

## Prevalence and biochemical changes in camels infested with gastro-intestinal nematodes with special reference to alterations of oxidant and antioxidant status

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**Received:** March 3, 2022; **Accepted:** November 23, 2022; **Published:** February 1, 2023

### Abstract

Parasitic infestation in camels is regarded a major factor in the performance status and optimum productivity in camels. The current study aimed to identify the gastrointestinal parasites load among camels in slaughterhouse in El-Beheira governorate, Egypt. The floatation technique was used for examination of the collected faecal samples. The findings revealed that out of 160 faecal samples collected from diseased (n=100) and healthy (n=60) camels, 55 (34.4%) were infested by various GIT nematodes and the *Trichuris*, *Haemonchus*, *Strongyloides* and *Trichostrongylus* were the most common genera with prevalence rates 12 (21.8%), 16 (29.1%), 18 (32.7%), and 9 (16.4%), respectively. The associated biochemical changes reported that serum total protein, albumin and glucose concentrations were decreased ( $P < 0.05$ ) in infested camels compared with the apparently healthy camels, while non-significant difference ( $P > 0.05$ ) was observed with globulin and A/G ratio. The glutathione peroxidase (GPx) activity was significantly reduced ( $p < 0.05$ ). Meanwhile, the hydrogen peroxide was significantly higher in infested camels compared to apparently healthy ones ( $P < 0.05$ ). In conclusion, these results provide a significant data about the most common GIT nematodes found in camels as well as highlights the alterations in oxidant and antioxidant status in camels associated with parasitic infestation.

**Keywords:** camels, gastrointestinal parasites, glutathione peroxidase, hydrogen peroxide.

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### Introduction

The camel is a unique biological model and promising livestock in the climate-change context. Dromedary camels can produce milk for a longer time than other animals reared under the same adverse environmental conditions (Zhu et al., 2019). Camels are considered as an important mode of transportation in the arid areas as well as a major source for meat and milk production is various African and Asian countries. Although, camels are less susceptible to infection but various parasites species resulting in significant

economic losses due to decrease in camel production and performance as well as mortalities in severe non-treated complicated cases may be occur (Sazmand and Joachim, 2017).

Despite the limited knowledge about camel's parasitic diseases around the world and only few studies focused on the desert locations where camels were raised as a part of their cultural civilization and traditional purpose. For examples, a comparative study in Algeria in dromedary camels confirmed that some parasites are identical to those found in

ruminants posing a major issue as well as financial and public health risk (Bouragba et al., 2020). In Egypt, (El-Khabaz and Arfa, 2019) revealed that a wide spectrum and high prevalence of internal parasites were detected among 120 clinically suspected males camels imported from Sudan through Abu-Simbel quarantine station, Aswan governorate from January till July 2016. Although some studies reported the high prevalence of parasitic infestation in adult camels than young but the intensity and severity in young is significantly higher than adult in China (Guowu et al., 2020).

Various tissues parameters are found associated with the parasitic embedding and penetration to the host tissues inducing inflammation such as reactive oxygen species (ROS) which are produced during the inflammatory reactions (Egwunyenga et al., 2004; Pal et al., 2006). Furthermore, superoxide radical ( $O_2^-$ ), hydroxyl radical (OH), and hydrogen peroxide ( $H_2O_2$ ) are forms of reactive oxygen species (ROS) that cause oxidative damage to macromolecules like membrane lipids containing polyunsaturated fatty acids, critical proteins like enzymes, and nucleic acids, particularly DNA (Belló et al., 2000). Moreover, antioxidants are molecules that aid in the effective scavenging of free radicals and the suppression of reactive oxygen substances' activities that are found across the body and comprise both enzymatic and non-enzymatic mechanisms. Superoxide dismutase, glutathione peroxidase, and catalase are the most important enzymatic antioxidants (Panda, 2012).

As results of the scarce local or international information about the camel diseases particular parasitic infestations with their economic impact. Many researchers have been inspired to investigate the most common parasitic infestation and biochemical parameters in camels. About 95.000 camels are present in Egypt and due to the limited knowledge about the biodiversity of camel's parasites, this study aimed to determine the

common gastrointestinal parasitic genera affecting camels as well as changes in the oxidant and antioxidant status among infested camels located at Beheira governorate, Egypt.

## **Materials and methods**

### ***Area of Study***

El-Beheira governorate estimated to be 9826.00 Km<sup>2</sup>. It is in the West of Delta region with 31° 2' 10" N, 30° 28' 10" E. The north coast of El-Beheira governorate is a natural extension to Alexandria coasts. Furthermore, one of the famous areas is Netroun Valley, which is famous for dry mild weather at which camels, sheep and goats are raised intensively. El-Beheira is one of the most important of land reclamation as the government has arable lands.

### ***Animals and samples collection***

One hundred and sixty camels from El-Beheira governorate slaughterhouse (100 diseased camels and 60 apparently healthy camels), aged 3-5 years were examined (December 2021- March 2022). Clinical examination was carried out according to Higgins and Kock (1984). Faecal samples were collected from all examined camels and all data were tabulated. About 100 of the examined camels were anaemic and/or with pale mucous membrane, rough coat and emaciated with diarrhoea. The faecal samples were collected in sterile faecal containers and transported in cold box to the Laboratory of Animal Medicine for Research Studies, Faculty of Veterinary Medicine, University of Sadat City for faecal and parasitological examination.

### ***Blood samples***

Blood samples were collected from the jugular vein using anticoagulant-free, dry-clean-labelled vacuum tubes. The blood was centrifuged for 10 minutes at 3000 g to obtain a clear non-haemolyzed serum. The serum was kept at -80 °C until biochemical analysis of protein profile, glucose and hydrogen peroxide.

### **Tissue samples**

For determination of glutathione peroxidase, intestinal tissues were collected and perfused with 4-8 volumes per weight of 50 mM cold buffer 0.1% composed from (Assay buffer pH 7.0, phosphate buffer and triton X - 100) and then the mixture was centrifuged at 4000 rpm for 10-20 minutes at 2-8 °C and the supernatant was obtained.

### **Faecal and Parasitological examination**

The collected faecal samples were examined individually by direct smear, simple floatation method using saturated salt solution as described by Cebra et al. (2007) and the sedimentation technique according to Zajac and Conboy (2012) to detect the presence of gastrointestinal eggs/ oocysts.

### **Measurement of the Serum protein profile, glucose oxidant and antioxidant system concentrations**

Using commercial kits (Bio-Diagnostics Ltd; Egypt), spectrophotometry was used to assess serum total protein, albumin and glucose concentrations. By subtracting serum albumin from total protein values, serum globulin concentrations were estimated, and division of albumin concentration into globulin concentration calculated A/G ratio.

### **Measurement of the Glutathione peroxidase (GPx)**

The activity of the Glutathione peroxidase was determined by the method described by Paglia and Valentine (1967) using a commercial kit (Bio Diagnostics Company Ltd; Egypt). Cumene hydroperoxide promotes the oxidation of glutathione (GSH) by glutathione peroxidase. The oxidized glutathione (GSSG) is rapidly converted to the reduced form in the presence of glutathione reductase (GR) and NADPH, resulting in the oxidation of NADPH to NADP<sup>+</sup>. At 340 nm, the decrease in absorbance was estimated.

### **Measurement of the Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**

Hydrogen peroxide was measured in blood plasma using a commercial kit (Bio Diagnostics Company Ltd; Egypt) according to Fossati et al. (1980) who described the process as in the presence of peroxidase (HRP), H<sub>2</sub>O<sub>2</sub> reacts with 3,5-dichloro-2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore.

### **Statistical analyses**

The collected data were expressed as means with standard errors (Mean  $\pm$  SE). Comparison between two groups was conducted using t-test. Significance was determined at P < 0.05. Data were analysed using IBM SPSS Statistics 16 (IBM Corporation, Armonk, NY, USA).

## **Results**

### **Prevalence and morphological identification of gastrointestinal parasites in camel at El- Beheira Governorate:**

Out of examination of 160 camels (100 diseased camels and 60 apparently healthy camels) at the slaughterhouse at El-Beheira governorate 55 (34.4%) were infested with gastrointestinal parasites. The affected camels showed pale mucus membrane, rough coat, emaciation, diarrhoea and poor body conditions. The dominant genera through parasitological examination were *Trichuris*, *Haemonchus*, *Strongyloides* and *Trichostrongylus* with prevalence rates of 12 (21.8%), 16 (29.1%), 18 (32.7%), and 9 (16.4%) respectively as shown in table1.

The Morphological description and measurements of the examined eggs showed that *Trichuris* eggs were barrel shaped, brown, measured 70-80 by 30-42  $\mu$ m including the plugs, containing an unsegmented embryo with transparent plugs at either pole (Figure 1A).

Meanwhile, *Haemonchus* eggs measured 70-85 by 41-48  $\mu\text{m}$ , thin shelled and containing an embryo of 16-32 cells (Figure 1B). *Strongyloides* eggs were oval, thin shelled, transparent, measured 40-60 by 20-25  $\mu\text{m}$ , and containing fully developed embryo (Figure 1C). *Trichostrongylus* eggs measured 60-70 by

25-35  $\mu\text{m}$ , oval, thin shelled and containing an embryo of 16-32 cells when passed in faeces (Figure 1D). All the affected cases showed mixed infection by two or three species of parasites as shown in the supplementary Table 1.

**Table 1.** Prevalence of different parasites genera recovered from camels at slaughterhouse at El-Beheira Governorate.

Total examined camels	Positive for parasites infestation		<i>Trichuris</i>		<i>Haemonchus</i>		<i>Strongyloides</i>		<i>Trichostrongylus</i>	
	n	%	n	%	n	%	n	%	n	%
160	55	34.4	12	21.8	16	29.1	18	32.7	9	16.4

n, number of camels; % was estimated according to the total positive samples from camels (n=55).

#### ***Alterations of some biomarkers, oxidant and antioxidant status in serum of apparently healthy and infested camels.***

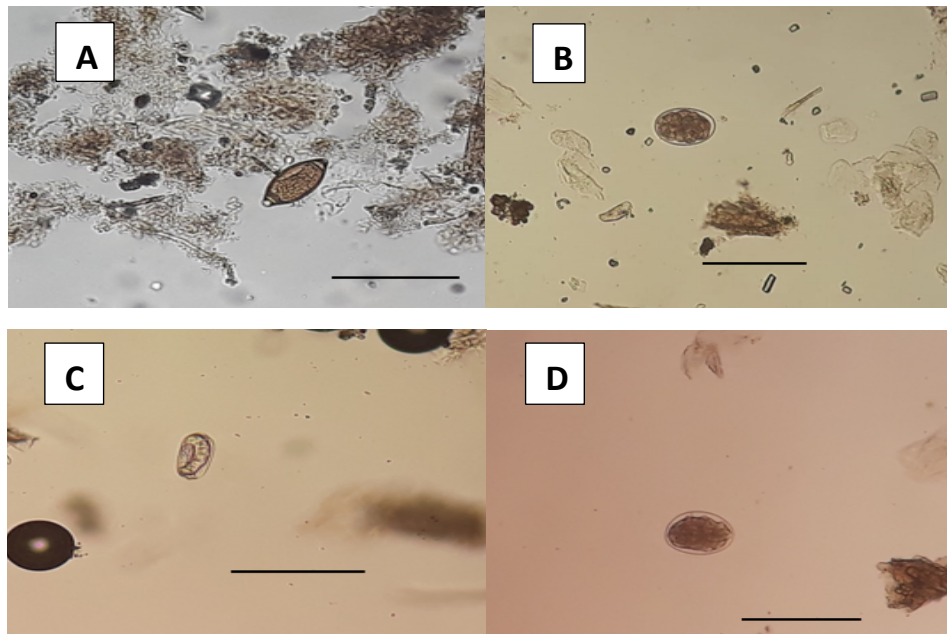
Our results revealed that serum total protein, albumin and glucose were decreased ( $P < 0.05$ ) in infested camels compared to

apparently healthy ones, while globulin and A/G ratio were reported non-significant difference ( $P > 0.05$ ). The glutathione peroxidase (GPx) activity was lower ( $P < 0.05$ ). Meanwhile, hydrogen peroxide was higher ( $P < 0.05$ ) in infested camels compared to the apparently healthy ones as shown in Table 2.

**Table 2.** Serum protein profile, glucose oxidant and antioxidant biomarkers in apparently healthy and infested camels (mean  $\pm$  SE).

Variables	Healthy camels (n=20)	Infested camels (n=30)
TP (gm/dl)	6.9 $\pm$ 0.1	5.97 $\pm$ 0.09*
Albumin (gm/dl)	4.3 $\pm$ 0.09	3.6 $\pm$ 0.06*
Globulin (gm/dl)	2.5 $\pm$ 0.16	3.3 $\pm$ 0.12
A/G ratio	1.9 $\pm$ 0.18	1.6 $\pm$ 0.13
Glucose	67.9 $\pm$ 0.93	60.76 $\pm$ 0.86*
GPx (U/gT)	51.2 $\pm$ 1.15	47.42 $\pm$ 0.9*
H <sub>2</sub> O <sub>2</sub> (mM / L)	14.87 $\pm$ 0.36	18.07 $\pm$ 0.4*

n, number; TP, Total protein; A/G, Albumin/globulin; GPx, Glutathione peroxidase; H<sub>2</sub>O<sub>2</sub>, Hydrogen peroxide; \*P < 0.05.



**Figure 1.** Microphotographs of eggs of gastrointestinal nematodes recovered from faeces of examined camels in El-Beheira governate, Egypt. A. *Trichuris*, B. *Haemonchus*, C. *Strongyloides*, D. *Trichostrongylus*.

## Discussion

Camelids are members of the biological family *Camelidae* that some of them have the ability to survive under hard climatic conditions of the dry regions. The interests in this animal family has increased considerably over the last few decades. For a long time, the main animal products gained from these animals are meat, milk, and hair fiber. In addition, they are used for race and perform work among other activities. In the tropics and subtropics, camelids may become an imperative protein source for humans (Zarrin et al., 2020). Camel parasitic infestation are major causes significant economic impacts and harmful effect on the camel's health status (Guowu et al., 2020; Locklear et al., 2021).

According to the current study, the overall prevalence rate of gastrointestinal parasites from 160 examined camels was (34.4%) and the identified parasitic genera were *Trichuris*, *Haemonchus*, *Strongyloides* and *Trichostrongylus*. Furthermore, all positive parasite- infested cases showed mixed infection

by two or more parasites. These findings were consistent with (Osman et al., 2014), who reported that the overall prevalence rate of nematode infestation in camels in Egypt was (36.15%), and the coproculture revealed that *Trichostrongylus*, *Nematodirus*, *Ostertagia*, *Osphegestomum*, and *Trichuris* genera were the most detectable parasite eggs. Furthermore, Abdel-Rady (2014) revealed that most nematodes recovered from camels were *Strongyloides*, *Trichuris*, and *Trichostrongylus* genera, with 55%, 25%, and 20% prevalence rate, respectively. However, investigation of 717 faecal samples from *dromedary camels* reported to be 48.26% infested rate with GIT parasites as well as 12 Nematoda infestation were found with high prevalence of genus *Strongyloides* and the low prevalence of genus *Cooperia* (Bouragba et al. 2020).

In a comparative study in China (Guowu et al., 2020) identified 15 gastrointestinal parasite genera out of 362 faecal samples with 100% while, *Ostertagia* and *Trichostrongylus* were the most dominant genera with 100% and

98.1% respectively and co-infection with several parasites species were evident. In a similar study in Somalia (Ibrahim et al., 2016) inspected 167 faecal samples and found that 50.3% were infested by gastro-intestinal parasites and the Nematodes were the most prevalent parasites (47.9%) particular *Dictyocaulus*, *Trichostrongylus*, *Parascaris equorum* and *Strongyloides* genera as well as co-infection and 35.7% of examined camels revealed heavy infection. On the other surveillance in Iran (Borji et al., 2010) revealed higher prevalence rate (75.1%) out of 306 faecal samples from *dromedaries camels* with regard to different types of nematode eggs including *Nematodirus*, *Strongyloides*, *Trishuris*, *Marshallagia*, and *Stongyle*-type. Additionally, (Wakil et al., 2017) demonstrated that the overall prevalence rate 69.3% and *Strongyle* 41.1% were the most identified, followed by *Strongyloides*, *Coccidia*, *Trichuris*, *Ciliates*, *Fasciola*, *Monezia*, *Balantidium*, *Amphistomes* and *Ascaris* genera with (9.5%), (7.4%), (4.5 %), (3.5%), (0.9%), (0.9%), (0.5%), (0.5%) and (0.5%) respectively. The wide range in the prevalence rates and prevalent parasites species may be due to the difference in the number of examined camels, geographic location, sampling time, managemental and environmental condition as well as camel's breeds.

Camel blood reflects normal physiological or unfavourable conditions. Haematological and biochemical indicators are caused by all these factors mentioned above. These variations could be utilized to demonstrate the usual physiological and health status in camels which is very crucial for camel welfare (Momenah, 2014).

Regarding the effect of the parasitic infestation on the biochemical profile, the current study assessed the serum total protein, albumin and glucose levels which were decreased in infested camels compared to apparently healthy ones with (P value < 0.05), while globulin and A/G ratio were reported with non-significant statistical difference (P

value > 0.05). These was agreed with previous reports of (Hassan et al., 2019; Momenah, 2014). They demonstrated that the protein profile had a significant drop in the mean values of the serum total protein and albumin in infested camels. This showed that the parasitic infestation caused digestive disturbances and hepatic dysfunction as well as decline of the serum total protein and albumin values (Baraka et al., 2000). Furthermore, during parasite infestation, the serum albumin has a negative acute phase and is thought to be a free radical scavenger (Sazmand and Joachim, 2017). Also, during parasites infestation, reduction in the food intake and absorption act as a cause for decline blood glucose level (Momenah, 2014).

In the same perspective, the GPx concentrations were significantly lowered (p value < 0.05), while the mean value of H<sub>2</sub>O<sub>2</sub> level was significantly higher in infested camels compared to apparently healthy ones (p < 0.05). Similarly, the significant increase of H<sub>2</sub>O<sub>2</sub> generation enhanced lipid peroxidation and indicates higher amounts of free radical in infested camels' plasma (Azma et al., 2015). In a related study (Abd Ellah, 2013) reported high level of reactive oxygen species in hosts infected with parasitic infestation that correlated basically to the nutritional status, degree of infestation, and the damaging effect on tissues. In addition, an association between lipid peroxidation and external and endo parasites infestation was evident.

Concerning the GPx activity, different parasite infestations have been linked to oxidative stress and variations in antioxidant levels (Heidarpour et al., 2013). The activity of GPx is a key factor in the intracellular breakdown of lipid peroxides (Brigelius-Flohé and Flohé, 2020) and inhibits oxidative damage to cell membranes caused by these metabolites (Huang et al., 2018). The increased consumption of GPx by the tissues could explain the decrease in antioxidant system for the competing the degenerative effect of intestinal parasites (Abd Ellah et al. 2008). In similar and comparative findings in sheep

(Siwela et al., 2010) recorded higher GPx activity, indicating that the infection had put the sheep under oxidative stress. The disturbance in antioxidants system is an indication for the presence of oxidative stress and intestinal membrane damage as well as imbalance between pro-oxidants and non-enzymatic antioxidants (Elmahallawy et al., 2020).

## Conclusion

Nematode's infestation in camels may play a key role in reduction of camel's overall performance and productivity. The current study revealed the relative high prevalence of GIT nematodes including, *Trichuris*, *Haemonchus*, *Strongyloides* and *Trichostrongylus* genera. Furthermore, the results demonstrated the disturbance in oxidant and antioxidant status as well as reduction in protein profile and glucose level in infested camels. This finding approved that abattoir survey may act as a significant tool to determine the prevalence of gastrointestinal helminths with insights about some serum alteration in infested camels in Egypt that help for the prevention and control of infections in camels. Further studies are needed for the epidemiological pattern related to the parasitic infestation.

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**Supplementary Table 1:** The distribution of mixed parasites species among examined camel's faecal samples

Sample ID	<i>Trichuris</i>	<i>Haemonchus</i>	<i>Strongyloides</i>	<i>Trichostrongylus</i>
1	+		+	+
2	+	+		
3		+	+	
4	+	+		
5 - 8				
9	+	+		+
10		+	+	
11-13				
14	+	+	+	
15				
16	+	+	+	
17	+	+	+	
18				
19	+	+	+	
20 - 21				
22		+	+	+
23 - 25				
26		+	+	+
27 -30				
31		+	+	
32 -33				
34	+	+	+	
35 - 37				
38 - 39			+	+
40			+	+
41			+	+
42 - 44				
45			+	+
46				
47			+	+
48	+	+		
49				
50				
51	+	+	+	
52 -53				
54	+	+	+	
55				