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**RESEARCH ARTICLE**

# Clinico-Histopathological Findings and PCR Based Diagnosis of Lumpy Skin Disease in the Sultanate of Oman

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**ABSTRACT**

Current report describes the clinical, histopathological and molecular diagnosis of lumpy skin disease (LSD) outbreak in the Sultanate of Oman during 2009. Outbreak was suspected on the basis of clinical picture in 13 cattle (n=201) farms belonging to Al-Batinah (7 holdings), Al-Dakhiliyah (2 holdings) and Ash Sharqiyah (4 holdings) regions. All suspected cases were clinically examined and a tentative diagnosis of LSD was made upon observation of classical signs. Morbidity and mortality rates were recorded as 27.9 and 5.5%, respectively. Apparent case fatality rate observed was 19.6%. Histopathological examination of the suspected skin biopsy samples revealed presence of ballooning degeneration and intracytoplasmic inclusion bodies characteristic of LSD. PCR reaction was carried out to confirm the presence of disease. Amplification of 221bp (base pair) PCR product from samples belonging to all 3 affected regions confirmed the presence of LSD virus DNA.



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## INTRODUCTION

Lumpy skin disease (LSD) is an infectious, eruptive, occasionally fatal disease of cattle caused by a virus of the family *Poxviridea* and genus *Capripox* which is sometimes also termed as Neethling virus (Salib and Osman, 2011). The disease is of significant economic importance to cattle industry due to reduction in milk production, abortion, temporary or permanent sterility, damaged hides and deaths (Anonymous, 2010; Tuppurainen and Oura, 2011). LSD presents itself as an acute, sub-acute or inapparent disease with variable severity depending upon capripoxvirus strain and the host breed.

Lumpy skin disease can be suspected whenever clinical signs indicate towards persistent fever (may exceed 105.8°F), wide spread skin nodules (lumps), enlarged peripheral lymph nodes, conjunctivitis, keratitis, corneal opacity, edema in the brisket and legs (Radostits *et al.*, 2007). Animals recover slowly from the severe disease and may suffer from mastitis, pneumonia, formation of necrotic skin plugs leaving deep holes in the hide (Tuppurainen *et al*., 2011). Histopathology can be an important tool to exclude viral, bacterial or fungal causes of nodular development in clinical cases and characteristic cytopathic effects (necrosed epidermis, ballooning degeneration of squamous epithelial cells and eosinophilic intracytoplasmic inclusion bodies) in cases of LSD are well documented (Ali *et al* 1990; Brenner *et al.*, 2006).

Epidemiological observations indicate that *Bos indicus* is less susceptible to clinical disease than *Bos taurus* (Radostits *et al.*, 2007) and cows in lactation are more at risk (Carn and Kitching, 1995). LSD is a less contagious disease with generally low mortality (less than 10%) and varying (1-90%) morbidity rate (Davis, 1991b; Coetzer, 2004; Salib and Osman, 2011). Biting flies (*Stomoxys calcitrans* and *Biomyia fasciata*) and mosquitoes (*Culex mirificens* and *Aedes* natrionus) can be a source for transmission of the disease (Chihota *et al.*, 2001). In a recent study researchers (Tuppurainen *et al.*, 2011) found molecular evidence suggesting that LSD can be transmitted through hard (Ixodid) ticks (*Rhipicephalus decoloratus*, *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*). Other risk factors associated with spread of LSD were found to be worm humid agroclimate, communal grazing/watering and introduction of new animals in a herd (Gari *et al.*, 2010).

Laboratory confirmation of LSD in a newly suspected area or region requires viral isolation on cell culture

(Davis, 1991a) or positive polymerase chain reaction (PCR) to confirm the LSD virus DNA (Anonymous, 2010). Both conventional (Gerrit *et al.,* 2005; Tuppurainen *et al.,* 2005) and quantitative real time PCR methods for diagnosis are well documented (Bowden *et al.,* 2008; Balinsky *et al.,* 2008) and hold an edge over other methods like electron microscopy, viral isolation and ELISA in terms of availability, sensitivity and specificity (Awad *et al.,* 2010).

To date, many vaccines had been tried to control the LSD and it is concluded that in the wake of an outbreak cattle can be protected against LSD by using the strains of capripoxvirus derived from sheep or goats (Kitching, 2003). Although, commercial live attenuated LSDV vaccines are available but their use in countries previously free from disease is not recommended because of the potential safety issues as skin lesions containing high titers of virus can develop in vaccinated animals that may disseminate the virus through vectors (Brenner *et al.,* 2009; Tuppurainen and Oura, 2012).

The disease was first described in Northern Rhodesia (currently Zambia) in 1929 and then a rapid spread was observed in cattle over most of the African continent (Davis, 1991b). LSD was first reported outside Africa in Middle East (Brenner *et al.,* 2006; El-Kholy *et al.,* 2008) in 1991. More recent outbreaks of LSD have been reported in Egypt (2006), Israel (2006 and 2007), Palestine (2007 and 2008) and Bahrain (2006-2009) (Anonymous, 2011). LSD was possibly introduced in the region through import of live animals from endemic countries of African continent (Shimshony and Economides, 2006). If not controlled properly, a further spread of LSD into Asia (east) and Europe (north) through Middle East and Turkey is possible (Tuppurainen and Oura, 2012). LSD is an OIE listed disease and is, therefore, subjected to continuous surveillance. Current report describes the occurrence of lumpy skin disease in the Sultanate of Oman in 2009, where both clinical and laboratory confirmations were made.

## MATERIALS AND METHODS

**Clinical and field investigations:** On April 7, 2009 a disease clinically resembling LSD was reported by local veterinarians in native cattle of Al-Batinah region. On April 13, 2009 the disease was also suspected in the (Ash Sharqiyah and Al-Dakhiliyah) regions (Fig. 1). All the affected cattle showed almost similar clinical picture. Following these reports, field and laboratory investigations were commenced by the Veterinary Research Center (VRC) to identify the cause. Clinical picture indicated towards a possible outbreak of LSD and as a control measure affected herds were vaccinated by using the sheep and goat pox vaccine Kenya strain as per recommendations i.e. $\log^{10}$ 3.7 $TCID_{50}$ (50% tissue culture infective dose) (Davis, 1991a; Brenner *et al.,* 2006; Anonymous, 2010). Clinical outbreaks that started with appearance of first cases are still ongoing and 113 outbreaks of LSD were reported to OIE in 2010 (Anonymous, 2011).

**Laboratory investigations:** Representative pieces of skin nodules from all clinical cases (n=56) were collected in sterilized containers. Samples were then fixed in 10% buffered formalin and transported to pathology section of VRC. The tissues were processed to obtain 4-5 µm thick paraffin embedded sections and were stained with hematoxylin and eosin (Anonymous, 2010).

A polymerase chain reaction (PCR) was carried out for the confirmation of disease by using commercial 'Lumpy Skin Disease Virus PCR kit' (Shanghai ZJ Bio Tech Co, Ltd, China) by strictly following manufacturer's recommendations. Briefly, 100 mg homogenized tissues from skin lesion was taken for DNA extraction through DNA extraction buffer supplied by the manufacturer. For each reaction master mix was prepared by adding 35µl reaction mix with 0.4µl of enzyme mix. Each reaction tube was then poured with 5µl of extracted DNA and 35µl of master mix. Thermocycler was set to following protocol: 94°C for 2 min, 1 cycle; 93°C for 15 sec, 55°C for 30 Sec, 72°C for 30 sec, 35 cycles. PCR product was electrophoresed in 1.5% agarose gel until the DNA samples have migrated a sufficient distance through gel. The PCR results were considered positive for LSD virus DNA when a 221bp product was observed.

## RESULTS

**Clinical and field investigations:** First suspected cases were observed by the local veterinarians during the month of April (2009) in Al-Batinah region. Outbreaks were recorded in 7 different holdings from wilayats of Rustaq (5 farms) and Al-Musanaa (2 farms). In total 94 cattle were present in these farms with 26 affected animals. Moreover, 4 deaths were also recorded in these farms due to same disease. Later in the same month outbreaks were also recorded in Al-Dakhiliyah (2 farms in wilayat Nizwa) and Ash Sharqiyah regions (4 farms in wilayat Ibra). Out of 55 susceptible cattle in Ash Sharqiyah region, 14 were found in clinical disease and one death was recorded. A total of 52 susceptible cattle were present in 2 affected farms in wilayat Nizwa (Al-Dakhiliyah region) where 16 animals were found in clinical disease and 6 deaths were recorded (Table 1).

Affected animals showed severe clinical signs characterized by generalized skin nodules of 2-3 cm in diameter (Fig. 2), enlarged peripheral lymph nodes, edema of the dependent parts (brisket and forelegs) (Fig. 3), lacrimation and corneal opacity (Fig. 4). With the progression of disease the nodules became necrotic, and eventually a deep scab formed (sit-fast) (Fig. 5). The small ruminants (sheep and goat) housed with disease affected cattle did not show any symptom of the disease.

Analysis of the outbreak statistics (Table 1) revealed a relatively consistent morbidity rate with highest value in Al-Dakhiliyah region (30.8%) followed by Al-Batinah (27.6%) and Ash Sharqiyah (25.4%) regions, $\chi^2$=0.38 (2df), p=0.38. Highest mortality rate was observed in Al Dakhiliyah region (11.5%) followed by Al-Batinah (4.3%) and Ash Sharqiyah (1.8%) regions, $\chi^2$=5.38 (2df), p=0.07. Case fatality rates were significantly different ($\chi^2$=17.48, df=2, p<0.001) among all the regions with highest values observed in Al-Dakhiliyah (37.5%) followed by Al-Batinah (15.4%) and Ash Sharqiyah (7.1%).

**Table 1:** Morbidity, mortality and case fatality statistics of lumpy skin disease outbreaks in the Sultanate of Oman during 2009

| Region | Wilayat | LSD Affected Farms | Susceptible Cattle | Confirmed Cases | Deaths | Morbidity Rate (%) | Mortality Rate (%) | Case Fatality Rate (%) |
|---|---|---|---|---|---|---|---|---|
| Al-Batinah | | 7 | 94 | 26 | 4 | 27.6 | 4.3 | 15.4[2] |
| 1 | Rustaq | 5 | 52 | 20 | 2 | | | |
| 2 | Musnaa | 2 | 42 | 6 | 2 | | | |
| Ash Sharqiyah | | 4 | 55 | 14 | 1 | 25.5 | 1.8 | 7.1[2] |
| 1 | Ibra | 4 | 55 | 14 | 1 | | | |
| Al-Dakhiliyah | | 2 | 52 | 16 | 6 | 30.8 | 11.5 | 37.5[1] |
| 1 | Nizwa | 2 | 52 | 16 | 6 | | | |
| Total | | 13 | 201 | 56 | 11 | 27.9 | 5.5 | 19.6 |

Values with different numeric superscripts differ significantly, $\chi^2$=17.48, df=2, P<0.001.

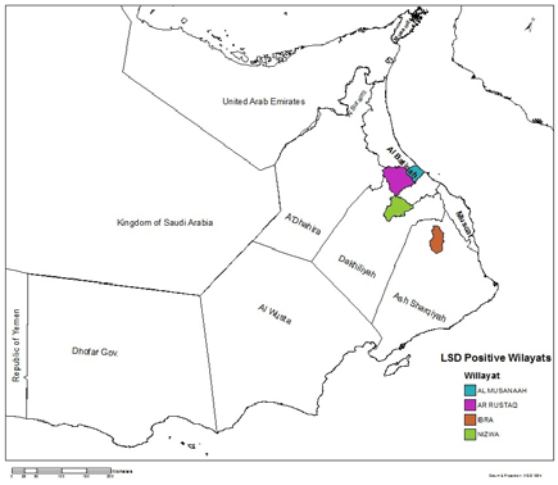


**Fig. 1:** Wilayats (n=4) and regions (n=3) of the Sultanate of Oman affected with lumpy skin disease outbreaks during 2009.

**Fig. 2:** Large circumscribed skin nodules in LSD affected cattle.

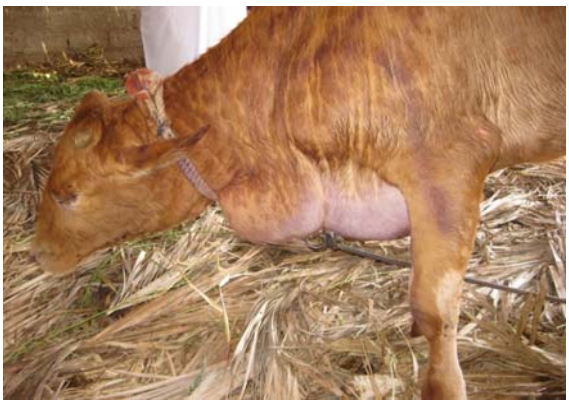

**Fig. 3:** Brisket edema in LSD affected cattle.

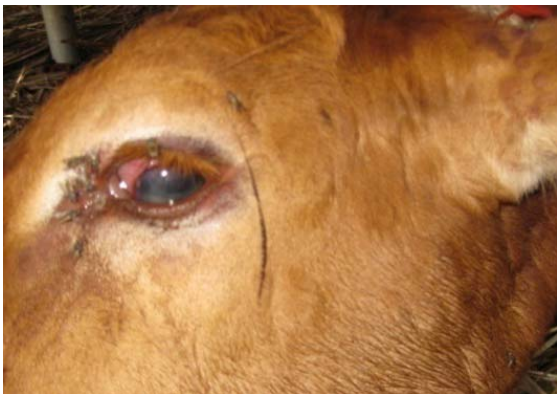

**Fig. 4:** Corneal opacity and lacrimation in LSD infected cow.

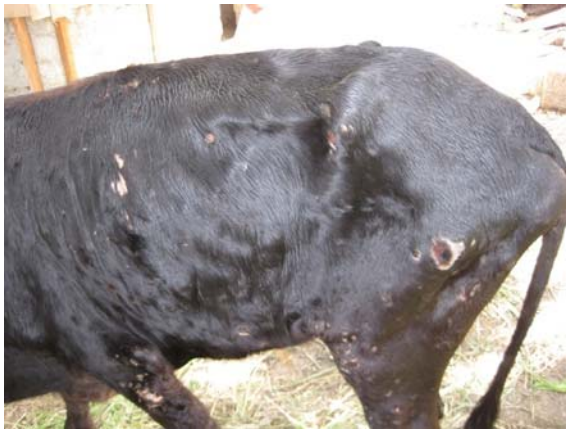

**Fig. 5:** Progression of lumpy skin disease: note the necrotic nodules and deep scab formation (sitfast).

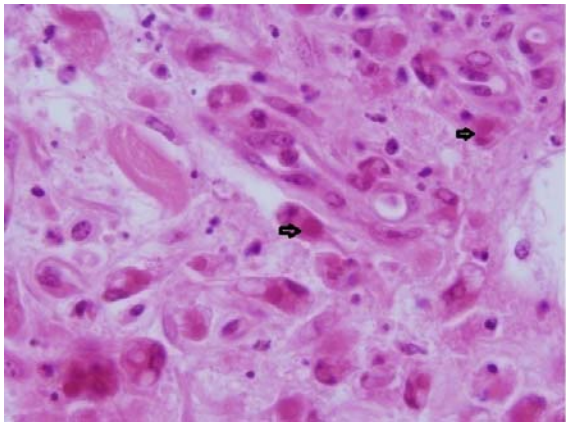

**Fig. 6:** Photomicrograph of skin showing granuloma with intracytoplasmic inclusions due to lumpy skin disease virus. H&E; X 400.

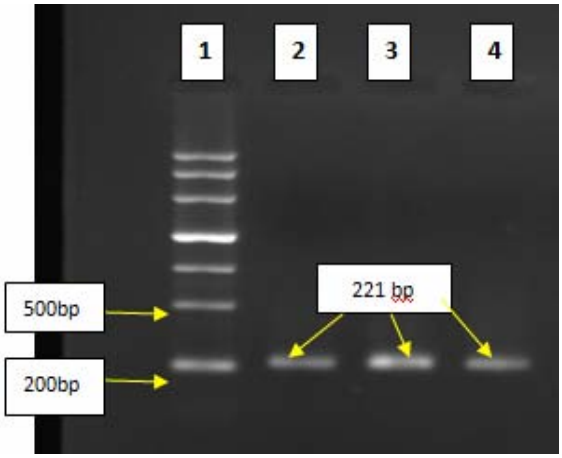


**Fig. 7:** PCR product (221bp) confirming the presence of LSD virus DNA in the samples taken from skin lesion of infected cow.
Line 1: DNA ladder
Line 2: Positive Sample from Al Batinah Region
Line 3: Positive sample from Ash Sharqiyah Region
Line 4: Positive sample from Al Dakhiliyah Region

**Laboratory Investigations:** The H and E stained section from different parts of the skin lesion showed ballooning degeneration of epithelial cells and presence of eosinophilic intracytoplasmic inclusion bodies due to infection with lumpy skin disease virus (Fig. 6) as demonstrated by positive PCR results. A 221bp size of PCR product was obtained from all suspected samples indicating the presence of LSD virus DNA (Fig. 7).

## DISCUSSION

Exotic pathogens are of a major threat to national economies as they tend to spread more rapidly because of initial confusions in field and laboratory diagnosis. A rapid, reliable laboratory diagnosis is required to confirm occurrence of an exotic disease along with the adaptation of a prompt control measures to limit its spread.

Clinical picture of the disease led to the speculations about occurrence of LSD at affected farms. Outstanding features of the disease i.e. characteristic skin nodules, enlarged lymph nodes, brisket edema and absence of disease in sheep and goats cohorts were in accord with those documented by Coetzer (2004), Brenner *et al*. (2006), Radostits *et al.,* (2007), Gari *et al*. (2010) and Salib and Osman (2011). Similarly, histopathological picture of epithelial degeneration with intracytoplasmic inclusion bodies also pointed towards presence of lumpy skin disease virus as reported in literature (Ali *et al.,* 1990; Brenner *et al.,* 2006; Tuppurainen *et al.,* 2011).

Morbidity (27.9%) and mortality (5.7%) observed during this outbreak were in agreement with other authors who report that the LSD is a disease with high morbidity (1-90%) and low mortality (<10%) rates (Davis, 1991a; Salib and Osman, 2011) and these values can fluctuate according to geography, climate, management conditions, immune status of animal, breed and strain of virus involved (Tuppurainen and Oura, 2012). Differences observed between the mortality and case fatality rates in 3 regions could be attributed to variations in the agro-ecological zones and husbandry practices. Similar was reported by the Gari *et al*. (2010) in a questionnaire based study of risk factors responsible for the spread of LSD in Ethiopia who concluded that the observed differences in prevalence and severity of disease could be linked to diversity of agro-climatic zones and farming practices.

Laboratory confirmation was made upon the results of a positive PCR reaction and this is a quick, sensitive, reliable method as antigenic resemblance of LSD virus with sheep and goat poxvirus makes the diagnosis through routine serological tests (virus neutralization, indirect fluorescent antibody test, ELISA etc.) impossible (Gerrit *et al.,* 2005; Tuppurainen *et al.,* 2005; Balinsky *et al.,* 2008; Anonymous, 2010). PCR is also preferred over other reliable methods of virus isolation and electron microscopy because these are not readily available and time consuming (El-Kholy *et al.,* 2008; Awad *et al.,* 2010).

Current report indicates towards occurrence of lumpy skin disease in the Sultanate of Oman, where diagnosis was made based upon the clinical picture, absence of disease in small ruminant cohorts, histopathological findings and PCR results. Introduction of disease in the Sultanate can be attributed to the infected livestock movement as Oman regularly imports cattle from African continent (where disease is endemic) to meet its food requirements (Shimshony and Economides, 2006). Prevailing farming conditions, uncontrolled animal movement, communal grazing and nomadism may spread LSD further in the affected regions and the country. Keeping in view the economic impact of LSD a strict surveillance and quarantine regulation is required by the animal disease control authorities in the Sultanate.

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