Serological, virological and molecular diagnosis of an outbreak of lumpy skin disease among cattle in Butana area, Eastern Sudan

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Abstract

Background: Lumpy skin disease (LSD) is a highly infectious disease of cattle caused by a virus of the Poxviridae family, genus Capripoxvirus. The disease has great economic effects on the global cattle industry. In this study, an outbreak of LSD among cattle which occurred in Nahr Atbara and Halfa El Jadida locations in Butana area, Kassala State in November, 2020 is described.

Methods: Lumpy skin disease virus (LSDV) antibody and nucleic acid were detected in specimens (n = 50) using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

Results: The virus was isolated on the chorioallantoic membrane (CAM) of embryonated chicken eggs and identified by PCR. The number of animals at risk was 295 with a morbidity rate of 4.1% and a mortality rate of 2.4%.

Conclusions: LSDV was diagnosed for the first time in the Butana region, Kassala State, Eastern Sudan in 2020.

KEYWORDS

cattle, ELISA, isolation, lumpy skin disease, PCR, Sudan

1 | INTRODUCTION

Lumpy skin disease (LSD) is a poxvirus disease of cattle (Bos indicus and Bos taurus), Buffalo and some wild animals caused by lumpy skin disease virus (LSDV or Neethling virus), a virus from the family Poxviridae, genus Capripoxvirus (Babiuk et al., 2008). The disease is of economic importance as it can cause a substantial reduction in milk production, temporary or permanent sterility in bulls, damage to hides and health due to secondary bacterial infections (OIE Terrestrial Manual, 2017). Being a transboundary disease, LSD could spread by way of production and marketing chains (Abutarbush, 2014). Mechanical transmission of LSDV is thought to be predominantly by biting arthropods. Some tick spp. has been proven to be able to mechanically and biologically transmit the virus (Abutarbush, 2017). LSD was first recognised in Zambia in 1929 (Morris, 1931). An episode in 1943–1944 involved other countries including South Africa. LSDV remained restricted to southern Africa until 1956, when it spread to central and eastern Africa. Since the 1950s, the virus has continued to spread progressively throughout Africa, first north to Sudan and subsequently westward, to appear by the mid-1970s in most countries of western Africa (Davies, 1981). In 1988, the disease was confirmed in Egypt (Ali et al., 1990) and in 1989 a single outbreak occurred in Israel, representing the first report outside the African continent (Davies, 1991).

In Sudan, LSD was observed in August, 1971 in Western Sudan in epizootic proportions and spread to the Eastern Sudan then the disease was confirmed by isolation and identification of the causative agent (Ali...
Outbreaks of LSD in imported Friesian and indigenous cattle in Khartoum State during the period between 1989 and 1991 were described by Khalafalla et al. (1993).

The current study reports a LSD outbreak in Kassala State and discusses serological, virological and molecular investigations of the disease.

2 MATERIALS AND METHODS

2.1 Outbreak investigation

LSD-like lesions in neck, brisket, abdomen, thighs and udder were observed affecting cattle of both local (Butana) and cross-bred animals in seven cattle herds in Nahr Atbara and Halfa El Jadida districts, Kassala State during November, 2020. Kassala State covers an area of 42.282 km², which is located between latitudes 14–17°N and longitudes 34–37°E. The State has an ecology that varies between poor savanna in the north and east and rich savanna in south and west. The outbreak occurred in cattle reared in an open grazing system in the west rich savannah region of the State. Cattle showing clinical signs consistent with LSD were recognised. The most clinical features observed were nodular lesions in the skin, lymphadenopathy, lacrimation and pyrexia (rectal temperature 40–41°C). The number of animals at risk was 295 with a morbidity rate of 4.1% (12/295) and a mortality rate of 2.4% (7/295).

2.2 Sample collection

Blood samples (n = 43) were collected in EDTA and plain vacutainer tubes (Nahr Atbara 34 and Halfa El Jadida 9). The serum was separated by centrifugation at 2000 rpm for 15 min and kept at –20°C until being tested for LSDV antibodies by ELISA. EDTA blood samples were kept in 4°C for viral DNA extraction. Skin nodule biopsies samples (n = 7) were collected aseptically from cattle of different ages exhibiting LSD-like lesions (Nahr Atbara 5 and Halfa El Jadida 2). The nodules with surrounding area were shaved by a sterile scalpel; a small incision was then created deeply into the skin to include dermis and epidermis then excised and sent to the laboratory in phosphate buffer saline (PBS). A 10% antibiotic mixture (penicillin G-sodium 100 IU/ml and streptomycin sulphate 100 mg/ml) was added to the collected samples, and samples were then homogenised using mortar and pestle, subjected to three cycles of freezing and thawing then centrifuged at 600 x g for 10 min at 4°C (Sigma, Germany). The resulting supernatant was stored at –80°C until viral DNA extraction and virus isolation attempts were performed.

2.3 Reference virus

Reference virus of LSDV (Neethling virus) was kindly provided by the Department of viral vaccines, Central Veterinary Research Laboratory (CVRL), Soba, Sudan.

2.4 Serological assay

The sera samples were screened for antibodies against the Capripox viruses (CPV) using the capripox double antigen ELISA (IDvet Innovative Diagnostics, France) according to the manufacturer’s instructions. The optical densities (OD) were measured using a microplate ELISA reader (Stat Fax 4200, USA) at 450 nm wavelength. Sera with ratios of sample to positive control antibody (S/P%) of titres greater than or equal to 30% were considered positive.

2.5 Virus isolation

Specific pathogen-free (SPF) 11-day-old embryonated chicken eggs (ECE) were inoculated in duplicate with the prepared skin nodule biopsies samples through the chorioallantoic membrane (CAM) route according to House et al. (1990). The eggs were then incubated at 37°C for 7 days and candling was conducted daily. Five eggs were used as negative controls.

2.6 DNA extraction

Viral DNA was extracted from EDTA blood samples, the supernatant of homogenised skin nodule biopsies samples and inoculated CAM using a commercial innuPREP virus DNA/RNA kit (Analytica Jena, Germany). The extraction was performed according to the manufacturer’s instructions and then stored at –20°C until tested.

2.7 Polymerase chain reaction (PCR)

PCR amplification was conducted using primer sets for detection of LSDV (forward: 5'-AAGTTACTTATATGGGAAAAGG-3') and (reverse: 5'-GTGTTATCATCTTCTATAAAC-3') according to Batra et al. (2015). The primers amplify conserved sequences of the P32 gene of Capripoxvirus. Amplifications were carried out on K960 Gradient thermocycler (Heal Force, China) at 94°C for 2 min followed by 40 cycles (94°C for 20 s, 55°C for 10 s and 72°C for 20 s) and a final extension at 72°C for 5 min. Finally, the PCR products were subjected to electrophoresis in 1% agarose gel, stained with ethidium bromide and visualised under ultraviolet (UV) light. The length of the amplicon was approximately 203 bp.

2.8 Statistical analysis

The statistical computation was performed using statistical package SPSS version 20 (SPSS Inc., Chicago, USA). Simple percentage (%) was used to determine numbers of infected animals, mortality and case fatality rates.
3 | RESULTS

3.1 | Epidemiological investigation

Cattle with typical signs of LSD were documented in Nahr Atbara and Halfa El Jadida locations (Figure 1). Affected animals revealed pyrexia, lymphadenopathy, lacrimation and firm round nodules (0.5–5 cm in diameter) were noticed along the neck, brisket, abdomen, thighs and udder (Figures 2 and 3). Epidemiological investigation indicated that a total of 295 at risk animals, of which 12 (4.1%) showed clinical disease and 7 (2.4%) died. The highest mortality and fatality rates were recorded in Nahr Atbara location (3.5% and 77.8%, respectively) while no death from LSD was recorded in Halfa El Jadida with 3.1% morbidity (Table 1).

3.2 | ELISA

Serological testing using ELISA indicated that 18 out of the tested 43 (41.9%) serum samples were positive for capripox antibodies. Twelve (35.3%) were positive in Nahr Atbara and 6 (66.7%) were positive in Halfa El Jadida (Table 2).

3.3 | Virus isolation

Isolation of LSDV showed the characteristic pock lesions with various sizes on CAM of embryonated chicken eggs (EGE) from all skin samples (Figure 4).

3.4 | PCR

A total of 43 whole blood, 7 skin samples from susceptible animals and 4 CAM lesions were examined for the presence of LSD virus using polymerase chain reaction (PCR). All tested whole blood samples were negative whereas all skin and CAM samples were positive (Figure 5).

4 | DISCUSSION

Lumpy skin disease was diagnosed for the first time in Sudan in 1971 from an outbreak in Western Sudan and spread to Gedarif district (Ali & Obeid, 1977) which is neighbouring area to the Butana region where the current outbreak occurs. Later, the disease was reported in 1989 in Khartoum State in the outbreaks among imported Friesian cattle based on histopathological and electron microscopical findings in addition to
virus neutralisation (Khalafalla et al., 1993). This report represents the first confirmation of the presence of LSDV (Neethling-type virus) infection in the Butana area, Kassala State, Eastern Sudan based on serological, virological and molecular diagnosis. The clinical signs noticed in the current outbreaks are similar to those described by Ali et al. (1990) in Egypt, Ali and Obeid (1977), Khalafalla et al. (1993) in Sudan and Kasem et al. (2018) in Saudi Arabia. In the present study, Nahr Atbara location recorded higher morbidity (4.5%), mortality (3.5%) and case fatality rate (77.8%) than Halfa El Jadida location which recorded 3.1% morbidity, 0% mortality and case fatality. The low morbidity and mortality in the current study are similar to the rates observed by Ali et al. (1990) in cattle and buffalo in Egypt and Kasem et al. (2018) in cattle.
in Saudi Arabia. However, Khalafalla et al. (1993) reported high mor-
bidity (37.9%) and low mortality (4.2%) among imported Friesian cat-
tle. The higher fatality rate in Nahr Atbara may be attributed to the 
fact that the majority of the dead animals were young animals. This
is in accordance with Khalafalla et al. (1993) who reported that death
rates due to LSD in newborn calves were relatively high. Moreover, LSD
morbidit and mortality rates differ according to geographical regions,
management system, virus virulence and population of biting insects
(Ahmed & Kawther, 2008; Ayelet et al., 2014; Brenner et al., 2006; Tuppurainen et al., 2011). Cattle affected in this outbreak were raised in
agroclimatic zone, which is suitable for the breeding of insect vectors
of LSD. The current outbreak was reported in November 2020 which
is the end of the rainy season and the ensuing of a colder weather
in Sudan and with a noticeable rise in the biting insects’ populations
that likely transmitted the virus mechanically between cattle. This sup-
ported by the findings that the appearance of clinical LSD in Sudan
occurred after the rainy season when there was a large number of
biting flies and mosquitoes (Khalafalla et al., 1993). Our investigation
indicates that females (46.9%) were more infected than males (27.3%),
which is in agreement with Kasem et al. (2018) in Saudi Arabia. This may
be attributed to the difference in the sample size of female and male or
due to the fact that majority of cattle in an investigation area are dairy
cattle where females’ number is high. The location of sampled cattle
was also identified as potential risk factor that affects LSDV seropos-
itivity among cattle. High LSDV infection was noted in cattle sampled
from Halfa El Jadida (66.7%) compared with Nahr Atbara (35.3%) loca-
tion. This might be attributed to the heavy rainfall during the rainy
season in this location and subsequent higher abundance of the biting
insects.

Control and prevention of LSDV rely on application of vaccination,
restricted animal movement and vector control (OIE Terrestrial Man-
ual, 2010). Ali (1973, unpublished data) inoculated 10 cattle with a
sheep pox strain that was isolated at the same time of the first outbreak
of LSD in Sudan in 1971 (Ali & Obeid, 1977) but failed to protect cattle
against intradermal inoculation with isolated LSDV. This lack of pro-
tection may indicate the necessity of a specific vaccine. The National
program of LSD control in Sudan has now successfully developed a
candidate vaccine from a local strain of LSD (Massalamia) through
serial passages in cell culture. This new vaccine has been tested under
laboratory conditions but still awaits field trials to confirm its efficacy
and safety.
CONCLUSION

LSDV was diagnosed for the first time in the Butana region, Kassala State, Eastern Sudan in 2020. The outbreak took place after the annual rainy season in Sudan. Sequencing of the isolated virus in this outbreak is recommended. Control measures including vaccination, movement restrictions and vector control are recommended in case of LSD outbreaks. Passive surveillance for new cases throughout the country and preventive measures as a routine practice are warranted.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ETHICAL STATEMENT

This study was approved by the ethics committee of the Central Veterinary Research Laboratory (CVRL), Animal Resources Research Corporation (ARRC). Sample collection was conducted according to the animal welfare code of Sudan.

AUTHOR CONTRIBUTIONS

Mohammed Hussien: Methodology; Writing original draft. Adam Osman: Investigation. Eman Bakri: Methodology. Amira Elhassan: Methodology. Molhima Elmahi: Funding acquisition. Shima Alfaki: Methodology. Abdel Rahim El Hussein: Supervision, Writing review & editing.

DATA AVAILABILITY STATEMENT

Data used to support the findings of this study are available from the corresponding author upon request.

FIGURE 5  Agarose gel electrophoresis of the products amplified with PCR using the specific primers for lumpy skin disease virus (LSDV). M: 100 bp DNA ladder, Lane 1: positive control, Lane 2: negative control, Lane 4–7: positive CAM samples, Lane 8-11: positive skin biopsy samples and Lane 3: negative sample

PEER REVIEW

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