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A REGIONAL OUTBREAK OF CONTAGIOUS CAMEL ECHTYMA IN IRAN

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ABSTRACT

The purpose of this report is to describe an outbreak of contagious camel echtyma in Iran. In May 2009, a pox like disease was investigated in Karaj province of Iran. At the time of investigation, Cutaneous lesions (scabs) were prominent and mostly confined on the lips. Samples from the lesion of lips were taken for genomic detection by PCR and for histopathologic examinations. PCR confirmed the presence of contagious camel echtyma virus in camel skin biopsy samples. Histopathological examination of the lesions revealed parakeratotic and orthokeratotic hyperkeratosis, acanthosis, intraepidermal pustule and subepidermal microabsecess formation. Marked hyperkeratosis, acanthosis with hydropic degeneration of spinosum cells and elongated rete ridges associated with dermatitis were prominent. Intracytoplasmic eosinophilic inclusion bodies were seen in the degenerating spinosum cells and keratinocytes.

Key words: Contagious camel ecthyma, histopathological examination, Iran, PCR

Contagious ecthyma was first reported in new world camelids in 1940 (Wernery and Kaaden, 2002) and in old world camelids in 1969 (Abubakr *et al,* 2007). Both bactrian and dromedary camels are susceptible to this disease (Wernery and Kaaden, 2002). Contagious camel ecthyma virus (CCEV) is a member of parapox genus (Munz, 1992). In some outbreaks of contagious camel ecthyma (CCE), parapox ovis (Robertson, 1976; Munz, 1986 and Gitao, 1994), in one occasion parapox camel strain (Manfield and Tinson, 1996) and in another occurrence pseudo-cowpox (Abubakr *et al,* 2007) viruses were isolated. CCEV infection may result in very severe, severe, mild and unapparent symptoms (Abubakr *et al,* 2007; Manfield and Tinson, 1996; Munz, 1992; Chaudhary and Akbar, 2000). Current strategies to control CCE involve the inhibition of direct or indirect contact with the source of parapox virus including carrier animals and fomite objects (Wernery and Kaaden, 2002; Munz, 1992). It might be possible, in future, to prevent or control this disease by vaccination (Wernery and Kaaden, 2002). Present report describes an outbreak of CCE in three camel herds in Karaj province (Abyek district) of Iran.

Clinical Study and Investigation

In May 2009 a report was received from camel owners of Karaj province of Iran about a pox like disease. At the time of investigation of the first herd (n=8), all animals, including a calf, were affected by CCE in which cutaneous lesions (scabs) were prominent. Lesions were mostly confined on the lips (Fig 1) and in one camel pendulous lower lip were attractive. In addition, there were some scab like lesions on the other parts of body such as muzzle, neck, trunk, perineum and legs. Nearly all camels of this herd showed different grades of emaciation. Prior to displaying CCE lesions, the calf of this herd was transferred to the second herd with camels of different ages. Subsequently, a number of camels in the second herd displayed CCE symptoms. In the latter herd, different stage of the disease was observed including the development of edema in face and mouth (Fig 2), proliferative papules, ulcers and scabs. The lesions were mostly confined on lips especially at lip commissure. This herd was grazing a pasture with plenty of thorny plants and because of these situation papules soon changed to painful ulcer. As a result, most of the affected camels had low body condition. Two weeks later, at the second visit of the second herd, in some affected camels, pendulous lower lips were noticed. At this time, another large camel herd was affected by CCE. The third camel herd had a separate pasture, not far from the second herd.

Samples from the lesion of lips were taken for genomic detection by PCR and histopathologic

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Fig 1. Cutaneous lesions (nodules and scabs) in affected camel by CCEV. Lesions were mostly confined on the lips.

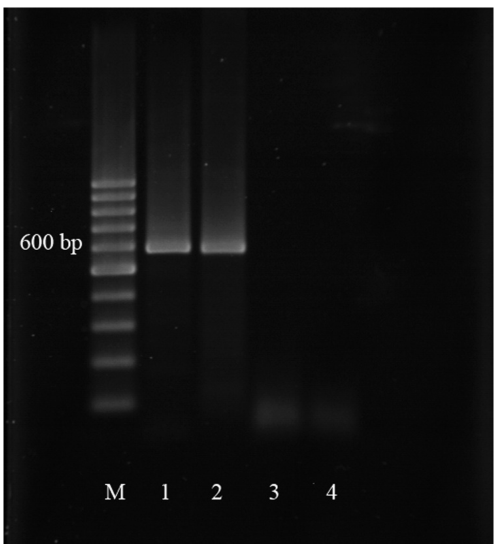

Fig 3. Identification of camel contagious ecthyma virus

Lane M: 100 bp marker

Lane 1: amplification of genomic LT cell DNA infected with CCEV, Kerman/2000 strain

Lane 2: amplification performed on skin biopsy with camel CEV infection

Lane 3: amplification performed on normal skin biopsy

Lane 4: amplification performed on uninfected LT cell DNA

examinations. Samples for PCR were frozen at -20°C. Samples for histopathologic examinations were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 micrometer, and stained with Heamatoxylin and Eosin (H&E).

An Iranian Contagious Ecthyma, Kerman/2000 strain, isolated on lamb testis (LT) cell cultures in its

Fig 2. Severe head oedema in an affected camel by CCEV.

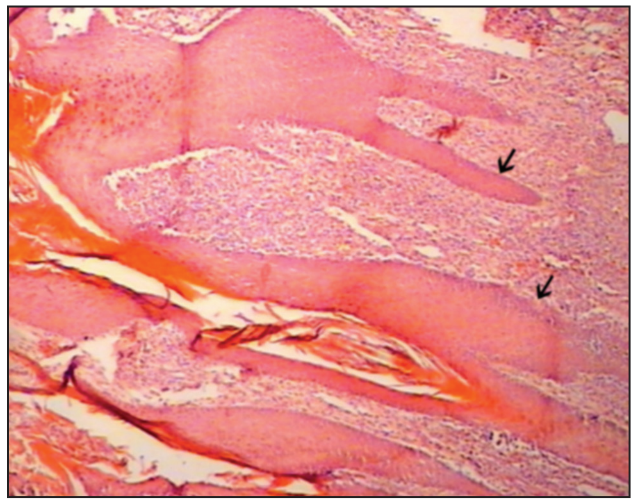

Fig 4. Camel contagious ecthyma skin. Hyperplastic epidermis including hyperkeratosis, pustule formation, acanthosis, elongated rete ridges, serocellular accumulations in the epidermis associated with wart like dermal papillae and dermatitis (H & E Stain).

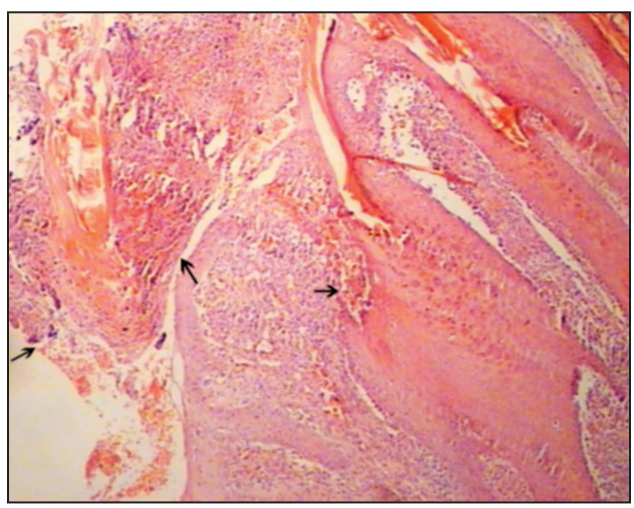

Fig 5. Camel contagious ecthyma skin. Hydropic degeneration of hyperplastic epidermal cells contained intracytoplasmic eosinophilic inclusion bodies (H & E Stain).

60 passage, was used as positive control (Varshovi *et al,* 2005). Uninfected LT cells and normal skin biopsy of camel were used as negative controls. Infected and uninfected cell suspensions and prepared biopsy sample were subjected to DNA extraction using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH). According to the manufacturer's instructions, DNAs were extracted via specific binding on glass fibers in presence of guanidium HCl. Total extracted DNA was used for PCR to amplify a part of B2L gene. The specific oligonucleotide primers were previously published (Inoshima *et al,* 2000).

The forward primer: 5′ - GTCGTCCACGAT GAGCAGCT – 3′, and the reverse primer: 5′–TACGTGGGAAGCGCCTCGCT -3′ were designed. One µl sample of each prepared genomic DNA were placed in PCR mixture (25 µl) contained 1.5 mM MgCl2, 10X PCR buffer (2.5 µl), 0.2 µM of each oligonucleotide primer, 0.2 mM of each dNTP (cinnagen, Iran) and 2 U of Taq DNA polymerase (Fermentas, Canada). The amplification reaction was performed in a DNA thermal cycler (Eppendorf, Germany). Temperature cycling for PCR consisted of 94^{o} C for 3 minutes (one cycle), 94^{o} C for 1 minute, 55^{o} C for 1 minute, and 72^{o} C for 1 minute, repeated for 35 cycles, followed by 72^{o} C for 10 minutes. 6 µl of the amplified product was electrophoresed in 1.5% agarose gel at 80 volts for 80 minutes using Tris-borate-EDTA buffer. The DNA bands were visualised by UV transluminator after ethidium bromide (0.5 µl/ml) staining.

Amplification of the B2L gene of CE, Kerman/2000 strain, and camel skin biopsy sample gave a positive result of DNA amplification with the expected size of 594 bp. No amplified product was observed when DNA of negative controls (uninfected LT cell culture and normal skin biopsy) was examined in PCR (Fig 3).

Histopathological examination of the lesions revealed parakeratotic and orthokeratotic hyperkeratosis, acanthosis, intraepidermal pustule and subepidermal microabsecess formation. Marked hyperkeratosis, acanthosis with hydropic degeneration of spinosum cells and elongated rete ridges associated with dermatitis were prominent (Fig 4). Intracytoplasmic eosinophilic inclusion bodies were seen in the degenerating spinosum cells and keratinocytes (Fig 5). Serocellular crusts contained bacterial colonies were seen on these lesions. Furthermore, hyperemia, oedema, diffused infiltration of mononuclear cells predominantly lymphocytes as lymphocytic dermatitis were diagnosed.

Discussion

The purpose of this case report was to elaborate a regional outbreak of contagious camel echtyma in Iran. Clinical symptoms together with histopathological findings and genomic detetion (PCR) of CCEV ascertained that CCE was the problem in the investigated herds.

Clinically, it might be difficult to differentiate true camelpox from CCE (Wernery and Kaaden, 2002; Munz, 1992). Although, ecthyma lesions are mostly confined to lips and during some weeks after the onset of disease, pendulous lower lip appearance is the prominent symptom for CCE (Ali *et al,* 1991; Abbas and Omar, 2005; Manfield and Tinson, 1996). Confirmation of CCEV by PCR provided definite approval for the presence of CCE in this outbreak. It is recommended that PCR is a valid tool to ascertain CCE (Wernery and Kaaden, 2002).

Based on the anamnesis, camels of the first herd were in direct and/or indirect contact with few goats. It is therefore, conceivable that *Parapox ovis* can be the cause of CCE outbreak of this camel herd (Gitao, 1994; Munz, 1986; Robertson, 1976; Azwai *et al,* 1995).

In the second herd, having no contact with goats, it is more likely that the transfer of the calf from the first herd might be the cause of infection. Engagement of the third herd were possibly took place by infected camel of the second herd, contaminated pasture and contact with exposed attendants (Abubakr *et al,* 2007).

In conclusion, to the best of our knowledge this the first report on CCE outbreak in camel in Iran in which the clinical symptoms were approved by histopathological findings as well as genomic detection of the virus by PCR.

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Functional properties of bioactive peptides produced from camel milk

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Milk proteins exert a wide range of nutritional, functional and biological activities. Bioactive peptides encrypted in milk proteins are a major source for novel functional food ingredients. These peptides can be produced in vivo during gastrointestinal digestion or in vitro throughout food processing using specific enzymes. Milk protein derived peptides have different functionality including antioxidant activity, antimicrobial activity and blood pressure–lowering effect. Most of these foods are produced using bovine's milk proteins but bovine milk allergy by far is the most prevalent food allergy especially in children and â-lactoglobulin (â-LG) is considered the dominant bovine milk allergen. Camel whey lacks â-LG and similar to human milk á-Lactalbumin is its predominant whey protein. It is well known that the oxidative stress due to free-radicals is considered to be responsible for many chronic diseases such as cardiovascular diseases, diabetes, cataracts, neurodegenerative disorders, and certain types of cancer and aging. The result of our studies showed that the bioactive peptides derived from camel milk protein had higher functionality including antioxidant activity, anti-hypertension effect and antimicrobial activity comparing to bioactive peptides from bovine milk proteins. Considering the health effect of camel milk proteins and its bioactive peptides, it could be the 'super food' of the future.

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