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EFFECT OF SOME DAILY MANAGEMENTAL FACTORS ON APPEARANCE OF ABNORMAL BEHAVIOR AND HEALTH STATUS OF DONKEYS

(With 4 Tables and 2 Figures)

By

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تأثير بعض المعاملات اليومية على ظهور السلوك الغير طبيعي وعلى الحالة الصحية للحمير

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تـم إجراء هذه التجربة على عدد ٥ من إناث الحمير والتي كان متوسط أعمارها ٥ سنوات وأوزانهـا حوالى ٢٥٠ كيلوجرام. سكنت هذه الحيوانات بالمزرعة التجريبية الخاصة بكلية الطـب البـيطري بجامعة أسيوط داخل حوش مفتوح ذو أرضية خرسانية دون وجود فراش وتحت الظروف البيئية السائدة. تم تغذية هذه الحيوانات بحرية على عليقة حافظة مكونة من مخلـوط مركـزات الأعلاف والتبن مع تقديم الماء لها بصورة مستمرة لمدة أسبوعين وذلك كفـترة ضـابطة. تلـت هـذه الفترة الفترات التجريبية والتى كانت مدة كل منها أسبوعين كالآتي:- ١- وضع فرشة من قش الأرز تحت الحيوانات. ٢- زيادة نسبة الطاقة والبروتين بتقديم البرسيم للحيوانات مع العليقة الحافظة. ٣- تقديم ماء الشرب للحيوانات كل يومين بدلا مـن السـقى اليومى. ٤- تسكين الحيوانات داخل غرفة مغلقة بدلا من الحوش المفتوح. تم تسـجيل سـلوكيات هـذه الحيوانات وكذلك تم فحصها وذلك لتحديد حالتها الصحية مع أخذ عيـنات من دم هذه الحيوانات لتقدير نسب بعض مكونات الدم ومستوى هرمون الكورتيزول بهـا. أثبتت النتائج التي تم الحصول عليها أن المعاملات اليومية التى ترتبط ببقاء الحيوانات داخل المساكن كان لها التأثير الأقوى فى ظهور السلوك الغير طبيعى بين الحمير وأن بعض من هذه العوامل كانت مسببة للإجهاد وإنعكست بصورة واضحة على الحالة الصحية وبعض مكونـات الـدم لهذه الحيوانات. وقد أوصت النتائج بملاحظة عدم حدوث أى تغييرات على برنامج الرعاية الخاص بالفصيلة الخيلية المرباه داخل المزارع وذلك من أجل الحصول على الحد الأقصى من الإراحة وبالتالى الإنتاج.

SUMMARY

Five non pregnant, non lactating she-donkeys of about 5 years in age and 250 kg in weight were used in this investigation. Animals were tied under shed in an open yard with a concrete floor belonging to the experimental farm of faculty of veterinary medicine, Assiut University. They were ad libitum fed a maintenance ration. Drinking water was freely available allover the experiment. Donkeys were subjected to four experimental trials, two weeks each (bedding the floor, offering Barseem hay with the ration, watering each two days and housing inside closed pens). Behavior of the experimented animals was recorded. Moreover, their health status and some of blood parameters were estimated. The obtained results indicated that, managemental factors related to the time spent in the stable showed the strongest associations with stereotypic and abnormal behavior. Some of these conditions were considered as a stressful factor and reflected prominently on the animal's health status and blood parameters. Therefore, changes in the management program within the equine farms should be avoided to provide the animals with a comfortable situation.

Key words: *Management, behavior, health, donkeys*

INTRODUCTION

A great knowledge on the effect of management factors is required to investigate the ontogney of abnormal behavior in the stabled equines. Stabled horses often exhibit behaviors which owners regard as unwelcomed. These include stereotypies, defined as repetitive, relatively invariant and apparently functionless activities (Mason, 1991) and redirected behaviors, which are directed towards an inappropriate target (Fraser and Broom, 1990). Cage design, isolation rearing and food deprivation have been implicated as proximate causes of stereotypic behaviors (Morgan, 1973; Odberg, 1986 and Appleby & Lawrence, 1987). Arousal generated by frustrated motivation is a possible shared cause (Duncan *et al.,* 1993). Heterogeneity also emphasized as a cause of different stereotypies (Mason, 1991).

Despite much work on farm and laboratory species, the cause of stereotypic and redirected behavior in equines remain unclear. Heritability plays some role but little is known about the relative importance of management factors that might frustrate motivation in equines. Feeding practice have a greater effect than housing practices on the incidence of abnormal behaviors (Marsden, 1993). Other possible causes of abnormal behaviors in equines include factors associated with weaning, social contact, crowding and training practices (Kiley-Worthington, 1983 & 1987). Exposure to stereotypic neighbor may also increase the likelihood of stereotypy development or performance.

The aim of the present study was to establish the relative influence of some management factors on the development and performance of abnormal behaviors in donkeys.

MATERIALS and METHODS

I- Animals, feeding and management: -

Five non pregnant, non lactating she-donkeys of about 5 years in age and 250 kg in weight were used in this investigation. Animals appeared to be clinically healthy and parasitological examination revealed no gastrointestinal affections. They were housed under the prevalent environmental conditions as they tied under shed in an open yard with a concrete floor belonging to the experimental farm of faculty of veterinary medicine, Assiut University. Animals were ad libitum fed a maintenance ration consists of commercial concentrate mixture and wheat straw. Drinking water was freely available allover the experiment.

II- Experimental trials: -

Animals were housed under the previously mentioned conditions for two weeks as a control trial. After that, they were subjected to four experimental trials as follows: -

- Trial one: -

Where the animals housed under the previously mentioned control conditions except that the floor was bedded with rice straw.

- Trial two: -

Where the animals housed under the previously mentioned control conditions except that the energy and protein contents of the diet increased by adding Barseem hay to the previously mentioned maintenance ration with a rate of 2 kg / head.

- Trial three: -

Where the animals housed under the previously mentioned control conditions except that they were watered each two days instead of daily watering.

- Trial four: -

Where the animals housed under the previously mentioned control conditions except that they were housed and tied inside closed pen rather than open yard.

Each experimental trial was consists of two weeks followed by one week as a preliminary period during which, animals were reposed the control conditions (McGreevy *et al.,* 1995).

- Behavioral observation: -

Behavior of the experimented animals was recorded following the method of Martin & Bateson (1988) using the scan sampling method where the observer can observe all the animals without being seen by them. Behavior was observed and analyzed according to McGreevy *et al.* (1995). During the control and experimental periods, animals were observed during the second week for 8hours / day (four hours in the morning and four hours in the afternoon). The behavioral observations were carried out in the morning between 9:00 and 13:00 and in the afternoon between 13:00 and 17:00.

Animals were observed for incidence of any of the following abnormal behavioral patterns: -

1. Kicking
2. Eating dung
3. Eating bedding
4. Throwing food out of manger
5. Licking the walls
6. Weaving
7. Crib-biting and wind-sucking

- Health status measurements: -

On the 1st, 7th and last day of all trials, experimented donkeys were examined clinically according to Blood and Henderson (1974) and Blood & Radostits (1990) to determine their average pulse rate, respiratory rate and their body temperature as well as the condition of their mucous membranes, fecal matters and coats.

- Blood parameters: -

During the last two days of all trials, three blood samples, 5 ml each were drawn from the jugular vein of each animal. The first one was drawn into glass test tubes contain EDTA for leucocytic count according to Franke and Reitman (1963). The second blood sample was drawn into centrifuge tubes and centrifuged for 30 minutes at 3000 r.p.m and the obtained sera were assayed within three hours for their glucose concentration according to Tinder (1969). The third blood sample was drawn into centrifuge tubes and centrifuged for 30 minutes at 3000 r.p.m and the obtained sera were freezed at –80 °C and kept for further analysis to determine their cortisol level using TDx FLx system according to Dandliker and Sassure (1973).

III- Statistical analysis: -

Statistical analyses of the collected data were carried out according to procedures of completely random design (SAS, 1995).

RESULTS

The results of this study were illustrated in tables 1,2,3,4 as well as figures 1 and 2.

DISCUSSION

I- Behavioral observations of experimented animals: -

The data represented in table (1) and assimilated on figure (1) showed the effect of the studied management conditions on the incidence of abnormal behavior among donkeys. These data revealed that, incidence and performance of abnormal behaviors by donkeys was significantly affected with changing the daily management conditions than the control one ($P<0.01$).

Bedding the floor of the yard with rice straw was reflected on the experimented donkeys with incidence of eating bedding with a rate of 60% as shown in trial one. However, increasing the energy and protein content of the diet during trial two by adding Barseem hay to their maintenance ration was associated with the incidence of kicking, throwing food out of manger, weaving and crib-biting & wind-sucking with rates of 80, 40, 40 and 40%, respectively. Kownacki *et al.* (1978); Duncan *et al.* (1993) and McGreevy *et al.* (1995) indicated that, both oral-based (crib-biting and wind-sucking) and locomotor (kicking and weaning) stereotypies are more prevalent among stabled equines that daily fed forage. This finding may be related to the enforced free time of the stabled animals which accompanied by high protein content of the offered food. At the same time, increased incidence of throwing food out of manger may be related to searching for their favorable food, Barseem hay.

Moreover, watering the animals every two days instead of every day during trial three was associated with the incidence of kicking, eating dung and licking the walls with rates of 40, 60 and 80%, respectively. This finding may be related to the stressful situation of water restriction which kept the animal nervous and searching for water even by eating their dung or licking the walls (Waring, 1983 and Kiley-Worthington, 1987). At the same time, housing and tying the experimented donkeys inside closed pens rather than open yards resulted in incidence of kicking and weaving with rates of 60 and 40%, respectively. This may be related to the fact that locomotor (kicking and weaning) stereotypies are more prevalent among stabled animals.

Moreover, weaving is the most likely behavior to be learnt among equines by observation. Stabled horses and donkeys may be more likely to encounter weavers however, large yards was generally associated with a reduced risk of these abnormal behaviors (Houpt, 1986 and McGreevy *et al.*, 1995).

II- Health status of the experimented animals: -

The data represented in table (2) showed the effect of the studied management conditions on pulse rate, respiratory rate (No. / min.) and body temperature (°C) as well as the conditions of mucous membrane, faecal matter and coat of the experimented donkeys. The average data were 38, 10, 37.4, normal, normal, normal following the control conditions; 38, 10, 37.3, normal, normal, normal after bedding the floor of the yard with rice straw; 40, 12, 37.4, normal, normal, normal after adding Barseem hay to the maintenance ration; 48, 18, 37.4, normal, firm & dark, normal following watering each two days and 38, 10, 37.4, normal, normal, normal after housing the animals inside closed pens, respectively. These results revealed that, among all experimented management conditions, watering the animals every two days was the only factor that had a significant effect ($p<0.01$) on health status of donkeys. Watering the animals every two days was associated with a significant increase in their pulse and respiratory rates with changing in the constancy of their faecal matter. This finding may be related to the physiological and biological adjustments and changes in the animal body to meet this new stressful situation (Hafez, 1975; Banerjee, 1982 and Radostits *et al.,* 1994). Moreover, it could be related to the fact that insufficient water intake affects significantly the blood water content and increases the blood viscosity which reflects on the animal with an obvious effect on their pulse rate (Blood and Radostitis, 1990).

III- Blood parameters of the experimented animals: -

The data represented in table (3) showed the effect of the studied management conditions on the differential leucocytic count of donkeys. These data showed that, the counts (10^{3} / µl) of total WBCs, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils during control conditions were 11.14, 6.36, 3.12, 1.12, 0.42, 0.120, respectively. However, it was 11.19, 6.19, 3.32, 1.10, 0.45, 0.130 after bedding the floor of the yard with rice straw; 10.94, 6.31, 2.97, 1.14, 0.40, 0.120 after increasing the energy and protein contents of the diet; 11.11, 6.22, 3.22, 1.13, 0.42, 0.120 following watering the animals each two days and 10.97, 6.16, 3.12, 1.16, 0.40, 0.130 after housing the animals inside closed pens, respectively. This result indicated that, leucocytic series of the experimented donkeys was not significantly affected by any of the studied management conditions.

At the same time, The data illustrated in table (4) and assimilated on figure (2) showed the effect of these studied management conditions on the blood levels of cortisol and glucose of donkeys. Serum cortisol level was 0.97, 0.97, 0.88, 1.91 and 1.43 μg / L while, serum glucose level was 5.87, 5.87, 5.34, 7.52 and 7.48 Mmol / L following control, bedding the floor, offering Barseem hay with the maintenance ration, watering each two days and housing inside closed pens, respectively. These findings indicated that, both watering the animals each two days and housing inside closed pens were significantly affected blood cortisol and glucose levels ($P<0.01$) however, other factors did not do so. The significant increase in the blood cortisol level indicated an occurrence of stress due to these conditions where acute stress causes an outpouring of ACTH which intern causes the adrenal cortex to increase its secretion of glucocorticoids including cortisol (McDonald, 1969; Burchfield *et al.,* 1980 and Stephens, 1981). However, the increase in blood glucose level may related to the fact that glucocorticoids, including cortisol, act mainly on the hepatocytes which induced to produce gluconegenic enzymes which in turn increase the rate of gluconeogenesis and enhance the conversion of protein to glucose. Moreover, cortisol causes a moderate reduction in the rate of glucose utilization by the body cells, which leads to a rise in blood glucose level (Guyton and Hall, 1996).

CONCLUSION

In conclusion, management factors related to the time spent in the stable showed the strongest associations with stereotypic and abnormal behavior. Some of these conditions are considered as a stressful factor and reflected prominently on the animal health status and serum level of both cortisol and glucose which are likely to upset its body homeostasis and so, its behavior. Therefore, changes in the management program within the equine farms should be avoided to provide the animals with a comfortable situation.

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Table 1: Incidence of abnormal behavioral patterns (%) of the experimented animals

Behavior	Control	Trials			
		One	Two	Three	Four
Kicking	0^{a}	0^{a}	80^{d}	40^{c}	60^{b}
Eating dung	0^{a}	0^{a}	0^{a}	60^{b}	0^{a}
Eating bedding	0^{a}	60^{b}	0^{a}	0^{a}	0^{a}
Throwing food	0^{a}	0^{a}	40^{b}	0^{a}	0^{a}
Licking the walls	0^{a}	0^{a}	0^{a}	80^{b}	0^{a}
Weaving	0^{a}	0^{a}	40^{b}	0^{a}	40^{b}
Crib-biting and wind-sucking	0^{a}	0^{a}	40^{b}	0^{a}	0^{a}

Trial one = Bedding the floor Trial two = Offering Barseem hay plus maintenance ration
Trial three = watering each two days Trial four = Housing inside closed pens
Figures in the same raw with different superscripts differs significantly ($p < 0.01$)

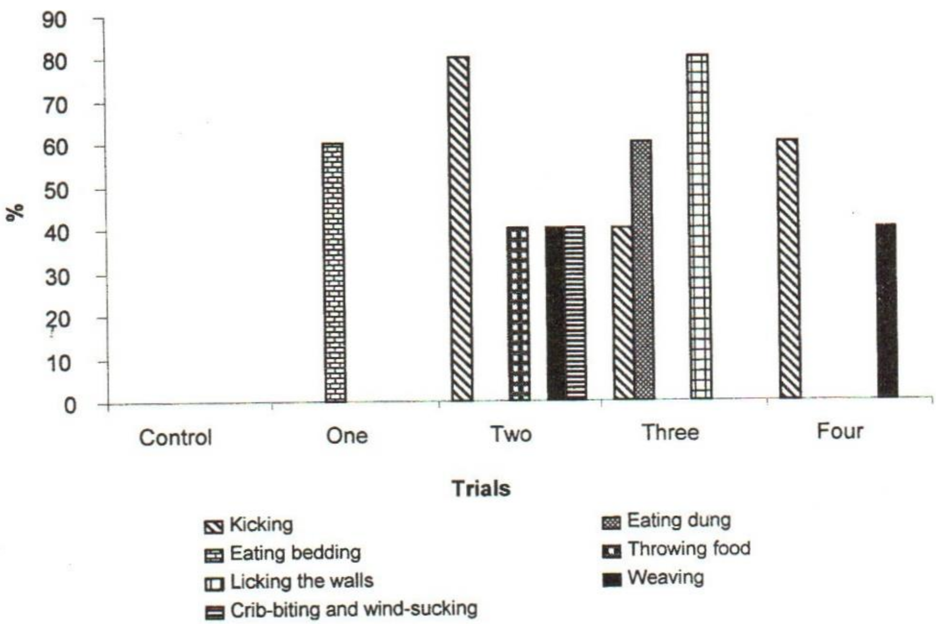

Fig. 1 : - Incedince of abnormal behavioral patterns of the experimented animals

Table 2: Health status measurements of the experimented animals

Item	Control	Trials			
		One	Two	Three	Four
Pulse rate (No./min)	38±2 [a]	38±1 [a]	40±2 [a]	48±2 [b]	38±2 [a]
Respiratory rate (No./min)	10±1 [a]	10±1 [a]	12±1 [a]	18±1 [b]	10±1 [a]
Body temperature (°C)	37.4±0.1 [a]	37.3±0.1 [a]	37.4±0.1 [a]	37.4±0.1 [a]	37.4±0.1 [a]
Mucous membrane	Normal	Normal	Normal	Normal	Normal
Fecal matter	Normal	Normal	Normal	Firm & Dark	Normal
Condition of the coat	Normal	Normal	Normal	Normal	Normal

Trial one = Bedding the floor Trial two = Offering Barseem hay plus maintenance ration
Trial three = watering each two days Trial four = Housing inside closed pens
Figures in the same raw with different superscripts differs significantly ($p < 0.01$)

Table 3:- Differential leucocytic count (10^3 / µl) of the experimented animals

Item	Control	Trials			
		One	Two	Three	Four
Total WBCs	11.14±0.12	11.19±0.10	10.94±0.16	11.11±0.12	10.97±0.10
Neutrophils	6.36±0.10	6.19±0.10	6.31±0.13	6.22±0.10	6.16±0.18
Lymphocytes	3.12±0.10	3.32±0.10	2.97±0.12	3.22±0.10	3.12±0.10
Monocytes	1.12±0.04	1.10±0.02	1.14±0.06	1.13±0.02	1.16±0.04
Eosinophils	0.42±0.03	0.45±0.03	0.40±0.01	0.42±0.02	0.40±0.03
Basophils	0.120±0.01	0.130±0.02	0.120±0.01	0.120±0.01	0.130±0.01

Trial one = Bedding the floor Trial two = Offering Barseem hay plus maintenance ration
Trial three = watering each two days Trial four = Housing inside closed pens

Table 4: Serum cortisol (µg / L) and glucose (Mmol / L) concentrations of the experimented animals

Item	Control	Trials			
		One	Two	Three	Four
Cortisol	0.97±0.02 [a]	0.97±0.02 [a]	0.88±0.01 [a]	1.91±0.01 [b]	1.43±0.02 [b]
Glucose	5.87±0.20 [a]	5.87±0.10 [a]	5.34±0.20 [a]	7.52±0.20 [b]	7.48±0.15 [b]

Trial one = Bedding the floor Trial two = Offering Barseem hay plus maintenance ration
Trial three = watering each two days Trial four = Housing inside closed pens
Figures in the same raw with different superscripts differs significantly ($p < 0.01$)

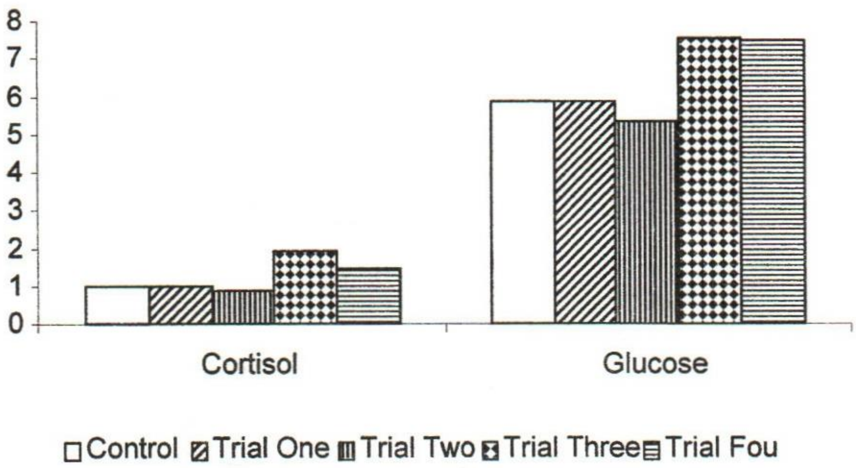

Fig.2: - Serum cortisol (µg / L) and glucose (Mmol / L) concentrations of the experim animals

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MYXOBOLUS INFESTATION IN OVARIES OF SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS*

(With 2 Tables and 5 Figures)

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الإصابة بطفيل ميكزوبولاس فى مبايض الأسماك القطية النيلية (القراميط)

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الهـدف مـن هذه الدراسة هو اجراء دراسة ميدانية وملاحظة الأعراض الإكلينيكية ونسب الإصـابة وكذلك التغيرات المرضية نتيجة اصابة مبايض الأسماك القطية النيلية (القراميط) بطفـيل الميكزوبولس. تم فحص عدد ١٢٠ سمكة على مدار عام ٢٠٠٤ بمعدل ١٠ سمكات شـهرياً ووجد أن عدد ١٥ سمكة كانت تحمل حويصلات طفيل الميكزوبولس فى المبايض. وكانـت معـدلات الإصابة فى آخر فصل الخريف قليلة ثم ازدادت تدريجيا فى فصل الشتاء حـتى وصـلت إلـى أعلى معدلاتها فى بداية فصل الربيع، ولم تسجل أى حالة من حالات الإصابة فى فصل الصيف. كما لوحظ أن الإصابة كانت فى مبيض واحد فقط فى ٦ أسماك (٤٠%) مـن الأسـماك المصـابة فى حين كانت الإصابة فى المبيضين معاً فى ٩ أسماك (٦٠%) مـن الأسماك المصابة. اما حدة الإصابة فقد ازدادت ايضاً خلال فصل الشتاء حتى وصـلت اعلى مستوى لها فى بداية فصل الربيع ثم تضاءلت سريعا مع بداية فصل الصيف. اظهـر الفحـص الميكروسـكوبى لحويصلات طفيل الميكزوبولس عدد كبير جداً من أبواغ (جراثـيم) الطفيل عند درجات مختلفة من التطور والنمو. كما اظهر الفحص الميكروسكوبى لمبـيض الأسـماك المصـابة أن حويصـلات طفيل الميكزوبولس قد قامت بالضغط على الويضـات القريـبة ممـا تسـبب فـى ضمورها و موتها فى بعض الأحيان، كما تسببت الحويصلات فى حدوث اضطرابات دموية فى الأنسجة المجاورة لها. ونظراً لأن هذا الطفيل وجـد فـى مـبايض القرامـيط فقد يكون نوعاً جديدا من الطفيليات والذى يحتاج لمزيد من الدراسة مستقبلاً.

SUMMARY

The main aim of this study was to investigate the clinical and postmortem findings, seasonal prevalence, and histopathological alterations that are caused by probably a new species of *Myxobolus* in ovaries of sharptooth catfish, *Clarias gariepinus*, in Assiut, Egypt. Out of 120 fish examined over one year (2004), ovaries of only 15 (12.5 %) fish were infested with macroscopic *Myxobolus* cysts (plasmodia and host cyst) that were embedded in the connective tissue among ova. Prevalence of infestation started low in late autumn and increased over winter and reached maximum in early spring. Infestation was not recorded in summer. Six (40%) out of the infested fish had *Myxobolus* cysts in only one ovary, meanwhile, the reminder (60%) of infested fish had both ovaries infested. Also, intensity of infestation gradually increased over winter and was maximal in early spring, but abruptly declined in summer. Microscopic examination of plasmodia showed numerous typical *Myxobolus* spores at various developmental stages. Mature spores are oval in shape with two anteriorly located polar capsules that have 4-5 coils of polar filaments. Microscopic examination of infested ovaries revealed that *Myxobolus* plasmodia were encapsulated within a thin connective tissue layer of host reaction. *Myxobolus* cysts compress neighboring tissues causing atrophy of ova and local circulatory disturbances. Based on the tissue location of plasmodia and morphological character of the mature spores, the parasite in the present study might be a new species.

Key words: *Clarias gariepinus, myxobolus, ovaries*

INTRODUCTION

Commercial farming of sharptooth catfish, *Clarias gariepinus*, is a rapidly growing aquaculture industry in Upper Egypt. *C. gariepinus*, has recently gained a consolidate position in the food fish market as it is widely accepted by consumers in Upper Egypt.

Myxosporea are economically important fish parasites which form an abundant and diverse group. They cause heavy infections, extensive lesions, and mortalities in cultured fish (Lom and Dykovâ, 1995). In Africa, about 100 species are currently known from the continent (Fomena and Bouix, 1997). In Egypt, myxosporean parasites were examined in River Nile fish by Aziza (1980), Imam *et al.* (1987), Abdel Ghaffar *et al.* (1998), and Ali (1999, 2000). Currently, study of myxosporean infections focuses on pathogenicity and significance of the parasite in both aquaculture and captured fish (Lom and Dykovâ, 1995).

Clarias gariepinus, a carnivorous bottom feeder, acts as a host for plenty of parasites that have tremendous effects on fish health and population throughout the River Nile. In Egypt, *C. gariepinus* had been found to be infested with *Myxobolus lazeri* (Aziza, 1980), and *Myxobolus clarii* (Mandour *et al.*, 1993). Histozoic Myxobolidae cause great destruction of the host tissues and are of serious concern to fish culture (Kabata, 1985).

There are scanty data on *Myxobolus* infestations and the pathology they cause in fish ovaries (Lom and Dykovâ, 1995; Gbankoto *et al.*, 1998; Reed *et al.*, 2003). In the present study, prevalence and intensity of infestation of *C. gariepinus* ovaries with probably a new species of *Myxobolus* have been investigated over one year. In addition, clinical and postmortem findings and histopathological alterations of *C. gariepinus* infested ovaries were studied.

MATERIALS and METHODS

Fish:

A total of 120 live, apparently healthy, female specimens of sharptooth catfish, *Clarias gariepinus*, of 300-800 g were collected from January 2004 to December 2004 (10 fish /month) from El-Ibrahemia canal and its tributaries, Assiut city.

Parasitological examination of samples

A-Clinical examination:

Fish were externally examined after capture for any apparent clinical signs or lesions. Fish were incised according to (Stoskopf, 1993) to examine ovaries for macroscopic *Myxobolus* cysts to determine prevalence of infestation (number of infested fish divided by the number of examined fish per month). Longitudinal incision was made in both ovaries to determine total numbers of *Myxobolus* cysts to determine intensity (number of cysts per infested fish) of infestation. Cysts were examined for size, consistency, and contents.

B-Microscopic examination:

Impression smears were made from cysts, air dried, and then fixed in 40% ethanol for 10 min. Fixed smears were stained with Methylene blue or Lugol's iodine solution.

Histopathological examination

Infested ovaries were excised from infested fish, fixed in 10% neutral buffered formalin for 48 hours, and then processed for microscopic examination. Thin paraffin sections were stained with

Haematoxylin and Eosin (H&E), Toluidine blue, and Periodic Acid Schiff's (PAS) stains.

RESULTS

Parasitological examination of samples

Longitudinal incision of infested ovaries showed whitish round to oval cysts (*Myxobolus* plasmodia and host cyst) that were randomly scattered and embedded in connective tissue among ova (Fig. 1). Cysts were seen by naked eyes and were 1.2- 1.5 mm in diameter. Cysts were located in ovaries of immature females and mature females at off-spawning seasons, while ovaries of mature females at spawning season needed more careful examination because cysts are of average size of mature ova but of different color. Interestingly, wall of cysts collected in spring, the primary spawning season, were fragile and readily ruptured releasing mature spores; in contrast, wall of cysts collected during late autumn and winter were relatively firm and resistant to rupture if compared to those collected in spring.

Seasonal prevalence and intensity of infestation

Out of the 120 fish examined, ovaries of only 15 (12.5%) fish were found to be infested with *Myxobolus* cysts. Infestations were not seen during summer, but were recorded at a relatively low rate when temperature started to drop in late autumn (Table 1). Prevalence gradually increased over winter and reached maximum when temperature started to rise in early spring, and then declined again in late spring (Fig. 2).

Intensity of infestation was determined according to number of cysts per ovary and whether one or both ovaries were infested (Table 2). Six (40%) out of the infested fish had *Myxobolus* cysts in only one ovary, meanwhile, the reminder (60%) of infested fish had both ovaries infested. Infestation was considered severe when 10 or more cysts were seen in one or both ovaries, while was considered moderate when 6-9 cysts were seen in one or both ovaries. Females were considered lightly infested when had 1-5 cysts in one or both ovaries.

Generally, during winter, when temperature is lowest in season, most cases of infestation were light. When temperature starts to rise, intensity of infestation gradually increases where moderate cases were recorded. Furthermore, Intensity of infestation continues to increase in spring when severe cases of infestation were seen and then rapidly declined and even disappeared in summer. In addition, during late autumn, when temperature starts to decline, infestations re-emerge when light cases of infestation were seen again.

Microscopic examination

Microscopically, plasmodia were encapsulated within a thin fibrous connective tissue capsule of host reaction and infiltrated with few lymphocytes with dilated blood capillaries (Fig.3). Plasmodia were filled with numerous typical *Myxobolus* spores where mature spores located centrally, while the developing ones were peripherally arranged. Furthermore, plasmodia collected in early spring had mainly mature spores, while plasmodia collected during autumn and winter had mainly developing spores.

Mature spores are oval in shape with slightly pointed anterior end and more rounded posterior end, and measuring 10.6 X 9.4 μ (Fig.4). Also, mature spores have at the anterior end two oval polar capsules with pointed anterior end and rounded posterior one. Polar capsules are of equal size, and measuring 4.8 X 3.5 μ. Each polar capsule has 4-5 coils of polar filament. Sporoplasm contains an iodinophilus vacuole that stain positively with lugol's iodine solution. Furthermore, thin sections of infested ovaries stained with PAS showed positively stained dark red spores.

Histopathological examination

Toluidine blue and H&E stained sections showed that *Myxobolus* cysts exert pressure atrophy over the adjacent ovarian tissues and cause disturbances in local circulation. Adjacent ova show degenerative changes in nuclei and cytoplasm and separation of the squamous cell layer that covers ova (Fig.5).

DISCUSSION

Present study revealed that *Myxobolus* infestation of ovaries of sharptooth catfish, *C. gariepinus*, is a mildly spread among wild population. Prevalence of infestation was 12.5% of all fish examined over one year. Water temperature has a great influence over seasonal prevalence of myxosporean infestations (Negm-Eldin *et al.*, 1999). The prevalence of ovarian infestations with *Myxobolus* cysts increased in winter and early spring, while decreased in autumn. Interestingly, during summer, there was no record of *Myxobolus* cysts in the ovaries of fish examined. Similar annual cycles were reported with other myxosporean infestations (Negm-Eldin *et al.*, 1999). In accordance with Clifton-Hadley *et al.*, (1986) who concluded that water temperature influences maturation of spores and development of myxosporean infestations in fish, in the present study, mature spores were seen in plasmodia collected in early spring, while developing spores were seen in plasmodia collected during autumn and winter.

Intensity of infestation has a cycle similar to that of prevalence. Intensity of infestation has gradually increased over winter and spring and then abruptly declined in late spring and summer. Sudden decline in prevalence and intensity of infestations during summer may be due to dispersing of intact cysts with eggs laid by infested mature females during spawning or, alternatively, rupture of cysts releasing mature spores in ovarian tissues. Ovarian contractions during egg lying might promote rupture of the cysts. This is supported by the fact that the cysts' walls are fragile and easily to be ruptured during egg laying season, but relatively harder during off-spawning seasons. Furthermore, it is supported by the fact that sporogenesis is completed during winter, and by spring plasmodia contain fully developed mature spores.

Dispersing of *Myxobolus* cysts or spores with laid eggs might be the primary route of spreading of infection and completing of the parasite's life cycle. It is not clear how this parasite reaches fish ovaries, its target organs. The exact mechanism of host invasion is unknown, but many freshwater myxosporeans have an alternate stage of development in oligochaetes (Oumouna *et al.,* 2002) or ploychaetes (Bartholomew *et al.*, 1997) which produces actinosporean spores that invade host. Oral route of transmission is also common route for myxosporean infestations (Lom and Dykovâ, 1995). In either case, the sporoplasms cross the epithelial barrier and are carried by the blood stream or lymphatic system to the target organ (Kabata, 1985).

Encapsulation of *Myxobolus* cysts within a thin connective tissue capsule of host reaction indicates that plasmodia severely irritate ovarian tissues stimulating a proliferative inflammatory response. This capsule is driven from the surrounding population of connective tissue cells and from compressed cells of the neighboring tissues (Lom and Dykovâ, 1995).

The extent of damage to tissues infested with Myxosporea depends on species of parasite and its life cycle stage, intensity of infestation and the host reaction (Lom and Dykovâ, 1995). Microscopic examination of infested ovaries revealed that the myxobolus cysts replaced original ovarian tissues, compressed neighboring ova, and caused disturbances in circulation in neighboring tissues. *Henneguya oviperda* causes similar lesions in ovaries of pike, *Esox lucius*, in

Europe, where atrophy of large number of ova was observed with local circulartory disorders (Lom and Dykovâ, 1995). *Myxobolus dahomeyensis* has been reported to hinder the successful breeding of several species of tilapia and their hybrids in Benin. *M. dahomeyensis* is found in ovaries of brooder tilapia where it penetrates inside the mature ova and liquefies the content causing total destruction of mature ova. In severe cases of infestation, ovaries become like sacs full of whitish fluid with spores and damaged ova (Gbankoto *et al.*, 1998).

Myxosporea are host, organ and tissue specific (Molnar, 1994). Myxosporean infestations had been reported in *C. gariepinus* in Egypt. Aziza (1980) described *Myxobolus lazeri* from kidneys, while Mandour *et al.* (1993) reported *Myxobolus clarii* from testis of *C. gariepinus*. Mature spores morphology is the key feature in identification of *Myxobolus* (Kabata, 1985). Mature spores of *M. lazeri* (9.8 X 6.1 μ) are smaller than those of the parasite of the present study (10.6 X 9.4 μ). In addition, polar capsule of *M. lazeri* spores are smaller (5.1 X 2.3 μ) than those of the parasite spores of the present study (4.8 X 3.5 μ). Morphological characters of the parasite's spores in the present study are close, but not similar, to those of *M. clarii* that is found in testis of *C. gariepinus* (Mandour *et al.*, 1993). Mature spores of the parasite in the present study are relatively larger but within the average size as mature spores of *Myxobolus clarii*. Size of the polar capsules of *M. clarii* spores (5.1 X 2.5 μ), however, is smaller than those of spores of the present study.

Plasmodia of *Myxobolus gariepinus* reported by Reed *et al.* (2003) in ovaries of *C. gariepinus* in Botswana were 2-3 mm in diameter, while fully mature plasmodia of the parasite in the present study was 1.2-1.5 mm. Mature spores of *M. gariepinus* (13.9 X 10.8 μ) are larger than those of the parasite the present study. Furthermore, polar capsules of *M. gariepinus* spores are measured (6.2 X 3.5 μ) and contain 5-6 coils of polar filaments, while polar capsules of the parasite of the present study were smaller and contain 4-5 coils of polar filament.

Based on its host species, tissue location, and mature spores morphology and dimensions, the parasite in the present study might be a new species. Classification of the parasite in the present study, however, needs further investigations including comparative ultra structure study and molecular identification.

Sharptooth catfish is widely accepted by consumers in Upper Egypt as a relatively cheaper choice of fish protein. Commercial farming of sharptooth catfish has significantly increased in Upper Egypt over the past few years. With no obvious method of treatment or control, ovarian infestation of sharptooth catfish with *Myxobolus* may affect fecundity (Lom and Dykovâ, 1995) and thus populations of wild and cultured fish.

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Table 1: Seasonal prevalence of *Myxobolus* infestation in *Clarias gariepinus* ovaries.

Month	Examined Fish	Prevalence	
		No. of infested fish	%
January	10	2	20
February	10	3	30
March	10	3	30
April	10	2	20
May	10	1	10
June	10	0	0
July	10	0	0
August	10	0	0
September	10	0	0
October	10	1	10
November	10	1	10
December	10	2	20
Total	120	15	12.5

Table 2: Intensity of *Myxobolus* infestation in *Clarias gariepinus* ovaries.

Infestation case	Month	Intensity		
		Ovaries infested	Total number of cysts	Severity
1	January	1	4	Light
2		2	5	Light
3	February	1	7	Moderate
4		2	8	Moderate
5		2	10	Severe
6	March	2	12	Severe
7		2	16	Severe
8		2	25	Severe
9	April	2	14	Severe
10		2	23	Severe
11	May	1	6	Moderate
-	June - September	-	-	-
12	October	1	1	Light
13	November	1	2	Light
14	December	1	2	Light
15		2	5	Light

Fig. 1: A photograph of *Clarias gariepinus* ovary incised longitudinally and infested with *Myxobolus* plasmodia (P) that are embedded in connective tissue among ova.

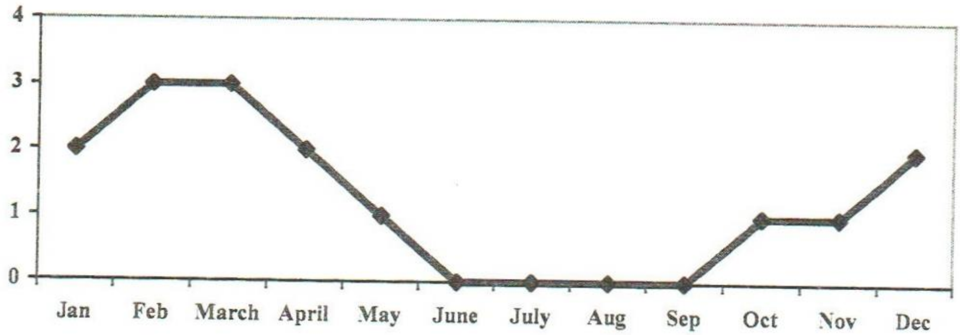

Fig. 2: Seasonal prevalence of *Myxobolus* infestation in *Clarias gariepinus* ovaries.

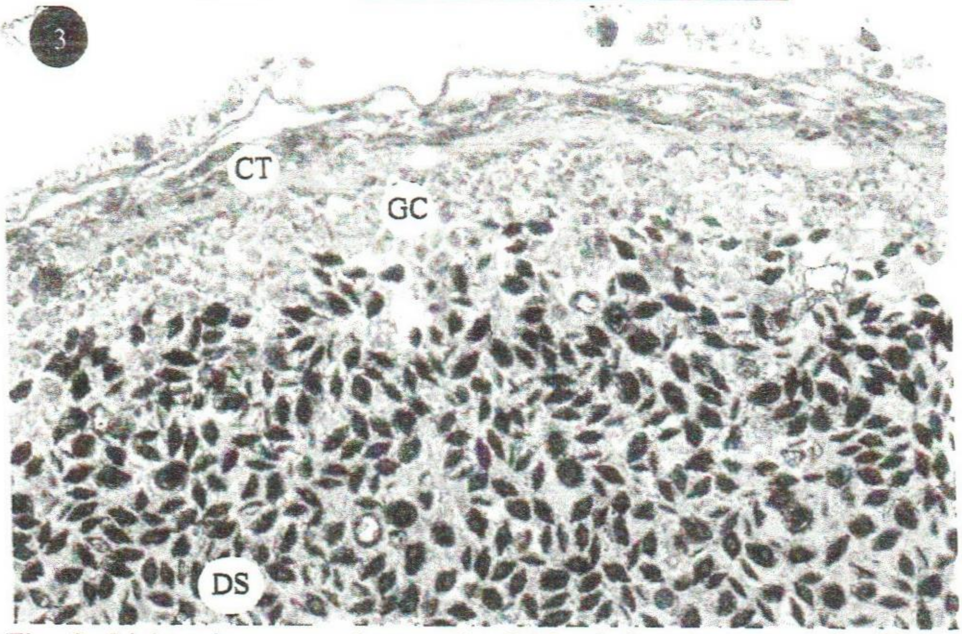

Fig. 3: Light microscope photograph of *Myxobolus* plasmodium in *Clarias gariepinus* ovary. Plasmodia are enclosed in fibrous connective tissue capsule (CT) infiltrated with few lymphocytes. Germinating cells (GC) are located peripherally, while developing spores (DS) are toward the center. Toluidine stain (400X).

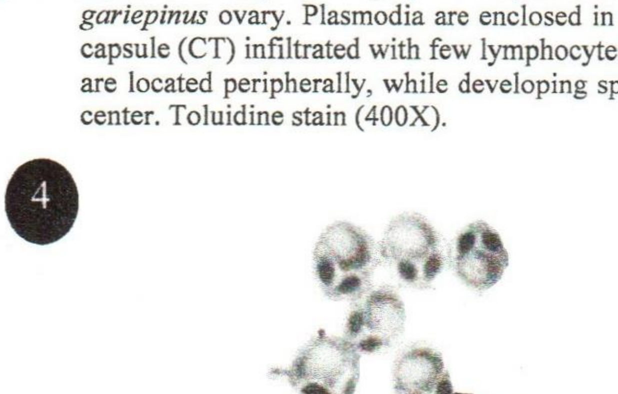

Fig. 4: Light microscope photograph of *Myxobolus* mature spores stained with methlyene blue. Spores are oval in shape with sporoplasm (SP) and two polar capsules (PC) (1000X).

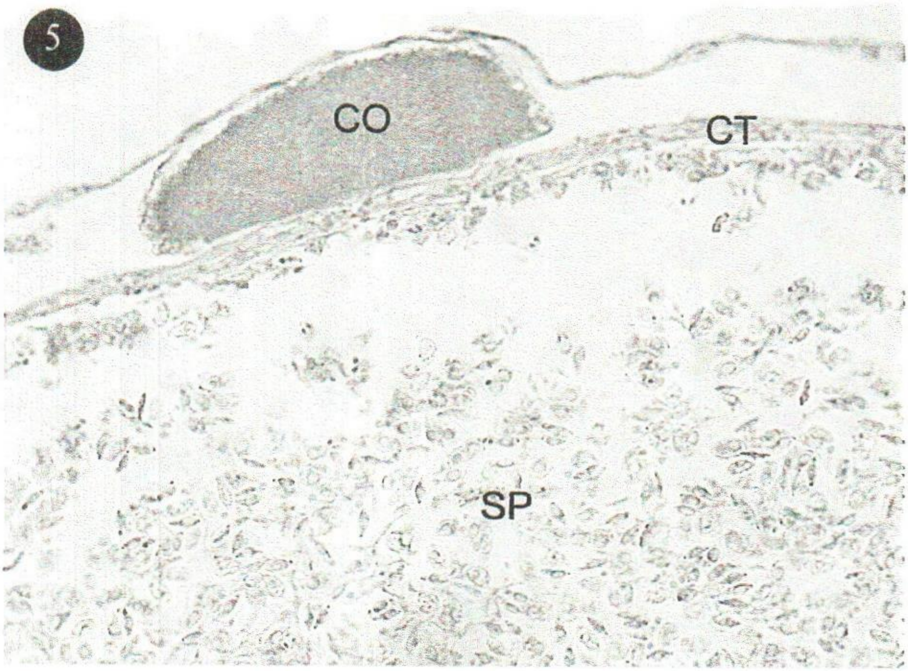

Fig. 5: Light microscope photograph of a plasmodium of *Myxobolus* full of spores (SP) encapsulated within a connective tissue capsule (CT) and compressing neighboring ova (CO) of *Clarias gariepinus* ovary. H&E (400X).