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RUMEN AND BLOOD CONSTITUENTS ALTERATIONS IN GASTROINTESTINAL PARASITES INFESTATION IN EGYPTIAN DROMEDARY CAMELS

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ABSTRACT

It was necessary to emphasize the rule of gastrointestinal parasites in clinical physical examination, prevalence and impact on rumen and blood constituents in apparently healthy, infected and treated dromedary camels. Complete clinical examination of 240 camels (including 60 infected cases); rumen and blood samples were examined during the affection and after treatment with Netobimin 15% (Hapadex®) as 5 ml/100kg body weight. High prevalence rate of gastrointestinal parasites was recorded as *Trichostrongylus* and *Nematodirus* were 53.7 and 52.5 %; while that of *Heamonchus, Trichuris, Cooperia* and *Monezia* were 22.1, 21.7, 21.3 and 12.5% respectively in camels examined around the year. The infected camels suffered from mild rumen acidosis with significant increases ($p<0.001$) in the level of acetic, propionic, butyric and isovaleric acids; macrocytic hypochromic anemia, hemodilution, moderate hypokinemia and hypercupremia. The increases in the rumen calcium, magnesium and zinc were significant ($p<0.001$). The study of the correlations confirmed the tight interaction between the rumen and blood constituents in health and disease.

Keywords:

Body fluids, camel, gastrointestinal parasites, Netobimin 15%.

INTRODUCTION

Camels are known to be susceptible to a wide variety of gastrointestinal helminthes **(El-Bahari 1985, Sharif *et al.,* 1997, Aypak *et al.*, 2013)**. There is little information about the metabolic disturbances which may occur in camels when infected with such parasites **(Zein El-Abdin *et al.,* 1975)**. The changes in ruminal and blood constituents in diseased camels have not been comprehensively studied **(Baraka *et al.,* 2000, Younus *et al.,* 2015)**. This study was carried out to investigate the prevalence of parasitic gastrointestinal parasites

in the camels in Giza Governorate in the different seasons; evaluation of the alterations in the ruminal and serum macro and micro-elements under the effect of the infection and after the treatment with Hapadex; representing the relationships and the interaction between those elements and other rumen and blood constituents; in order to explain these interactions to be put in consideration during the treatment of such disease conditions.

MATERIAL AND METHODS

This work was applied on 240 camels (60 in each season); belonging to Giza Governorate (at Imbaba, Warrak and Nahia localities). Each camel was exposed to a complete clinical examination. Rumen juice, Blood, serum and fecal samples were collected. These camels were kept by sporadic owners and fed on random, unmeasured quantities of food stuffs and mainly depending on the crops of the season. Fecal samples were examined by gross inspection, microscopically for direct smears,floatation and sedimentation techniques according to the method described by **Kaufmann (1996)**;while fecal egg count per gram (EPG) was applied according to the method of **Monning (1962)**. Selected 60 infested cases were examined for ruminal and hemato-biochemical constituents (15 positive cases in each season); these infected camels were treated with Netobimin 15% (Hapadex-Scher) as oral suspension (5 ml/100kg body weight) in a single dose given by the stomach tube; the rumen and blood samples were collected after 4 weeks; while 37 clinically healthy camels were used as the control group. The rumen juice samples were strained and fixed using methylene green formal saline according to the method of **Ogimoto and Imai (1981)** and kept in dark. The counting of forestomach protozoa was carried out according to the method described by **Dehority (1984)**. The forestomach strained liquor pH was determined immediately after collection of the samples by using electrical digital pH-meter (Model SMP1). Ammonia concentration was determined in samples preserved under a cover of paraffin oil according to the method described by **Zapletal (1967)**. Volatile fatty acids levels were determined using gas liquid chromatography, according to the method by **Cottyne *et al.* (1968)**; the individual volatile fatty acids were calculated by comparing with a standard solution. The level of hemoglobin, the packed cell volume (PCV), total erythrocytes count (RBCs), total leucocytes count (WBCs) were determined using the method described by **Jain (2000)**.The levels of serum total protein, calcium, inorganic phosphorus were measured with the Pye-Unicum Spectrophotometer using the specific kits. Serum and rumen sodium, potassium, copper,

magnesium and zinc estimated using the Atomspek Flame Photometer. The obtained data were statistically analyzed using the SXW statistical computer Software. Copy writes 1996 version 1.0.

RESULT AND DISCUSSION

The clinical examination showed that, the gastrointestinal parasites infested camels were generally emaciated, having pallor visible mucous membranes, weak, apathy, showing signs of restlessness, abdominal pain on deep palpation, with hind quarters moisten with diarrheic matter. The physical examination showed that, the body temperature in most cases was decreased (37.00±0.55 °C while in healthy camels was 37.93±0.65 °C in the same time of the day). The pulse rate, respiratory rate and rumen motility were 30.92±6.62/ min, 11.20±2.00/ min and 1.17±0.79/ 2min and in healthy camels were 34.00±4.40/ min, 11.00±1.35/ min and 2.80±0.76/ 2 min respectively. These results were in agreement with that reported by Kohler-**Rollefson *et al.* (2001); Baraka (2002) and Abdel-Rady (2014)**. The rumen motility rate was markedly reduced to 1.17±0.79\ 2min in comparison with healthy camels (2.80±0.73/ 2min); which can be explained by the reduced activity and appetite of infected camels. The fecal examination and egg count per gram revealed that, the infestations in the localities of Imbaba, Warrak and Nahia localities ranged between mild (50 - 400 EPG) to moderate infestations (400 - 1000 EPG); while no heavy infestations (1000 - 5000 EPG) were detected. These grades of infestations were reported after **Abubakr *et al.* (2002)**. Monitoring the prevalence of gastrointestinal parasites infestations (Table 1) it was clear that, the highest rate of infection of camels was with *trichostrongylus spp*. (53.7%) followed by *nematodirus spp*. (52.5%); then *hemonchus spp*. (22.1%) and *trichuris spp* (21.7%); while the lowest rate of infection was with *monezia spp.* (12.5%); these rates of infestations were in agreement with that mentioned by .**Abdel Salam and Farah (1988); Nafie, *et al.* (1992); Haroun, *et al.* (1996); El-Manyawe and Iskandar (1994) and Radfar *et al.,* (2013)**. Regarding to the effect of seasons on the infestations rates; it realized that highest infestations were in the spring (*trichostrongylus spp*. 70%, *cooperia spp.* 36.7% and *hemonchus spp*. 25%). In autumn the infestations with *nematodirus spp*. was the highest (65%) and trichuris *spp* (26.3%); while in winter *monezia spp* recorded its highest level of infestations in camels (15%). Similar results of prevalence were recorded by **Ukashatua *et al.* (2012) and Al-Megrin (2015).** The high prevalence of helminthes reported to be during the rainy seasons **(Nwosu *et al.,***

2007). Similar reports have been documented in Camels at Zaria **(Mohammed *et al.,* 2007).** The high prevalence rate for *Haemonchus specie* is consistent with previous findings of other studies **(Abdul - Salam and Farah, 1988; Kamani *et al.,* 2008 and Ibrahim and Arzoun, 2015).** This distribution of the incidence of infestations of camels with these types of gastrointestinal parasites should be put in recognition during the planning of their control and treatment. It was clear that camels under natural condition are practically never infected with just a single species of gastrointestinal parasites; multiple parasitism are the rule **(Osman *et al.,* 2014 and Kaufmann 1996)**.This study supports previous findings that nematodes are the commonest helminths in camels **(Abdul-Salam and Farah, 1988; Mohammed *et al.,* 2007; Kamani *et al.,* 2008 and Ukashatua *et al.,* 2012)**. The examination of the forestomach liquor (Table 2) revealed that; there was no significant decrease in the level of pH which was associated with marked decrease in the total number of the rumen protozoa; which can be referred to the direct relationship between total protozoal count and the pH **(Baraka and Dehority 2003)**.The significant decrease ($p<0.01$) in the *Entodinium spp*. and significant increase in *Diplodinium spp*. ($p<0.01$) can be explained on the basis of the secondary indigestion due to the gastrointestinal infection, inapptance and reduction in the amount of ingested food. It was obvious that, the reduction in the pH and the significant increase in the total volatile fatty acids concentrations were not enough to cause the superiority of *Entodinium spp* which has a negative relationship with *Diplodinium spp*. **(Baraka and Dehority 2003 and Baraka, *et al.* 2005)**. The decrease in the rumen liquor pH associated with a significant increase ($p<0.001$) in the volatile fatty acids with a marked increase in the ammonia concentration which changes according to the rate of re-cycling of ammonia in saliva, rumen, liver and nitrogenous compounds which are used in the formation of complicated bacterial protein **(Payne and Payne, 1987)**. The infested camels suffered from mild rumen acidosis which was confirmed by the significant increases ($p<0.001$) in the level of acetic, probionic, biotyric and isovaleric acids; these changes confirms that lactic acidosis is not the type of acidosis in camels **(Baraka 2004)**.The significant increases of rumen sodium ($p<0.01$), potassium and magnesium ($p<0.001$) can referred to the reduction in the rate of absorption from the rumen due to the secondary indigestion **(Schmidt-Nielsen *et al.*1956 and Baraka 1995)**. The increases in the level of copper and zinc ($p<0.001$) can be explained on the basis of increases in the calcium concentration which causes a hindrance in their absorption from the

rumen **(Baraka *et al.* 1999)**. The stratified squamous epithelium of reticulum, rumen and omasum represents the major site of net magnesium absorption; and transmitted across the epithelium by an active sodium-linked, ATP-ase dependent pump. When magnesium absorption is impaired, dietary magnesium is accumulated in the forestomach; which can explain the significant increase ($p<0.001$) in its level **(McCaughan 1992)**. The hemato-biochemical examination (Table 3) showed a marked reduction in the hemoglobin level which can be referred to the dramatic effect of the blood sucker parasites on the host. **(Kohler-Rollefson *et al.* 2001)** mentioned that it can cause about 60% of deaths during rainy seasons in Sudan. This can explain the dramatic changes in the blood picture of affected camels which revealed a low hemoglobin level because of blood losses; the significant increase ($p<0.001$) in the PCV as a result of continuous water losses in the diarrhea, dehydration and reduction in food intake; the apparent increase in the RBCs count was a result of the dehydration. The water-restricted camels showed an increase in the plasma osmolality **(Ben-Goumi *et al.* 1996)** and the water is trapped from the alimentary tract in the plasma by the albumin, urea, sodium and glucose **(Yagil and Etzion 1979)**. The affected camels suffered from macrocytic hypochromic anemia. These changes in the blood indexes confirm the occurrence of hemodilution **(Esievo and Saror 1991 and Barak *et al.* 2006**). The marked increase in the total count of WBCs can be referred to the defense reaction against the secondary infection may affect the camel under this stressor condition. These findings were explained and confirmed by **(Wilson 1984, Payne and Payne 1987, Kaneko 1989, Smith 1990, Chandel *et al.* 1992 and Baraka 2003)**.The serum total protein level increased in order to maintain the water level in the plasma. Regarding to the ration between albumin and globulin it was clear that an increase in globulin level was achieved to increase the level of antibodies to face the secondary complications which may affect the camels under the stress of parasitic infection **(Partani *et al.* 1995)**. The serum sodium level showed a mild increase can be referred to the effect of reduced food and water intake causing increased parathyroid hormones activity to restore the water level in plasma and in the same time cause the significant retention of calcium ($p<0.01$) and magnesium in the plasma and consequently a reduction in the serum inorganic phosphorus level **(McDwell, *et al.* 1983; Payne and Payne 1987 and Church 1988**). An increase in the potassium level can be explained on the basis of shifting of potassium ions from intracellular to extracellular fluid in cases of indigestion which enhanced by the effect of dehydration and reduction in the level of

effective circulatory volume **(Tasker 1980)**. The ratio between calcium and magnesium was within the normal levels; which confirm that camels have a unique mechanism to keep the proper bone metabolism **(Ben-Goumi *et al*. 1996)** and the neuromuscular balance even under anorexic and stress conditions **(Baraka *et al.* 2005)**. Mild increase in the serum copper was recorded; this hypercupremia may be due to the stimulation of ceruloplasmin synthesis and its release from the liver **(Clegg *et al.* 1987)**; Similar results were recorded by **Corrigal *et al.* (1976)** who mentioned that, the plasma zinc in sheep decreased in case of inapptance with rumen stasis in comparison with clinically normal sheep, and the plasma copper showed an increase in its level. The moderate reduction in the serum zinc may be explained depending on that in a number of disease states hypozincemia can occur as a result of secondary stimulation of liver metallothionein synthesis and the subsequestration of zinc in the liver. Similar alterations intheserumcopperandzincwere recorded by **Baraka *et al*. (1999).** Monitoring the level of ruminal and blood constituents in the camels after treatment (Tables 2, 3) with Hapadex 15% suspension; it was obvious that, the recovery was obtained in most cases and the physical and biochemical characters of the forestomach fluid returned toward the normal level. On the other hand, the cellular and biochemical constituents of the blood were within the normal or slightly elevated. The study of the correlation between the macro and micro elements levels in the rumen and serum of gastrointestinal parasites infested camels (Table 4) and their relation to the rumen pH; revealed that there is significant negative relationship between the rumen pH and calcium in both serum and rumen liquor, potassium in both serum and rumen liquor and rumen sodium; while a significant positive relationship was present between rumen pH and serum sodium. The serum calcium had a significant positive relationship with serum copper and ruminal calcium, potassium and magnesium. The serum inorganic phosphorus showed a significant negative relationship with ruminal calcium and copper. Significant positive relationship between serum sodium and negative one with serum zinc and ruminal copper was recorded. The serum potassium was significantly related positively to ruminal calcium, potassium and magnesium. The serum magnesium level significantly showed a positive relationship with rumen calcium, potassium and magnesium while negatively related to serum zinc and rumen copper. The relationship between serum copper and ruminal calcium, potassium and magnesium was significantly positive. Highly significant inverse relationship was observed between serum zinc and rumen copper; while a positive one

was with rumen sodium. In the ruminal Fluid there was a significant positive relationship between calcium, potassium and magnesium.These finding confirms and explains the tight interaction between the blood and rumen constituents under the control of rumen pH, type of feed stuffs gained by the animal, stress factors accompany the infections and their own pathological alterations in the different systems of the camels. The obtained data gives a great recommendation of Hapadex as a broad spectrum anthelmintic in the treatment of gastrointestinal parasites infection in camels.

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Table (1): Prevalence of gastrointestinal parasites species in camels in different seasons.

Spp. / Season	Trichostrogylus			Cooperia			Haemoncus			Nematoirus			Trichuris			Monezia		
	+ve	No.*	(%)	+ve	No.	(%)	+ve	No.	(%)	+ve	No.	(%)	+ve	No.	(%)	+ve	No.	(%)
Winter	31	60	51.7	12	60	20	15	60	25	25	60	41.7	11	60	18.3	9	60	15
Spring	42	60	70	22	60	36.7	15	60	25	33	60	55	13	60	21.6	8	60	13.3
Summer	34	60	56.7	6	60	10	13	60	21.6	29	60	48.3	12	60	20	8	60	13.3
Autumn	22	60	36.7	11	60	18.3	10	60	16.7	39	60	65	16	60	26.3	7	60	11.7
Total	129	240	53.7	51	240	21.3	53	240	22.1	126	240	52.5	52	240	21.7	32	240	12.5

*No.: Total No of examined camels in each season

Table (2): Fore-stomach liquor examination.

Parameters \ Ex. Camels	Healthy camels (37)	Infested camels (60)	After treatment (60)
pH	6.975±0.065	6.308±0.140	6.645±0.180
Total protozoa ($x10^4$/ml)	25.581±3.401	21.640±3.774	23.724±8.012
Entodinium (%)	35.693±5.785	15.840±12.360 [b]	36.720±15.51
Diplodinium (%)	45.115±3.156	64.748±17.700 [b]	35.250±3.068
Epidinium (%)	4.817±0.624	4.213±1.434	2.950±0.581
Dasytricha (%)	6.835±0.834	6.411±1.637	5.492±0.870
Isotricha (%)	0.077±0.035	0.010±0.000	0.000±0.000
Caloscolex (%)	5.527±1.129	5.931±3.207	4.504±0.961
Buetschlia (%)	1.862±0.387	1.295±0.756	1.563±0.302
TVFAs (mmol/L)	44.728±6.852	84.206±10.379 [a]	58.119±7.136
Acetic acid (mmol/L)	31.827±4.869	58.132±6.880 [a]	43.050±4.481
Propionic acid (mmol/L)	8.682±1.505	14.513±2.841 [a]	10.643±1.258
Isobutyric acid (mmol/L)	0.373±0.033	1.244±0.063 [a]	0.600±0.018
Butyric acid (mmol/L)	2.982±0.564	7.975±1.378 [a]	4.036±0.717
Isovaleric acid (mmol/L)	0.482±0.044	1.170±0.189 [a]	0.600±0.717
Valeric acid (mmol/L)	0.300±0.041	0.591±0.077	0.371±0.059
Lactic acid (mmol/L)	0.021±0.004	0.070±0.002	0.074±0.029
Ammonia (mmol/L)	8.692±1.148	10.007±0.616	7.653±2.363
Sodium (mmol/L)	92.727±5.414	115.380±4.752 [b]	97.071±5.908
Potassium (mmol/L)	11.143±1.509	18.412±1.803	14.854±1.730
Calcium (mmol/L)	0.421±0.169	1.460±0.702 [a]	0.981±0.120
Magnesium (mmol/L)	0.326±0.199	1.443±0.342 [a]	0.554±0.017
Copper (µmol/L)	0.321±0.032	0.509±0.092	0.307±0.043
Zinc (µmol/L)	0.752±0.154	1.402±0.271 [a]	0.865±0.097

a: (p<0.001) b: (p<0.01) c: (p<0.05).

Table (3): Haemato-biochemical examination.

Parameters \ Ex. Camels	Healthy camels (37)	Infested camels (60)	After treatment (60)
Hemoglobin (g/L)	132.550±9.930	129.887±2.605	133.140±3.440
RBCs (10^{12}/L)	13.020±1.091	14.044±2.720	14.519±4.436
WBCs (10^{9}/L)	4.374±0.506	7.515±1.073	5.672±1.330
PCV (L/L)	24.976±1.388	30.280±2.221 [a]	28.368±2.045
Total protein (g/L)	53.273±2.563	65.025±2.914	59.236±9.932
Albumin: globulin	1:0.704	1:0.982	1:0.753
Sodium (mmol/L)	140.180±1.525	148.300±0.071	144.560±4.010
Potassium (mmol/L)	4.881±0.208	5.980±0.379	5.311±0.920
Calcium (mmol/L)	1.758±0.208	2.466±0.071 [b]	1.867±0.590
Inorg. Phos. (mmol/L)	1.700±0.154	1.459±0.100	1.443±0.805
Magnesium (mmol/L)	0.539±0.073	0.738±0.043	0.588±0.150
Calcium : Magnesium	1:0.307	1:0.299	1:0.315
Copper (µmol/L)	8.848±1.033	9.436±0.988	8.359±2.300
Zinc (µmol/L)	10.235±1.940	8.314±1.084	9.364±1.013

a: (p<0.001) **b: (p<0.01)** **c: (p<0.05).**

Table (4): Correlation between macro and micro elements levels in the rumen and serum of gastrointestinal parasites infested camels.

	Ca	I.ph*	Na	K	Mg	Cu	Zn	r**Ca	rNa	rK	rMg	rCu	rZn
rpH	-0.59	-0.05	0.58	-0.54	-0.12	-0.29	-0.43	-0.55	-0.83	-0.72	-0.24	-0.22	0.31
Ca		0.45	-0.37	0.37	0.22	0.64	-0.05	0.61	0.10	0.71	0.57	-0.10	-0.41
I.ph			0.10	0.07	0.06	-0.10	0.52	-0.54	-0.26	0.06	0.02	-0.54	0.05
Na				0.22	0.54	0.10	-0.70	-0.09	-0.47	-0.01	0.22	-0.68	0.48
K					0.81	0.30	-0.38	0.84	0.46	0.83	0.65	-0.52	0.13
Mag						0.36	-0.65	0.63	0.06	0.68	0.52	-0.65	-0.07
Cu							0.04	0.54	-0.07	0.71	0.71	0.00	-0.26
Zn								-0.20	0.55	-0.70	-0.30	-0.93	-0.28
rCa									0.31	0.85	0.79	-0.30	-0.41
rNa										0.44	-0.02	0.39	-0.14
rK											0.71	-0.23	-0.24
rMg												-0.32	0.26
rCu													-0.34

I.ph*: inorganic phosphorus **r**: rumen**