CASE REPORT

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Outbreak of lumpy skin disease in a dairy farm in Keffi, Nasarawa State, Nigeria

AJ Adedeji[1], OB Akanbi[2], JA Adole[1], NC Chima[1] & M Baje[3]

[1.] *National Veterinary Research Institute, Vom, Nigeria*

[2.] *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria*

[3.] *Nagari Integrated Dairy Farms, Keffi, Nasarawa State, Nigeria*

*Correspondence: Tel.: +2348033559785; E-mail: yinkadeji@yahoo.com

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Abstract

A dairy farm in Keffi, Nasarawa State, North Central Nigeria reported cases of skin infections in cattle suggestive of lumpy skin disease (LSD) in the years 2014 and 2016. During the 2016 outbreak, skin biopsies were collected for laboratory investigation using histopathology and polymerase chain reaction (PCR). The samples were subjected to PCR using two sets of primers which amplified the G-protein-coupled chemokine receptor (GPCR) gene and RNA polymerase subunit gene (RPO30) of the LSD virus (LSDV). Clinical signs observed in the two outbreaks were similar and included ocular and nasal discharges, nodular skin lesion, and pyrexia and reduced milk production. Morbidity rate was 6.25% (25/400) in 2014 and the mortality rate was 2.5% (10/400). Furthermore, the second outbreak in 2016 had morbidity and mortality rates of 5% (20/400) and 0.5 % (2/400) respectively. From animals that died in the 2014 outbreak, necropsy findings included nodular lesions on the skin, muzzle, in the buccal cavity and nasal mucosa. Also, congested and oedematous lungs and enlarged lymph nodes were observed. The nodular skin lesions observed during the 2016 outbreak were larger and more numerous in the Sokoto Gudali, while the Holstein Friesian cross had smaller gross skin nodules with acute to sub-acute histopathologic changes. The histopathological examination of the skin biopsy of the HF was consistent with LSD viral lesions, while PCR detected the RPO30 and GPCR gene fragments of LSDV.

Keywords: Dairy, histopathology, Lumpy skin disease, polymerase chain reaction, Nigeria

Introduction

Lumpy skin disease (LSD) is an economically important viral disease of cattle caused by LSD virus (LSDV) a member of the genus *Capripoxvirus* (Woods, 1988; Tulman *et al.,* 2001). LSDV is highly host specific infecting cattle, buffalo and closely related-wild ruminants (Sharawi & El-Rahim, 2014; Beard, 2016). The first description of LSD was in Zambia in 1929 and since then, the disease has been reported in many countries in Africa and Asia (Davies, 1982; Tuppurainen *et al.,* 2015). Recently, LSD was also reported in several European countries thereby raising concern of the threat of the disease to livestock farmers in Europe (Tuppurainen & Oura, 2014; Beard, 2016; Mercier *et al.,* 2017). The clinical signs of LSD include lacrimation, fever, anorexia, skin nodules which appear after the initial increase in body temperature (Hunter and Wallace, 2001). The skin nodules which are distributed all over the body are very characteristic and easily recognizable (Beard, 2016). Other clinical signs include enlarged

lymph nodes and ulcerative lesions in the mucous membranes of the mouth and nose, subcutaneous swellings on the legs (Hunter and Wallace, 2001; Tuppurainen *et al.,* 2005). Morbidity rate associated with LSD are usually high with low mortality, however mortality is usually high in exotic breeds (Tuppurainen *et al.,* 2005). The LSDV is transmitted mechanically by insects, but feeding troughs and formites have also been incriminated in the transmission of the virus (Chihota *et al.,* 2001; Lubinga *et al.,* 2013). The diagnosis of LSD is usually confirmed by viral isolation, electron microscopy, histopathology, and polymerase chain reaction (PCR) (Walid *et al.,* 2010; Tuppurainen & Oura, 2012). Prevention and control is by vaccination of susceptible animals in endemic regions, restriction of movement of cattle and insect control during outbreaks (Tuppurainen *et al.,* 2017).

The first report of the occurrence of LSD in Nigeria was by Woods (1974) with later reports by Bida *et al.* (1976), Nawathe *et al*. (1978), Nawathe *et al*. *(*1982), and Adedeji *et al.* (2017). Hence, epidemiological data on the status of LSD in Nigeria is very scanty. Field reports of suspected cases of LSD in Nigeria abound but laboratory diagnosis is hardly carried out and hence limited reporting is done. Moreover, LSD can be confused with other skin diseases such as pseudo-lumpy skin disease, insect bites, demodicosis, and dermatophilosis (Tuppurainen *et al.,* 2017). Therefore, rapid laboratory confirmation is essential for control of LSD in commercial farms to avert economic losses and spread due to the transboundary nature of the disease. This study reports the diagnosis of LSD based on clinical signs, PCR and histopathology of two outbreaks of the disease in a dairy farm in Keffi, Nasarawa State, Nigeria.

Case Report

Case location

The case was in Keffi, a town located in Nasarawa State. Keffi is located on Latitude 8.8471° N and longitude 7.8776° E. It has an area of 138 Km² and a population of 92,664.

Case history and sample collection

A dairy farm in Keffi, Nasarawa State, Nigeria reported the presence of nodular skin eruptions in their herd suspected to be LSD in July 2014 and September 2016. Prior to the year 2014, the herd was not vaccinated against the LSD. But thereafter the animals were vaccinated using foreign LSD vaccine and in 2015 there were no cases of LSD on the farm. However, in the year 2016 there was a second suspected outbreak of LSD among the local breed (Sokoto Gudali) and the cross breed (Sokoto Gudali/ Holstein Friesian). The two suspected LSD outbreaks were noticed following heavy fly infestation during the raining season. Skin biopsies were surgically collected during the second outbreak in 2016 and the site was sutured after which antibiotics were administered. Of the samples collected, one part was placed on ice and the other part was placed in 10% phosphate buffered formalin fixative and transported to the National Veterinary Research Institute (NVRI), Vom Nigeria for laboratory investigation. All animals showing clinical signs suggestive of LSD were isolated. Animals with ulcerated skin lesions were cleaned, disinfected after which topical antibiotics were administered and flies control was carried out on the dairy farm using acaricide spray.

Gross and histopathology

Skin biopsies of 4x4 cm in diameter were taken from the cutaneous nodules on a Sokoto Gudali bull and a Holstein Friesian cross heifer and placed in 10% phosphate buffered formalin fixative for 48-72hours. These tissues were later cut into 1x1cm in diameter each using scalpel blade and processed using an automatic tissue processor. The tissues were dehydrated in ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five micron thickness of paraffin sections were prepared and labeled appropriately and thereafter, routinely stained with hematoxylin and eosin (H and E) dyes as described by Neamat-Allah (2015). For histopathological examination, 100X and 400X of Carl Zeiss® binocular microscope was used.

Primers for the detection of lumpy skin disease virus genes

Two sets of published specific primers were used for the detection of LSDV genes using PCR as listed in Table 1. The primers target two genes [the G-protein-coupled chemokine receptor (GPCR) gene and RNA polymerase subunit gene (RPO30] of the LSDV which are conserved regions of the LSDV (Table 1) and can differentiate the virus from other members of the genus *Capripox* (Lamien *et al.,* 2011).

Detection of lumpy skin disease virus polymerase chain reaction

The samples were homogenized to 20% solution using pestle and mortar and subsequently subjected

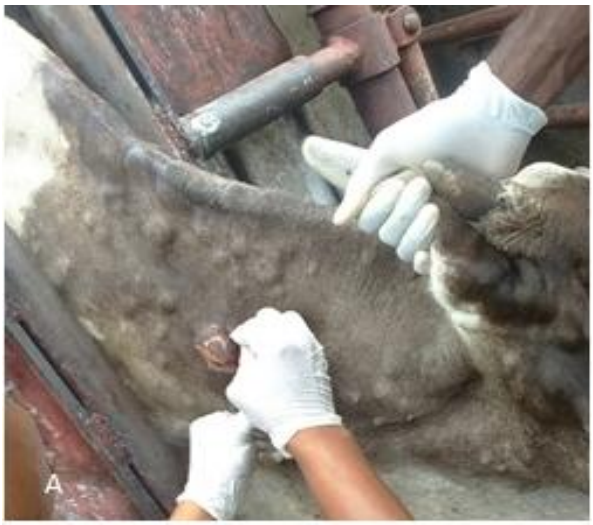
Table 1: Primer sequences used for amplification of the G-protein-coupled chemokine receptor (GPCR) gene and RNA polymerase subunit gene (RPO30) of the Lumpy skin disease virus

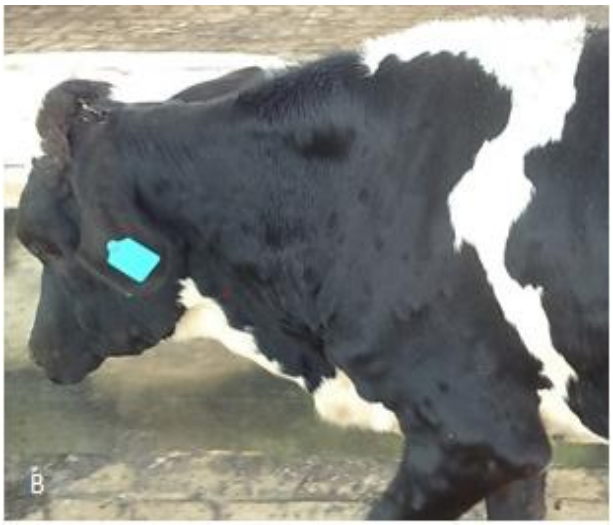
Gene Target	Sequences (5'– 3')	Size (bps)	Reference
RNA polymerase subunit (RPO30)	TCTATGTCTTGATATGTGGTGGTAG AGTGATTAGGTGGTGTATTATTTTCC	172bps	Lamien *et al.,* 2011
G-protein-coupled chemokine receptor (GPCR)	TTAAGTAAAGCATAACTCCAACAAAAATG TTTTTTTATTTTTTATCCAATGCTAATACT	1150 bps	Le Goff *et al*., 2009

to DNA extraction using QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The PCR reaction mix for the GPCR gene was carried out in a 25µl volume reaction mix comprising of 10 mMTris–HCl, $MgCl_2$, 2.5mM dNTPs mix 1µl, 20pmol of each primer, 2.5 units of Taq polymerase, nuclease free water 14.0 µl and DNA template 2.5µl. The PCR thermal cycling condition was as follows; Initial denaturation for 2 min at 94 °C followed by 35 cycles for 40s at 94 °C, 30s at 55 °C and 72°C for 30s, then final extension at 72 °C for 5 min. The samples were held at 4 °C. The PCR mix for the RPO30 gene was carried in 25 µl consisting of 2X master mix 12.5 µl, 20 pmol of each primer, nuclease free water 8.0 µl and DNA template 2.5µl. The thermal cycling conditions were 95^0 C for 4 min followed by 40 cycles of $95\ ^0$C for 30s, $55\ ^0$C for 30 s and $72\ ^0$C for 30s. Electrophoresis of the amplified products was then carried out on a 1.5% agarose gel stained with ethidium bromide and run on 80 volts for 60 min. A 1Kb Plus ladder (Thermo Scientific) was added alongside the amplicons. The gel was then viewed under UV light in a Syngene Bio-imaging system. The positive control was the LSD vaccine (National Veterinary Research Institute Vom, Nigeria) and the negative control is molecular grade nuclease free water. Both were subjected to DNA extraction and PCR.

Results and Discussion

Farm records revealed the farm had 400 dairy cattle [100 Holstein Friesian (HF) and 300 indigenous breed] which included Sokoto Gudali (SG) breed (Table II). During the 2014 outbreak the clinical signs observed were fever, ocular discharges and nodular skin lesions on the neck and shoulder and reduced milk production. In 2014, morbidity and mortality rates was 6.25% (25/400) and 2.5% (10/400) respectively. But, HF were the only animals affected with breed morbidity rate of 40% (25/100) and 10% (10/100) mortality rate. The post mortem findings were nodular lesions on skin, nodules on the muzzle, buccal cavity and nasal mucosa, congested and oedematous lungs, enlarged liver and lymph nodes were also observed (Table 2). LSD diagnosis was based on clinical signs and postmortem findings. In the year 2015 the whole herd was vaccinated against LSD and later the HF cattle were culled and replaced with SG breeds. As at 2016, the dairy farm records revealed that there were 350 SG and 50 cross breeds of Holstein Friesian and SG cross. The clinical signs observed in the year 2016 outbreak were fever, salivation, lacrimation, ulcers in oral cavity, nodular skin lesion on the neck and shoulder (Plate IA & B), with 5% (20/400) morbidity rate and 0.5% (2/400) mortality rate. Grossly, the SG bull (Plate IA) had numerous, hard, well circumscribed varying sizes 1-5 cm in diameter of skin nodules around submandibular, neck and shoulder regions. These nodules were often times coalesced, having a flat-top surface. While the HF heifer (Plate IB) had similar but moderately sized, numerous well circumscribed skin nodules around the neck. After the 2016 outbreak, the whole herd was vaccinated with LSD vaccine (NVRI, Vom). The clinical signs of the LSD in this study were pathognomonic and consistent with a recent report of LSD outbreak by Adedeji *et al.* (2017) in Jos, Nigeria, although, higher mortality rates of 1.5%-5.3% were reported in three outbreaks in HF in the aforementioned study. The morbidities, mortalities and reduced milk production associated with LSD in the dairy farm in this study were of dare economic consequences to the farm. In a report in Nigeria, the economic losses attributed to LSD in a dairy were estimated to be $17,377.05; however, in this study the direct economic losses were not estimated (Adedeji *et al.,* 2017). Vaccination of the herd in 2015 may have protected against LSD outbreak, but culling most of the vaccinated animals and restocking may have led to the 2016 outbreak. Moreover, LSD can occur in cycles with periods of lull which gives the impression that the disease has been controlled only for outbreaks to occur again (Tuppurainen & Oura, 2012). Microscopically, the lesions in the HF heifer were acute to subacute with





Plates I A & B: A Sokoto Gudali bull with numerous severe well circumscribed skin nodules around the neck (A), A Holstein Friesian cow in the dairy farm in Keffi, Nassarawa State with similar numerous well circumscribed skin nodules around the neck (B)

Table 2: Clinical manifestation of suspected lumpy skin disease outbreaks on the farm, 2014-2016

Year	Herd size	Breed	Clinical manifestation	Post mortem findings	Samples collected	Diagnosis
2014	400	Holstein Friesian Sokoto Gudali	Fever, salivation, lacrimation, nodular skin lesion on the neck and shoulder and herd morbidity was 6.25% (25/400), mortality of 2.5% (10/400). The only affected were the Holstein Friesian with breed morbidity and mortality of 40% (25/100) and 10 (10/100), respectively.	Nodules on skin muzzle, buccal cavity & nasal mucosa, congested and oedematous lungs, enlarged lymph nodes and liver	None	Based on clinical signs and Postmortem findings
2016	400	Holstein Friesian crosses, Sokoto Gudali	Fever, salivation, lacrimation, ulcers in oral cavity, nodular skin lesion on the neck and shoulder, morbidity 5% (20/400) mortality 0.5% (2/400). After the disease was controlled the animals was vaccinated with Local LSD vaccine, After the disease was controlled, the animals was vaccinated with LSD vaccine (NVRI, Vom).		Skin biopsies (n=2)	Based on clinical sign, histopathology and PCR, with 100% detection rate (2/2)

severe neutrophilic dermatitis, dermal edema and ulceration (Plates IIA-D). The SG breed lesions were chronic with intradermal vasculitis and presence of polymorpho-histiocytic cellular infiltration. The microscopic lesion observed particularly was cellular infiltration which is suggestive of LSD (Al-Salihi & Hassan, 2015). There was a positive correlation between size of gross pathologic lesions (i.e 1-5cm) of skin nodules with the severity of microscopic lesions in the in the HF. The GCPR and RPO30 gene fragments of LSDV was detected by PCR from the skin biopsies collected during the 2016 LSD outbreak in 100% (2/2) of the samples analyzed (Table II). The RPO30 (Plate IIIA) and GPCR (Plate IIIB) gene fragments were amplified at 172bps and 1150bps respectively which is the expected PCR product size.

The positive control was also amplified at the expected product size, while the negative control was not amplified. Lumpy skin disease outbreaks were confirmed in this dairy farm based clinical signs, postmortem findings, histopathological examinations and PCR results. The confirmation of the diagnosis of LSD is usually by performing virus isolation, electron microscopy, and histopathological examination and PCR (Tuppurainen & Oura, 2012). Similarly, our PCR results is in agreement with reports by Tuppurainen *et al.* (2005) and Walid *et al.* (2010) that concluded skin biopsies/samples can be used for detection of LSDV using PCR. Also, Lamien *et al.* (2011) confirmed that the RPO30 gene fragments can be used to differentiate LSDV from sheep pox virus. Furthermore, the GCPR gene fragment has been shown to be one of the most variable genes within viruses of the genus capripox and is a suitable target for genetic discrimination (Le Goff *et al.,* 2009; El-Tholoth & El-Kenawy, 2014). The two outbreaks of LSD on the farm coincided with high infestation of flies on the farm corroborating the findings that

LSDV may be mechanically transmission by insects (Chihota *et al.,* 2001; Lubinga *et al.,* 2013). It seems, therefore, that insect control may offer a viable preventive measure against field outbreaks of LSD in cattle herds. This study represents the first report of confirmation of LSD outbreak in Nigeria based on PCR and histopathological examination in Nigeria.

In conclusion, Lumpy skin disease outbreak was confirmed in a dairy farm in Keffi based on clinical signs, histopathological examination and PCR results. For proper control of LSD in Nigeria, prompt laboratory investigation, vaccination and insect control should be carried out.

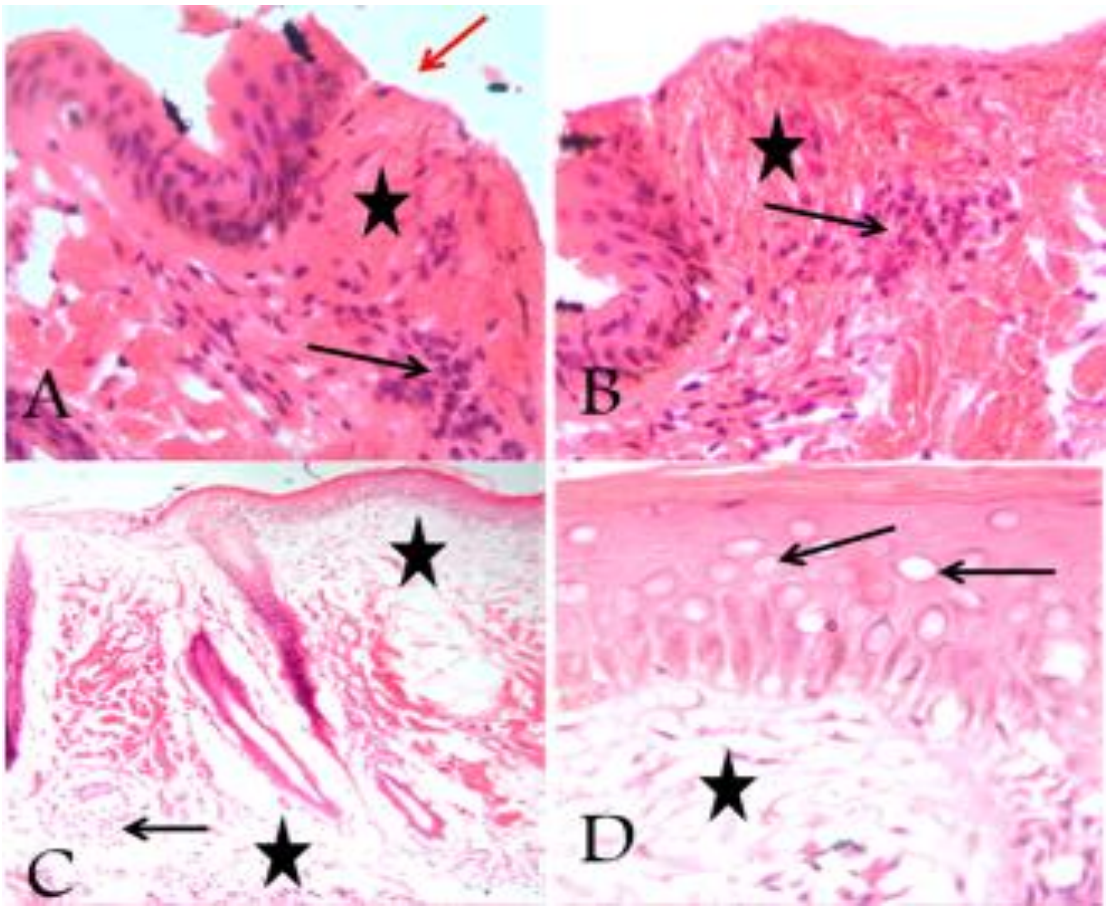
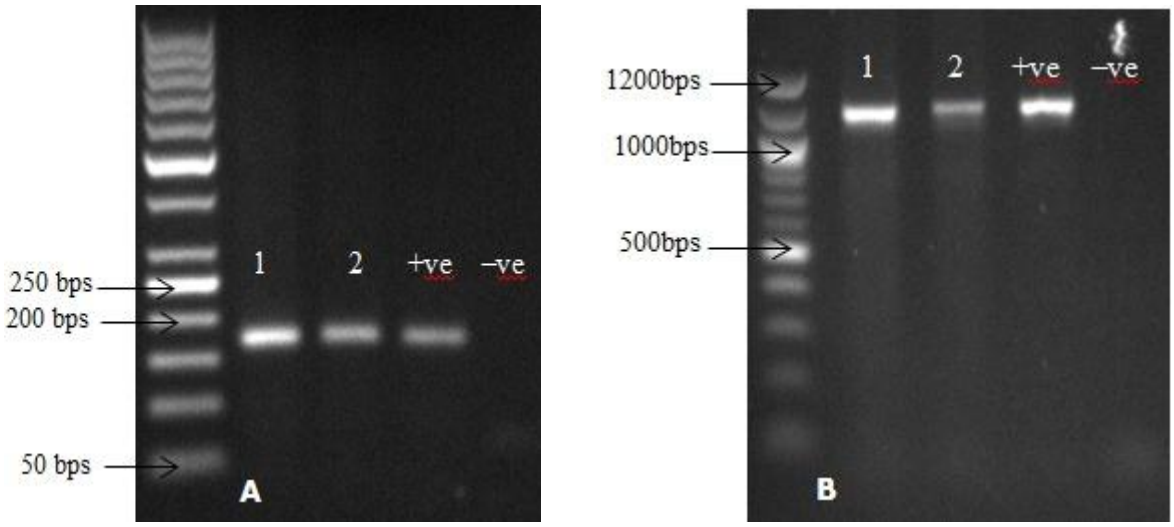


Plate IIA: Skin; Severe focal ulcerative flat-top nodular dermatitis and granulation tissue formation (black star) with severe neutrophilic cellular infiltration (black arrow). The epidermis is separated and sloughed off exposing the dermal granulation tissue (red arrow). H&E stain X400 HPF; **B:** Skin, close up of 2A. H&E stain X400; **C:** Skin; diffuse severe superficial and deep dermal edema (black stars), collagenolysis and focal perivascular mononuclear cuffing with perivascular edema (black arrow). H&E stain X 100 HPF; **D**: Skin; higher magnification of 2C, epidermal basal keratinocytes hydropic degeneration (black arrow) with basal cell layer spongiosis and intradermal edema (black star). H&E stain X 400 HPF



Plates IIIA & B: 1.5% agar gel electrophoresis amplified product of RPO30 gene of lumpy skin disease virus from samples collected from a dairy farm in Keffi. 1-2 are the skin biopsies samples collected from a dairy farm in Keffi. Positive samples were amplified at 172bps (Plate IIIA), 1.5% agar gel electrophoresis of GCPR gene of lumpy skin virus amplified from samples collected in a dairy farm in Keffi, Nasarawa State. 1-2 are the skin biopsy samples (Plate IIIB). Positive samples were amplified at 1150bps.
Key: +ve is the positive control and –ve is the negative control

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