PREVALENCE OF GASTROINTESINAL PARASITES AMONG IMPORTED CAMELS WITH A TRIAL OF LARVAL ELECTROPHORETIC AND MORPHOLOGICAL DIFFERENTITAION

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ABSTRACT

On examination of 929 samples from imported camels from Geboty for gastrointestinal parasites, 82.9 % were found infected with two *Eimeria* species (*E.rajasthani & E.cameli*) and 3 nematode eggs (*Nematodirus sp. Trichuris sp* and a *Trichostrongyloid* egg *sp.*).The infection was common in all seasons but prevalent in autmn. Two morphologically identified third stage larvae recovered from similar *Trichostrongyloid* eggs were differentiated through electrophoresis proving it as a tool of diagnosis of 3rd Stage larva.

INTRODUCTION

With the excessive demand of subsidiary resources of animal protein other than beef and mutton, the number of imported camels increased especially with their considerable cheap prices and having no record of BSE (Bovine Spongiform -Encephalopathy. Imported camels are subjected to general examination including parasitological one, which reveals that camels are liable to many species of gastrointestinal nematodes and *Eimeria* with varied rates. Recently in Egypt, **El-Manyawe & Iskander (1994)** recorded 6 nematode egg species and 2 *Eimeria* species from camel then, **Morrsy (1997)** described 4 species of *Eimeria* from camel. While **Wahba and El-Refaii (2003)** found one *Eimeria* species besides 10 species of nematode larvae, also **Eid (2002)** recorded 10 genera of Trichostrongyloid larvae. Both single and mixed infection with Trichostrongyloids was recorded (**Njiru *et al.*, 2002**). This study was to record the gastrointestinal parasites infecting imported camels in different seasons and diagnosis of 3rd Stage larvae of simialr egg pattern morphologically and electrophoretically.

MATERIAL AND METHODS

929 fecal samples from imported camels were examined for gastrointestinal parasites using floatation and sedimentation technique. Positive fecal samples for nematodes other than *Nematodirus* and *Trichuris* were cultured for 1 week to collect 3rd stage larvae. The recovered larvae were collected by Baremann technique. The unsporulated eimerian oocysts were kept in 2.5% potassium dichromate for sporulation (**Soulsby, 1986**). All detected eggs as well as oocysts were identified. The more frequent larvae were morphometrically identified following (**Eckert, 1989**) and (**Borgsteede & Hendriks, 1974**) and collected under binocular dissecting microscope then kept each species separately with equal volume of (phosphate buffer saline) PBS, labeled and frozen at (-20 °C) for electrophoresis analysis.

Electrophoretic differentiation of 3rd stage larvae using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): The two more frequent identified *Trichostrongyloid* larvae species were sonicated and prepared for SDS-PAGE using the discontinuous system of **Laemmli (1970)**. The gel used consisted of a stacking gel (4 % acrylamide) and a separating gel (10 % acrylamide). Samples were denatured by boiling for 90 second in Laemmli sample buffer and electrophoresed at room temperature at a constant voltage of 120 V in a running buffer. The molecular weights of the larva proteins were estimated by comparing their electrophoretic mobilities with standard molecular weight marker (SIGMA) after electrophoresis in the same gel.

RESULTS

Table 1 summarized the results of parasitological examination of 929 fecal samples from imported camels to Egypt. The total infection rate was 82.9% existing in all seasons but little higher in autmn (88.4%) followed by winter, spring and summer.

Two *Eimeria* species and three nematode eggs on the generic level were detected. The first *Eimeria* species (*E.rajasthani*) was found more frequent than the other species and sporulated in 3-5 days. The unsporulated oocyst (plate1,A) was ovoid in shape and averaged 31 x 26.5um in size, pale brown in color . double layered wall with a micropyle. and a micropylar cap. The sporulated oocyst (plate 1,B) contains 4 sporocysts each was ellipsoidal and averaged 11.3 x 8.1um . Both steida body and sporocystic residium were present. The other *Eimeria sp.* was *E cameli* (plate1, C) was found in too few number to sporulate. The unsporulated oocyst was truncated, blackish to dark brown with three layered wall and of large size measuring 85.2 x 67.1 um in average.The

Trichostrongyloid egg sp. (plate 1, D) was light brown measuring 70- 90 x 45-57.5 um mean (75.3 x 50.5 um). The *Nematodirus* egg *sp.* (plate 1, E) was distinguished by its large size (184 x 73.6 um) and having 8 brown embryonic cells. The *Trichuris* egg *sp.* (plate 1.F) was brown in color and barrel in shape with bipolar plug. It measured 62.5-72.5 x 27-32.5 um avareged 67.5 x 30.4 um. Many 3rd larval stage species were encountered but 2 species were chosen for electrophoresis trial as those two species were frequently more than other species and can be collected and distinguished from each other . 3rd stage larvae of *Trichostongylus sp.* (plate 2, A&B) averaged 619.6 um in total length and 34.3 um in maximum width containing 16 triangular intestinal cells and its tail sheath was 37.5 um in average. The other 3rd L.S was *Cooperia sp.* (plate 2 C&D) measured 702 um in length average and 25.6 um in maximum width , containing 16 intestinal cells and considerably long tail sheath measuring 66.5 um in average.

The first group of 3rd stage larvae (plate 3: 1,1) *Trichostrongylus sp.* revealed indistinctive bands greater than 205 Kd in addition to three distinctive bands, one between 97.4 and 116 Kd, second at 97.4 and the third one at 45 Kd. The second group larvae (plate 3: 2,2) (*Cooperia sp.*) gave two indistinctive bands greater than 205 Kd, in addition to two distinctive bands, the first at 97.4 Kd and the second between 97.4 Kd and 66 Kd.

DISCUSSION

Increase in consumption of camel meat associating with rising import of such animals gives a good opportunity of transmission of the incoming parasites to the native habitat. Besides the effect of the parasites on the general status of the animals, nematodes have been incriminated in haematological and biological changes in the infected animals **(Haroun *et al.*, 1996)**. In the present study, the total infection rate was 82.9% ranged from 75.8% in summer to 88.4% in autumn supporting the positive correlation between rainfall intensity of infection with gastrointestinal nematodes in countries like Sudan and South eastern African area **(Fadli *et al.*, 1992)**. The present rate is lower than 93.3% recorded by **Abdel-Wahed *et al.*, 1995**) which probably due to the low number of examined animals (30). In the present study Infection with *Eimeria* species was (3.9%) which is lower than what recorded either from native camels (40%) by **Morrsy (1997)** or from imported camels (31.4%) by **Wahba & El-Refaii (2003)**. Although the infection was low but spread all over the year with a little higher rate in summer 4.7% as recorded by **Morrsy (1997)** where the highest rate was in August 76.5%. This variation could be due to type of species detected. As in 2003 no infection was recorded in native animals and only one

species was found (*E.dromedari*) from imported ones according to **Wahba & El-Refaii**. In the mean time, the present species were *E. rajasthani & E. cameli* detected previously in native camels **(Morrsy, 1997)**. Concerning the helminthic infection, Single infections were higher than mixed ones and *Trichostrongyloid* sp. predominate the rest of helminthes in all seasons as indicated by **Najiru *et al.*, (2002).** The highest infection rate with *Nematodirus sp.* eggs was 3% in winter and total infection rate was 1.7% which is lower than the record in 1994 (3.7%) in native camel. **(El-Manyawe & Iskander)** and 3.3% in imported camel **(Wahba & El-Refaii 2003)**. The infection with *Trichuris sp.* eggs was 1.8% with highest rate in winter 4% lower than what recorded either by **El-Manyawe & Iskander, (1994)** (4.6%) or by **Wahba & El-Refaii (2003)** (6.6%). Meanwhile, **Wahba & El-Refaii (2003)** record neither *Nematodirus* nor *Trichuris sp* eggs from native camels. 3rd S.L of *Trichostrongylus* and *Cooperia* were prevalent other species of nematodes which supported by **(Eid, 2002)** and **(Najiru *et al.*, 2002).** the morph metric data of the present species of eggs or larvae are in the range given by the previous authors **(Abdel-Gawad, 1974; El-Manyawe & Iskander, 1994; Eid, 2002** and **Wahba & El-Refaii, 2003)**.The variation in the parasitic fauna from one year to the other indicate the necessity of the regular examination of the imported animals to protect native ones.

The trial for electrophoretic differentiation between the two chosen identified *Trichostrongyloid* larvae succeeded in demarcation between them. The pattern of both groups denoted to have the same kind of protein (97.4 Kd) which is common cuticular protein of parasitic nematodes **(Kennedy & Harnett, 2001)**. Proteins bands less than 97.4 Kd in *Cooperia spp.* (Plate 3: 2,2) is somewhat closed to that given for the cysteine proteases enzymes of *Hemonchus, Trichostrongylus colubriformis* and *Ostertagia sp.* **(Jesmer *et al.*, 1993)**. While for *Trichostrongylus sp.* (Plate 3: 1,1) there is a band between 97.4 and 116 (about 108 Kd) which is most likely a contortin-like protein (110 Kd) that of *Hemonchus contortus*. But the occurrence of the 45 Kd band is differentiating the *Trichostrongylus* from *Hemonchus* larvae which is also could be considered as one of the gut enzymes for *Trichostrongylus* larvae. Consequently, the electrophoretic analysis of larvae from morphologically similar eggs of the Trichostrongyloid species is applicable. These results are of value from not only the biochemical criteria but also to the immunological intervention by vaccine.

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Table (1): Seasonal prevalence of internal parasites infesting imported Camels to Egypt.

Season	Tricchostrongyloid	Eimeria	Trichuris	Nematodirus	Trichostrongyloid + Trichuris	Trichostrongloid+ Eimeria	Trichostrongyloid+ Trichuris + Eimeria	Trichostrongyloid + Nematodirus + Eimeria	Negative	positive	Total Number Examined
Winter	129	8	8	6	7	6	-	3	31	167	198
%	65	4	4	3	3.5	3	-	1.5	15.7	84.3	
Spring	124	8	5	1	4	21	-	-	43	163	206
%	60	3.9	2.4	0.49	1.9	10.2	-	-	20.9	79.1	
Summer	99	9	2	5	8	15	6	-	46	144	190
%	52	4.7	1.1	2.6	4.2	7.9	3.2	-	24.2	75.8	
Autumn	218	11	2	4	23	35	3	-	39	296	335
%	65.1	3.3	0.6	1.2	6.9	10.4	0.90	-	11.6	88.4	
Total	570	36	17	16	42	77	9	3	159	770	929
%	61.4	3.9	1.8	1.7	4.5	8.3	0.97	0.3	17.1	82.9	

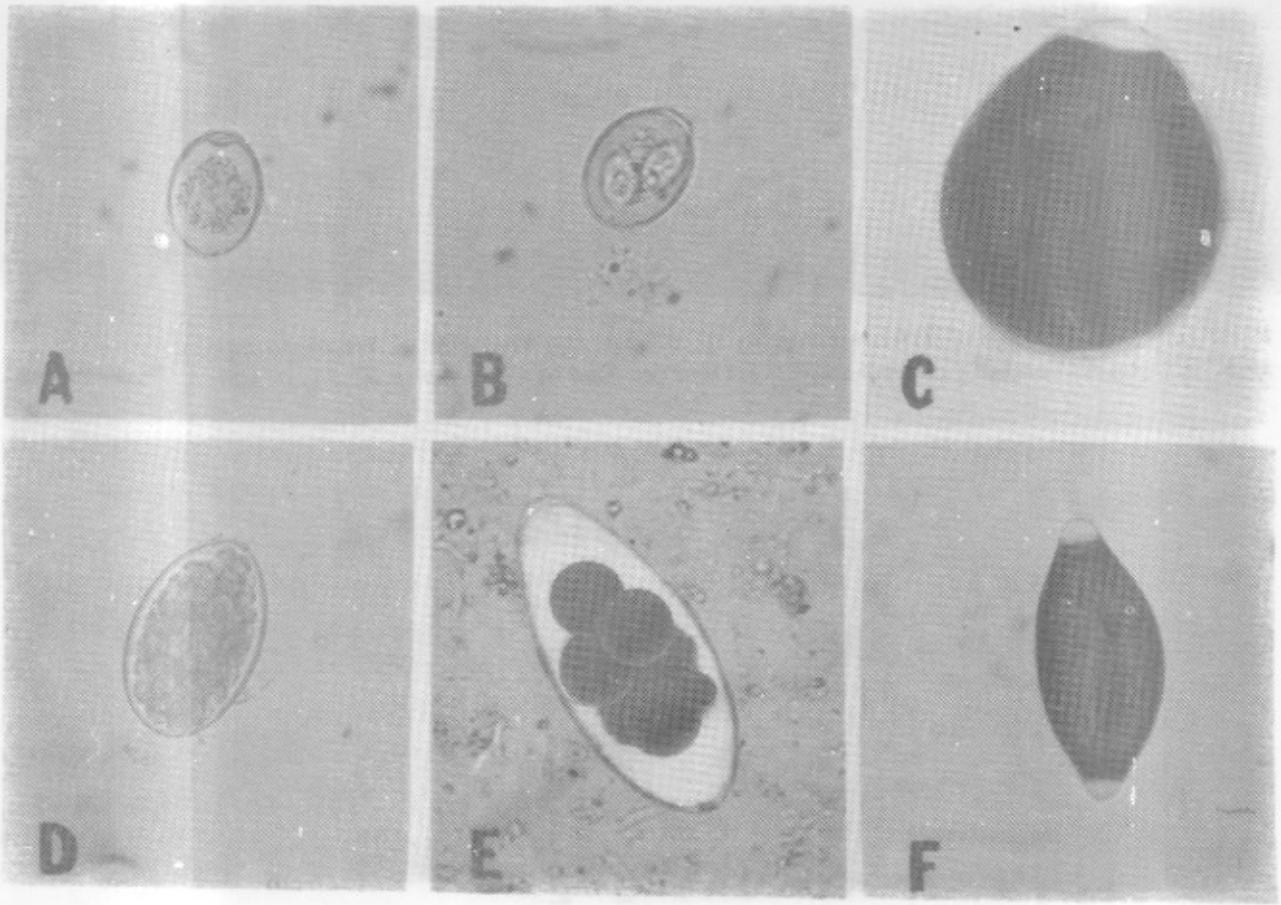

Plate (1): (A) Unsporulated oocyst of *E. rajasthani* (B) sporulated oocyst of *E.rajasthani* (C) Unsporulated oocyst of *E. cameli* (D) *Trichostrongyloid sp.*egg. (E) *Nematodirus sp.* egg (F) *Trichuris sp.* egg (x 400) D&E (x100)

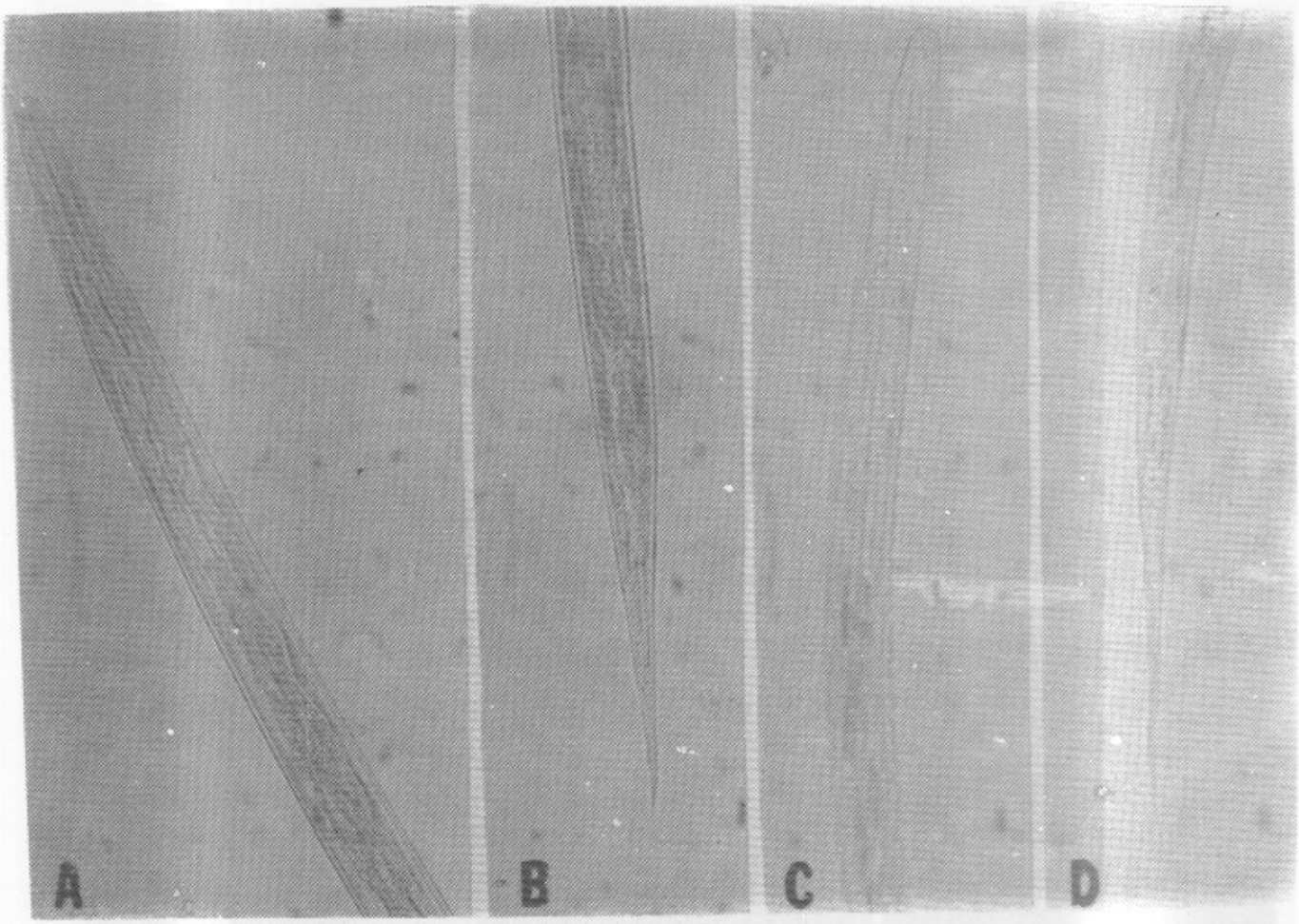

Plate (2): (A) Ant. end of *Trichostrongylus sp.* 3rd S.L (B) Post end of *Trichostrongylus sp.*3rd S.L (C) Ant end of *Cooperia sp.*3rd S.L (D) Post. End of *Cooperia sp* 3rd S L (x 400)

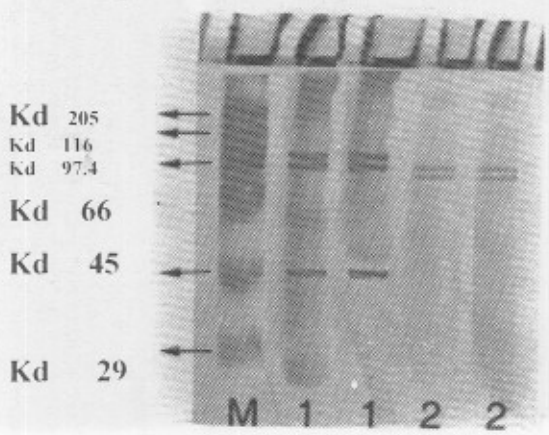

Plate (3): (1,1) *Trichostrongylus sp.* (2,2) *Cooperia sp.*

الملخص العربى

عند فحص عدد 929 عينة براز للطفيليات المعدمعوية من جمال مستوردة من جيبوتى وجد ان 82.9% مصابة بنوعين من الأيميريا وثلاثة انواع من بويضات الديدان الخيطية. شاعت الاصابة فى كل فصول السنة ولكن بنسبة اكبر فى الخريف تم التمييز بين نوعين من الطور اليرقى الثالث ناتجين من زرع بوضات ترايكوسترونجلويد متشابهة باستخدام الفصل الكهربى مما يجعلها طريقة اضافية للتشخيص بجانب التشخيص الوصفى