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PATHOLOGICAL DIAGNOSIS OF SKIN BIOPSIES FOR DIFFERENT OUTBREAKS OF CAMEL POX VIRUS INFECTION IN EGYPT AND UAE

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ABSTRACT

In this study, skin biopsies of two outbreaks of camel pox infection in camels were collected from Belbeis, Sharkeya governorate, Egypt and Al Ain city, Abu Dhabi, United Arab Emirates (UAE) for histopathological diagnosis and polymerase chain reaction (PCR) characterization and identification. Camel pox virus (CPV) reported as a specific viral disease of camel. CPV is highly contagious mainly in calf camels and usually mild in intensity.The mild form, characterized by papular, pustules on the lips, nostrils, cheeks and different parts of skin. CPV usually associated by lymphadenopathy and nasal discharge .

This study declared numerous outbreaks of mild pathogenicity for camel pox virus and the causative agent was identified and confirmed as camel pox virus (CPV) by PCR.

***Keywords**: Camel pox .Virus Identification. Lymphadenopathy. Characterization. PCR. Pathogenicity.*

Abbreviations:

CPV Camel pox virus
PCR polymerase chain reaction
ATI A-type inclusion
UAE United Arab Emirates
OPV Orthopoxvirus

INTRODUCTION

Mastitis may be defined as an inflammation of all structures forming the mammary tissue and the surrounding connective tissue. The disease is the reaction of the mammary gland to irritants and significantly influences the quality and quantity of mammary tissue and milk **(Yüksel *et al.*, 2009).** It is mainly resulting in pathological, physical and chemical changes in milk and glandular tissues, resulting from an invasion of mammary tissues by pathogenes through the teat canal as mentioned by **Quinn *et al.*, (2002) andRadostits *et al.*, (2007).** The disease results in some changes in the composition of milk and shortening of the productive lives of cows **(Gürbulak *et al.*, 2009).** It is important to improve productivity and quality of milkby reducing mastitis incidence **(Halasa *et al.*, 2007;Cha *et al.*, 2011andHogeveen *et al.*, 2011).**

Mastitis was found to induce economic losses including value of discarded milk as milk from affected cows may become unsuitable for human consumption due to bacterial contamination resulting in food poisoning, interference with manufacturing process and in rare cases providing mechanism of spread of zoonotic diseases amongpopulation. Zoonotic diseases transmitted via raw cow milk included Staphylococcal food poisoning, brucellosis, tuberculosis, toxoplasmosis and leptospirosis as previously stated by**Mungube *et al.*, (2005) and Radostits *et al.*, (2007)**.

Oxidative stress is usually described as an imbalance between antioxidant and oxidant levelsas mentioned by**Lykkesfeldt and Svendsen, (2007).** A condition of oxidative stress was produced when the production of oxidants exceeded the capacity of antioxidant defense resulting in oxidative damage to macromolecules such as lipid, proteins and DNA**(Sordillo and Aitken, 2009).**

In this regard, **Jin *et al.*, (2014)** mentioned thatMammary epithelial cells exhibit a high metabolic rate during lactation and therefore large amounts of lipid peroxides and reactive oxygen species (ROS) are produced in vivo**.**

A few hours after the udder infection with pathogenic microorganisms, the number of somatic cells in the milk, was found to be increased in response to activation of the inflammatory processes. When the mammary gland was invaded and was colonized by bacteria, the macrophages responded by initiating the inflammatory response, one that attracted polymorphonuclear cells in milk to kill the bacteria. More than 90% of SCC in infected glands was composed of neutrophils **(Groza, 2006; Andrieu, 2008).**More over **Rinaldi *et al.*, (2007)** stated thatReactive oxygen species (ROS) mediatedthe antibacterial activity of neutrophils**.**

An excess of oxidative reactions of bacterial processes might cause damage to the tissues. The absence of optimal amounts of antioxidants and an excess of ROS resulted in oxidative stress development **(Andrei, 2010b)**. Free radicals are natural end products of the intensive metabolism in cells of the living organism, including high-yielding dairy cows. When the homeostasis was disturbed mainly by generation and accumulation of these free radicals, oxidative processes resulted in oxidative stress causing mastitis in dairy cows **(Poławska *et al.*, 2012)**.

The objective of the present study was toevaluate the role of oxidative stress biomarkers, antioxidant enzymes and metabolites as possible biomarkers for clinical mastitis in dairycows.

MATERIALS AND METHODS

A total number of 116 dairy cows aged between three to ten years were included in this study. Off all, 100 cows exhibited the clinical signs of mastitis. Besides, 16 apparently healthy cows were selected as a control group. All samples were collected in the period between October, 2014 and January, 2015, where 96 samples were collected from Dakahlia while 20 samples were collected from Damietta Governorate, Egypt.

According to **Radostits *et al.*, (2007),** detailed clinical examination of the animals was carried out. Physical examination of the cows was concerned with evidence of systemic illness (on which fever, ruminal stasis, inappetence to anorexia, tachycardia, dullness and recumbency was observed and recorded). Also, local physical examination of the udder

via palpation and inspection of the quarters as well as the supramammary lymph nodes was conducted. It was directed toward detection of the inflammatory cardinal signs or evidence of fibrosis or atrophy. For observation of any abnormalities in the examined milk, visual examination of the milk was done including (milk flakes, clots, pus, watery and bloody secretion).

Sampling:

1. Milk sample

Under complete aseptic precautions, sampling of the examined milk was taken using standard methods described by **Hogan *et al.* (1999).**About (20 ml) of milk was obtained from each affected quarter in separate sterile and well corked falkon tubes. The obtained milk samples were incubated at 37 ^{o}c for 12-24hours, then centrifugate the tubes at 3000 rpm for 15 minutes for concentration of the bacterial cells at the sediment and discard the supernatant. From the sediment, a loopful of about (0.01ml) was mixed with 5 ml of TryptoneSoya Broth **(Oxoid, LTD, Batingstock, Hampshire, England)** in sterile corked test tubes. Then, the tubes were incubated for 18 hours. Each sample was streaked on three different selective media. For isolation of *Staphylococcus aureus*, Baird parker (Bp.) medium **(Oxoid)** supplemented with 5% egg yolk- telluriteemulsion was used. Edward's medium (EDW.) **(Oxoid)** supplemented with 6% defibrinated sheep blood was used for the selective isolation *of Streptococcus spp.* For *Esherichiacoli*selective isolation, Eosin methelene blue medium (EMB.) was used **(Quinn *et al.*, 2002)**.

2. Blood Samples Collection

Two types of venous blood samples (ten ml for each) were collected via puncture of jugular vein from each cow. **Blood plasma samples,** were collected into Eppendorf tube which was mixed with Ethylenediaminetetraacetic acid (EDTA) as anticoagulant for biochemical estimation of level and activity of SOD, CAT and GR. hematological studies.**Blood serum sample,** were collected in clean dry tube without anti-coagulant. The collected blood samples were left to coagulate, thencentrifuged at 3000 rpm for 10 minutes to obtain blood serum forbiochemical analysis of TAC, MDA, Zn and Cu levels according to the method described by manufacture **(Biogiagnostic, Cairo,Egypt).**

3. Statistical analysis

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA). Means and standard deviation for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Correlations between different parameters were carried out using Pearson correlation coefficient. Differences between means at $p < 0.05$ were considered significant.

RESULTS

Concerning the bacteriological isolation and identification, *Staphylococcus aureus* appeared as black shiny colonies surround by hallow zone on Baired parker. *Escherichia coli (E. coli)* appeared as green metallic sheen colonies on the Eosin Methelene Blue media. While *Streptococcus agalactiae (S. agalactiae)* appeared on Edward's media (B hemolysis)as

colorless colonies with bluish hue and surrounded with complete zone of hemolysis. In the current study, the bacteriological isolation profile showed that about (159) bacterial isolates were obtained from the (100) cultured milk samples. The mixed infection 118/159 (74.21%) between *Staphylococcus spp*., *Streptococcus spp*. and *E.coli* was the largest percentage of isolation, then isolation percentage, came to be for *Staphylococcus spp.* 21/159 (13.22%) then for *E. coli* 15/159 (9.43%) and Streptococcus *spp.* 5/159 (3.14%).

Regarding the level of different oxidative stress markers and enzymatic antioxidants activities in clinical mastitis, there was a significant($p < 0.05$) decrease in Super oxide dismutase (SOD), Catalase (CAT) and Total antioxidant capacity (TAC) compared to those of the control group. Whereas there was a significant ($p < 0.05$) increase in Malondialdehyde (MDA) and Glutathione reductase (GR) compared to control group.

Regarding different antioxidant trace elements levels in clinical mastitis, there was a significant ($p< 0.05$) decrease in Zn level compared to control group. Whereas there was a significant ($p< 0.05$) increase in Cu level compared to those of the control group.

Table (1): Showing prevalence of camel Pox infection among herds in Belbeis, Sharkeya governorate, Egypt and Al Ain city, Abu Dhabi, United Arab Emirates (UAE)

Serial	Flock number	Number of infected camels	Young camels	Adult camels	Percentage of morbidity	Percentage of mortality
1	250	35	6	29	14%	0
2	640	90	28	62	14.06%	0

Fig 1: A camel showing numerous cutaneous papules all over the body
Fig 2: Mucopurulant nasal discharge from infected camel
Fig 3: Enlargement of the mandibular lymph nodes

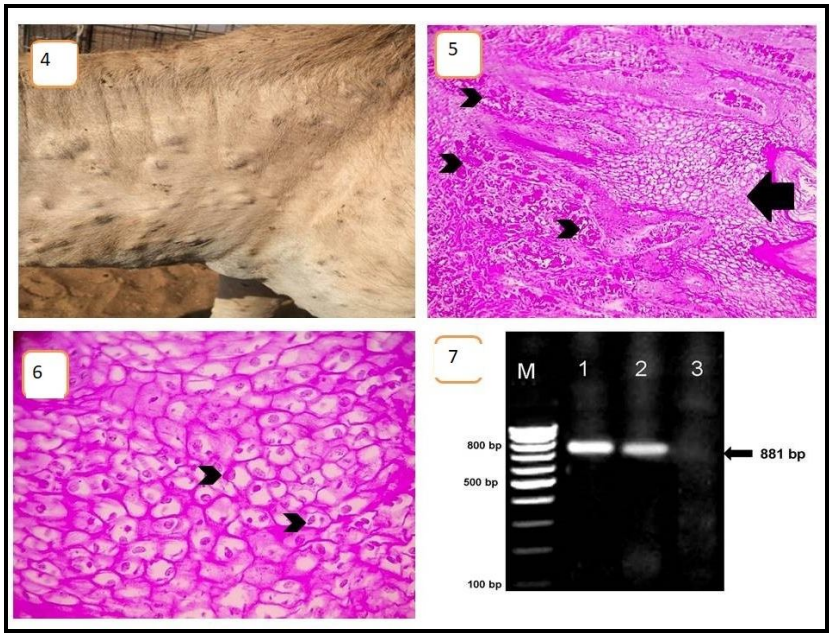

Fig 4: Enlargement of the pre-scapular lymph node beside multiple cutaneous papules
Fig 5: Skin of camel showing acanthosis with hydropic degeneration (arrow) of the prickle cells with presences of homogenous round eosinophilic intracytoplasmic inclusion body beside congested dermal blood vessels (arrow head) H&E., original magnification X52
Fig 6: High power to fig 6 to show inclusion bodies(arrowheads) (H & E., original magnification X520)
Fig 7: Gel electrophoresis of PCR products of 881 of camel poxvirus genome. M: DNA marker, Lane 1 and 2: The amplified products prepared from skin lesions, Lane 3: negative control sample.

DISCUSSION

Camel pox is one of the most important viral diseases encountered dairy and racing camels in United Arab Emirates **(Wernery and Kaaden 1995; Gahlot, 2000; Abdulwahhab, Yas 2003).** Its considerable economic importance is due to a high degree of morbidity, loss of condition and weight and may affect their physiological racing performance. It was further observed that young camels (2-3 years old) are more susceptible to the disease, and occurs mainly in summer months our results are in agreement with **Manefield and Tinson 1997; Muhammed et al., 2005; Abdulwahhab et al., 2012.**

The severity of infection differ according to the age of the animal, it's nutritional and immunological status, our results are in agreement with **Abu-Elzein, 2004.**

Camels infected with Pox disease showed different skin lesions starting as papules of 1-3mm in diameter followed by vesicles and then turning into pustules .these lesions primary appear on the head, eyelids, nostrils and could be seen on the thigh, inguinal, perineal regions. Our results are inagreement with **Higgins 1983; Wernery et al 2002** and **OIE Terrestrial manual, 2010** who revealed fever, lacrimation, mucopurulant nasal discharge and enlargement of lymph nodes.

Microscopically, Acanthosis with hydropic degeneration of the prickle cells with

presences of homogenous round eosinophilic intracytoplasmic inculsion body. Our results are in harmony with (**Maysa et al., 1998 and Zaitoun et al., 2000).** Skin lesions of CPV were confirmed and the virus identified by Genetic analyses.

This study declared numerous outbreaks of mild pathogenicity for camel pox virus and the causative agent was identified and confirmed.

REFERENCES

Abdulwahhab A., Maher M.B. and El Sayed E. (2012): Clinical Studies on Skin Diseases in the Arabian Camels (Camelus dromedarius) in UAE. Proceedings of the XXVII world Buiatrics congress, Lisbon Portugal, 03-08/06/2012, PP:10.

Abdulwahhab, Yas. (2003): Camels: Diseases & Treatment. First Edition. Amrit Advertising, UAE, ISBN – 9948 – 03 – 059 – 1.

Abu Elzein, E.M.E. (2004). J. Sc. Tech. 29:28 – 32.

Afonso CL, Tulman ER, Lu Z, Zsak L, Sandybaev NT, Kermbekova UZ and Rock DL (2002): The genome of camelpox virus. Virology, 295(1)1-9

Awol N Kiros S, Tsegaye Y, Ali M, and Hadush B(2014): **Study on mange mite of camel in Raya-Azebo district, northern Ethiopia Veterinary Research Forum 5 (1) 61 – 64**

Bancroft, J.D., Stevens, A. (1996) **Theory and Practice of Histological Techniques. 4th Edition, Churchill Livingstone, New York**

BekeleM(2010): **An epidemiological study on Major camel disease in Borana lowland, southern Ethiopia DCG report No. 58 Drylands coordination group Oslo P 67-98.**

Gahlot, T.K. (2000). Selected Topics on Camelids. The Camelid Publishers, Bikaner, India. pp 1 – 614.

Higgins, A. J. (1983) : Observations on the diseases of the Arabian camel (Camelus dromedarius) and their control. Vet. Bull. 53(12): 1089 – 1100.

Higgins AJ(1984): The camel in health and disease. Br. Vet. J., 140(5): 482-504.

Higgins A.J. (1985): the camel in health and disease. 4. Common ectoparasites of the camel and their control. Br. Vet. J., 141, 197.

Kewan, K. Z. E. (2003): Studies on camel nutrition Ph.D. Thesis, Fac. Agric., Alex. Univ., Egypt

Khalafalla AI (1998): Epizootiology of camelpox, camel contagious ecthyma and camel papillomatosis in the Sudan. Proc. 3rd. Annual Meeting for Animal Production under Arid Condition vol. 2, 115-131 (UAE).

Kinne J and Wernery U(1999): Experimental camelpox infection. In: Meeting of the European Soc. of vet. Pathol, Nantes, France, pp: 14-17.

Mahmoud MA, Abo-Elnag TR, Osman WA, Bassiouny AI and Goda AS(2012): Epidemiology and Characterization of Camel Poxvirus in Northwest Costal Area of Egypt Global Veterinaria 9 (6): 738-744.

Mamo ezahegne, Bayleyegn, Gizachew, Tessema, Tesfaye Sisay , Legesse, Mengistu , Medhin, Girmay , Bjune, Gunnar, Abebe Fekadu and Ameni Gobena(2011): **Pathology of Camel Tuberculosis and Molecular Characterization of Its Causative Agents in Pastoral Regions of**

Ethiopia PLoS ONE journal, Volume 6, Issue 1, e15862.

Manefield, G. W. and Tinson, A. H. (1997). Camels – A Compendium Sydney Post Graduate Foundation Vade Mecum Series C No. 22.

Mayer A. & Czerny C.-P. (1990). Chapter 4, Camelpox virus in : virus Infections of Vertebrates, Vol. 3, Virus Infections of Ruminants, Dinter Z. & Morein B., Elsevier Science Publisher B.V., Amsterdam, The Netherlands, 19-22.

Meyer, H., P. Martin and R. Hanns-Joachin, 1994. **Sequence alterations within and downstream of the. A type inclusion protein genes allow differentiation of Orthopoxvirus species by polymerase chain reaction journal of general virology, 75: 1975-1981.**

Maysa HS, Ahmed MH, Salem SA and Elkabany MM (1998): Some virological and pathological studies on camel pox. Egypt, J. Agric. Res., Vol. 76 (4): 1671-1686.

Muhammad G, Khan MZ, Hussain MH, Iqbal Z, Iqbal M and Athar M (2005): Ethnoveterinary practices of owners of pneumatic-cart pulling camels in Faisalabad City (Pakistan). J. Ethnopharmacol. 28 97 (2): 241-246.

OIE Terrestrial manual, 2010 Report of the second meeting of the OIE ad hoc Group on Diseases of Camelids,Paris, 3-5 May 2010.

Sooud AO (1995): Some aspects of camel nutrition in a workshop on the development of animal production in the desert and newly reclaimed areas. Desert Research Center, Cairo 52-60.

VILJOEN, G. J., L. H. NEL, J. R. CROWTHER (2005). Molecular Diagnostic Polymerase Chain Reaction Handbook. IAEA, Springer, Netherlands. pp. 57-62.

Wernery KU and Zachariah, R (2000): Experimental camelpox infection in vaccinated and unvaccinated guanacos. J. Camel Prac. and Res. 7: 153-157.

Wernery, L. I. and Kaaden, O.R. (1995): Infectious Diseases of Camel.English Translation by Buzanoski JH p 68.

Wernery, U., Kaaden, O.R. (2002): Bacterial diseases and viral diseases.In: Infectious diseases in camelids, ed. Wernery U, 2nd ed., pp.19–236. Blackwell Science, Berlin, Germany.

Zaitoun AM, Ali HS and Abdel-Ghaffar SH(2000): Studies on camel pox in Assiut Governate, Egypt. Assiut Vet. Med. J., Vol. 43 (85): 140-146.

الملخص العربى

التشخيص الباثولوجي على عينات جلدية لمرض جدري الجمال

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يعتبر جدري الجمال من أخطر الامراض الفيروسية التي تصيب الابل واكثرها انتشارا وخاصة في الاعمار الصغيرة (الحيران) التى تتراوح بين عمر سنة وثلاث سنوات وكانت نسبة الاصابة فى القطعان (١٤%) ونسبة الوفيات (٠%) نظرا لكون الاصابة من النوع الاولي.

في هذه الدراسة، تم جمع عينات من الجلد من منطقتين حدث فيهما تفشي عدوى جدري الجمال الاولي من مدينة بلبيس، محافظة الشرقية، مصر، والثانية من مدينة العين، أبو ظبي، الإمارات العربية المتحدة وذلك لتشخيص مرض جدري الجمال عن طريق الانسجة وتأكيد التشخيص عن طريق تفاعل البلمرة المتسلسل. وكانت الاعراض فى اغلب الحالات تتمثل فى بثور صغيرة على الشفاه العلوية والسفلية ،الأنف والخدين (النوع الاولي البدائي) الي ظهور الاعراض علي بقية الجسم مما يترتب عليها فقدان الشهية، الحمى، الإسهال مع إفرازات الآنف المخاطية، كبر في العقد الليمفاوية، وتم التشخيص المعملي للعينات والتي استخلصت النتائج الى تأكيد اصابة الحالات المريضة والتى تظهر عليها الاعراض بمرض جدري الجمال .