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USE OF BLOOD ANALYTES IN ASSESSMENT OF STRESS DUE TO DROUGHT IN CAMEL

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ABSTRACT

A study was carried out to assess the stress due to drought in dromedaries on the basis of variations in the values of some blood analytes. The animals screened belonged to farmers' stock. The serum analytes included cortisol, aldosterone, sodium, potassium, chloride, calcium, phosphorus, proteins, urea, creatinine and vitamin A. The haematological analytes included red blood cell and white blood cell indices. The mean values of serum cortisol and aldosterone were significantly ($p \leq 0.05$) higher in camels belonging to drought affected areas than those of other areas. The drought affected camels had lowered eosinophil and lymphocyte counts and haemoglobin concentration. Mean serum vitamin A level and total proteins were non-significantly ($p \geq 0.05$) lower in animals of drought affected areas. The animals did not reveal any occult bacterial infection in blood smears. The affected animals did not show apparent changes in the physical health except pica in few cases.

Key words : Aldosterone, blood analytes, cortisol, dromedaries, drought and stress

The camel is well adapted to extremes of desert environs due to several strategies evolved by it and is resistant to many of the infections even during the stress (Kataria *et al*, 2001). Though the camel resists sickness to a great extent due to stress but the production potential may get at times reduced. Because of camel not getting inflicted with diseases during such periods, not much attention is paid to either stress factors and nor to the health of the animal though stress may alter the physiological status of the individual (Kataria *et al*, 2000).

Drought conditions may severely affect the productivity because of drastic changes in the environment . In arid and semi-arid tract the changes in external environment includes scarcity of water and scanty or no vegetation. Disturbances in *milieu interior* results in stress on the animals. The use of haematological norms is one of the easy means to evaluate the adaptability and productive efficiency of the animals. Further it helps to assess the health status of the animals. It is well documented that the chemical composition of blood in any living animal is greatly influenced by physical as well as metabolic factors. There is scarcity of data to observe effect of the drought on the dromedaries. Therefore the present study was carried out for the stress assessment in dromedaries of drought affected areas through some blood analytes. In addition the camels were also screened for some occult bacterial infection.

Materials and Methods

For the assessment of stress through blood analytes 83 adult dromedaries of either sex belonging to farmers' stock were screened out of which 40 belonged to drought affected areas and 43 animals belonged to other areas. The animals were kept for the purpose of farming and light load carrying with almost similar feeding and watering conditions. The blood analytes included some serum parameters (cortisol, aldosterone, sodium, potassium, chloride, calcium, phosphorus, proteins, urea, creatinine and vitamin A) and haematological indices (total erythrocytic count (TEC), haemoglobin, packed cell volume, viscosity, total leucocytic count (TEC), differential leucocytic count (DLC) and osmotic fragility). The animals were categorised as healthy males (20), healthy females (23), drought affected males (18) and drought affected females (22).

The serum cortisol and aldosterone levels were determined by using RIA kit (DPC, USA) as per the manual and manufacturer's instructions supplied with the kit. For the measurement of activity, ^{125}I Gamma counter (ECIL) was used. Serum sodium, potassium, and chloride were determined as per

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the standard methods given by Oser (1976). Serum calcium, phosphorus, proteins, creatinine and urea were determined by using colorimetric kits (Wipro) and serum vitamin A was determined by Carr and Price method (Varley, 1988). Viscosity was determined by using viscometer (Oser, 1976) and osmotic fragility as per the method described by Perk (1963). The other haematological indices were determined by the standard methods described by Schalm *et al* (1975). The sampling was carried out during moderate environmental conditions and the mean maximum temperature during the period was 38. 01± 0.41°C.

Paired - t test (Snedecor and Cochran, 1967) was carried out to determine the statistical significance for individual parameters between healthy animals and drought affected animals, healthy males and healthy females and drought affected males and drought affected females.

Results and Discussion

The mean ± SEM values of serum cortisol, aldosterone, sodium, potassium and chloride are presented in table 1, of calcium, phosphorus, proteins, urea, creatinine and vitamin A are presented in table 2, of total erythrocytic count (TEC), haemoglobin, packed cell volume, viscosity and osmotic fragility in table 3, and of total leucocytic count (TEC) and differential leucocytic count (DLC) in table 4.

The overall mean values of serum cortisol and aldosterone were significantly ($p \leq 0.05$) higher in the animals of drought affected areas. Serum cortisol was 5.27 and aldosterone was 1.75 times higher than in the animals of other areas, respectively.The overall mean values of cortisol and aldosterone in the animals of other area were more or less similar to those reported by earlier workers in the camels (Agarwal *et al*, 1991; Ziv *et al*, 1997 and Kataria *et al*, 2000).

The increase in the values of the two hormones during drought indicated stress on the animals. Kataria *et al* (2000) reported a 4-fold rise in the mean values of serum cortisol due to dehydration in winter months and pointed towards the stress to the animals. The stress is an important stimulus for the release of cortisol through CRH and ACTH (McDonald, 1980).

Table 1. Serum levels of hormones and electrolytes in dromedaries.

S.No.	Category (No. of animals)	Cortisol ng/ml	Aldosterone ng/ml	Sodium mmol/l	Potassium mmol/l	Chloride mmol/l
1.	Overall (Other area)	9.11 ± 1.01	2.80 ± 0.091	160 ± 5.01	5.81 ± 0.23	108.01 ± 4.01
(i)	Male	9.98 ± 0.99	2.91 ± 0.054	165 ± 4.90	5.61 ± 0.41	110 ±5.20
(ii)	Female	9.77 ± 1.11	3.78 ± 0.082	156 ± 6.1	5.99 ± 0.25	107 ± 4.40
2.	Overall (Drought affected area)	48.01 ± 3.00[b]	4.91 ± 0.072[b]	158 ± 4.93	5.00 ± 0.31	106 ± 5.90
(i)	Male	42.2 ±2.99	4.99 ± 0.068	159 ± 5.65	4.91 ± 0.22	104 ±4.70
(ii)	Female	45.9 ± 3.21	4.01 ± 0.088	154 ± 4.91	5.01 ± 0.30	107 ±4.30

1. Overall means (drought affecetd area) within a given parameter superscribed by letter 'b' differ significantly ($p \leq 0.05$) from respective overall means (Other area). No superscription indicates non significant ($p > 0.05$) differences.
2. Within each class the means have been compared from each other. No superscription indicates non significant ($p > 0.05$) differences.

Table 2. Serum levels of electrolytes, metabolites and vitamin A in dromedaries.

S.No.	Category (No. of animals)	Calcium mg/dl	Phosphorus mg/dl	Total Proteins g/l	Creatinine mg/dl	Urea mg/dl	Vitamin A µg/dl
1.	Overall (Other area)	10.34 ± 0.99	4.98 ± 0.57	73.21 ± 5.98	1.43 ± 0.09	23.89 ± 1.12	75 ± 7.81
(i)	Male	11.21 ± 1.21	5.12 ± 0.61	75.12 ± 6.00	1.45 ± 0.05	25.19 ± 1.31	78 ± 6.09
(ii)	Female	9.98 ± 1.00	4.64 ± 0.56	71.31 ± 5.51	1.41 ± 0.09	21.78 ± 1.01	72 ± 6.97
2.	Overall (Drought affected area)	9.12 ± 1.12	1.73[b] ± 0.32	69.23 ± 4.91	1.45 ± 0.04	24.08 ± 1.12	66 ± 6.12
(i)	Male	9.31 ± 1.09	1.75 ± 0.51	70.21 ± 5.0	1.46 ± 0.089	26.7 ± 1.4	69 ± 6.32
(ii)	Female	9.00 ± 1.17	1.68 ± 0.71	68.12 ± 4.98	1.42 ± 0.06	20.9 ± 1.07	63.51 ± 7.23

1. Overall means (drought affecetd area) within a given parameter superscribed by letter 'b' differ significantly ($p \leq 0.05$) from respective overall means (other area). No superscription indicates non significant ($p > 0.05$) differences.
2. Within each class the means have been compared from each other. No superscription indicates non significant ($p > 0.05$) differences.

Table 3. Red blood cell indices in dromedaries.

S.No.	Category (No. of animals)	TEC $X10^{12}/l$	Hb g/dl	PCV %	Viscosity	Osmotic Fragility	
						Min.	Max.
1.	Overall (Other area)	8.99 ± 0.91	13.01 ± 1.01	31.01 ± 1.91	4.20 ± 0.10	0.29 ± 0.019	0.19 ± 0.017
(i)	Male	9.1 ± 0.89	14.01 ± 0.91	32.41 ± 1.76	4.21 ± 0.09	0.30 ± 0.016	0.20 ± 0.013
(ii)	Female	8.88 ± 1.00	12.92 ± 1.02	30.01 ± 1.41	4.19 ± 0.08	0.28 ± 0.013	0.18 ± 0.015
2.	Overall (Drought affected area)	7.79 ± 0.97	12.41 ± 1.07	29.91 ± 1.66	4.32 ±0.09	0.30 ± 0.019	0.20 ± 0.017
(i)	Male	7.91 ± 0.891	12.99 ± 1.02	30.01 ± 1.71	4.28 ±0.08	0.29 ± 0.014	0.19 ± 0.019
(ii)	Female	7.68 ± 0.82	12.01 ± 1.05	29.09 ± 1.51	4.33 ± 0.10	0.31 ± 0.012	0.21 ± 0.014

1. Overall means (drought affecetd area) within a given parameter have been compared with respective overall means (other area). No superscription indicates non significant ($p > 0.05$) differences.
2. Within each class the means have been compared from each other. No superscription indicates non significant ($p > 0.05$) differences.

Table 4. White blood cell indices in dromedaries.

S.No.	Category (No. of animals)	TLC $x10^9/l$	Neutrophil %	Lymphocyte %	Monocyte %	Eosinophil %	Basophil %
1.	Overall (Other area)	18.99 ± 1.01	61.01 ± 2.01	30.01 ± 1.90	5.20 ± 0.10	4.20 ±0.030	1.0 ± 0.001
(i)	Male	19.1 ± 1.05	64.01 ± 2.91	32.41 ± 1.96	4.91 ± 0.19	4.10 ± 0.20	1.0 ± 0.001
(ii)	Female	18.88 ± 1.00	58.92 ±4.02	29.01 ± 1.41	5.49 ± 0.18	4.30 ± 0.11	1.0 ± 0.001
2.	Overall (Drought affected area)	15.09 ± 1.01[b]	69.41 ± 2.07[b]	23.91 ± 1.56[b]	4.22 ± 0.29	2.20 ±0.28[b]	1.0 ± 0.001
(i)	Male	15.00 ± 1.02	62.99 ± 3.02	30.01 ± 1.71	4.28 ± 0.28	2.30 ± 0.15	1.0 ± 0.001
(ii)	Female	15.18 ± 1.08	59.01 ± 2.05	29.09 ± 1.51	4.43 ± 0.31	2.10 ± 0.13	1.0 ± 0.001

1. Overall means (drought affecetd area) within a given parameter have been compared with respective overall means (other area). No superscription indicates non significant ($p > 0.05$) differences.
2. Within each class the means have been compared from each other. No superscription indicates non significant ($p > 0.05$) differences.

Higher cortisol concentration is probably required to meet the energy crisis during physical stress to the animals. By the glucogenolytic and gluconeogenetic properties cortisol enhances glucose levels (Tharp, 1975).

Higher aldosterone levels in the animals of drought affected areas were probably to absorb salt and water from the gastrointestinal tract. In the present investigation the sodium levels were in the normal range in drought affected animals. Probably it was because of the higher aldosterone levels. During drought conditions mineral deficiency is a common feature (Kumar, 2004) but higher aldosterone levels helped the animals to maintain the normal sodium levels. Water scarcity occurs during drought. It may produce mild to severe dehydration to the animals. Yagil (1985) opined that aldosterone was important for the dehydrated camels. Non significant ($p > 0.05$) changes were observed in the overall mean values of sodium, potassium and chloride. This showed the modulation of physiological mechanisms by the camel during adverse conditions (Kataria, 2000) like drought in present investigation.

The overall mean values of serum calcium and phosphorus in the present study in healthy male and female animals corroborated the earlier findings in dromedaries (Sarwar and Majeed, 1997 and Kataria *et al*, 2002a). The overall mean values of calcium did not show any significant ($p \leq 0.05$) changes due to drought. However, serum phosphorus levels were significantly ($p \leq 0.05$) lower in the drought affected animals. This was probably due to scanty vegetation or dietary deficiency of the phosphorus. The pica was observed in many camels of the drought affected areas.

Mean values of total proteins, urea and creatinine in the camels of other areas were similar to those reported by Kataria *et al* (2002a). Nonsignificant changes were observed in the mean values of total proteins, creatinine, urea and vitamin A. Kumar

(2004) and Gottam (2004) reported vitamin A deficiencies in the drought affected cattle, sheep and goat. This was evident by the history, symptoms as well as by the laboratory findings. However, in the present investigation no signs of vitamin A deficiency were observed in the camels of drought affected areas. This showed the sufficient storage of vitamin A by the liver in camels which would have kept the serum levels higher during scarcity periods. Generally the serum levels lower than 30 µg/dl indicates depletion of vitamin A from the liver (Kaneko *et al*, 1999).

The mean values of red blood and white blood indices in the camels of other area were similar to those reported for the camels by earlier workers (Soni and Aggarwala, 1958; Perk, 1963; Yagil *et al*, 1974; Wilson, 1989; Nyang'o *et al*, 1997; Kataria *et al*, 2001 and Kataria *et al*, 2002b). Nonsignificant ($p > 0.05$) changes were observed in the overall mean values of all the red blood cell indices. However, total leucocytic counts were significantly ($p \leq 0.05$) lower in the drought affected animals. A marked lymphocytopaenia, monocytopaenia and eosinopaenia was observed in the drought affected animals. However, the neutrophil percentage was signifiacntly higher in the later. This was probably due to the effect of higher cortisol levels. McDonald (1980) described that higher cortisol values may produce low lymphocyte, monocyte and eosinophil counts. It is probably because of redistribution of circulating cells to other body compartments.

The mean values for each analyte were compared according to sex within each class i.e. camels of other area and camels of drought affected areas. The changes were non significant ($p > 0.05$) in each case.

The bacteriology carried out on blood smears did not reveal presence of any occult bacterial infection.

Conclusion

The results of the present investigation indicated that serum cortisol and aldosterone levels were good indicators to assess the stress to the animals who did not exhibit apparent signs of stress. The picture of other analytes may appear normal but there interpretation should be made on the basis of the other related parameters. The deficiency signs together with the laboratory analyses will be helpful in the proper management of the animals during drought conditions.

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Natural (clinical) dermatophilosis in the one-humped camels (*Camelus dromedarius*) slaughtered in Sokotio abattoir, Nigeria

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Skin scabs from the back, flanks, neck, legs, lips and other parts of the body of 528 camels (C. *dromedarius*) presented for slaughter at the Sokoto abattoir, Nigeria were examined for *Dermatophilus congolensis* by the direct Gram staining and culture methods. It was shown that 62 (11.7%) of the camels examined between March 2001 and February 2002 were infected by *D. Congelensis*. All of the infected camels were detected by culture method whiel 60 of them were detected by gram-staining. However, there was no significant difference (df=1; x^2=3.2, p>0.05) between the two methods in detecting dermatophilosis in camels. Of the 365 camels examined during the dry season, 33 (9.1%) were affected while 29 (17.8%) of the 163 examined during rainy season were affected. The prevalence rates for dermatophilosis were significantly higher (df=1; x^2=8.3, p>0.001) during the rainy season than in the dry season. To the author's knowledge, this is the first documented report of natural (clinical) dermatophilosis in camels in Nigeria.

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THESIS ABSTRACT

Title of Thesis : A study on heavy chain antibodies of Indian desert camel (*Camelus dromedarius*) with particular reference to their natural antibody activity.

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The present study was aimed at studying immunoglobulins of Indian desert camels (*Camelus dromedarius*). IgG molecules devoid of light chains in their structure ("Heavy chain antibodies" : HCAbs) have recently been reported from other parts of the world to occur in camels, llamas and other species in the family Camelidae. But there is no report on the HCAbs in the Indian dromedary camels. Therefore, the present study was conducted to identify, purify and characterise heavy chain antibodies (HCAbs) from the sera samples of Indian desert camels (*Camelus dromedarius*). Physico-chemical properties (molecular composition, size and charge dispersity), antigenic properties (relatedness with IgG from other animal species) and functional/biological properties (natural antibody activty against erythrocytes from different animal species) were studied.

Blood samples from adult Indian desert camels were collected aseptically. Serum obtained was inactivated for complement. HCAbs (IgG_2 and IgG_3 subtypes) in camel sera samples were isolated and purified by ammoniuim sulphate precipitation followed by ion-exchange chromatography in DEAE-cellulose, and affinity chromatography in protein A-sepharose and protein G-sepharose. Purified HCAbs were detected and characterised by SDS-polyacrylamide gel electrophoresis, agar gel immunodiffusion, counter and immunoelectrophoresis, indirect Coombs' test, ELISA immunoblotting.

In ion-exchange chromatography, IgG_3 (HCAb) was eluted in pure form at 260 mM NaCl concentration. However, its elution started at 220 mM NaCl along with IgG_1 (the full-length conventional antibody). Elution pattern in ion-exchange chromatography indicated its charge heterogeneity. The charge heterogeneity was also indicated by immunoelectrophoresis wherein they migrated in b-region. Because of the smaller size and more net negative charge, they could be detected in counterimmunoelectrophoresis using anti-IgG3. Purification of HCAbs was also done from serum, ammonium sulphate precipitated Igs and ion-exchange purified fractions by protein A and/or protein G-sepharose affinity chromatography. Their molecular masses were determined by SDS-PAGE to be 46.77 kDa for IgG_2 and 46.65 kDa for IgG_3 heavy chain in reducing conditions. The molecular masses in non-reducing PAGE were found to be more than expected, indicating their anomalous migration.

Antigenic relatedness of camel IgG was investigated employing three different tests viz., AGID, ELISA and immunoblotting. These tests using anti-camel IgG_3 revealed the presence of cross-reactive epitopes on IgGs of pig and ruminants (cattle, buffalo, sheep, goat) but absence of any reactivity with Igs from horse, dog, guinea pigs, mice, fish, poultry and human. This finding further confirmed the present viewpoint on the phylogenetic relationship of camels with pigs and ruminants.

For studying the natural antibody activity of camel sera and purified HCAbs against erythrocytes, blood samples from six animals each of ten different animal species viz. cattle, buffalo, sheep, goat, pig, horse, dog, mouse, poultry and frog were collected in Alsever's anticoagulant solution. Indirect Coombs' test proved to be a better test than HA, for studying the natural antibody activity in HCAbs since they behaved as incomplete antibodies. Indirect Coombs' test could detect 'natural' antibody activity in camel sera and isolated HCAbs against erythrocyte surface antigens from diverse animal species; a finding indicating their contribution in the Ig repertoire diversity.