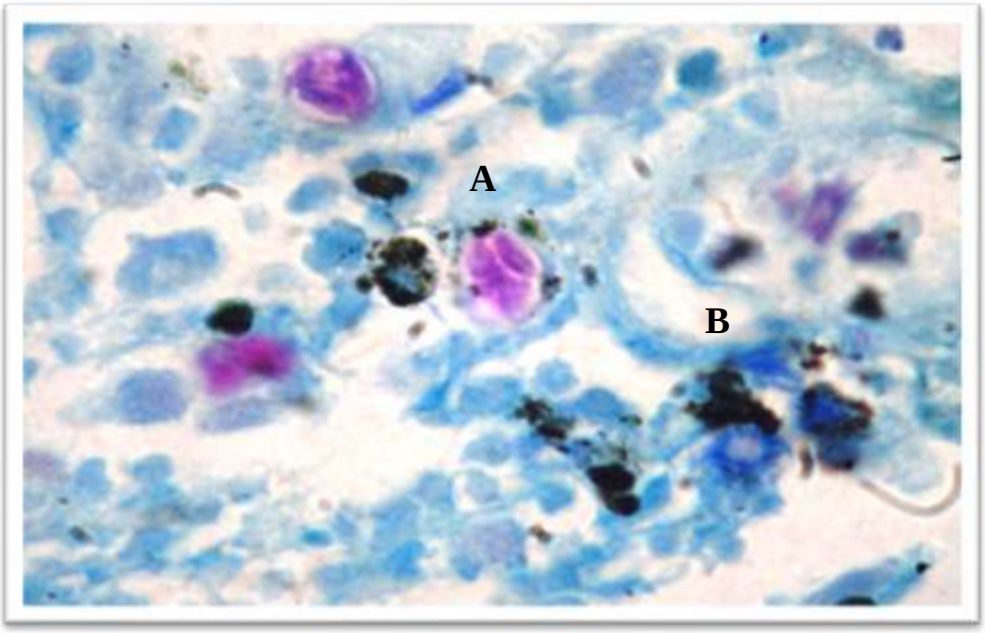

Figure 9. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed mature spore observed within lumen of degenerated tubules (A) and large accumulation of melanomacrophages (B) within immature unstained spores (Acid fast stain x100).

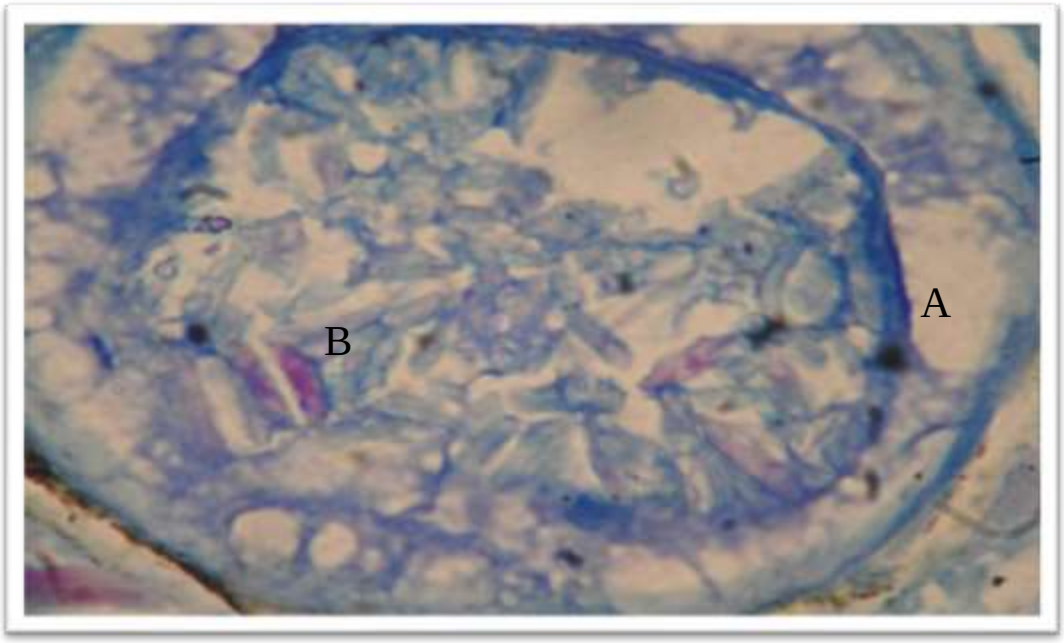

Figure 10. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed large plasmodia stage (A) filled with immature spore and polar filaments (B) (Acid fast stain 100x).

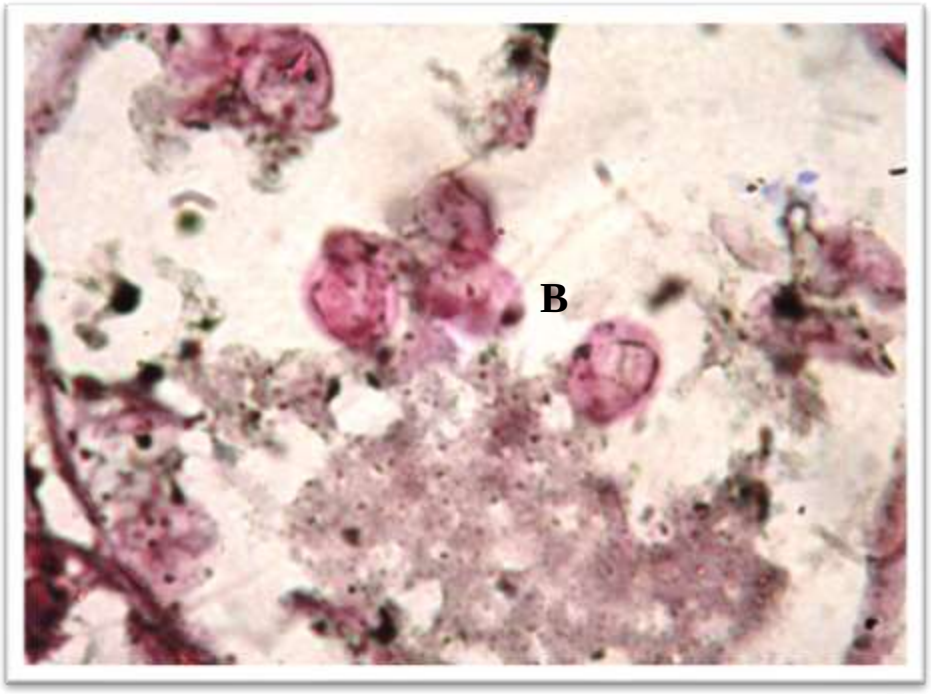

Figure 11. Histopathological section in the kidneys tissue from *C. luteus* infected with *M. bramae* showed large plasmodia cyst (A) with number of mature spores stained positive with acid fast stain (B) (Acid fast stain 100x).

There were more than 1350 species of myxosoridia that parasitized on fishes, but few of them can cause serious affected lesions due to both of partners (host and parasite can live with other with less severity (26). Major of recent studies referred that risk of myxosporidia infection may be relatively mild when the host exhibits immune response through continuous infections. Other researcher found that myxosporidia parasites have the ability to escape the host immune response via host antigenic variation and masking resulting in accepting balance between the host and parasite for prolong commensalism (34). The Myxosporidia parasites cause obvious clinical signs and

severe pathological changes of infected fishes. The main clinical presentation of present investigated infected fish mainly with *Myxobolus* species revealed fishes become pale and weak, rest often near the surface of the water, with difficult problems in movements and the fishes die from complete collapse (6). Myxosporidia parasites histozoic (in tissue) or coelozoic (in internal cavity), histozoic infection characterized by whitish or brown cysts with milky substance containing spores that are found on the external surface of internal organs. Coelozoic infection of the urinary and bile cavity has small plasmodium and produces few spores, frequently only two spores (32).These similar observation with (1, 22, 31, 40). The results of histopathological changes by *M. bramae* in the present study revealed the occurrence of severe vacuolar changes with renal tubular degeneration and necrosis of infected fishes and accumulation of melano-macrophages especially in the hemopoitic tissues, together with inflammatory cells infiltration, typically melano-macrophages due to immune response of infected tissue in the site of parasite attachment (28, 33). Also, myxosporidia parasites cause mechanical damage occurring from extruded of polar filaments and attached to host tissue. These changes are agreeable with those of (24) in consistence with present study. Moderate inflammatory response associated with melanomacrohpage cells infiltration mainly in renal tissue. These inflammatory due to various forms of *Myxobolus* development, including mature and immature spores together with evidence of plasmodial cyst that invaded several viscera. Active proportion of these spores accumulating in the macrophage centers of the kidney apparently represents damaged and deformed specimens have agreement with (3, 28, 35). Phagocytosis play vital role in pathogenesis of Myxosporidia especially *Myxobolus* species when trophozoites of myxosporidia attach myocytes and *Myxobolus cerebralis* attach chondrocytes finally phagocytized the whole tissue causing tissue necrosis. Also, evidence of erythrophogocytosis reported by other Myxosporidia species. Other species like *Sphaerospora renicola* that phagocytic of erythrocytes during infection of renal parenchyma of common carp with possible intense bloody effects (12). Mature parasite induce tissue disruption when localized in an aberrant site that lead to tissue damage also these myxosporidia may invade and attack other unsuitable host tissue and induced various host immune response. The pathogenicity of Myxosporeans depends on several factors including types, development stage of life cycle parasite together with number of Myxosporidia and host immune response. Many researcher suggest that these Myxosporidia competed the host nutrition by active transport (via osmotrophy) of trophozoites which consider the active stage while localized plasmodium supplied by high specialized cellular membrane that facilitate it is nutrition (pinocytosis). This explains why food vacuoles are not found within their cytoplasm (40). Several previous observations mentioned that both of *Myxobolus* species and *Henneguya* species that have the ability to be encapsulated with cell membrane that surrounded around plasmodia and protected them from environment effects with evidence of localized effect. The capsule arises from accumulated of host's connective tissue or from stressed cells from adjacent tissue. Also, capsule prevents lesion dissemination or limiting diffusing of infection. In addition, manifestation of plasmodia encapsulation mainly in *M. musculi* has been recorded in renal tissue and these data in correlation with several previous observations (35). The majority of these myxosporeans have ability to invade many visceral tissues and induced several tissue adaptation such as atrophy, hyperplasia, hypertrophy, necrosis, inflammation (usually proliferation) and tissue damage (17), together with dystrophic calcification, hyalinazation and mineralization of affected organs, while other like *Myxobolus cerebralis* and *M. sandrae* cause spinal cords distortion with neuron distortion in Salmon fishes and *Perca fluviatitis* (27). Also, the current study showed hyalinization especially with *M. bramae* that infected renal tissue of *C. luteus* this finding have in agreement with previous manifestation by (27). The granulation tissue appears due to plasmodia stage surrounded by encapsulation of

connective tissue from host tissue itself, these findings agreeable to (2, 4, 25).

REFERENCES

1. Abbas, A.A.-K. 2007. Histopathological Studies of some Parasites of the Asian Catfish, *Silurus triostegus* (Heckel, 1843) and Potassium Permanganate on the Black Molly, *Poecilia sphenops* (Valenciennes, 1846). Ph. D. Dissertation, Coll. Educ., Univ. Basrah. pp: 144

2. Abdel-Baki, A.A.S.; H.M. Abdel-Haleem,; T. Sakran,; E. Zayed,; K.E. Ibrahim and S. Al-Qurashy, 2015. Two *Myxobolus* spp. infecting the kidneys of Nile tilapia (*Oreochromis niloticus*) in the River Nile at Beni-Suef governorate, Egypt, and the associated renal changes. Parasitol. Res. 114: 1107-1112.

3. Adriano, E.A.; S. Arana,; M.M. Carriero,; J. Naldoni,; P.S. Ceccarelli. and A.A. Maia, 2009. Light, electron microscopy and histopatholgy of *Myxobolus salminus* n. sp., a parasite of *Salminus brasiliensis* from the Brazilian Pantanal. Vet. Parasitol. 165: 25-29.

4. Al-Dosary, S.H. 1999. A Study on Some Protozoan Parasites of Six Species of Freshwater Fishes of Qarmat Ali River, Basrah. M. Sc. Thesis, Coll. Agric., Univ. Basrah: pp: 61.

5. Ali, A.H. 2019. First record of endoparasite *Colobomatus asiaticus* Hayward, 1996 (Copepoda: Poecilostomatoida: Pilichthyidae) from *Sillago sihama* (Forsskål, 1775) from Iraqi marine waters. Iraqi J. Agric. Sci. 50 (2): 534-540

6. Amlacher, E. 1970. Textbook of Fish Diseases (Engl. Transl.). T.F.H. Publ., Jersey City. pp: 302

7. Arkush, K.D.; Jr.S. Frasca, and R.P. Hedrick, 1998. Pathology associated with the rosette agent, a systemic protest infecting salmonid fish. J. Aguat. Anim. Health, 10: 1-11

8. Bassey, S.E. 2011. A Concise Dictionary of Parasitology. 1st ed. Zetus Concepts, Port Harcourt. pp: 115

9. Bilqees, F.M. 1995. Histopathology of the liver of Hilsa (Ham.) Proc. Parasitol. 19: 1-20

10. Bruno, D.W.; B. Nowak, and D.G. Elliott, 2006. Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. Dis. Aquat. Org. 70: 1-36

11. Bykhovskaya-Pavlovskaya, I.E,; A.V. Gusev,; M.N. Dubinina,; N.A. Izyumova,; T.S. Smirnova,; I.L. Sokolovskaya,; G.A. Shtein,; S.S. Shul'man, and V.M. Epshtein, 1962. Key to Parasites of Freshwater Fish of the U.S.S.R. Akad. Nauk, S.S.S.R., Moscow. pp:727 (In Russian).

12. Csaba, G.; E. Kovac-Geyer,; L. Bekesi,; M. Bucsek, and K. Molnår, 1984. Studies into the possible aetiology of swimbladder inflammation in carp fly. J. Fish Dis. 7: 39-56

13. Duijn, Van C., Jnr. 1973. Diseases of Fishes, 3rd ed., Iliffe Books, London. pp: 372

14. Dykova, I. and J. Lom, 1978. Histopathological changes in the gills infected with myxosporidian parasites of the genus *Henneguya*. J. Fish Biol. 12: 197-202.

15. Eschmeyer, W.N. 2018. Species by family/ subfamily in the Catalog of Fishes. http://research.calacademy.org/research/ichthyology/Catalog/SpeciesByFamily.as P. (Updated 2 July 2018).

16. El-Matbouli, M.; T. Fischer-Scherl, and R.W. Hoffmann, 1992. Present knowledge on the life cycle, taxonomy, pathology, and therapy of some Myxosporea spp. important for freshwater fish. Anuu. Rev. Fish Dis. 3: 367-402.

17. Feist, S.W. 1997. Pathogenicity of renal myxosporeans of fish. Bull. Eur. Assoc. Fish Pathol. 17: 209-214.

18. Franzen, C. and A. Müller, 1999. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. clin. Microbial Rev. 12: 243-285.

19. Froese, R. and E. Pauly, 2014. FishBase. World Wide Web electronic publications, www.fishbase.org. (Accessed December).

20. Gilbert, M.A. and W.O. Granath, 2003. Whirling disease of salmonid fish: life cycle, biology, and disease. J. Parasitol. 89: 658-667.

21. Hammood, N.W. 2017. Investigation of Some Parasitic and Bacterial Infections in Some Fish Species of Tigris River at Baghdad City. M. Sc. Thesis, Coll., Sci. Univ. Tikrit. pp: 141

22. Hoffman, G. L. 1998. Parasites of North American Freshwater Fishes, 2nd ed. Cornell Univ. Press, London, pp: 539.

23. Kaur, H. 2014. Myxozoan infestation in freshwater fishes in Wetlands and aquaculture

in Punjab (India). Adv. Anim. Vet. Sci. 2(9): 488-502

24. Kaur, H. and R. Singh, 2010. A new myxosporean species *Myxobolus sclerii* sp. nov. and one known species *M. stomum* Ali *et al*. 2003 from two Indian major carp fishes. J. Parasit. Dis. 34(1): 33-39.

25. Khalifa, K.A. and G. Post, 1976. Histopathological effect of *Lernaea cyprinacea* (a copepod parasite) on fish. Prog. Fish Cult. 38: 110-113.

26. Lom, J. and I. Dykova, 1994. Studies on protozoan parasites of Australian fishes III. species of the genus *Myxobolus* Butschli, 1882. Eur. J. Protistol. 30: 431-439.

27. Lom, J. and I. Dykova, 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol. 53: 1-36

28. Manrique, W.G.; G.S. Claudiano,; T.R. Petrillo,; M.P. Casto,; M.P. Figueiredo,; M.A.A. Belo,; J.R.E. Moraes, and F.R. Moraes, 2014. Response of splenic melanomacrophage centers of *Oreochromes niloticus* (Linnaeus, 1758) to inflammatory stimuli by BCG and foreign bodies. J. Appl. Ictthyol. 30: 1001-1006

29. Manrique, W.G.; M.A.P. Figueiredo,; M.A.A. Belo,; M.L. Martins, and K. Molnår, 2017. *Myxobolus* sp. and *Henneguya s*p. (Cnidaria: Myxobolidae) natural co-infection in the kidney of *Piaractus mesopotamicus* (Characiformes: Serrasalmidae). Parasitol. Res. pp: 9

30. Margolis, L.; G.W. Esch,; J.C. Holmes,; A.M. Kuris, and G.A. Schad, 1982. The use of ecological terms in parasitology (report of an adhoc committee of the American society of parasitologists). J. Parasitol. 68(1): 131-133.

31. Mohammed, H.J. 2017. Parasitic Fauna of Some Fish Species from Diyala River in Diyala Province. M. Sc. Thesis, Coll. Educ. Pure Sci. (Ibn Al-Haitham), Univ. Baghdad, pp: 122.

32. Möller, H. and K. Anders, 1986. Diseases and Parasites of Marine Fishes. Möller, Kiel. pp: 365.

33. Molnår, K. 2000. *Myxobolus intrachondrealis* sp. n. (Myxosporea: Myxobolidae), a parasite of the gill cartilage of the common carp, *Cyprinus carpio*. Folia Parasitol. 47: 167-171

34. Molnår, K. and E. Kovåcs-Geyer, 1985. The pathogenicity and development within the fish host of *Myxobolus cyprini* Doflein, 1898, Parasitology 90: 549-555.

35. Molnår, K. and Cs. Szėkely, 2014. Tissue preference of some myxobolids (Myxozoa: Myxosporea) from the musculature of European freshwater fish. Dis. Aquat. Org. 107: 191-198

36. Molnár, K.; S. Marton, and Cs. Székely, 2010. Differentiation of *Myxobolus* spp. (Myxozoa: Myxobolidae) infecting roach (*Rutilus rutilus*) in Hungary. Parasitol Res. Org. pp: 14

37. Mustafa, S.A. 2019. Assessment of hydrogen peroxide on histopathology and survival rate in common carp *Cyprinus carpio* L. infected with saprolegniasis. Iraqi J. Agric. Sci. 50(2): 697-704.

38. Shul'man, S.S. 1988. Myxosporidia of the U.S.S.R. Nauka, Moscow (English Translation). Amerind Publ., New Delhi: pp: 632

39. Sofia 2004. World review of fisheries and aquaculture. Part (1).

40. Stoskopf, M.K. 1993. Fish Medicine. W.B. Saunders Co., Philadelphia. pp: 882.

41. Stanković, M.B.; Z.P. Dulić, and Z.Z. Marković, 2011. Protein sources and their significance in carp (*Cyprinus carpio* L.) Nutrition J. of Agri. Sci. 56 (1): 75-86.