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THE DISCOVERY OF MYXOBOLUS SP. IN THE TESTIS OF THE EGYPTIAN TOAD BUFO REGULARIS, WITH DETAILED DESCRIPTION OF THE CYST AND SPORES.

(With 7 Fig.)

By

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(Received at 21/7/1993)

أكتشاف طفيل ميكزوبولس / ميكزوسبوريديا) لأول مره في خصية الضفدعه المصريه بوفو رجيولارس مع دراسة تفصيليه للأكياس والجراثيم

جمال عابد ، أحمد جلال

تم وصف هذا النوع من طفيليات الميكزوسبوريديا من الحويصلات التى جمعت من الخصيه للضفدعه المصريه بوفو رجيولارس هذا وقد وصفت جراثيم هذا النوع وصفاً تفصيلياً شملت دراسة هستولوجيه وهستوكيميائيه وقورنت بالانواع الأخرى القريبه لها من نفس الجنس. وقد بينت دراسة كيمياء الانسجه على وجود كميات كبيره من الجليكوجين والبروتين فى الخلايا الولوده هذا يفسر مدى أحتياج هذه الخلايا للطاقه التى تساعد على تكوين الجراثيم. هذا وقد أتضح أن الفراغ اليودى فى أسبوروبلازم الجرثومه دائرى الشكل محتوياً على جليكوجين.

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SUMMARY

One species of Myxosporidia (Sporozoa) was discovered from the testis of a toad *Bufo regularis* in the form of cysts. The cysts were 1-4X0.7-3.5mm. (average 2.5X2.1mm). The cysts were filled with a fluid, containing mature spores which appears milky white to the naked eye. The spores were 9.4-10.6 X 9.2 μ (average 10X9.1 μ) with two equal polar capsules each measuring 3.5-4.1X2.4-2.7 μ (average 3.8X2.6 μ (average 3.8x2.6 μ). The spores contained iodinophilous vacuoles within their sporoplasm. The morphological and histochemical studies were carried out on the cyst and spores.

INTRODUCTION

Myxosporidian spores were rare in amphibians being firstly described from the kidneys of frogs and toads in North America during the last century by *OHLMACHER (1893); WHINERY (1893) and GURLEY (1894)*, and in Europe by *THELOHAN (1895); LABBE (1899) and MORELLE (1929)*. Descriptions of these parasites were based solely on mature spores found in the kidneys of adult amphibians. *KUDO (1920)* provisionally included all of the European paraites in the genus *Wardia* as one species, *Wardia* Ohlmacheri. *EWERS (1973)* described. *M. chimbuensis* from the testis of the hylid frog *litoria darlingtoni*, from New Guinea; *THEODORIDES* et al. *(1982 a,b)* reportedf testicular *Myxobolus spp.* from *Bufo regularis* and the ranid, *ptychadena maccarthyensis,* in Togo (A frica). The only other *Myxobolus sp.* reported in Anurans is *M. ranae,* which forms tumors in the skin of the common grass frog, *rana temporaria*, in Europe. *GUYENOT and NOVILLE (1922)* , *UPTON* et al. *(1992)* reported *Myobolus bufonis sp.* n. from the testis of *Bufo maculatus,* from Cameroon (Africa). They found that the *myxobolus sp.* most closely resembles that reported by *THEODORIDES* et al. *(1981 a,b)* from *Bufo regularis* in Togo, although it currently is not possible to determine whether the two represent the same species. However, no named *Myxobolus sp.* from amphibians matches. The measurements reported in their study and they gave the name *Myxobolus bufonis sp.* n. to the parasite of *Bufo maculatus* from Cameroon (West Agrica).

MATERIAL and METHODS

Two hundred egyptian toads *bufo regularis* were collected from different parts in Assiut locality. Cysts were discovered in testis, some of the cysts were squashed between two slides to produce free spores. Lugol's iodine was added to some of these films to stain the iodinophilous vacuole.

The polar filaments were extruded by addition of saturated urea solution. Gimesa's stained smears were also prepared. Other cysts were fixed either in formal-alcohol or bouin's solution. The cysts were then embedded in paraffin wax as usual. Paraffin sections were stained either with haematoxylin and eosin or with some histochemical reagents (Periodic acid Schifff's - P.A.S. reaction with diastase control, Best's carmine and Bromophenol blue) were also used to study the structure of the cyst wall, metrocytes, spores and to study the chemical nature of the iodinophilous vacuole.

RESULTS

The parasite was found in 4.5% of the Egyptian toad examined. The cysts were white in colour, oval or rounded in shape measuring 1-4xo. 7-3.5 mm. (average 2.5x2.1 mm.). The spores (Fig. 1,2) were more or less rounded in shape, measuring 9.4-10. 6x9-9.2 u (average 10.x9.1 u). Two equal polar capsules were situated at the anterior end, each measuring 3.5-4.1x2.4-2.7 u (average 3.8x2.6 u.), with a polar filament measuring 24.6-47.8 u. (average 36.2 u.) in length when fully extruded but when it is resting inside the polar capsules, it consisted of 4-5 coils. The ratio of polar capsule/spore length 0.38 u. when lugol's iodine was added a brownish rounded mass was detected within the sporoplasm ususlly known as iodinophilous vacuole measuring 2.7 u. The sporoplasm few coarse granules. One sporoplasmic nucleus was visible, it measured 1.5 u.

Haematoxylin and eosin stained paraffin sections showed that the cyst (Fig. 3) was composed of two layers. The outer one consisted of a very thin collagen (persumably of host origin). Fibroblasts were present surrounding the collagen layer. The inner layer consisted of the cyst wall proper of the parasite which is very thin & smooth. Nucleated layer containing sporoblasts (metrocytes) was observed lining the cyst wall proper. The fully developed spores were present inwards.

Periodic acid Shiff's stained sections showed that the outer layer of the cyst wall and cyst wall proper were red in colour (P.A.S. positive). The iodinophilous vacuole and sporoblasts were deep red in colour (P.A.S. positive) (Fig.4).

Some sections were exposed to diastase to be digested for 30-60 minutes at 37°C followed by P.A.S. stain. The outer layer of cyst wall became P.A.S. positive indicating that it contained mucopolysaccharides or mucoproteins. The iodinophilous vacuole and the sporoblasts changed their red colour as a result of digestion of the glycogen present in these structures (Fig.5).

Best's carmine stained sections, showed that the outer layer of the cyst wall stained pale red, while the sporoblasts and iodinophilous vacuole stained deep red in colour. The presence of a red rounded structure (Fig. 6) in the sproplasm below the polar capsule may indicate that this structure is a store house for glycogen.

Bromophenol blue for proteins showed that the outer layer of the cyst wall (Fig. 7), the cyst wall proper, sporoblasts and spores stained bluish in colour. This indicates that these structures contained proteins.

DISCUSSION

Since the spores contained two polar capsules at their anterior end, and the sporoplasm contained an iodinophilous vacuole, then the parasite is related to the family Myxobolidae Thelohan, 1892. Moreover, the characters of th present parasite is identical with those of the genus *Myxobolus ButsCHLI, 1882.*

Theodorides et al., (1981 b) described spores of *Myxobolus* sp. from *Bufo regularis* in Togo, Africa, which measure about 10 um. Long, and 9 to 10 um wide. Although no specific meaurements were given to the from found in *Ptychadena maccarthyensis,* the authors reported that spores from *Ptychadena maccarthyensis (and B.regularis)* were about to um by 8 um and had polar capsules about 4 um. by 2.5 um. (Theodorides et al., 1981). The *Myxobolus* spp. from these two hosts are depicted as having slightly different internal details, which suggests that two distinct species may be involved.

Upton et al., (1992) reported *Myxobolus* sp. form the testis of *bufo maculatus* from Cameroon, Africa. He found that the *Myxobolus* sp. in his work most closely resembles that reported by Theodorides et al., (1981 a, b) from *Bufo regularis* in Togo. They mentioned that although it currently is not

possible to determine whether the two represent the same species. However, no named *myxobolus* sp. from amphibians matches the measurements reported in their study and they assigned the name *Myxobolus bufonis* sp. n. to the parasite of *Bufo maculatus* from Cameroon (West Africa).

MANDOUR et al., *(1993)* reported *Myxobolus clarii* from the testis of the fish *Clarias lazera*. They described the iodinophilous vacuole as a crescent-like body between the wall of the spore and the posterior border of the sporoblasm. In the present study it is rounded, which may indicate the taxonomic importance of the iodinophilous vacuole.

The present material cannot be compared with that described from fish which is a different host. Moreover, the morphological characters and measurements of the parasite are quite different.

From the present study, it appears that our material is morphologically identical with *Myxobolus bufonis UPTON* et al., *(1992)*. However, future work may reveal biological differences.

For this reason, the species under discussion is suggested to be indentified as Myxobolus sp.

Moreover, studies on the life cycle of this parasite may show that show that our material differs from that described by *UPTON* et al. *(1992)*.

The histochemicol study throws light on the structure & chemical nature of cyst wall & spores.

The presence of large amount of glycogen and protein in metrocytes (nucleated layer containing sporoblasts) my show the function of these nutrient substances to help metrocytes to produce spores.

The iodinophilous vacuoles in the sporoblasm of spores contained glycogen as shown by Best's carmine stain, thus the iodinophilous vacuole acts as a store house for glycogen wich may be used as a source of energy for further development of the parasite.

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Fig.1 :
Spores of *Myxobolus* sp. stained with Giemsa stai n (X 1250).

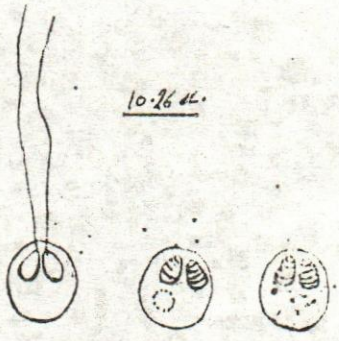

Fig.2 :
Spores of *Myxobolus* sp. (Camera-Lucida drawings)

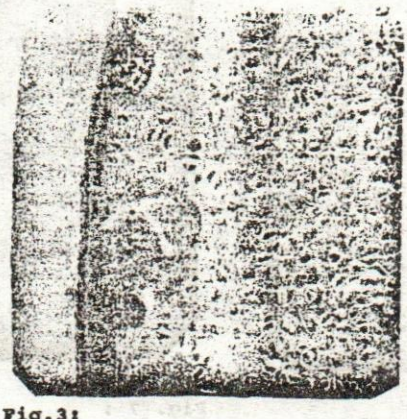

Fig.3:
T.s. through the cyst wall of *Myxobolus* sp. stained with H. & E. (X 256).

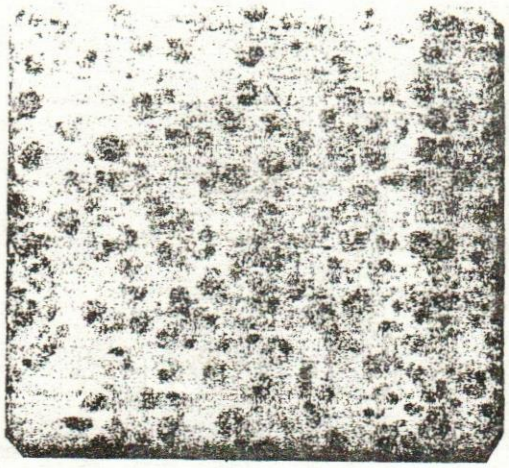

Fig. 4 :
The iodinophilous vacuole (V) of *Myxobolus* sp. stained with P.A.S. (X 500).

Fig. 5:
The iodinophilous vacuole (V) of *Myxobolus* sp. after digestion with diastase (X 200).

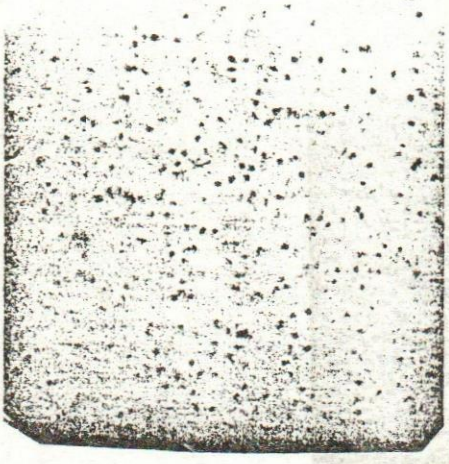

Fig. 6:
The rounded structure of iodinophilous vacuole (V) of *Myxobolus* sp.stained with Best's carmine (X 200)

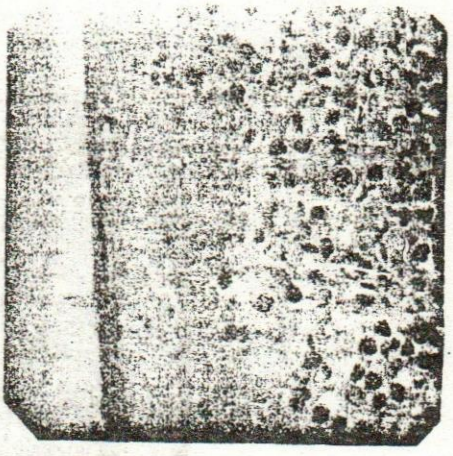

Fig. 7 :
T.S. through the cyst of *Myxobolus* sp. stained with Bromophenol blue (X 320).