

Prevalence of some Protozoan Parasites in the Egyptian Tortoise (*Testudo kleinmanni*)

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Abstract:

A total of 50 apparently healthy Egyptian tortoises were collected from five different localities in Egypt, Port Saied, Elsayeda Aesha market, Ismailia, Cairo and Giza. Parasitological examination revealed the presence of five protozoan parasites namely, *Entamoeba invadens*, *Cryptosporidium serpentis*, *Nyctotherus* species, *Balantidium* species and *Rickettsia like organism*. The prevalence of protozoan parasites in the 50 tested tortoises was; *Entamoeba invadens* 8 (16%), *Cryptosporidium serpentis* 48 (96%), *Nyctotherus* species 50 (100%), *Balantidium* species 7 (14%) and *Rickettsia like organism* 3 (6%). The presence of *Entamoeba invadens*, *Cryptosporidium serpentis* and *Balantidium* species pose a threat to human being handling these pet animals, so public health awareness should be considered and regular parasitological examinations should be done.

Introduction:

There are approximately 6,000 extant species of reptiles. Currently 78 are listed as threatened/ endangered internationally including the Egyptian tortoise (*Fish and Wildlife Service, 1992*).

Tortoises are hosts to a wide variety of non enteric protozoan and metazoan parasites. If the enteric protozoa are included, virtually 100% of free-ranging reptiles harbor some kind of parasites acting as reservoirs for the infective agent, these distributing the infection

among the healthy individuals. However, the presence of parasites in an individual is not necessarily associated with a disease state (*Mcfarlen, 1991 and Barnard and Upton, 1994*).

Parasitic infestation in reptiles may be increased by stressful life; concentration of animals and the presence of different species in a small living space actuate multiplication and spreading of parasites, which in nature live in cohabitation with their hosts (*Rataj et al, 2011*).

Material and methods:

50 Egyptian tortoises (*Testudo kleinmanni*) were collected from five different localities in Egypt (10 from Portsaid, 10 Ismailia, 10 Cairo, 10 Giza pet animal shops and 10 Elsayeda Aesha market).

These tortoises were examined carefully both antemortem and postmortem for the detection of protozoan parasites. Fecal examinations were carried out using direct smear method and concentration flotation technique by Sheather's sugar or zinc sulphate solution according to **Levine (1985)**.

Fecal and mucosal scraps smears were fixed in absolute methanol and stained by modified Zeihl Neelsen technique for the detection of *Cryptosporidium* oocyst according to **Pohjola (1984)**. Thin and thick blood smears were carried out, air-dried, fixed in absolute methanol, stained in Geimsa stain and examined microscopically for detection of blood protozoan parasites according to **Foreyt (1989)**.

Results and Discussion:**Prevalence of the detected protozoan parasites:**

Parasitological findings of the examined tortoises revealed the identification of five protozoan parasites namely; *Entamoeba invadens*, *Cryptosporidium serpentis*, *Nyctotherus* species, *Balantidium* species and *Rickettsia* like organism. The prevalence of

these protozoan parasites was *Entamoeba invadens* 8 (16%), *Cryptosporidium serpentis* was 48 (96%), *Nyctotherus* species 50 (100%), *Balantidium* species 7 (14%) and *Rickettsia* like organism 3 (6%).

In their survey with 1251 tortoises, **Pasmans et al (2008)** found *Nyctotherus* species in 3.4%, in contrary to the current study (100%), *Balantidium* was found in 12.8% nearly similar to the present results (14%) and finally, *Entamoeba* species in 12.8% and *Entamoeba invadens* (2.6%) which is low (16%) in the current study.

The current results related to *Cryptosporidium serpentis*; were much higher (96%) than that obtained by **Rataj et al (2011)** where results were 96% but their results were 61.6% in Corn snakes (16 snakes).

The importance of *Cryptosporidium* came from the work of **Traversa et al (2008)** who confirmed that the potential zoonotic risk of *Cryptosporidium* isolated from reptiles and not only from mammals. **Pasmans et al (2008)** reported that, unlike other animals in which infection with *Cryptosporidium* species is usually self limiting in immunocompetent individuals, Cryptosporidiosis in reptiles is frequently chronic and sometimes lethal.

In addition, the importance of Cryptosporidiosis is explained by **Terrell (2003)** and **Traversa et al (2008)** that Cryptosporidiosis in

companion and captive exotic animals (snakes and lizards) has received particular attention in the present years due to the public health concerns.

Concerning the ciliate protozoa *Balantidium* and *Nyctotherus* species, **Satorhelyi and Sreter (1993)** found both ciliates in 38% of examined tortoises which appeared higher than the current study findings for *Balantidium* (14%) and lower for *Nyctotherus* species (100%).

Rataj et al (2011) found *Balantidium* species in 26.2% of tortoises (including one *Testudo kleinmanni*), while this study found it in 14%.

The importance of the detected *Balantidium* species in tested Egyptian tortoises is due to finding of **Girling and Raiti (2004)** that *Balantidium* species is an important commensal organism but may reach high levels in the presence of gastrointestinal diseases.

The high percentage of ciliates in this study may come in agreement with **Keymer (1978); Holt et al (1979) and Satorhelyi and Sreter (1993)** who reported that the abundance and prevalence of ciliates in tortoises was much higher than other endoparasites. In addition to this high percentage, the tested tortoises were apparently healthy, this concurs with **Telford (1971); Keymer (1978) and Holt et al (1979)** who reported that ciliates are considered slightly pathogenic or non pathogenic at all to reptiles.

Moreover, the high prevalence of *Nyctotherus* in the present tested tortoises without the appearance of clinical signs is declared by **Barnard and Upton (1994) and Greiner and Schumacher (2000)** who reported that *Nyctotherus* and *Balantidium* species were commonly found in herbivorous lizards and also in turtles and snakes, and were not considered as pathogens.

Also, from data shown in Table (1), the prevalence of *Balantidium* (14%) nearly the same as the finding of **Pasmans et al (2008)** who found *Balantidium* species in 12.8% of captive chelonians. The highly pathogenic, *Entamoeba invadens* was found in 16% of examined tortoises during this study, higher than the finding of **Pasmans et al (2008)** 2.6%.

The importance of *Entamoeba invadens* is to be lethal reported by **Kaneene et al (1985)** who attributed the deaths in reptiles at the Detroit Zoo to parasitic infestation with *Entamoeba* species.

Morphological criteria of the protozoon parasites

a- Entamoeba invadens:

Cysts are found in fresh faecal samples and contained four nuclei. The cyst ranging in size from 12-18µm by 9-11 µm in diameter (Fig. 1). The size of the cyst was similar to that obtained by **Pasmans et al (2008)**.

b- Cryptosporidium serpentis

The Modified Ziehl-Neelsen staining technique revealed the

presence of oocysts which ranging in size from 4 to 6 μm in diameter (Fig. 2). The size of the oocyst was similar to that obtained by *Rataj et al (2011)*.

c- *Nyctotherus* species:

The trophozoite was oval in shape with a thickening of the wall at one end where there is a nipple-like protuberance. The anterior extremity is more pointed than the posterior one. Length is 98-121 μm and width 62-87 μm (Fig. 3). The size of the trophozoite was similar to the unidentified species obtained by *Rataj et al (2011)*.

d- *Balantidium* species:

Balantidium cysts are spherical and covered with a heavy cyst wall

about 2 μm in thickness. The size observed was 41 μm to 75 μm . (Fig. 4). The small micronucleus usually occupies a space at one side and near the middle of the macronucleus. The size of the cyst was nearly similar to the unidentified species recorded by *Satorhelyi and Sreter (1993)*.

e- *Rickettsia* like organism:

A *Rickettsia*-like organism was found within erythrocytes. Multiple invasions of cells were common, the organism was small and reddish dots or rods with a large range of morphological forms (Fig. 5). It measured 2-3 μm , the size of this protozoan was similar to that obtained by *Barry (2011)*.

Table (1): The prevalence of detected protozoan parasites in examined tortoises

Protozoan species	No. of examined tortoises	No. of infested tortoises	Prevalence %
<i>Entamoeba invadens</i>	50	8	16%
<i>Cryptosporidium serpentis</i>	50	48	96%
<i>Nyctotherus</i> species	50	50	100%
<i>Balantidium</i> species	50	7	14%
<i>Rickettsia</i> like organism	50	3	6%

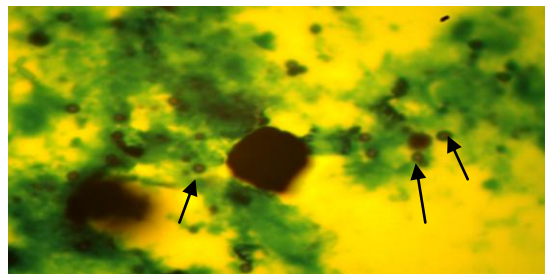
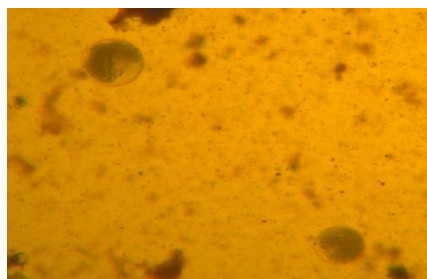
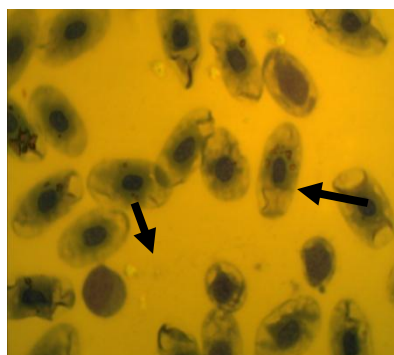


Fig. (2): *Cryptosporidium serpentis* oocyst

Fig. (1): Cyst of *Entamoeba invadens*

Fig. (3): *Nyctotherus* species trophozoiteFig. (4): *Balantidium* speciesFig. (5): *Rickettsia* like organism in RBCs

Recommendations:

- 1- Parasitological examination should be regularly done for Egyptian tortoises.
- 2- Egyptian tortoises should be provided with clean suitable food and improvement of their environmental conditions to minimize stress factors which enhance parasitic infestations.
- 3- Regular usage of suitable anthelmintic drugs in pet tortoises.
- 4- Public awareness should be considered during handling of pet tortoises due to the zoonotic importance of some parasites such as *Cryptosporidium*, *Entamoeba* and *Balantidium*.

Conclusion:

In this study, all the 50 tested Egyptian tortoises were apparently healthy, although, they were collected from different localities in Egypt and vary wildly in management. This agreed with *Ellis and Seal (1996)* who reported that, the wild animals become very susceptible to many sorts of infection in captivity. So Egyptian tortoises should be provided with clean suitable food and improve their environmental conditions. Regular parasitological examination for Egyptian tortoises should be carried out. Finally, public health awareness should be considered during handling the pet tortoises

because of the zoonotic importance of *Cryptosporidium*, *Entamoeba* and *Balantidium* infestation.

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الملخص العربي

مدى إنتشار الإصابة ببعض الأوليات في السلاحف المصرية (تستودو كلاينماني)

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تم تجميع ٥٠ سلحفاة من السلاحف المصرية و كانوا بحالة صحية ظاهرية جيدة من مناطق مختلفة في مصر ، بورسعيد ، سوق السيدة عائشة ، الإسماعيلية ، القاهرة و الجيزة لمتاجر الحيوانات الأليفة. كشف الفحص الطفيلي عن وجود خمسة أنواع من الأوليات ، كان معدل الإصابة بها كالتالي: إنتاميبا إنفيدانس في ٨ من أصل ٥٠ سلحفاة (١٦ ٪) ، و عثر على الكريبتوسبورديوم سيربينتس في ٤٨ من أصل ٥٠ سلحفاة (٩٦ ٪) ، و نوع نيكتوثيراس في جميع السلاحف من أصل ٥٠ سلحفاة (١٠٠ ٪) ، تم العثور على نوع البلانتيديوم في ٧ من أصل ٥٠ سلحفاة (١٤ ٪) و تم العثور على كائنات شبيهة بالريكتيسيا في ٣ من أصل ٥٠ سلحفاة (٦ ٪) . و توصي الدراسة بضرورة تحسين بيئة السلاحف في الأسر و الفحص الدوري الطفيلي لها. كما توصي الدراسة بضرورة توخي الحذر عند التعامل مع هذه السلاحف لوجود بعض الطفيليات الأولية التي تنتقل من هذه السلاحف إلى الإنسان مسببة أمراضا خطيرة له و التي تم عزلها أثناء هذه الدراسة مثل إنتاميبا و الكريبتوسبورديوم و البلانتيديوم.