Comparative Clinical Pathology (2018) 27:1497–1500
https://doi.org/10.1007/s00580-018-2763-9

ORIGINAL ARTICLE

Clinicopathological investigations during an outbreak of camelpox in a dromedary camel herd in India

Shirish D. Narnaware[1] · Rakesh Ranjan[1] · Shyam S. Dahiya[2]

Received: 16 September 2017 / Accepted: 5 June 2018 / Published online: 14 June 2018

Abstract

Camelpox is an important infectious viral skin disease of camelids. However, clinicopathological aspect of the disease has not been studied in detail so far. This study was carried out to investigate the clinicopathological changes associated with camelpox outbreak in a dromedary camel herd in India. The clinical signs, pathological lesions and haematological and blood biochemical parameters were studied in infected camels. For diagnosis, the scab samples were subjected to PCR for amplification of haemagglutinin (HA) gene of camelpox virus (CMLV). The camelpox infection was reported in total 55 (17.02%) camels of the herd. The age of infected camels varied from 1 to 13 years with significantly more incidence in camels of age group 1–3 years (61.81%) than camels of age group more than 3 years (38.18%). The infected camels showed clinical signs of fever, anorexia, lacrimation and characteristic pock lesions on the skin of lips, mouth, nostrils, head, neck, thighs, legs, abdomen and inguinal region. The haematological and serum biochemical parameters revealed anaemia and hypoproteinemia in infected camels. The histopathology of the scabs revealed hyperplasia of epidermis, hydropic degeneration of the keratinocytes and intracytoplasmic eosinophilic inclusion bodies. The PCR revealed amplification of HA gene of CMLV in all the scab samples collected from infected camels. The clinicopathological studies in camelpox infection will give further insight into the pathogenesis of the disease and can help clinicians in the effective management of the disease.

Keywords Camelpox · Haematology · Serum biochemistry · Clinicopathology · India · PCR

Introduction

Camelpox is an important wide-spread viral skin disease of camels, which is mainly characterised by skin lesions or systemic infections depending upon the virus strain and the immune status of the animal (Kaaden 2002; OIE 2014). The disease causes considerable loss to camel farmers in terms of morbidity, mortality, loss of weight and reduction in milk yield (Bhanuprakash et al. 2010). The characteristic skin lesions first appear on the head, eyelids, nostrils and the ears, and later may spread to the neck, legs, genitalia, mammary glands and perineum (Wernery et al. 1997). In the systemic form, pox lesions may cover the entire body including the mucous membranes of the mouth and respiratory tract (Wernery and Kaaden 2002; OIE 2014). The camelpox virus (CMLV) is secreted in milk, saliva and nasal and ocular secretions of the infected camels, and the transmission usually occurs by direct or indirect contact through inhalation or skin abrasions (OIE 2014).

Sporadic outbreaks of camelpox have been reported among camels in India and other countries in the recent past (Bhanuprakash et al. 2010; Kachhawaha et al. 2014; Dahiya et al. 2017), Iraq (Gatie 2016), Iran (Mosadeghhesari et al. 2014) and Sudan (Motalab and Ahmed 2014; Khalafalla and Abdelazim 2017). However, detailed clinicopathological studies in infected camels during these outbreaks are lacking. This is the first systematic study that documents haematological and serum biochemical parameters associated with natural infection of camelpox.

Material and methods

In the month of December 2014, an outbreak of camelpox was reported in an organised dromedary camel farm having total

✉ Shirish D. Narnaware
drshirish009@gmail.com

[1] ICAR- National Research Centre on Camel, Post Bag No. 07, Jorbeer, Bikaner, Rajasthan 334001, India

[2] ICAR-Directorate of Foot and Mouth Disease, Mukteswar, Nainital, Uttarakhand, India

herd strength of 323 camels. This farm was situated at Bikaner district of Rajasthan, India which is having tropical climatic conditions. During the outbreak, the infected camels were observed for clinical signs, and blood samples were collected from ten infected and ten non-infected camels for haematological and serum biochemical analysis. Blood samples were analysed immediately after collection for haematological parameters, viz., haemoglobin (Hb), total red blood cell count (RBC), total white blood cell count (WBC) and differential leucocyte count (DLC). Sera samples were subjected to biochemical parameters, viz., total protein, albumin, calcium, cholesterol, triglycerides, alkaline phosphatase, lactate dehydrogemnase (LDH), aspartate aminotransferase (AST) and alanine transaminase (ALT) using the commercial kits (Span diagnosics™).

The scab samples were collected from affected camels in 10% formal saline for histopathology and also in sterile vials for DNA extraction and PCR. The formalin fixed tissue samples were embedded in paraffin, cut into 4–5 μ sections and stained with haematoxylin and eosin stain using the standard procedure.

Total genomic DNA was extracted from collected scab samples using PureLink® Genomic DNA Kit (Invitrogen, USA). The genomic DNA was used to amplify the haemagglutinin (HA) gene of camelpox virus (CMLV) by using the primers designed and based on CMLV gene sequences (GenBank accession No. DQ853384) forward primer (HA-F) (5' CGGTGGGGATCCATGGCACGATTGTC AATA 3′), reverse primer (HA-R) (5' TGGCAG CTCGAGTTATGTTTTGTATTTACG 3′) (Nagarajan et al. 2013). Reaction volumes for the PCR of 50 μl were used and contained 5 μl of 10× buffer with 15 mM $MgCl_2$, 10 mM of each dNTPs, 100 pmol of each oligonucleotide primer, 100 ng of DNA sample and 3 U Taq DNA polymerase. The reaction mixture was subjected to initial denaturation of the template at 94 °C for 5 min in thermal cycler (Eppendorf, Germany). Cycling conditions for PCR were 35 cycles each at 94 °C for 60 s, 50 °C for 60 s and 72 °C for 60 s, followed by a final extension for 10 min at 72 °C. The PCR amplified products were checked in 1% agarose gel. The size of the PCR product, specific for camelpox virus, was approximately 948 bp. The primers specific for topoisomerase gene of contagious ecthyma were used as negative control for differential diagnosis (Nagarajan et al. 2011).

The data obtained for haematological and biochemical parameters were expressed as mean ± standard deviation, and analysed by *t* test using SPSS16 statistical software (SAS Institute Inc., Cary, North Carolina, USA).

Results

The total incidence of camelpox in the herd was recorded as 17.02% ($n = 55$). The camels of age ranging from 1 to 13 years

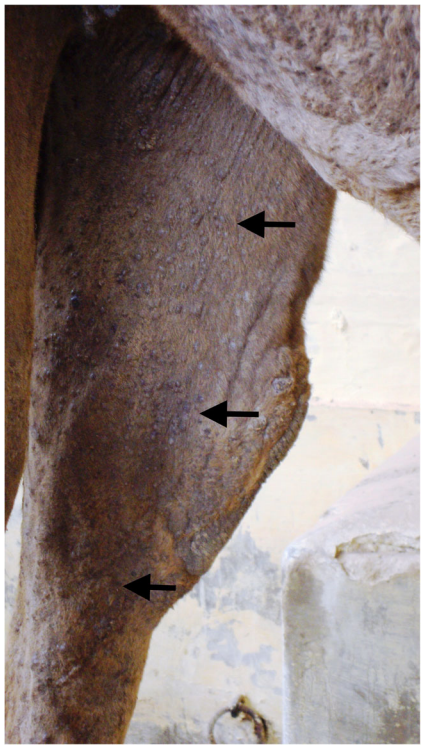

Fig. 1 Pock lesions on skin of inner thigh and legs (arrow)

of either sex were found infected with significantly more incidence in camels of age group 1 to 3 years (61.81%) than camels of age group more than 3 years (38.18%). There was no significant difference in incidence of camelpox infection in male ($n = 24$) and female ($n = 31$) camels. No mortality was reported in any of the infected camel. The symptoms in infected camels were anorexia, fever, lacrimation and pustular skin lesion on skin of lips, mouth, nostrils, head, neck, abdomen, inguinal region, thighs and legs (Fig. 1). In addition to these lesions, one camel also showed sign of corneal opacity and pustules on tongue and buccal cavity (Fig. 2). These pustular lesions later progressed into dry and scaly scabs which tend to bleed when removed.

The haematological parameters revealed significantly reduced haemoglobin in infected camels compared to non-infected camels ($P < 0.05$) (Table 1). The other

Fig. 2 Pustular lesions on the mouth, lips and nostrils. Also note corneal opacity

Table 1 Haematological parameters in camelpox infected and non-infected control camels

Parameter	Infected group (mean ± S.D.)	Control group (mean ± S.D.)
Hb (g/dl)	7.83 ± 0.79	9.04 ± 1.06*
RBC (10^6/μl)	5.08 ± 1.03	6.14 ± 1.08*
WBC (10^3/μl)	10.37 ± 3.72	11.08 ± 2.13
Neutrophil (%)	43.10 ± 15.07	50.30 ± 10.29
Lymphocyte (%)	51.10 ± 14.57	41.4 ± 9.77
Eosinophil (%)	3.00 ± 1.33	3.90 ± 1.44
Monocyte (%)	2.80 ± 2.04	4.40 ± 1.71

*The indices indicate a significant difference within row ($P < 0.05$)

haematological parameters showed no significant difference between infected and non-infected camels (Table 1). The serum biochemical parameters revealed significantly reduced total protein in infected camels as compare to non infected camels ($P < 0.05$) (Table 2). The other serum biochemical parameters showed no significant difference between infected and non-infected camels. The histopathological examination of the collected scab samples revealed hydropic degeneration of the keratinocytes and presence of characteristic intracytoplasmic eosinophilic inclusion bodies (Fig. 3). In several cases, the epidermis also revealed hyperplasia and underlying haemorrhages along with infiltration of mononuclear cells (Fig. 4). The PCR revealed amplification of HA gene of CMLV in all the scab samples collected from infected camels.

Discussion and conclusion

In the present study, higher incidence of camelpox was recorded in camels of less than 3 years age than adults or older animals. This finding is in agreement with a previous study which reported that the disease frequently affect young camels

Table 2 Serum biochemical parameters in camelpox infected and non-infected control camels

Parameters	Infected group (mean ± S.D.)	Control group (mean ± S.D.)
Total protein (g/dl)	6.90 ± 0.65	7.71 ± 0.64*
Albumin (g/dl)	3.36 + 0.18	3.43 + 0.23
Calcium (mg/dl)	9.82 ± 1.31	9.79 + 1.50
Triglycerides (mg/dl)	16.07 ± 3.35	15.98 ± 3.75
Alkaline Phosphatase (IU/L)	186.66 + 61.68	190.72 + 52.29
LDH (IU/L)	420.41 ± 143.25	435.11 ± 131.01
AST (IU/L)	109.76 ± 26.65	115.59 ± 25.83
ALT (IU/L)	11.59 ± 3.01	12.25 ± 2.59

*The indices indicate a significant difference within row ($P < 0.05$)

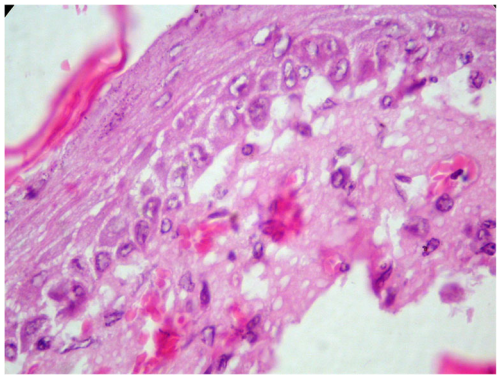

Fig. 3 Hydropic degeneration of the keratinocytes in the epidermis (HE × 400)

with severe form and high mortality (Nothelfer et al. 1995). However, in the present study, no mortality was reported in any of the infected camels, and mild lesions were observed in majority of the camels. This variation in disease manifestation may be due to the difference in virulence of the infecting virus strain and the immune status of animals (Kaaden 2002). The present outbreak occurred in mid December which is a dry and cold month in the Bikaner region of India. Several studies have reported that the incidence of camelpox outbreaks increases in rainy season with more severe form, whereas milder form was observed during the dry season (Wernery et al. 1997; Wernery and Kaaden 2002; Kachhawaha et al. 2014). Septicemia due to secondary bacterial infections is the major cause of mortality in infected camels (Wernery and Kaaden 2002). Since the camels of the present study were from an organised herd where infected camels were timely and appropriately treated, hence the spread of lesions was limited and no mortality was reported.

In the current study, typical symptoms of camelpox such as anorexia, fever, lacrimation and pustular skin lesions were observed which coincided with several reports by other investigators (Wernery and Kaaden 2002; Kachhawaha et al. 2014; Motalab and Ahmed 2014; Mosadeghhesari et al. 2014; Gatie 2016; Aregawi and Feyissa 2016). The histopathological lesions were also found characteristic to the camelpox infection

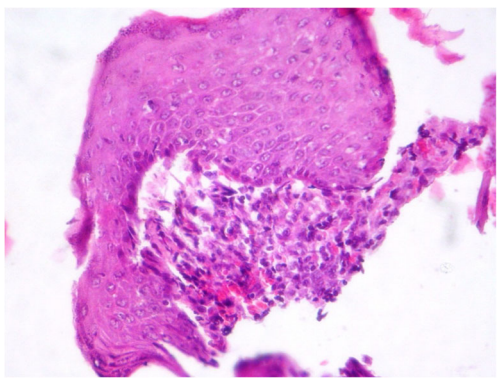

Fig. 4 Hyperplasia of epidermis with infiltration of mononuclear cells and haemorrhages (HE × 200)

and in agreement with previous studies (Motalab and Ahmed 2014; Dahiya et al. 2017).

Similar to the findings of the present study, anaemia and hypoproteinemia was frequently reported in camelpox infection (Hussein and Al-Mufarrej 1999), sheep pox infection (Issi et al. 2008) and other viral skin diseases akin to camelpox such as camel papillomatosis (Barakat et al. 2013) and camel contagious ecthyma (Narnaware et al. 2015). The anaemia and hypoproteinemia is mainly attributed to the marked decrease in food intake due to the painful lesions in the mouth and inappetance during the course of the disease. However, in the present study other haematological and serum biochemical parameters did not alter. Similar haematological findings were reported in a case of camelpox in India (Kachhawaha et al. 2014). This can be attributed to the supportive treatment and supplementary feed provided to the infected camels of the present study during the initial stages of the disease.

In agreement with a previous study, the PCR for amplification of HA gene of CMLV from scab samples was found to be a rapid and sensitive assay for detection of camelpox infection in the initial stage of the disease (Nagarajan et al. 2013). This assay was also reported to be useful in distinguishing orthopoxvirus from all other pox viruses (Nagarajan et al. 2013).

In conclusion, understanding the blood profile picture of camel affected by camelpox will give further insight into the pathogenesis of the disease and can help clinicians in the effective management of the disease.

Acknowledgements The authors are grateful to Director, ICAR- National Research Centre on Camel for providing necessary facilities to carry out the work.

Compliance with ethical standards

Conflict of interest The authors declared that they have no competing interests.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

Aregawi WG, Feyissa PT (2016) Diagnostic approaches towards camelpox disease. J Vet Sci Anim Husb 4:303

Barakat SEM, AL Hizab FA, El-Bahr SM (2013) Clinicopathological and serobiochemical investigation of naturally occurring cutaneous papillomatosis in dromedary camels (*Camelus dromedarius*). Sci Int 6:212–216

Bhanuprakash V, Balamurugan V, Hosamani M, Venkatesan G, Chauhan B, Srinivasan VA, Chauhan RS, Pathak KM, Singh RK (2010) Isolation and characterization of Indian isolates of camelpox viruses. Trop Anim Health Prod 42(6):1271–1275

Dahiya SS, Kumar S, Mehta SC, Singh R, Nath K, Narnaware SD, Tuteja FC (2017) Molecular characterization of camelpox virus isolates from Bikaner, India: evidence of its endemicity. Acta Trop 171:1–5

Gatie JA (2016) Recurrent outbreaks of camel pox in *Camelus dromedarius* in Dhi-Qar governorate/Iraq MRVSA 5 (special issue) 1st Iraqi colloquium on camel diseases and management, pp 58–63

Hussein MF, Al-Mufarrej SL (1999) Some clinicopathological aspects of camelpox in Saudi Arabia. J King Saud Univ Agric Sci 11(2):113–120

Issi M, Gul Y, Yilmaz S (2008) Clinical, haematological and antioxidant status in naturally poxvirus infected sheep. Revue Méd Vét 159(1): 54–58

Kaaden WU (2002) Camel pox. In: Infectious diseases in camelids, 2nd edn. Blackwell Science, Berlin, pp 176–185

Kachhawaha S, Srivastava M, Kachhawa JP, Tanwar M, Sharma A, Singh NK, Kachwaha K, Rathore SS, Tanwar RK (2014) Therapeutic management of camel pox—a case report. Adv Anim Vet Sci 2(4): 239–241

Khalafalla AI, Abdelazim F (2017) Human and dromedary camel infection with camelpox virus in Eastern Sudan. Vector Borne Zoonotic Dis 17(4):281–284

Mosadeghhesari M, Oryan A, Zibaee S, Varshovi HR (2014) Molecular investigation and cultivation of camelpox virus in Iran. Arch Virol 159(11):3005–3011

Motalab YMA, Ahmed AB (2014) Isolation and identification of camelpox virus in eastern Sudan. SUST J Agri Vet Sci 15(2):73–81

Nagarajan G, Swami SK, Dahiya SS, Sivakumar G, Narnaware SD, Tuteja FC, Patil NV (2011) Sequence analysis of topoisomerase gene of pseudocowpoxvirus isolates from camels (*Camelus dromedarius*). Virus Res 158:277–280

Nagarajan G, Swami SK, Dahiya SS, Sivakumar G, Yadav VK, Tuteja FC, Narnaware SD, Patil NV (2013) Phylogenetic analysis of immunomodulatory protein genes of camelpox virus obtained from India. Comp Immunol Microbiol Infect Dis 36:415–424

Narnaware SD, Nagarajan G, Dahiya SS (2015) Hemato-biochemical studies in Indian camel (*Camelus dromedarius*) affected with contagious ecthyma. Indian J Vet Pathol 39(2):168–170

Nothelfer HB, Wernery U, Czerny CP (1995) Camel pox: antigen detection within skin lesions—immunohistochemistry as a simple method of etiological diagnosis. J Camel Pract Res 2:119–121

OIE Terrestrial Manual (2014) Camelpox. Chapter 2.9.2

Wernery U, Meyer H, Pfeffer M (1997) Camelpox in the United Arab Emirates and its prevention. J Camel Pract Res 4(2):135–139

Wernery U, Kaaden OR (2002) Camelpox. In: Infectious diseases in camelids, 2nd edn. Blackwell Science, Berlin, pp 176–185