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# CHRONOLOGICAL CLASSIFICATION OF PATHOMORPHOLOGICAL LESIONS IN DROMEDARY CONTAGIOUS ECTHYMA INFECTION

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## ABSTRACT

An outbreak of camel contagious ecthyma (CCE) occurred in a camel herd of the National Research Centre on Camel, Bikaner, India. The pathomorphological lesions of CCE were classified into five different stages on the basis of their sequence of occurrence, nature and duration. In the initial stage, development of erythema and papules which lasted for 1 week, were observed. This was followed by facial swelling stage which remained for another 1 week, in which swelling on face due to enlargement of mandibular and cervical lymph nodes was observed. In the pustular stage, swelling was subsided and pustules developed on lips, nose, face, eyes and neck region. This stage lasted for 4-5 weeks. In the scab stage, the pustular lesions dried and remained as scab for 1-2 weeks. In the recovery stage, the scab lesions started detaching with formation of grey spots at the affected areas. This stage lasted for 1-2 weeks followed by complete recovery. Histopathology of pustular and scab lesions revealed hydropic degeneration of keratinocytes, hyperplasia of epidermis, hyperkeratosis and intraepidermal pustule with mononuclear cellular infiltration. Intracytoplasmic eosinophilic inclusion bodies were observed in keratinocytes in the epidermis. The total course of the disease in individual camel varied from 8 to 11 weeks. This is the first report on sequential classification of pathomorphological lesions of CCE.

**Key words:** Camels contagious ecthyma, histopathology, lesion classification, pathomorphology

Contagious ecthyma (CE) also known as orf, contagious pustular dermatitis, ecthyma contagiosum, contagious pustular stomatitis, infectious labial dermatitis, auzdyk, sore mouth and scabby mouth, is the most common viral disease of sheep and goats all over the world. In addition, the disease is also reported from other animals like deer, camels, alpacas, reindeer, musk oxen, bighorn sheep, antelopes, wapiti, dogs eating infected carcasses and occasionally human beings handling infected animals (CFSPH, 2007). The disease camel contagious ecthyma (CCE), is widely recognised in camel-rearing regions of the world. The clinical signs of CCE are often indistinguishable from lesions caused by camelpox virus or papilloma virus (Munz, 1992). CCE is characterised by localised lesions, mainly around the mouth and nose. This disease results in high morbidity, but it is not fatal.

The etiological agent of CCE in Indian dromedaries (*Camelus dromedarius*) is pseudocowpox virus (PCPV) which comes under the genus parapoxvirus, subfamily *Chordopoxvirinae* and family *Poxviridae* (Nagarajan *et al,* 2011). The transmission of the PCPV is thought to occur through direct or indirect contact. The virus can enter the skin through cuts and abrasions caused by grazing on thorny plants. The clinical signs in CCE are described as inappetence or starvation due to painful proliferative lesions causing inability of affected animals to graze or to suckle their dams leading to emaciation and anaemia (Khalafalla, 1998). Lesions are described as acute pustular and nodular lesions in and around the skin of lips, eyes, nose and sometimes around neck, trunk, perineum and legs (Bazargani *et al,* 2010). The histopathology showed vacuolar changes of the epithelial layers. Affected areas were ulcerated, haemorrhagic and with frequent secondary bacterial infection (Ali *et al,* 1991). Morbidity in affected herds was reported as 100% in young camels, with no adult involvement (Azwai *et al,* 1995). Despite its highly contagious nature, rapid spread and wide prevalence, sequential pathomorphological classification of lesions in infected camels has not been described so far. Lesion classification will help in the identification and treatment of CCE infected camels on the basis of nature of lesions and disease progression.

## Materials and Methods

The present outbreak of CCE occurred in August month (monsoon season) in 42 young camels

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of 5 months to 2 years belonging to a camel herd of the National Research Centre on Camel (NRCC), Bikaner, Rajasthan, India. These infected camels were kept in an isolated corral for treatment and were not allowed free grazing. They were given soft and ground feed during the entire period of disease. These camels were daily observed for development and spread of lesions from the day of their first appearance till complete recovery.

The pustular and scab lesions were collected from 15 CE infected camels with sterile blades and immediately kept in 10% neutral buffered formalin for histopathology and in sterile vials for DNA extraction and polymerase chain reaction (PCR). After fixation in formalin, dehydrated through graded series of alcohol and xylene, these scabs were then embedded in paraffin. The slides were made from paraffin blocks by cutting in to 4 μm thin sections. The slides were then stained with haematoxylin and eosin (HE).

Total DNA was extracted from skin scabs using Axy Prep multisource Genomic DNA miniprep kit (Geneaxy Scientific Pvt. Ltd.) according to the manufacturer's instructions. DNA extracted from skin scab of camel-pox lesion was also included in the PCR as negative control and for differential diagnosis. For PCR, the nucleotide primers were used as designed by Nagarajan *et al* (2010b) for amplification of the envelope gene of pseudocowpox virus (PCPV) using the following primer sequences: forward primer (5'-TTAATTTATTGGCTTGCAGAAC TCCGAGCGC-3'), reverse primer (5'-ATGTGG CCGTTCTCCTCCATC-3'). PCR amplification of the envelope gene was performed using the following thermal profiles: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR-amplified products were checked by electrophoresis in a 1% agarose gel.

**Results**

The present outbreak of CCE occurred during the monsoon season in 42 young camels of NRCC farm. Age wise the morbidity was 100% in camels of 5 months to 2 years. However, no mortality was reported in any of the infected camels. The lesions were classified into 5 different stages on the basis of sequence of occurrence, nature and duration of the lesions. In the initial stage, skin lesions were mild and characterised by development of erythema with reddening and papules which were found on lips and muzzle. In addition, these camels also showed frequent lacrimation (Fig 1). These lesions remained for a week.

After one week facial swelling stage occurred in which swelling of lips, face and jaw was observed due to enlargement of mandibular and cervical lymph nodes (Fig 2). This swelling was hard and remained for a week and then slowly started subsiding.

After 2 weeks the swelling disappeared and lesions progressed into pustular stage. During this stage topical treatment (washing of lesions with 0.2% potassium permanganate solution and application of antiseptic ointment) was started but despite this treatment, the pustular lesions became more severe. These severe pustular and ulcerative lesions were found on skin around lips, muzzle, eyes and sometimes on the upper part of the neck region (Fig 3). Pustular and ulcerative lesions on eyelids caused swelling of eyelids, lacrimation and pus formation leading to complete closure of eyes in some affected camels (Fig 4). The lesions were of variable size from a few mm up to 3 cm in diameter/length, multifocal to coalescing, and sometimes had a papilloma-like appearance. The lesions were painful and affected camels showed profuse salivation from mouth while chewing. The pain and distortion of the lips and mouth prevented the camels from suckling the dam or chewing the feed. The damaged or distorted lips gave the typical pendulous appearance to the lower lips (Fig 4). However, lesions were not found inside the buccal cavity mucosa in any of the affected camels. Some pustular lesions developed into hard nodules which were found on skin of face, head, neck and sometimes on shoulder and lower part of abdomen (Fig 5). These nodular and pustular lesions remained for upto 4-5 weeks. The affected camels started rubbing of nodular lesions against the wall of the corrals due to pruritus which led to sloughing, ulceration and oozing of blood from the skin of affected areas. At this stage these ulcerated lesions in some camels showed secondary bacterial infection which led to pus formation. The pus forming lesions also attracted flies and in some camels severe maggoted wound were formed due to myiasis.

After 4-5 weeks pustular lesions started healing and progressed to scab stage with drying and scab formation which remained for 2 weeks. After 2 weeks recovery stage occurred in which the scabs started detaching from the skin surface leaving dark brown or grey hairless spots at the affected areas (Fig 6). These spots remained for another 1-2 weeks and later showed complete disappearance without any scar formation.

**Fig 1.** Initial skin lesions in a camel showing development of erythema and papules on lips and muzzle and frequent lacrimation.

**Fig 3.** Severe pustular lesions on face and neck region.

**Fig 2.** Facial swellings on lips, muzzle and jaw.

**Fig 4.** Severe pustular and ulcerative lesions on lips and eyelids with pendulous lower lip appearance.

The course of the disease in individual camels varied between 8-11 weeks. Those camels with secondary bacterial infection and myiasis showed delayed healing of lesions and took a maximum of 11 weeks for complete healing even after intensive treatment. The dams were not showing any lesions around teats and udder although suckling continued by some of the less affected calves. Lesions were not reported on the skin of hands of camel keepers handling infected camels.

Histopathology revealed hydropic or ballooning degeneration of keratinocytes, hyperplasia of epidermis and hyperkeratosis (Fig 7) as well as intra epidermal pustule formation with the presence of mononuclear cellular infiltration and eosinophilic proteinous fluid in dermis (Fig 8). In cases with secondary bacterial infection the dermis revealed leucocyte infiltration containing mixed population of lymphocytes and neutrophils. Papillomatous proliferation of epidermis was also seen in cases with secondary bacterial infection of the lesions. Typical oval shaped eosinophilic intracytoplasmic inclusion bodies were observed inside keratinocytes in epidermis.

The DNA extracted from pustular and scab material from infected camels amplified DNA fragment of the expected size of 1170 bp for envelope gene of pseudocowpox virus in PCR. There was no amplification in the PCR using DNA from camelpox positive scab material (negative control).

## Discussion

The present outbreak of CCE occurred during the monsoon season in the camel herd of NRCC,

**Fig 5.** CE infected camel showing pustules and nodules on face, head, neck and shoulder region.

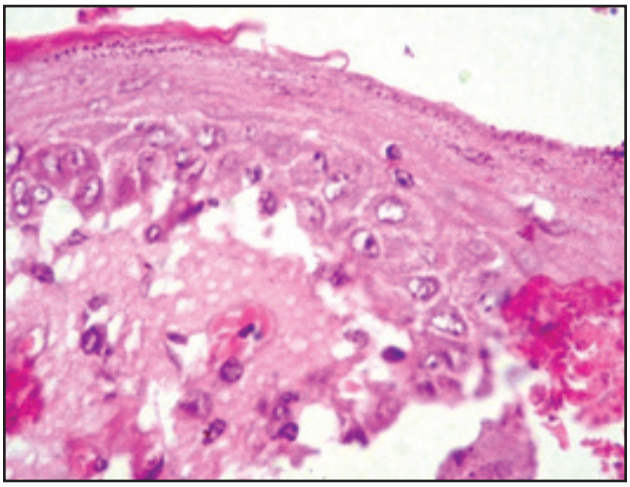

**Fig 7.** Hydropic degeneration of keratinocytes (HE stain. X 400).

**Fig 6.** Healing of the lesions with formation of dark brown or grey hairless spots at the affected areas.

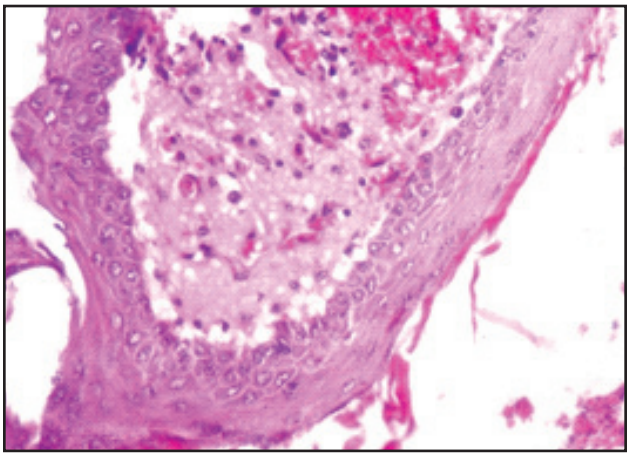

**Fig 8.** Intra epidermal pustule showing presence of eosinophilic proteinous fluid with mononuclear cellular infiltration and RBCs (HE stain. X 400).

Bikaner which substantiate the findings of the previous reports on the seasonality of CCE and its association with rainy season either from India or other countries (Khalafalla, 1998; Nagarajan *et al,* 2010a). The reason for its more frequent occurrence during the rainy season is the increased presence of insects or flies during this period (Wernery and Kaaden, 2002). Previously, it was thought that the abundance of thorny plants during rainy season causes injuries to lips or mouth making camels more susceptible for contracting this disease (Khalafalla, 2000) but this may not be true since some outbreaks occurred in with no access to thorny plants. Some calves were also infected at suckling stage (Housawi *et al,* 2004). The source of infection of the CCE outbreak in the NRCC farm with no contact to sheep or goats or infected camels, could be due to grazing on previously contaminated pastures or transmission through fomites. Moreover, the orf virus is reported to be resistant to inactivation in the environment and has been recovered from dried crusts even after 12 years (CFSPH, 2007).

The present outbreak occurred in camels between the age of 5 months to 2 years with no involvement of any adult animal. So far other outbreaks of CCE were also reported mainly in young camels up to 3 years of age (Khalafalla, 1998; Wernery and Kaaden, 2002). The absence of CCE in adult animals is probably due to an immune response which is absent in very young camels. In the present study 100% morbidity was reported in camels of 5 months to 2 years of age with no mortality. Previous reports showed a varying overall morbidity rates between 10-100% with no mortality (Ali *et al,* 1991; Khalafalla, 1998; Abu Elzein *et al,* 1998).

In the present study the pathomorphological lesions of CCE were classified into 5 different stages viz. initial stage, facial swelling stage, pustular stage, scab stage and recovery stage. The lesions were observed mainly on face, muzzle, lips, eyes and sometimes on neck and lower part of abdomen, whereas other body parts were found unaffected. However, previous studies on CCE reported lesions on nasal and oral mucosa, mucous membranes of the buccal cavity such as gingiva, palate and gums, tongue, and also on trunk, perineum, inner thighs, distal parts of the legs and vagina (Khalafalla, 1998; Bazargani *et al,* 2010). The difference in development and progression of lesions in the present study as compared to previous studies may be due to difference in the strain of the infecting virus.

Skin is the main site of predilection and essential for establishment and development of lesions in CCE. The initial clinical signs in affected camels were erythema and papules around lips and nose. These lesions were followed by swelling on lips, face and jaw along with swelling of lymph nodes in head region. After one week the swelling subsided and lesions developed into pustules, nodules and scabs involving facial and neck region. However, Khalafalla (1998) reported gross findings with nodules on lips initially followed by swelling of face. Papules and vesicles appeared later and within a few days developed into thick scabs and fissured crusts. The scabs were friable and bled easily. In affected animal, the condition was highly aggravated by myiasis which appeared to have been favoured by the abundance of flies during the hot and humid season (Wernery and Kaaden, 2002).

The course of the disease in individual camels in the present study varied from 8-11 weeks. Khalafalla (1998) reported healing of CCE lesions within 20-30 days in most cases but sometimes the course of the disease extended up to three months. The longer duration of the disease in camels in the present study might be due to more severe lesions and involvement of secondary bacterial infection and myiasis. The presence of lesions on teats and udder as reported in contagious ecthyma of sheep and goats (Nandi *et al,* 2011) has not been observed in the present study. Similarly, Wernery and Kaaden (2002) did not detect any lesions on the udders of the dams or on the skin of any adult camel. No lesions were observed on hands or other body parts of camel handlers in the present study indicating that the disease CCE is most probably not zoonotic in nature and is different for CE of sheep and goats (Nandi *et al,* 2011).

It is known that the epitheliotropic orf virus infects damaged skin and replicates in epidermal keratinocytes (McKeever *et al,* 1988). The microscopic examination of the affected skin in CCE revealed parakeratosis, acanthosis, ballooning degeneration of keratinocytes, intra-cytoplasmic eosinophilic inclusion bodies in swollen epidermal cells, focal ulcerations, inflammation and oedema of the dermis, epidermal necrosis with lymphocytic, neutrophilic and eosinophilic infiltrations and superficial bacterial and fungal colonies (Wernery and Kaaden, 2002; Housawi *et al,* 2004; Bazargani *et al,* 2010). The mononuclear cell infiltration in histology during pustular stage indicates a cellular immune response at the site of infection.

The laboratory diagnosis of CE is generally based on electron microscopy but PCR has been considered as a rapid and highly sensitive method for the diagnosis of CCE (Issi *et al,* 2010). The PCR used in the present study was found to be rapid and confirmatory for the detection of CCE infection.

In conclusion, the results of the present study provide important insights in to the sequential pathomorphological stages during an outbreak of CCE infection.

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