Journal of Animal and Veterinary Advances 11 (2): 284-288, 2012
ISSN: 1680-5593

Effect of Contagious Skin Necrosis and Trypanosomosis on Health Status of Camels

Maha I. Hamed and Mahmoud R. Abd Ellah
Department of Animal Medicine, Faculty of Veterinary Medicine,
Assiut University, 71526 Assiut, Egypt

Abstract: The present study was undertaken to evaluate the effect of Contagious Skin Necrosis (CSN) with or without trypanosomosis on blood oxidative status in camels. A total of 15 camels were subjected to the study. Out of them, 10 camels were suffered from CSN. Sterile bacteriological swabs from skin necrosed area whole blood samples for hematological analysis and for diagnosis of trypanosomosis and serum samples for measuring lipid peroxidation product (Malondialdehyde) were collected. The bacteriological examination revealed that *Staphylococcus aureus* was the predominant bacterial isolate alone in 6 cases and coupled with other bacteria in the remained 4 cases, the latter was coupled with coagulase negative staphylococci in 3 cases and coupled with *Streptococcus agalactiae* in one case. *Trypanosoma evansi* infection was identified using polymerase chain reaction in 5 camels that had CSN. Malondialdehyde showed significant increase in camels affected with CSN that associated with trypanosomosis. The current study revealed that *Staphylococcus aureus* was the predominant bacterial isolate in camels with CSN. Lipid peroxidation products increased in the blood of camels with CSN that associated with trypanosomosis, It is recommended to supply camels suffering from CSN with antioxidants to overcome the deterioration of blood oxidative status.

Key words: Camels, CSN, trypanosoma, MDA, blood, Egypt

INTRODUCTION

Camel remains an integral part of the culture and agriculture in many countries of the Arab world. The camels were classified into two species *Camelus dromedaries* (one humped, dromedary or Arabian camel) and *Camelus bacterianus* (two humped camel) (Higgins, 1986). The camel (Dromedary) is an important livestock species uniquely adapted to hot and arid environments. It produces meat, milk, hair, wool and hides, serves for riding (Schwartz and Dioli, 1992).

Contagious skin necrosis is a chronic inflammation of the skin primarily caused by *Staphylococcus aureus* and occurred mainly in young dromedaries and localized mostly in the shoulder and neck regions. (Domenech *et al*., 1977). The disease began with signs of folliculitis which frequently progressed to a furunculosis with individual or grouped small abscesses.

These abscesses could become large and when lanced yield whitish-green pus and the disease could be chronic and difficult to treat medically depending on the pathogenicity of the staphylococcal strain present (Higgins, 1986; Ismail *et al*., 1990; Dioli and Stimmelmayr, 1992; Wernery and Kaaden, 1995). Arthropods were incriminated as transmitting vectors of the various types of the isolated bacteria (Qureshi *et al*., 2002).

Trypanosomosis in camel caused by *Trypanosoma evansi* is still a serious problem in camel husbandry causes considerable economic losses in many camel-rearing regions of the world (Olaho-Mukani and Nyango'ao, 1996; Derakhshanfar *et al*., 2010). The course of the infection is often chronic and the parasitological diagnosis is usually difficult because the parasitaemia is low or no trypanosomes are found in the blood (Zweygarth *et al*., 1984).

Diagnosis of trypanosomosis depends on detection of the parasite in the blood or tissue fluids of the infected animals (Boid *et al*., 1986). With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by Polymerase Chain Reaction (PCR) have been developed (Abdel-Rady, 2008). Free radicals are highly reactive substances produced continuously during metabolic processes.

They mainly participate in physiological events such as immune response, metabolism of unsaturated fatty acids and inflammatory reaction. The most important free radicals include superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$) and hypochlorous acid (Stohs, 1995; Ellah, 2010). Free radical excess results in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers

Corresponding Author: Maha I. Hamed, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

which in turn, may be related to mutagenesis and aging processes (Poli, 1993). Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms (Sies, 1991) and/or from a decrease in antioxidant defense which may lead to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan *et al*., 2001). This condition can contribute and/or lead to the onset of health disorders in animals (Miller *et al*., 1993).

Lipid peroxidation is a well established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate Malondialdehyde (MDA) upon decomposition (Esterbauer *et al*., 1991). The goal of the present study evaluate the effect of Contagious Skin Necrosis (CSN) with or without trypanosomosis on blood oxidative status in camels.

MATERIALS AND METHODS

Animals: A total number of 15 camels were subjected to study. Out of them, 10 camels were suffered from CSN. The remained camels (N = 5) were healthy and kept as control. The investigated camels were clinically inspected for the presence of lesions of skin necrosis or abscesses according to Rollefson *et al*. (2001) and the suspected cases were subjected to detailed clinical examination and samples collection.

Diagnosis of contagious skin necrosis: Sterile bacteriological swabs were taken from the opened cutaneous abscesses of the infected camels after disinfection also the necrosed skin was detached and the underlying tissue was swabbed. Bacterial swabs were taken from abscesses and wounds and then inoculated into tube containing brain heart infusion broth and incubated at 37°C for 24-48 h.

The incubated tubes were streaked onto blood agar base enriched with 10% citrated sheep's blood and onto MacConkey agar plates.

The inoculated plates were aerobically incubated at 37°C for 24-48 h. The suspected colonies were picked up, purified and morphologically and biochemically indentified. Identification of the bacterial pathogens was made following standard bacteriological techniques (Quinn *et al*., 1994; Carter *et al*., 1995).

Diagnosis of trypanosomosis

Microscopical examination of blood films: For detection of trypanosoma infection 2-5 thin blood films from each camel were prepared, fixed with absolute methanol for 5 min then left for dryness and stained for 35-45 min by 10% Giemsa stain. Smears were carefully examined by using the oil immersion lens (Coles, 1986).

PCR technique: The PCR technique was performed according to the method described by Artama *et al*. (1992) using primer 1: 5′-CGAATGAATATTAAACAA TGCGCAGT-3′, primer 2: 5′- AGAACCATTTATTAGCTT TGTTGC-3′. Polymerase chain reaction was carried out in 25 μ reaction volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCI, 2 mM $MgCl_2$, 250 μM each of the four dNTP′s, 1 unit of Ampli Taq Gold® polymerase, 5 μL template and 2 μM of each of the oligonucleotide primers. The amplifications were conducted for 20 cycle in a DNA Thermal Cycler in which each cycle contained successive incubations of 94°C for 1 min (denature), 55°C for 2 min (anneal) and 72°C for 2 min (extension). Products of PCR were visualized by gel electrophoresis using a 2% agarose and ethidium bromide-staining. The PCR product was detected at approximately 177 bp.

Haematological analysis: Blood samples were collected from the jugular vein of the investigated camels into vacutainer tubes containing EDTA (Ethylene-Diamin-Tetra-Acetic-acid-di-sodium) as anticoagulant. Total red blood cells count was determined using haemocytometer while hemoglobin was determined by using Sahl's apparatus. Packed cell volume was determined by Microhaematocrite centrifuge apparatus. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated mathematically according to the method described by Coles (1986).

Measurement of serum MDA level: Blood samples for separation of serum were collected from the jugular vein into plain vacutainer tube and processed for separation of serum according to Coles (1986). Serum samples were used for measuring MDA concentration using commercial kit supplied by Bio-diagnostics (Cairo, Egypt).

Statistical analysis: Data were presented as mean and standard deviation. Statistical significance was determined by the Analysis of Variance (ANOVA) using Statistical Package for the Social Sciences for Windows (SPSS, Version 10.0, Chicago, IL, USA). Data from studied groups were tested for difference using Dunnett's t-test. Statistically significant differences were determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Ten camels showed characteristic dermal lesions of CSN, these lesions were demonstrated on different parts of the animal body as an area of skin necrosis in which the skin looked black in color and not covered with hair (Fig. 1). These areas sharply separated from the surrounding healthy skin and they were cold and very hard in consistency. When an area of necrosed skin was detached, circular ulcer of varying diameter, usually 2-10 cm remained and clearly demarcated from surrounding healthy tissue.

This ulcer was filled with large amounts of whitish pussy material tinged with blood and may reach up to 10 cm in depth (Fig. 2). The bacteriological examination of collected swabs from dermal lesion of CSN revealed that *Staphylococcus aureus* was the predominant bacterial isolate alone in 6 cases and coupled with other bacteria in the remained 4 cases, coupled with coagulase negative staphylococci in 3 cases and coupled with *Streptococcus agalactiae* in one case.

Trypanosoma evansi infection was identified using polymerase chain reaction in 5 camels that had CSN. Comparing data from camels with CSN groups with the control group revealed significant decrease in total RBCs count ($p<0.05$) in camels suffering from CSN alone. On the other hand, there were insignificant changes in Hb concentration, PCV, MCV, MCH and MCHC among studied groups. Serum malondialdehyde showed significant increase in camels affected with CSN with trypanosomosis ($p<0.01$) when compared with the control healthy camels (Table 1).

The main clinical findings in camels suffered from contagious skin necrosis were similar to that recorded by Higgins (1985), Dioli and Stimmelmayr (1992), Wernery and Kaaden (1995) and Rollefson *et al.* (2001). In the current study, lesions of contagious skin necrosis were found on the back of the animal, sides, shoulder region, gluteal region and in the ventral aspect of the neck. Similar field observation was previously recorded by Higgins (1985) who noted that lesions of staphylococcal dermatitis were usually situated in the gluteal, perineal and lower cervical regions. Dioli and Stimmelmayr (1992) observed that the most common affected sites were the head, neck and shoulder region. The obtained results revealed that the main isolated bacterial species from Contagious Skin Necrosis (CSN) affected camels was *S. aureus* (60.61%) while coagulase negative staphylococci represented 27.27% and *Streptococcus agalactiae* was 12.12%, these results agreed with Wernery and Kaaden (1995) who stated that staphylococcal dermatitis primarily caused by *S. aureus*. The obtained results slightly similar to Domenech *et al.* (1977), Dioli and Stimmelmayr (1992) and Rollefson *et al.* (2001) where they reported that contagious skin necrosis in camels caused by a number of bacteria including *S. aureus*. In contrast to the results of Edelstein and Pegram (1974) who stated that the main isolated bacteria

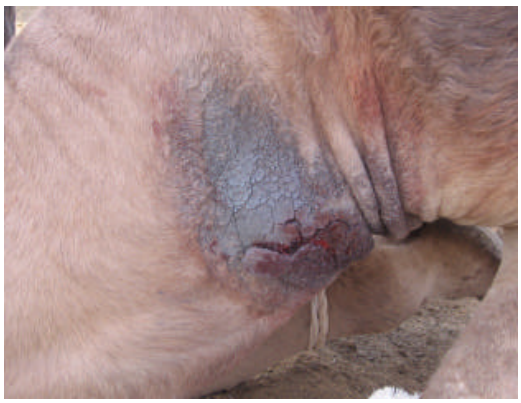

Fig. 1: Camel with CSN, skin looked black in colour and not covered with hair

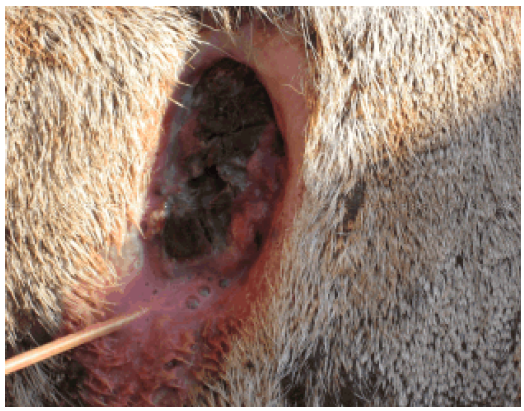

Fig. 2: Camel with CSN showing ulcer filled with large amounts of whitish pussy material tinged with blood

Table 1: Measured blood constituents and serum MDA levels in the investigated camels

Blood constituents	RBCs ($\times 10^6$ uL^{-1})	Hb (g dL^{-1})	PCV (%)	MCV (pg)	MCH (fL)	MCHC (g dL^{-1})	MDA (nmol mL^{-1})
Control	8.35±1.21^{a}	12.2±1.73^{a}	28.5±2.3^{a}	34.7±5.6^{a}	14.7±1.6^{a}	43.3±7.5^{a}	1.0±0.6^{a}
CSN	6.20±1.80^{b}	10.7±2.20^{a}	25.4±3.8^{a}	40.0±6.5^{a}	15.7±3.3^{a}	39.4±5.3^{a}	3.2±1.7^{ab}
Tryp.+CSN	7.20±1.30^{ab}	11.2±2.20^{a}	26.8±3.3^{a}	37.9±7.5^{a}	15.8±4.1^{a}	41.6±5.3^{a}	5.0±2.6^{b}

In each column, different letter means significant, Tryp: Trypanosomosis

from contagious skin necrosis was *Streptococcus agalactiae* followed by *S. aureus*. Lipid peroxidation in biological samples (Slater, 1982) and the metabolic fate of malondialdehyde has been extensively studied (Bird and Draper, 1982; Siu and Draper; 1982; Hjelle and Petersen, 1983). Unlike reactive free radicals, aldehydes are rather long lived and therefore can diffuse from their site of origin (i.e., membranes) to reach and attack other targets intracellularly or extracellularly (Esterbauer *et al*., 1982).

The increased MDA in the current study indicated increased oxidative stress in blood of camels with CSN coupled with trypanosomosis and this may attributed to decreased antioxidant levels or may be due to excessive release of free radicals. Anemia in the present study may be attribute to the increased reactive oxygen species (especially H_2O_2) which result in accumulation of hydrogen peroxide and causes oxidation of the sulfhydryl groups of the globin chains, the erythrocyte cell membrane may be damaged resulting in the erythrocyte being removed from circulation (Robbins and Kumar, 1994).

CONCLUSION

The current study revealed that *Staphylococcus aureus* was the predominant bacterial isolate. Lipid peroxidation products increased in the blood of camels with CSN and it is recommended to supply camels suffering from CSN with source for antioxidants to overcome the deterioration in blood oxidatuve status.

REFERENCES

Abdel-Rady, A., 2008. Epidemiological studies (parasitological, serological and molecular techniques) of *Trypanosoma evansi* infection in camels (*Camelus dromedarius*) in Egypt. Vet. World, 1: 325-328.

Artama, W.T., M.W. Agay and J.E. Donelson, 1992. DNA comparisons of *Trypanosoma evansi* (Indonesia) and *Trypanosoma brucei* spp. Parasitology, 104: 67-74.

Bird, R.P. and H.H. Draper, 1982. Uptake and oxidation of malonaldehyde by cultured mammalian cells. Lipids, 17: 519-523.

Boid, R., T.W. Jones and A.G. Luckins, 1986. 3. Protozoal diseases of camels. Br. Vet. J., 141: 87-105.

Carter, G.R., M.M. Chengappa and A.W. Robbers, 1995. Essential of Veterinary Microbiology. 5th Edn., Williams and Wilkins, USA., ISBN: 9780683014730, Pages: 394.

Coles, E.H., 1986. Veterinary Clinical Pathology. 4th Edn., W.B. Saunders Co., Philadelphia, London, ISBN: 978-0721618289, Pages: 486.

Derakhshanfar, A., A.A. Mozaffari and A.M. Zadeh, 2010. An Outbreak of trypanosomiasis (surra) in camels in the southern fars Province of Iran: Clinical, hematological and pathological findings. Res. J. Parasitol., 5: 23-26.

Dioli, M. and R. Stimmelmayr, 1992. Important Camel Diseases. In: The One-Humped Camel in Eastern Africa: A Pictorial Guide to Diseases, Health Care and Management, Schwartz, H.J. and M. Dioli (Eds.). Josef Margrave, Germany, ISBN: 9783823612186.

Domenech, J., G. Guidot and D. Richard, 1977. Les maladies pyogenses du dromadaire en Ethiope. Symptomatologie. Etiologie. Rev. Elev. Med. Vet. Pays Trop., 30: 251-258.

Edelstein, R.M. and R.G. Pegram, 1974. Contagious skin necrosis of Somali camels associated with *Streptococcus agalactiae*. Trop. Anim. Health Prod., 6: 255-256.

Ellah, M.R.A., 2010. Involvement of free radicals in animal diseases. Comp. Clin. Pathol., 19: 615-619.

Esterbauer, H., K.H. Cheeseman, M.U. Dianzani, G. Poli and T.F. Slater, 1982. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. Biochem. J., 208: 129-140.

Esterbauer, H., R.J. Schaur and H. Zollner, 1991. Chemistry and biochemistry of 4-hydroxyalkenal, malondialdehyde and related aldehydes. Free Radical Biol. Med., 11: 81-128.

Higgins, A.J., 1985. 4. Common ectoparasites on the camel and their control. Br. Vet. J., 114: 197-216.

Higgins, A., 1986. The Camel in Health and Disease. 1st Edn., Baillaire Tindall, London, ISBN: 9780702011672, Pages:168.

Hjelle, J.J. and D.R. Petersen, 1983. Metabolism of malondialdehyde by rat liver aldehyde dehydrogenase. Toxicol. Applied Pharmacol., 70: 57-66.

Ismail, M., M. Ezzat, J. El-Jakee, Z. El-Sayed and M.A. El-Rahman, 1990. Micro-organisms associated with closed abscesses of camels in Egypt. J. Vet. Med. J. Giza, 38: 53-62.

Miller, J.K., E. Brzezinska-Slebodzinska and F.C. Madsen, 1993. Oxidative stress, antioxidants and animal function. J. Dairy Sci., 76: 2812-2823.

Olaho-Mukani, W. and J.M. Nyango'ao, 1996. Comparison of suratex, parasite detection and antigen-ELISA for the evaluation of treatment efficacy and diagnosis of surra in dromedary camels. J. Camel Parasitolol. Res., 3: 1-5.

Poli, G., 1993. Liver damage due to free radicals. Br. Med. Bull., 49: 604-620.

Quinn, P.J., M.E. Carter, B.M. Maarkey and G.R. Carter, 1994. Clinical Veterinary Microbiology. Wolfe Publication Company, UK.

Qureshi, S., A.K. Kataria and T.K. Gahlot, 2002. Bacterial microflora associated with wounds and abscesses on camel (*Camelus dromedaries*) skin. J. Camel Pract. Res., 9: 129-134.

Robbins, S.L. and V. Kumar, 1994. Robbins Pathologic Basis of Disease. 5th Edn., WB Saunders Co., Philedelphia, London, Pages: 591.

Rollefson, K., P. Mundy and E. Mathias, 2001. Skin Problems. In: A Field Manual of Camel Diseases: Traditional and Modern Health Care for the Dromedary, Mundy, P., I. Kohler-Rollefson and E. Mathias (Eds.). ITDG Publishing, UK., ISBN: 9781853395031, pp: 69-98.

Schwartz, H.J. and M. Dioli, 1992. The One-Humped Camel in Eastern Africa. A Pictorial Guide to Diseases, Health Care and Management. Varlag Josef-Margraf, Germany, ISBN: 9783823612186, Pages: 282.

Sies, H., 1991. Oxidative Stress: Oxidants and Antioxidants. Academic Press, New York, ISBN: 978-0126427622, Pages: 650.

Siu, G.M. and H.H. Draper, 1982. Metabolism of malonaldehyde *in vivo* and *in vitro*. Lipids, 17: 349-355.

Slater, T.F., 1982. Lipid peroxidation. Biochem. Soc. Trans., 10: 70-71.

Stohs, S.J., 1995. The role of free radicals in toxicity and disease. J. Basic Clin. Physiol. Pharmacol., 6: 205-228.

Trevisan, M., R. Browne, M. Ram, P. Muti, J. Freudenheim, A.M. Carosella and D. Armstrong, 2001. Correlates of markers of oxidative status in the general population. Am. J. Epidemiol., 154: 348-356.

Wernery, U. and O.R. Kaaden, 1995. Infectious Diseases of Camelids. 2nd Edn., Wiley Blackwell, Berlin, USA., ISBN: 978-3826333040, Pages: 418.

Zweygarth, E., C. Sabwa and D. Rottcher, 1984. Serodiagnosis of trypanosomiasis in dromedary camels using a card agglutination test set (Testryp CATT). Ann. Soc. Belge. Med. Trop., 64: 309-313.