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Occurrence and molecular confirmation of lumpy skin disease among Vechur cattle in Kerala#

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Abstract

Lumpy skin disease (LSD) is a notifiable disease caused by LSD virus belonging to the family Poxviridae and Capripoxvirus genus. The disease is characterised by fever, lymphadenopathy and presence of skin nodules. This article reports the occurrence of lumpy skin disease among Vechur cattle and its molecular confirmation. Two heifers in a Vechur farm were presented with elevated body temperature and nodules of varying sizes on the skin of abdomen and thorax region. Polymerase chain reaction was performed with primers targeting the viral attachment protein, P32 using DNA extracted from skin nodules which yielded amplicons of 192 bp size specific for LSD virus. Haematological and serum biochemical parameters were found to be within in the normal ranges. Histopathology of skin nodules revealed pyogranulomatous myositis and fibrinous vasculitis. Successful treatment was provided with broad spectrum antibiotic, anti-inflammatory drugs and topical antiseptic application. The findings of present study warrant the need for adopting control measures against LSD, including vector control and vaccination in the indigenous breeds of cattle like Vechur.

Keywords: *Haematology, serum biochemistry, histopathology, P32 gene*, p*olymerase chain reaction*

Lumpy skin disease (LSD) is a contagious viral disease of cattle caused by lumpy skin disease virus (LSDV) belonging to Poxviridae family. In the recent years, the disease has become transboundary spreading all the way from Arab countries to Europe and all over Asia, reaching the Indian subcontinent (Khan *et al*., 2021). It affects the economy of the nation largely due to the reduction in milk production, death of the affected animals, weight loss, loss of hide, abortion, male and female infertility and reduction in draught power. The disease is known to be efficiently transmitted mechanically *via* arthropods *viz.,* flies and ticks, even though other means of transmission were also recorded (Tuppurainen *et al.*, 2017). Classical signs of the disease include pyrexia, lymphadenopathy and skin nodules (Tuppurainen, 2022) which later develop into hard, fibrotic plugs which ulcerate and predispose to various complications like myasis and bacterial dermatitis (Al-Salihi, 2014). Other sequelae include sepsis, pneumonia, cellulitis, mastitis and abortions (WOAH, 2021).

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Confirmatory diagnosis is usually done by molecular diagnostic techniques such as polymerase chain reaction (PCR) (WOAH, 2021). Even though specific treatment for the condition is not available, the use of broad-spectrum antibiotics and non-steroidal anti-inflammatory drugs along with topical antiseptics was found to be useful for better cures avoiding complications (Xavier *et al.,* 2020; Islam *et al.,* 2021). Vechur, the smallest cattle breed in the world, is an indigenous cattle variety of Kerala, well adapted to the hot and humid tropical climate of Kerala and is highly disease resistant (Anilkumar and Raghunandan, 2003). This paper reports occurrence of lumpy skin disease in Vechur cattle and its molecular confirmation.

Two Vechur cattle belonging to Vechur Cattle Farm, Kerala Veterinary and Animal Sciences University were presented with the history of elevated body temperature and appearance of skin nodules in the last two days. Detailed clinical examination of the animals was performed and the observations/ vital parameters were recorded. Blood sample were collected for haematobiochemical analysis and molecular studies. Skin biopsy samples were collected using a biopsy punch for molecular diagnosis and histopathology.

Haematological parameters such as haemoglobin (Hb), volume of packed red cells (VPRC), total erythrocyte count (TEC), total leucocyte count (TLC), absolute granulocyte, lymphocyte and monocyte counts and thrombocyte count were evaluated. Serum analysis for creatinine, alkaline phosphatase (ALP) and aspartate aminotransferase (AST) was performed using biochemical analyser.

Extraction of DNA from blood and skin biopsy samples was performed using Qiagen® DNeasy blood and tissue DNA extraction kit following the manufacturer's instructions and the extracted DNA was subjected to PCR using primers targeting viral attachment protein P32 gene (Forward: 5′-TCC-GAG-CTC-TTT-CCT-GAT-TTT-TCT-TAC-TAT-3′ Reverse:5′-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-3′) (Ireland and Binepal, 1998). PCR reaction mixture was prepared by mixing 12.5 µL of TaKaRa® master mix, 5 µL of extracted DNA sample and 1 µL each of 10 µM primers prepared and was made up to 25 µl by adding nuclease free water. Amplification of DNA was conducted with an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 30 seconds and extension at 72°C for 1 min. Final extension was performed at 72°C for 5 min. A positive control obtained after sequencing

Fig 1. Heifer 1 affected with LSD with nodules on abdomen and thorax region

Fig. 2. Heifer 2 affected with LSD with nodules on abdomen

Table 1. Haematological parameters of LSD affected animals

Parameter	Normal range	Heifer 1	Heifer 2
Total leucocyte count($\times10^3$/µL)	4.0-12.0	9.7	7.6
Lymphocytes count($\times10^3$/µL)	2.5-7.5	7.1	5.8
Monocytes count ($\times10^3$/µL)	0-0.8	0.8	0.6
Granulocytes count ($\times10^3$/µL)	0.6-4.0	1.8	1.2
Total red cell count($\times10^6$/µL)	5.0-10.0	10.48	6.38
Volume packed red cells (%)	24.0 -46.0	37.4	28.9
Haemoglobin (g/dl)	8.0 -15.0	13.5	10.4
Platelets ($\times10^3$/µL)	100 - 800	214	330

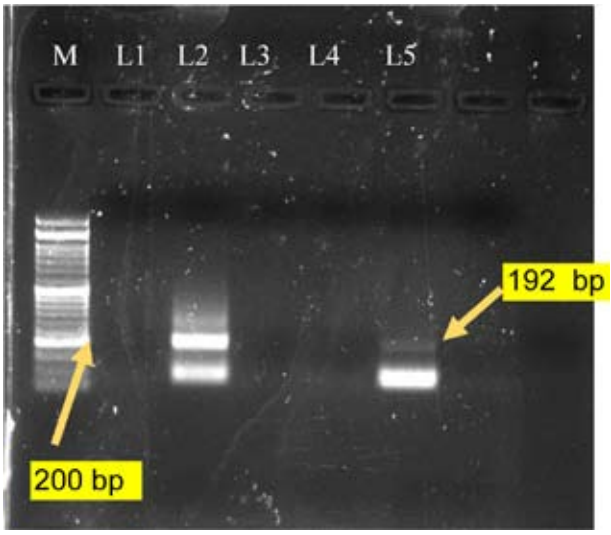

Fig. 3. Agarose gel electrophoresis of P32 specific PCR of LSDV

M : Ladder 50bp
L1: Negative control
L2: Positive control (Sequenced sample)
L3: Sample 1 (DNA- blood- Heifer 1)
L4: Sample 2 (DNA- blood- Heifer 2)
L5: Sample 3 (DNA- skin- Heifer 1)

the PCR product, confirming the presence of p32 gene was employed for diagnosis. Skin biopsy samples were subjected to histopathological processing and stained using haematoxylin and eosin.

Detailed clinical examination of animals revealed nodules of varied sizes over the skin especially of abdomen and thorax region. Fever and lymphadenopathy were also noted in the affected animals. Even though the indigenous breeds are reported to be resistant to diseases, LSD had been reported in them. Pandey *et al*. (2022) reported occurrence of LSD in indigenous *B. indicus* cattle and Asian water buffaloes in Central India. According to WOAH (2021), *Bos indicus* and *Bubalus bubalis* exhibit a lower susceptibility to the disease compared to *B. taurus*. Within *B. taurus*, thin-skinned breeds, particularly those from the Channel Islands, are predominantly affected. Hunter and Wallace (2001) on the other hand, suggested that *B. taurus and B. indicus* are equally susceptible to the infection of LSD virus. The findings of the present study are in agreement with them.

The presence of raised, firm, well-circumscribed skin nodules (Fig. 1 and 2) along with pyrexia (103.46 °F) and lymphadenopathy were the major clinical signs noticed in the affected animals. Similar clinical signs were also described by Salib and Osman (2011) and Yadav *et al*. (2024). Suwankitwat *et al*. (2022) also observed skin nodules, lymphadenopathy and fever as the major clinical signs of LSD affected animals. Skin nodules of varying size and number were observed as the classical clinical sign in LSD affected animals (Tuppurainen *et al*., 2005; Tuppurainen and Oura, 2012).

Table 2. Serum biochemical parameters of affected animals

Parameter	Normal Value	Heifer 1	Heifer 2
Creatinine (mg/dl)	0.5-2.2	0.8	1.3
ALP (IU/L)	0-200	79	112
AST (IU/L)	60-125	58	97

The haematological analysis showed no significant alteration (Table 1) in these cases when compared with normal values as per Susan (2016). Similarly, Abutarbush (2015) reported no significant changes in the haemogram and leucogram of LSD affected animals in the early stage of infection. Nevertheless, Xavier *et al*. (2020) reported significant changes in leukogram and haemogram in LSD affected animals. Neamat-Allah (2015) reported significant leucopenia, with lymphocytopenia, monocytopenia and insignificant granulocytosis after one to two days of infection with LSDV which later changed to significant leucocytosis with insignificant lymphocytosis, significant monocytosis and granulocytosis. They also reported insignificant anaemic changes in animals affected with LSDV post one to two days of infection which became significant after 10 to 14 days.

Serum biochemical parameters *viz*., creatinine, alkaline phosphatase (ALP) and aspartate amino transferase (AST) of affected animals were found to be within normal ranges (Table 2). Neamat-Allah (2011) reported significant elevated creatinine and AST levels in LSD affected animals. Abutarbush (2015) reported no significant changes in serum ALP levels in animals affected with LSD but significant reduction of creatinine was noticed. Sevik *et al*. (2016) reported elevated serum AST and ALP in LSD affected cattle which were attributed to the ability of the virus to cause hepatic damage. Absence of significant changes in haemato-biochemical parameters in the present study might be due to the collection of blood during early stage of infection.

PCR with DNA extracted from blood and skin biopsy samples targeting viral attachment protein, P32 gene yielded amplicons of 192 bp from the skin biopsy samples only (Fig. 3). Similar findings were reported by Jaferin *et al*. (2020), Nayakvadi *et al*. (2021) and Sudhakar *et al*. (2019). PCR was reported to be a simple, fast and sensitive diagnostic test for detection of capripoxvirus genome in blood and tissue samples (Tuppurainen *et al*., 2005). Blood samples failed to give positive reaction in PCR, which might be due to the transient phase of viraemia in affected animals as suggested by Al-Salihi (2014). Skin scab or skin nodule was reported as a sample of diagnostic value for Capripoxvirus as it is having predilection to the skin tissue (Bowden *et al*., 2008). Similar findings were also reported by Sudhakar *et al*. (2020), Nayakvadi *et al*. (2021) and Parvin *et al*. (2022). Babiuk *et al*. (2008) reported persistence of LSDV in the skin nodule for several months.

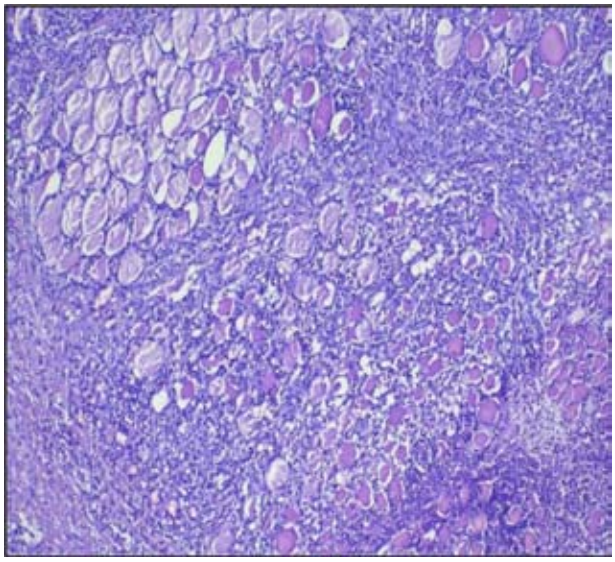

Fig. 4. Pyogranulomatous myositis in skin biopsy (H&E, x100)

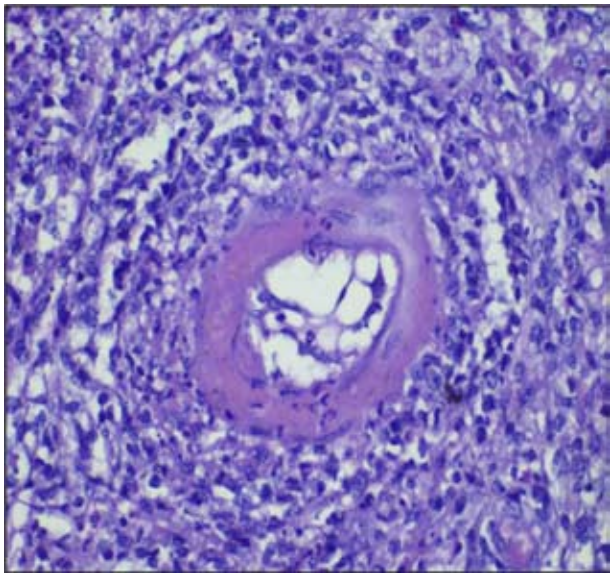

Fig. 5. Vasculitis-fibrinoid neurosis (H&E, x400)

Histopathology of skin biopsy sample revealed pyogranulomatous myositis (Fig. 4), fibrinous vasculitis (Fig. 5) and cystic dilation of sweat glands (Fig. 6). Amin *et al*. (2021) reported hydropic degeneration of epidermal cell layers with granulomatous infiltration of plasma cells, macrophages, lymphocytes, epithelioid cells and fibrocytes at ulcerated areas, blood vessels and dermal muscles in LSD affected animals. Mathewos *et al.* (2022) observed fibrin deposits in the vasculature of the dermal layer along with infiltration of mononuclear inflammatory cells in LSD affected skin.

The animals were isolated immediately and treated symptomatically using enrofloxacin @ 5 mg/kg BW IM and meloxicam @ 0.2 mg/kg BW SC for a period of one week. A parenteral administration of vitamins was given using Lavitone-H®. Ulcerated nodules were treated with

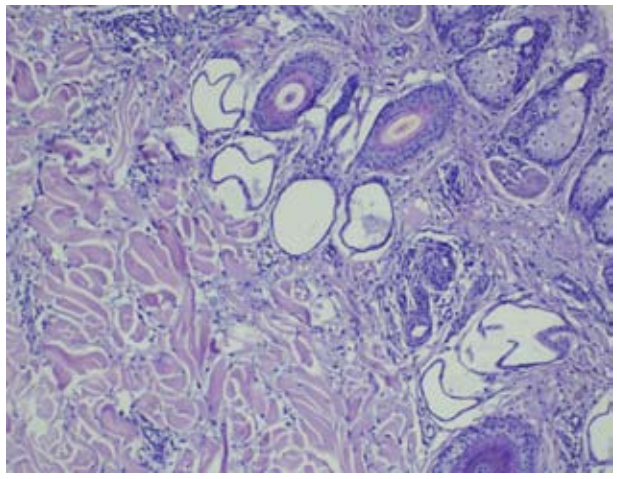

Fig. 6. Cystic dilation of sweat glands (H&E, x100)

topical application of boric acid, until granulation tissue could be appreciated. Salim and Osman (2011) (reference missing) reported the use of a combination of antibiotics, anti-inflammatory drugs and supportive therapeutic procedures for LSD. Topically anti-septic solutions and ointments were used for treatment of ulcerated skin lesions. Animals involved in the study reverted to normal body temperature within four days of initiation of treatment. They showed return to normal appetite within two weeks of treatment. Skin nodules were completely healed within one month period. Further control measures including vaccination, vector control and biosecurity measures such as isolation of affected animals, maintenance of separate feed mangers and water troughs and cleaning and disinfection of contaminated areas were advised.

Summary

The occurrence of lumpy skin disease among Vechur cattle in Kerala and its molecular confirmation by PCR targeting viral attachment protein gene P32 is described. The findings of the present study warrant the need for adopting the control measures against LSD including vaccination in the indigenous breeds of cattle like Vechur.

Conflict of Interest

The authors declare no conflict of interest.

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