Haematological, Biochemical and Histopathological Studies of Camels Experimentally Infected with *Eimeria cameli*

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ملخص البحث

جُمِعت عينات دم لهذه الدراسة من خمسة إبل أصيبت تجريبيًا بجرعة من بيوض متكيسة متبوغة للأيميرية الإبلية. جُمِعت عينات الدم في مانع للتجلط لعمل مسحات دم رقيقة وصبغها بالجمسا لإجراء العد التفاضلي للدم. حُدِدَتْ النسبة المئوية لحجم الخلايا المكدسة (PCV%) وتركيز خضاب الدم و العد الإجمالى لكل من كريات الدم الحمراء و البيضاء. أُستخدمت الأمصال المتحصل عليها من عينات الدم المتخثر لتحديد مستوى البروتين الكلي والزلال والكالسيوم والفوسفور غير العضوي. أُجريت أيضاً معايرة لمغنيسيوم ونحاس المصل. أظهرت نسبة الخلايا المكدسة إنخفاضاً معنوياً بينما لم تتأثر مستويات خضاب الدم. وسجل أيضاً تَغَيُرًا غير معنوى في مستويات العد الإجمالى لكل من كريات الدم الحمراء و البيضاء. من ناحية أخرى زاد عدد الخلايا الليمفاوية زيادة معنوية. كان الإنخفاض المعنوى فى مستوي البروتين الكلي إنعكاساً لإنخفاض مستوى الزلال. أظهرت هذه الدراسة أيضًا إنخفاضاً عالياً في مستوي الكالسيوم والفوسفور. أظهر فحص التشريح بعد الموت وجود بؤر ذات لون رمادى مبيض فى معى الصائم و معى اللفائفي وتورم وإحمرار فى الأغشية المخاطية للأمعاء الدقيقة. أوضح فحص الأنسجة المريضة مراحل تطور المتقسمة إضافة إلى كل من العرسية الكبروية و المكروية فى الظهائر و الغدد المخاطية للصائم و اللفائفى.

Summary

Blood samples for this study were collected from five camels experimentally infected with *Eimeria cameli* sporulated oocysts. Whole blood was obtained in EDTA; thin blood smears were made and stained with Giemsa stain for the differential blood counts. Packed cell volume (PCV), haemoglobin concentration (Hb) and total red blood cells (TRBCs) and white blood cells (TWBCs) counts were determined from the same blood samples. Sera were obtained from coagulated blood and used for the determination of total serum protein, serum albumin, calcium and inorganic phosphorus levels. Serum magnesium (Mg) and serum copper (Cu) were also titrated. The PCV of infected camels showed a significant drop, while Hb levels were not affected. An insignificant change in levels of TRBCs and TWBCs counts was recorded. Lymphocytes, on the other hand, showed a significant increase. The significant drop in total protein level is a reflection of a drop in albumin. Also a very significant reduction in the levels of Ca and P was demonstrated in this study. The postmortem finding showed pinhead-sized whitish-grey foci in the jejunum and ileum. The mucosa of the small intestine was swollen and reddened. Histopathological examination revealed stages of developing schizonts plus macro-and microgametocytes in the epithelia and mucosal glands of the jejunum and ileum.

Introduction

Healthy and productive camels always show normal haematological parameters and biochemical blood constituents. These normal values have been investigated by many authors worldwide and locally. Jackar *et al* (1962) gave quantitative values of the Indian camel blood. Bokari (1974) reported the normal haemogram of Iraqi camels. Observation on normal blood and biochemical values of Sudanese

camels were early reported by Hassan *et al* (1968) from western Sudan. Abdelgadir *et al* (1979) studied the haematology of camels grazing around Tumbool area of central Sudan and Yagoub (1988) reported the normal haematological and biochemical blood constituents of camels from eastern Sudan.

Coccidial infection, in all animals, is always associated with enteritis. The developmental stages of coccidia occur in the mucosa, mucosal glands and epithial cells of the intestinal tract (Soulsby, 1982). When the developing schizonts reach maturity, they burst releasing merozoites that can invade new intestinal cells to form new generations. This rupture of the schizonts may lead to damage of the intestinal wall resulting in severe internal bleeding, impaired absorption capacity and bacterial invasion. Chineme (1980) reported a detailed histopathological lesions caused by natural infection with *E. cameli* in a camel in Nigeria. Similar studies of Kawasmeh and El Bihari (1982) and Hussein *et al* (1987) were carried out in natural *E. cameli* infection in Saudi Arabia.

The present study was conducted in camels experimentally infected with *Eimeria cameli* to compare the haematological and biochemical blood values before and after infection. Intestinal portions obtained from these experimentally infected camels were further processed to elucidate the histopathological findings.

Materials and Methods

Experimental design

Five young camel, 18 to 24 month of age, were purchased from El Obied camel market and used as experimental animals. Animals were kept in a fence and zero grazed, examined for internal and blood parasites before infection. Sporulated oocysts of *E. cameli* were given per os to infect four camels and the fifth was kept as uninfected control.

1. Blood samples

Samples were collected weekly from five camels for six weeks before and ten weeks after infection with *E. cameli*. Blood was obtained from the jugular vein in tubes containing EDTA. In the laboratory, a thin blood smear was made, fixed with methanol and stained with Giemsa. This smear was used for the differential leukocyte count. The packed cell volume (PCV %) was determined using haematocrit centrifuge tubes. Haemoglobin concentration (Hb) was also determined by the cyanmeth-aemoglobin method using a colorimeter. The total erythrocytes count (TRBCs) and total leukocytes counts (TWBCs) were done by the improved Neubour haemocytometer.

2. Serum samples

Camel sera were separated by centrifugation of clotted blood collected from the jugular vein in plain vaccutainers. The tubes were left to stand for three hours before centrifugation to obtain clear sera. The total serum protein was determined by the Biuret method using a spectrophotometer and serum albumen was done by the modified method of Northam and Widdowson (1967). Serum calcium (Ca) was estimated by the murexide indicator to form calcium-murexide complex. This complex was back titrated with EDTA using the EEL titrator and Unigalvo for photoelectric detection of colour change from red to blue purple. The inorganic phosphorus (P) was detected in blood samples containing oxalate + fluoride. This was done after precipitation of blood protein with trichloroacetic acid. The protein free filtrate was treated with molybdic acid to form phosphomolybdate which was then reduced by amino-naphthol-sulphonic acid to produce a blue colour. The

intensity of this colour was detected on the EEL colorimeter.

Serum magnesium (Mg) and serum copper (Cu) as trace elements were also determined. To estimate serum Mg, 0.2 ml of the test serum was added to 2.8 ml of distilled water, then 0.5 ml of polyvinyl alcohol + 0.5 ml of tiller yellow were mixed with the solution to which 1ml of 7.5% sodium hydroxide was added. This mixture was thoroughly mixed, transferred to a colorimeter and read at 540 nm. For serum Cu, the method included heating, adding 2ml of H_2So_4, cooling and then adding 2 ml of concentrated nitric acid to the test serum. Then 0.2 ml of saturated potassium persulphate and 1ml sodium thiocyanate were added, mixed with the contents of the tube before the addition of 5ml of amyl alcohol and centrifugation. The supernatant was read at 480 nm.

3. Histopathology

Portions from the duodenum, jejunum, ileum and colon from the experimentally infected camels were collected and preserved in 10% buffered formalin. These tissues were collected immediately after camel slaughter or death from a peracute coccidiosis. Pieces from the jejunum and ileum were cut from areas showing giant schizonts. Then processed in paraffin, and 5µm sections were cut with a microtome, fixed in glass slides and stained with Haematoxylin and Eosin (H & E).

Statistical analysis

The paired t-test was carried out to detect the significance of any change in values before and after infection.

Results

1. Haematological values

Results of haematological parameters for the five experimentally infected camels are shown, for each camel separately, in Table 1. Normal values for each camel were obtained after sampling of all camels for 6 weeks before infection. The PCV % showed a significant drop ($P< 0.05$), while the Hb of infected camels showed insignificant decrease ($P> 0.05$). The change in TRBCs and TWBCs was detected as insignificant by the t-test ($P> 0.05$). For the differential leukocyte counts, the lymphocytes were significantly increased in number ($P < 0.01$) accompanied by a significant decrease in neutrophils ($P< 0.05$). Monocytes, basophils and eosinophils showed insignificant changes ($P> 0.05$).

2. Biochemical blood constituents

Values of blood constituents of the coccidian-infected camels are shown in Table 2. The drop in total protein was significant ($P < 0.05$), Albumen value showed insignificant increase while that of the globulin was highly significant ($P < 0.01$). The infected camels' serum Ca level showed a significant drop ($P < 0.05$). Magnesium was significantly reduced ($P < 0.05$) while copper showed the same levels before and after infection ($P > 0.05$).

3. Histopathological findings

The most prominent lesions were observed in the small intestine, particularly in the jejunum and ileum. They showed atrophic villi, degeneration, necrosis and denudation of the lining epithelium. In some areas the ulceration was very deep exposing the lamina propria. Most of the mucosal glands, crypts of Lieberkuhn in the affected areas were vacuolated and many of their epithelial cells were fragmented and disintegrated.

Occasionally, the crypts epithelium collapsed and detached from the basement membrane and found in the centre of the gland.

Infrequently, haemorrhages and congestion of the superficial vessels were detected. The changes in the epithelial tissue were accompanied by oedema, proliferation of fibrous tissue and profuse effusion of cellular exudation in the villi, lamina propria and between degenerated,

Table 1: Mean values of haematological parameters of five camels experimentally infected with *E. cameli*.

	Haematological parameters																	
	Before infection									After infection								
Camel	PCV	Hb	TRBCs	TWBCs	L	N	M	B	E	PCV	Hb	TRBCs	TWBCs	L	N	M	B	E
1	26.7	13.1	8.4	8.7	41.5	50.8	2.2	1.7	3.8	19.9	8.96	5.32	9.17	54.9	33.9	3.2	0.9	7.1
2	31.7	13.9	8.4	7.6	42.2	50.3	2.7	1.7	3.2	24.7	10.85	6.15	9.64	52.9	33.8	1.6	1.6	9.1
3	23.7	10.9	8.4	7.7	45	48	2.2	1.3	3.3	20.6	9.16	6.15	10.99	51.6	35.2	1.8	0.9	10.7
4	31.3	14.2	8.45	10.8	46.8	45.7	3.7	1	2.8	26.7	11.9	7.8	11.1	52.5	36.6	3.1	1.4	6.4
5*	28.4	13.4	7.1	11.1	51.9	33.8	4.6	2.1	7	30	14.18	9.7	11.1	55	39.8	1.3	0.3	3.8

*Camel no. 5 died on week 4 post infection.
Before = 6 weeks before infection with *Eimeria cameli*.
After = 10 weeks after infection with *Eimeria cameli*.
PCV = % ; TRBCs and TWBCs = X10ul; Hb = g/dl; = X10ul;
L= Lymphocyte; N= Neutrophils; M= Monocytes; B= Basophils; E= Eosinophils.

Table 2: Mean values of some biochemical blood constituents of camels experimentally infected with *E. cameli*.

	Biochemical blood constituents													
	Before infection							After infection						
Camel	T.S.P.	Alb	Globulin	Ca	P	Mg	Cu	T.S.P.	Alb	Globulin	Ca	P	Mg	Cu
1	8.1	3.83	4.27	8.12	5.42	2.17	4.58	5.74	4.25	1.49	7.13	5.03	1.49	4.86
2	8.2	3.78	4.4	8.23	5.42	2.12	4.8	6.63	3.95	2.68	7.06	4.78	1.53	4.86
3	8.18	4.17	4.02	8.4	5.37	2.15	4.58	6.88	4.53	2.35	7.71	5.28	1.5	4.58
4	8.23	4.23	4.03	8.52	5.32	2.2	4.92	7.68	3.98	3.7	8.22	5.16	2.15	4.54
5*	8.05	4.1	3.95	8.38	5.16	2.3	4.47	7.95	3.95	4	7.9	5.05	2	4.38

*Camel no. 5 died on week 4 post infection.
Before = 6 weeks before infection with *Eimeria cameli*.
After = 10 weeks after infection with *Eimeria cameli*.
T.S.P. = Total serum protein
Alb = Albumen
Ca = Serum Calcium
P= Inorganic phosphorus
Mg = Serum magnesium
Cu= Serum Copper

necrotized and dissociated mucosal glands.

The majority of infiltrated cells were eosinophils intermixed with some lymphoid, macrophages and plasma cells. Oedema and aggregated cells were seen in the submucosa.

Different developmental stages of *E. cameli* were trapped in denuded fragmented glands. Large mature and developing schizonts were also observed. These schizonts which filled the dilated atrophic glands and pushed the cellular debris to the periphery, were concomitantly detected with undifferentiated gamonts. Mature micro-and macrogametocytes were clearly seen. Moreover, mature oocysts of *E. cameli* could easily be spotted in the denuded epithelium of crypts of Lieberkuhn.

Discussion

Although no experimental infection of camels with coccidia was recorded before this study, all investigators who studied the natural coccidial infections of camels stated that, the disease produces pale mucus membranes (Hussein *et al*, 1987), progressive wasting (Chineme, 1980) and emaciation (Stepanova, 1982). All these observations indicate that coccidial infection in camel is always associated with anaemia. In this study, the anaemia is expressed as a significant decrease in the value of PCV % of the three camels (Nos. 1, 2 and 3) that survived until slaughtered. The haemoglobin value had also decreased in these camels and the total number of erythrocytes dropped.

The inflammatory cellular response in coccidial infection in camels was recorded as predominantly mononuclear and eosinophilic in nature (Kawasmeh and El Bihari, 1982). In this study, the differential leukocyte counts results reveal a highly significant increase in number of both lymphocytes and eosinophils which agrees completely with the findings of the latter authors.

It seems that the loss of body weight and emaciation consequent upon coccidial infection in camels is not expressed as decreases in haematological parameters only. The biochemical blood constituents had also become very much affected. The total serum protein and globulin decreased to the level that can easily be noticed during the post-mortem of infected camels. The carcasses of these camels looked severely emaciated, deprived of any fat or edible meat and oedematous in some parts.

Although no nervous symptoms were observed in this experiment, the highly significant decrease in serum magnesium and calcium levels of the infected camels seems to agree with the finding of Fanelli (1983). The author encountered cases of nervous symptom similar to those produced by hypomagnesaemia in naturally coccidia-infected bovine calves. He attributed these nervous symptoms to depletion of tissue magnesium and decrease in serum calcium sub-sequent to the intestinal damage caused by the coccidial infection.

The histopathological study clearly demonstrates that all developmental stages of the parasite occur in the jejunum and ileum. Chineme (1980) and Kawasmeh and El Bihari (1982) had found giant schizonts and other developmental stages in the lamina propria of the jejunum but they made no mention for their presence in the ileum. The associated inflammatory cellular response is agreed upon by all these authors and also in the present study as predominantly mononuclear and eosinophilic cells in character.

The haemorrhages and congestion of superficial vessels together with complete destruction of intestinal villi explain the peracute nature of coccidial infection. Hence, young camel calves

subjected to heavy doses of sporulated oocysts may suddenly die, and infrequently with blood oozing from their anuses. This condition should be differentiated from anthrax and other causes of sudden death.

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