New-Valley Lab.,
Animal Health Research Institute, ARC, Egypt

# A PRELIMINARY STUDY ON CRYPTOSPORIDIOSIS IN DROMEDARY CAMELS AT SHALATIN AREA, EGYPT

(With 2 Tables & One Figure and One Photo)

By

***M.A. SALEH and O.M. MAHRAN****

* Shalatin Lab., Animal Health Research Institute, ARC, Egypt

(Received at 17/12/2006)

## دراسة مبدئية علي الإصابة بالكريبتوسبوريديا في الإبل وحيدة السنم في منطقة شلاتين بمصر

***مصطفى أحمد صالح ، عثمان محمد مهران***

تصيب الكريبتوسبوريديا الطبقة الهدبية للغشاء الطلائي المبطن لجدار المعدة والأمعاء في الفقاريات ولها أهمية خاصة على الصحة العامة للإنسان. بالرغم من ذلك لا توجد بيانات متاحة لإصابة الجمال وحيدة السنم بهذا الطفيل. لذلك يهدف هذا العمل إلى التعرف على طفيل الكريبتوسبوريديا ومدي انتشاره في الجمال وحيدة السنم وتقييم بعض الخصائص البيوكيميائية في مصل دم الجمال المصابة كمؤشر للخطر الذي تسببه العدوى على صحة هذه الجمال. وبعمل مسح ميكروسكوبي على عينات براز من ١٠٩٧ من الجمال وحيدة السنم التي يتراوح عمرها من أقل من ٦ أشهر إلى أكثر من ٨ سنوات تبين أن ٣٧ (٣.٣٧%) من هذه الجمال كانت إيجابية لحويصلات الكريبتوسبوريديا وذلك باستخدام صبغة الزيل نيلسين المعدلة. وأوضح معامل الانحدار الخطي وجود علاقة إيجابية بين عمر الجمال ومدى الإصابة. وقد أوضح الفحص الميكروسكوبي للمسحات الصامدة للحمض وجود حويصلات بيضاوية محببة محاطة بطبقة مفردة تأخذ اللون الأحمر أو الوردي. وكان متوسط حجم هذه الحويصلات ٨.٣±١.٢٢ x ٦.١±٠.٨٨ ميكروملليلتر. هذة الخصائص الشكلية تتماشى مع المواصفات الخاصة بنوع الكريبتوسبوريديا ميورس. لذلك يمكن مبدئيا تسمية هذه الحويصلات "الكريبتوسبوريديا الشبيهة بالميورس". وقد تم عمل التحاليل البيوكيميائية لمصل دم عدد ٨ من الجمال المصابة وعدد ٨ جمال سليمة إكلينيكيا (يتراوح عمرها من ٣-٧ سنوات وخالية من الطفيليات الأخرى) وأوضحت النتائج نقص معنوي في متوسط (± خطأ معياري) تركيز الألبيومين (٢.٨٨٨ ±٠.١٠٤ ضد ٣.١٨٦±٠.٠٩١ جم /١٠٠ملليلتر بمستوى معنوية ٠.٠٤٩) الألفاتوكوفيرول (١.٣٥١±٠.٠٩٣ ضد ١.٦٥٨ ±٠.٠٨٢ مكجم /١٠٠ملليلتر بمستوى معنوية ٠.٠٠٣) ، بينما تضاعفت قيمة البيبسينوجين (٨٦٦.٥±٤٦.٤٢ ضد ٤٠٦.٢ ± ٣٢.٦١mU بمستوى معنوية ٠.٠٠٩) وذلك في الجمال المصابة مقارنة بالمجموعة السليمة. ومن ناحية أخرى كانت هناك تغيرات غير معنوية في متوسطات تركيز البروتين الكلي والجلوبيولين والصوديوم والبوتاسيوم والكلوريد في الجمال المصابة مقارنة بالمجموعة السليمة. وبذلك يمكن أن نستخلص من هذه الدراسة أن الجمال وحيدة السنم حساسة للإصابة بالكريبتوسبوريديا مع وجود خطر معنوي علي صحتها. وعليه يجب النظر إلى هذه الدراسة

على أنها خطوة نحو تمييز الكريبتوسبوريديا ميورس كمسبب للإلتهاب المعدي في الجمال وحيدة السنم. وذلك يتطلب دراسات أخرى للتعرف على الطفيل وإيضاح خط سير المرض الذي يسببة.


## SUMMARY

Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium* species that infect the microvillus border of the gastrointestinal epithelium of a wide range of vertebrates with a sparked great public health interest in humans. There are no available reports on cryptosporidiosis in dromedary camels. This work aimed to identify the cryptosporidial oocyst and its prevalence in dromedary camels and to estimate some serum biochemical characteristics in infected camels as an index of the risk arisen from the infection on the health of dromedaries. Microscopic survey on faecal samples from 1097 dromedary camels (aged from <6 months up to >8 years) revealed that 37 (3.37%) were positive for *Cryptosporidium* oocysts by using modified Ziehl-Neelsen stain. Linear regression analysis was positive between incidence of cryptosporidial infection and age. Microscopic examination of the acid-fast stained faecal smears revealed ovoid oocysts with single layer wall stained red or pink with a granular appearance. The average size (±SE) of the oocysts was 8.3±1.22 x 6.1±0.88 µm. These morphological characters fit the description of *C. muris*. So, these oocysts could be primarily called *Cryptosporidium muris* like oocysts. Serum biochemical analysis of 8 infected and 8 age-matched apparently healthy camels (3-7 years, free from other parasites) revealed significant reduction in the mean concentrations (±SE) of serum albumin (2.89±0.104 vs 3.19±0.091 g/dl, P=0.049) and α-Tocopherol (1.35±0.093 vs 1.66±0.082 µg/ml, P=0.009), whereas serum pepsinogin was doubled (866.5±46.42 vs 406.2±32.61 mU, P=0.003) in infected camels in comparison with controls. On the other hand, there were non-significant variations in the mean values of blood serum total protein, globulin, sodium, potassium and chloride of infected camels in comparison with controls. In conclusion, dromedary camels are susceptible to cryptosporidial infection with significant risk on their health. The present study should be regarded as a first step towards recognition of *C. muris* as a possible cause of gastritis in dromedary herds. More studies are needed for more identification of the parasite and to clarify its pathogenicity.


***Key words:*** *Cryptosporidiosis, dromedary camels, serum proteins electrolytes, pepsinogn, Egypt*

## INTRODUCTION

*Cryptosporidium* spp. are apicomplexan protozoan parasites of humans and animals (Xiao *et al.,* 2004). Unlike other coccidian parasites, cryptosporidia are found only in the microvillus border of the gastrointestinal epithelium (Ramirez *et al.,* 2004).

Two main *Cryptosporidium* spp. infecting mammals have been early reported: *C. muris* (Tyzzer, 1910) and *C. parvum* (Tyzzer, 1912). *C. muris* (*C. andersoni*) resides in the stomach of non ruminants and was discovered in the abomasum of feedlot cattle (Anderson, 1987). Unlikely, *C. parvum* is a parasite of the small intestine and it is usually the zoonotic genotype that can cause gastroenteritis in animals and humans (Morgan *et al.,* 1998). Recently *C. andersoni* n. sp. was isolated from the abomasum of cattle (Lindsay *et al.,* 2000 and Fayer, *et al.,* 2005).

The prevalence of *C. parvum, C. muris and C. andersoni* infection in ruminants and non ruminants has been reported elsewhere (Chalmers *et al.,* 2002; Dubey *et al.,* 2002; Enemark *et al.,* 2002; Kvac and Vitovec, 2003; Xiao *et al.,* 2004; Fayer *et al.,* 2005 and Masuno *et al.,* 2006). In Egypt, the prevalence of *C. parvum* was reported in ruminants (Nassif *et al.,* 2002 and El-Dessouky & El-Masry, 2005).

Pathophysiological studies on bovine and ovine intestinal cryptosporidiosis (*C. parvum*) revealed haematological changes and variations in serum proteins and electrolyte concentrations (Molina *et al.,* 1994; Nassif, *et al.,* 2002 and El-Dessouky & El-Masry, 2005). On the other hand, abomasal cryptosporidiosis (*C. muris*) has been associated with chronic weight loss in mountain gazelles and cattle (Pospischil *et al.,* 1987 and Anderson, 1987) and a detrimental effect on mean daily milk production in dairy cattle (Esteban and Anderson, 1995).

Studies of Anderson (1991) and Fayer *et al.* (1991) identified *C. muris* from bacterian camel. There are no available reports on the cryptosporidial infection in dromedary camels. The present work aimed to evaluate the prevalence of cryptosporidial infection in dromedary camels at Shalatin area with a preliminary identification of the cryptosporidial oocyst depending on microscopic morphological characteristics, in addition to the evaluation of the effect of the parasite on some health indices in camels by the determination of some serum biochemical characteristics including the concentrations of serum proteins, electrolytes, vitamin E and pepsinogin in the affected dromedaries.

## MATERIALS and METHODS

**Study area:**

The study was carried out at Shalatin City, Red Sea Governorate, Egypt. This area is a part of the Egyptian eastern desert and is considered the southern east border of Egypt. It is classified as a dry arid desert zone. The temperature ranges from 20-46°C. The watering in this area depends mainly on ground wells. The number of camels in this area (40 thousands) constitutes the third of the total number (133 thousands) of camels in Egypt (GOVS, 2005).

**Animals:**

The study was carried out during the period extended from November 2005 to march 2006. A total of 1097 dromedary camels of both sexes at different ages were examined for the detection of cryptosporidial oocysts. According to age, the faecal samples were classified into six categories from less than six months up to more than 8 years (Table 1).

**Sampling:**

**A-Faecal sample:** Faecal specimens were collected directly from the rectum of each animal into labeled screw- top specimen containers, which were placed in an insulated portable cooler, taken to the laboratory within few hours of collection and stored at 4°C until examined within 48 h. The consistency of the samples was scored as diarrhoeic or non-diarrhoeic.

**B-Blood samples:** Blood samples were collected in vacutainer tubes without anticoagulant from 8 camels (3-7 years) infected with cryptosporidiosis. The selected camels contained >2 oocysts per field and was considered as moderately to severely infected individuals (Peeters and Villacorta, 1995). These camels were not affected with diarrhoea and free from other GIT parasites. Another 8 blood samples were collected from age-matched apparently healthy and parasite free camels which were selected as controls. Serum was separated by centrifugation at 2000 rpm for 10 minutes. Serum samples were stored in clean disposable plastic tubes and frozen at -20°C until used.

**Parasitological investigations:**

Faecal specimens were analysed for the presence of *Cryptosporidium*. Smears prepared from the faecal samples were stained by the modified Ziehl–Neelsen technique described by Henriksen and Pohlenz (1981). Microscopic slide floatation (wet mount) examination was carried out by Sheather's sugar flotation procedure as described by Georgi and Georgi (1990).

Other GIT parasites were also examined by the direct floatation sedimentation techniques (Georgi and Georgi, 1990) to select parasite free individuals (as control group) or those harbouring only cryptosporidial infection (cryptosporidial infected group). The selected groups were used for further biochemical assay.

The identification of *Cryptosporidium* oocysts depended on the correct morphology, optical properties, internal structure, size and shape (Upton and Current, 1985 and Peeters and Villacorta, 1995). The size of oocysts was measured using eyepiece micrometer and illustrated by photomicrographs.

**Serum biochemical analysis:**

Blood serum was used for determination of total protein and albumin (Henry *et al.,* 1974). Globulin concentration was calculated mathematically by the difference between total protein and albumin. Sodium and potassium were estimated by using flame photometer and chloride by using chloride meter. Colourometric methods were used for determination of pepsinogen (Scott *et al.,* 1995) and α-tocopherol (Quaife *et al.,* 1949).

**Statistical analysis:**

Firstly, general linear model Analysis of Variance (GLM-ANOVA) was performed on the pooled biochemical data using SPSS 10 software package (SPSS, Chicago, IL) according to Borenstein *et al.* (1997) and SPSS (1999). Least Square Means (LSM) were compared with comparison-wise standard error rate. Student "t" test was used to compare the differences between the dependent means. Linear equation and linear Regression Analysis (LRA) was performed between the incidence of cryptosporidial infection and the age of camels. The regression factor ($R^2$) and the significance level (p) presented the linear regression analysis.

## RESULTS

**Prevalence of *Cryptosporidium* oocyst:**

Microscopic analysis of 1097 faecal specimens revealed that 37 (3.37%) were positive for *Cryptosporidium* oocysts. Based on detection of oocysts, the prevalence of infection for camels with diarrhoea was 2.30% (4 out of 147) and for those without diarrhoea was 3.47% (33 out of 950) as shown in Table (1).

**Table 1:** Prevalence and effect of age of camels on faecal excretion of cryptosporoidal oocysts.

| Age categories (Years) | Camels with diarrhoea | | | Camels without diarrhoea | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exam | Infec. | % | Exam | Infec. | % | Exam | Infec. | % |
| <0.5 | 26 | 0 | 0.00 | 98 | 3 | 3.06 | 124 | 3 | 2.42 |
| 0.5-1 | 18 | 1 | 5.56 | 87 | 2 | 2.30 | 105 | 3 | 2.85 |
| 1-2 | 34 | 1 | 2.94 | 261 | 8 | 3.07 | 295 | 9 | 3.05 |
| 2-5 | 21 | 0 | 0.00 | 253 | 10 | 3.95 | 274 | 10 | 3.65 |
| 5-8 | 32 | 1 | 3.13 | 178 | 7 | 3.93 | 210 | 8 | 3.81 |
| >8 | 16 | 1 | 6.25 | 73 | 3 | 4.11 | 89 | 4 | 4.49 |
| Total | 147 | 4 | 2.30 | 950 | 33 | 3.47 | 1097 | 37 | 3.37 |

The results showed that age had a marked effect on the prevalence of cryptosporidial infection (Table 1). Linear equation as assessed by the linear regression analysis (LRA: $y = 0.395x + 1.996$) indicated a positive linear relationship between the incidence of cryptosporidial infection and age (Fig. 1). The recorded regression factor value ($R^2$=0.976) between the incidence of cryptosporidial infection and age was highly significant (P<0.001).

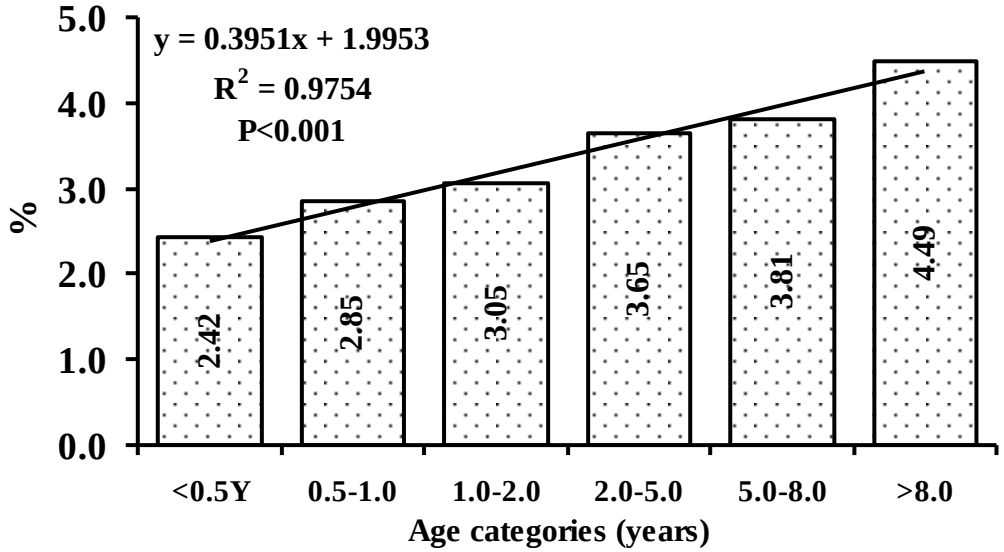


**Fig. 1:** Linear regression analysis as assessed by the linear equation, regression factor ($R^2$) and the significance level (p) showing the effect of age on the prevalence of cryptosporidiosis in dromedary camels

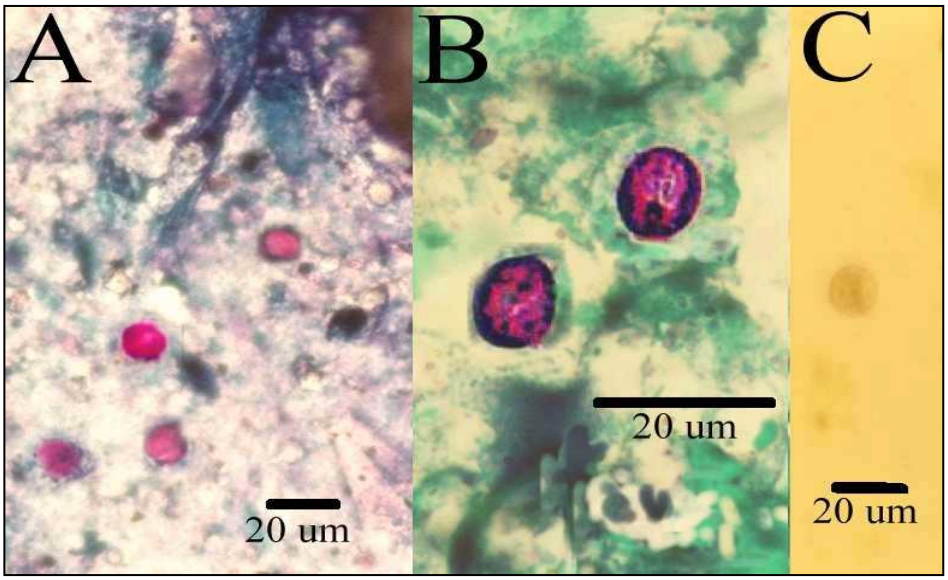


**Photo 1:** Faecal smear showing *Cryptosporidium muris* like oocyst stained by modified Zielhl-Neelsen stain (a x40, bx100), Unstained *C. muris* like oocyst (c x40).

**Oocysts morphology:**

Microscopic examination of the acid-fast stained faecal smears revealed ovoid oocysts (Photo 1) that were an average size of 7.5-10.1 x 5.5-7.3 μm with a mean ± SD of 8.3±1.22 x 6.1±0.88 μm (n=36). The oocysts were ovoid with single layer wall, stained red or pink with a granular appearance against a green background. The sporozoites were clearly visible (4-8 sporozoites) inside the oocyst. The mean ± SD of length to width ratio was 1.36 ± 0.041 (1.21-1.52).

**Serum biochemical findings:**

Serum biochemical analysis (Table 2) revealed significant reduction in the mean concentrations ±SE of blood serum albumin (2.89±0.104 vs 3.19±0.091 g/dl, P=0.049) and α-Tocopherol (1.35±0.093 vs 1.66±0.082 μg/ml, P=0.003) and significant elevation in the mean concentration of pepsinogin (866.5±46.42 vs 406.2±32.61 mU, P=0.009) of infected camels in comparison with controls. On the other hand, there were non significant variations in the mean (±SE) values of blood serum total protein (6.35±0.173 vs 6.50±0.181 g/dl, P=0.497), globulin (3.46±0.138 vs 3.32±0.144 g/dl, P=0.841) sodium (139.9±2.11 vs 142.1±2.01 mmol/l, P=0.447), potassium (4.65±0.102 vs 4.5±0.097 mmol/l, P=0.311) and chloride (95.8±2.101 vs 97.1±1.889 mmol/l, P=0.501) of infected camels in comparison with controls (Table 2).

**Table 2:** Serum biochemical changes (means± SE) in cryptosporidial infected camels

| Serum constituents | Infected camels | Control camels | P-value |
|---|---|---|---|
| Total protein (g/dl) | 6.351±0.173 | 6.501±0.181 | 0.497 |
| Albumin (g/dl) | 2.888±0.104 | 3.186±0.091 | 0.049* |
| Globulin (g/dl) | 3.463±0.138 | 3.315±0.144 | 0.841 |
| Sodium (mmol/l) | 139.86±2.11 | 142.11±2.01 | 0.447 |
| Potassium (mmol/l) | 4.651±0.102 | 4.497±0.097 | 0.311 |
| Chloride (mmol/l) | 95.79±2.101 | 97.12±1.889 | 0.501 |
| Pepsinogin (mU) | 866.5±46.42 | 406.2±32.61 | 0.009** |
| α-Tocopherol (μg/ml) | 1.351±0.093 | 1.658±0.082 | 0.026* |

# DISCUSSION

The epidemiology of cryptosporidiosis has been dynamically changing over the past decade. The emergence of cryptosporidiosis in animals, including parasite biology, environmental spread, and livestock production trends, presence of new animal hosts and potential risk of transmission from animals to humans was recently highlighted (Ramirez *et al.,* 2004 and Xiao *et al.,* 2004).

In the current work, 3.37% (37 out of 1097) of dromedary camels were shedding cryptosporidial oocysts. By tracing the available literature, there were no cited reports on cryptosporidial prevalence in camels. However, several reports had evaluated the prevalence of cryptosporidiosis in other ruminants. Anderson (1991) found that the prevalence of *C. andersoni* within cattle populations in the USA appeared to be low (1.4%). More recent studies have demonstrated overall prevalences below 5% in cattle irrespective of age (Fayer *et al.,* 2000; Wade *et al.,* 2000). Higher prevalence was reported by Enemark *et al.,* (2002) who found that 28.0% of cattle excreted *C. andersoni*, while 16.7% were positive for *C. parvum*. McAllister *et al.,* (2005) found that the incidence of *C. muris*, and *C. parvum* in cows were 10.6%, and 18.4% respectively. These differences possibly related to the variation in the parasite species, host susceptibility, methods of investigation, management and environment (Tzipori and Ward, 2002). It was noticed that the prevalence of cryptosporidiosis was not related to diarrhoea in camels. The prevalence was 2.30% in camels with diarrhoea whereas it was 3.47% in camels without diarrhoea. These results differ than those reported for animals infected with *C. parvum*, in which diarrhoea is a cardinal sign due to intestinal hyper-motility (Enemark *et al.,* 2003 and Lise *et al.,* 2005). However, our results concur with the

reports of Anderson (1998) and McAllister *et al.* (2005) which clarified that the infection with *C. muris* is primarily in the abomasum and the detection of oocysts in faeces was not necessarily correlated with diarrhoea.

Several studies had explored the relationship between cryptosporidial infections and age of the host. *C. parvum* was detected in ruminant intestine in the first few weeks of live indicating that the infection occurred in the neonatal period (Nydam *et al.,* 2001). The percentage distribution of positive *C. parvum* samples is negatively correlated with increase in age (Olson *et al.,* 2004). *C. muris* however, can persist for months and even years in the abomasums of weaned or adult animals causing chronic abomasal infection (Esteban and Anderson, 1995). The shedding of the oocysts persists in older animals is continued until slaughter (Anderson, 1998 and Enemark *et al.*, 2002). The positive linear relationship between the incidence of cryptosporidial infection and age in the present work agrees with the findings of Esteban and Anderson (1995), Anderson (1998) and Enemark *et al.,* (2002) for *C. muris* infection in cattle.

Previous studies revealed substantial differences in the size and shape between *C. parvum* (5.0 x 4.5 µm and spherical) and *C. muris* (7.5–9.8 x 5.5–7.0 µm and ovoid) oocysts (Upton and Current, 1985; Anderson, 1991 and Fayer *et al.,* 1991). These differences enable the two species to be distinguished readily on microscopical examination (Anderson, 1998). Recently, a new species, *C. andersoni* (6.0–8.1 x 5.0–6.5 µm) was proposed in the abomasum of cattle (Lindsay *et al.*, 2000). In the current study, microscopic examination revealed ovoid oocysts that were an average size of 7.5-10.1 x 5.5-7.3 µm with a mean ±SD of 8.3±1.22 x 6.1±0.88 µm. The oocysts were ovoid with single layer wall, stained red or pink with a granular appearance against a green background. The sporozoites were clearly visible (4-8 sporozoites) inside the oocyst. The mean± SD of length to width ratio was 1.36±0.041 (1.21-1.52). All the morphological characters fit the description of *C. muris* isolated from bacterian camel (Andeson, 1991 and Fayer *et al.,* 1991). Because faecal oocysts from dromedary camels were not available for molecular and structural studies, we were unable to fully recognize the parasite so it could be called *Cryptosporidium muris* like oocysts. Further researches for abomasal histopathological investigations supported by genotyping and molecular identification are required.

Molina *et al.* (1994), Nassif *et al.* (2002); El-Dessouky and El-Masry (2005) and Omran *et al.* (2005) found a significant effect of the intestinal cryptosporidiosis (*C. parvum*) infection on the metabolic indices that related to neonatal diarrhoea syndrome of ruminants. Up till now the effect of abomasal cryptosporidiosis (*C. muris*) on the health and metabolic profile of the host is not clear. Our results showed that blood serum albumin decreased in infected camels without significant change in total serum protein concentration. The reduced serum albumin concentrations might be related to the retardation of protein digestion in the abomasum as a result of the loss of membrane-bound digestive enzymes (Anderson, 1998) and the reduced gastric absorption of amino acids due to the loss of epithelium, villous atrophy and crypt hyperplasia (Taylor *et al.,* 1999; Dubey *et al.,* 2002 and Topouchian *et al.,* 2003).

Omran *et al.* (2005) and El-Dessouky and El-Masry (2005) found a significant effect of the intestinal cryptosporidiosis (*C. parvum*) infection on the circulating electrolytes concentrations due to prolonged diarrhoea in the new born ruminants. In the current study, however, cryptosporidiosis did not affect serum electrolyte concentrations in affected camels. These results differed than those reported for animals infected with *C. parvum* in which the infection disturbed electrolyte concentrations and interfered with acid base homeostasis or osmotic balance in the blood (Walker *et al.* 1998).

The hyperactivity of abomasal mucosa (Dubey *et al.,* 2002) might be responsible for the increased serum pepsinogin concentration in the current work. Anderson (1998) found that plasma pepsinogen of cattle affected with *C. muris* averaged about twice the normal concentration, which was also similar to cattle with type 2 ostertagiasis.

The decreased α-Tocopherol concentration agree with the results of Simpson (1992) who found decreased vitamin E concentrations in intestinal cryptosporidiosis in calves. Lightbody *et al.* (2001) found that abomasal parasitic infection was associated with a transient decrease in total antioxidant capacity in sheep and goats. Takeda *et al.* (2003) found oxidative stress and cytokine expression in peripheral blood lymphocytes of calves experimentally infected with *C. parvum*. If this increase in oxidative stress and cytokine production is true for abomasal cryotosporidiosis, so that the decrease in the antioxidant capacity and hence α-Tocopherol reduction is acceptable in the current study.

In conclusion, dromedary camels are susceptible to cryptosporidial infection with significant risk on their health. The present study should be regarded as a first step towards recognition of *C.*

*muris* as a possible cause of gastritis in dromedary herds. More studies are needed to identify the parasite and to clarify the pathogenicity of this organism.

## REFERENCES

*Anderson, B.C. (1987):* Abomasal cryptosporidiosis in cattle. Vet. Pathol., 24: 235-238.

*Anderson, B.C. (1991):* Experimental infection in mice of *Cryptosporidium muris* isolated from a camel. J. Protozool., 38: 16S-17S.

*Anderson, B.C. (1998):* Cryptosporidiosis in Bovine and Human Health. J. Dairy. Sci., 81: 3036–3041.

*Borenstein, M.; Rothstein, H. and Cohen, J. (1997):* Sample Power Statistics 1.0. SPSS Inc., Chicago.

*Chalmers, R.M.; Elwin, K.; Reilly, W.J.; Irvine, H.; Thomas, A.L. and Hunter, P.R. (2002): Cryptosporidium* in farmed animals: The detection of a novel isolate in sheep. Internat. J. Parasit., 32: 21-26.

*Dubey, J.P.; Markovits, J.E. and Killary, K.A. (2002): Cryptosporidium muris*–like Infection in Stomach of Cynomolgus Monkeys (*Macaca fascicularis*). Vet. Pathol., 39: 363-371.

*El-Dessouky, S.A. and El-Masry, N.M. (2005):* Effect of *Cryptosporidium parvum* infection on the haematological and blood biochemical changes of buffalo calves with special reference to the prevalence of infection among buffaloes. Assiut Vet. Med. J., 51: 108-123.

*Enemark, H.L.; Ahrens, P.; Lowery, C.J.; Thamsborg, S.M.; Enemark, J.M.D.; Bille-Hansen, V. and Lind, P. (2002): Cryptosporidium andersoni* from a Danish cattle herd: identification and preliminary characterization. Vet. Parasit., 107: 37–49.

*Enemark, H.L.; Bille-Hansen, V.; Lind, P.; Heegaard, P.M.H.; Vigre, H.; Ahrens, P. and Thamsborg, S.M. (2003):* Pathogenicity of *Cryptosporidium parvum* - evaluation of an animal infection model. Vet. Parasitol., 113: 35–57.

*Esteban, E. and Anderson, B.C. (1995): Cryptosporidium muris*: Prevalence, persistency and detrimental effects on milk production in a dry lot dairy. J. Dairy Sci., 78: 1068-1072.

*Fayer, R.; Phillips, L.; Anderson, C.V. and Bush, M. (1991):* Chronic cryptosporidiosis in a bactrian camel (*Camelus bactrianus*). J. Zoo Wildl. Med., 22: 228-232.

*Fayer, R.; Santín, M. and Xiao, L. (2005): Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). J. Parasitol., 91: 624-629.

*Fayer, R.; Trout, J.M.; Graczyk, T.K. and Lewis, E.J. (2000):* Prevalence of *Cryptosporidium, Giardia and Eimeria* infections in post-weaned and adult cattle on three Maryland farms. Vet. Parasitol., 93, 103–112.

*Georgi, J.R. and Georgi, M.E. (1990):* Parasitology for Veterinarians 5th ed. W. B. Saunders Company, New York.

*GOVS (2005):* Technical veterinary report 2005, General Organization of Veterinary Service, Cairo, Egypt.

*Henriksen, S.A. Pohlenz, J.F.L. (1981):* Staining of *Cryptosporidia* by a modified Ziehl–Neelsen technique. Acta Vet. Scand., 22: 594–596.

*Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974):* Clinical Chemistry: Principles and Techniques. 2nd Ed. Harper and Row; Hagerstown M D. pp. 473.

*Kvac, M. and Vitovec, J. (2003):* Prevalence and pathogenicity of *Cryptosporidium andersoni* in one herd of beef. J. Vet. Med., series B, 50: 451-457.

*Lightbody, J.H.; Stevenson, L.M.; Jackson, F.; Donaldson, K. and Jones, D.G. (2001):* Comparative aspects of plasma antioxidant status in sheep and goats, and the influence of experimental abomasal nematode infection. J. Comp. Pathol., 124: 192-199.

*Lindsay, D.S.; Upton, S.J.; Owens, D.S; Morgan, U.M.; Mead, J.R. and Blagburn, B.L. (2000): Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from Cattle, *Bos Taurus*. J. Eukar. Microbiol., 47: 91-95.

*Lise, A.T.W.; Brenna, D.J.; Martin, S.W.; Knneth, E.L. and Andrew, S.P. (2005):* Prevalence of *Cryptosporidium parvum* infections in southwestern Canada and its association with diarrhea in neonatal dairy calves. Can. Vet. J., 46: 349–351.

*Masuno, K.; Yanai, T.; Hirata, A.; Yonemaru, K.; Sakai, H.; Satoh, M.; Masegi, T. and Nakai, Y. (2006):* Morphological and immunohistochemical features of *Cryptosporidium andersoni* in cattle. Vet. Pathol., 43: 202-207.

*McAllister, T.A.; Olson, M.E.; Fletch, A.; Wetzstein, M. and Entz, T. (2005):* Prevalence of *Giardia* and *Cryptosporidium* in beef cows in southern Ontario and in beef calves in southern British Columbia. Can. Vet. J., 46: 47–55.

*Molina, J.M.; Rodriguez-Ponce, E.; Ferrer, O.; Gutierrez, A.C. and Hernandez, S. (1994):* Biopathological data of goat kids with cryptosporidiosis. Vet. Rec., 135: 67-68.

*Morgan, U.M.; Sargent, K.D.; Deplazes, P.; Forbes, D.A.; Spano, F.; Hertzberg, H.; Elliot, A. and Thompson, R.C.A. (1998):* Molecular characterization of *Cryptosporidium* from various hosts. Parasitol., 117: 31–37.

*Nassif, M.N.; Amer, S.A. and Osman, S.A. (2002):* Some studies on ovine and caprine cryptosporidiosis concerning prevalence and electophoretic pattern of blood serum protein. Assiut Vet. Med. J., 47: 249-262.

*Nydam, D.V.; Wade, S.E.; Schaaf, S.L. and Mohammed, H.O. (2001):* Number of *Cryptosporidium parvum* oocysts shed by dairy calves after natural infection. Am. J. Vet. Res., 62: 1612–1615.

*Olson, M.E.; Handley, R.M.; B. Ralston, J.; Mcallister, T.A. and Thompson, R.C.A. (2004):* Update on *Cryptosporidium* and *Giardia* infection of cattle. Trends Parasitol., 20, 185 -191.

*Peeters, J.E. and Villacorta, I. (1995): Cryptosporidium*. In: Eckert, J., Braun, R., Shirley, M. W., and Coudert, P. (Eds.), Biotechonolgy guidelines on techniques in Coccidiosis Research. Office for official publication of the European communities, Luxembourg. pp 202 -240.

*Pospischil, A.; Striglmair-Herb, M.T.; von Hegel, G. and Wiesner, H. (1987):* Abomasal cryptosporidiosis in mountain gazelles. Vet. Rec., 121: 379–380.

*Quaife, M.L.; Scrimshaw, N.S. and Lowry 0.H. (1949):* A micromethod for assay of total tocopherols in blood serum. J. Biol. Chem., 180: 1229-1235.

*Ramirez, N.E.; Ward, L. and Sreevatsan, S. (2004):* A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes and Infection, 6: 773–785.

*Scott, I.; Stear, M.J. McKellar, Q.A. (1995):* Comparison of four methods for the determination of plasma pepsinogen concentration. Res. Vet. Sci., 59: 234-237.

*Simpson, V.R. (1992):* Cryptosporidiosis in newborn red deer (*Cervus elaphus*). Vet. Rec., 130: 116-118.

*SPSS, (1999):* ANOVA and LRA in Sample Power Statistics, SPSS, 10.0.1 Syntax Reference Guide for SPSS Base. SPSS Inc., 233 South Wacker Drive, Chicago, IL.

*Takeda, K.; Omata, Y.; Koyama, T.; Ohtani, M.; Kobayashi, Y.; Furuoka, H.; Matsui, T. Maeda, R. and Saito, A. (2003):* Increase of Th1 type cytokine mRNA expression in peripheral blood lymphocytes of calves experimentally infected with *Cryptosporidium parvum*. Vet. Parasitol., 113: 327–331.

*Taylor, M.A.; Marshall, R.N. Green, J.A. and Catchpole, J. (1999):* The pathogenesis of experimental infections of *Cryptosporidium muris* (strain RN 66) in outbred nude mice. Vet. Parasitol., 86: 41–48.

*Topouchian, A.; Huneau, J.F.; Barbot, L.; Rome, S.; Gobert, J.G.; Tome, D. and Kapel, N. (2003):* Evidence for the absence of an intestinal adaptive mechanism to compensate for *C. parvum*-induced amino acid malabsorption in suckling rats. Parasitol. Res., 91: 197-203.

*Tyzzer, E.E. (1910):* An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. J. Med. Res., 23: 487-511.

*Tyzzer, E.E. (1912): Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. Arch. Protistenkd., 26: 394-412.

*Tzipori, S. and Ward, H. (2002):* Cryptosporidiosis: Biology, pathogenesis and disease. Microbes and Infection, 4: 1047-1058.

*Upton, S.J. and Current, W.L. (1985):* The species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) infecting mammals. J. Parasitol., 71: 620-625.

*Wade, S.E.; Mohammed, H.O. and Schaaf, S.L. (2000):* Prevalence of *Giardia* sp., *Cryptosporidium parvum* and *Cryptosporidium andersoni* (syn. *C. muris*) in 109 dairy herds in five counties of southeastern New York. Vet. Parasitol., 93: 1–11.

*Walker, P.J.; Constable, P.D.; Morin, D.E.; Drackley, J.K.; Foreman, J.H. and Thurmon, J.C. (1998):* A Reliable, Practical, and Economical Protocol for Inducing Diarrhea and Severe Dehydration in the Neonatal Calf. Can J Vet Res; 62: 205-213.

*Xiao, L.; Fayer, R.; Ryan, U. and Upton, S.J. (2004): Cryptosporidium* Taxonomy: Recent advances and implications for public health. Clin. Microbiol. Rev., 17: 72-97.