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Sanjeevakumar Lalasangi
Ph.D. Scholar, Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore, Karnataka, India

Suguna Rao
Professor and Former Head of the Department, Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Byregowda SM
Former Director, IAH&VB, KVAFSU, Bangalore, Karnataka, India

Chethan Kumar HB
Scientist, ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru, Karnataka, India

Yogishardya R
ACTO, National institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru, Karnataka, India

Girish MH
Associate Professor (i/c) and Head, Department of Veterinary Anatomy and Histology, Hebbal, Veterinary College, KVAFSU, Bangalore, Karnataka, India

Manjunatha Reddy GB
Senior Scientist National institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru, Karnataka, India

Corresponding Author:
Suguna Rao
Professor and Former Head of the Department, Veterinary Pathology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Molecular detection of lumpy skin disease virus (LSDV) in different organs of post-mortem cases in naturally affected cattle

Sanjeevakumar Lalasangi, Suguna Rao, Byregowda SM, Chethan Kumar HB, Yogishardya R, Girish MH and Manjunatha Reddy GB

Abstract
Lumpy skin disease (LSD) is a economic significant disease of cattle and buffaloes caused by lumpy skin disease virus (LSDV) of the genus Capripoxvirus, family *Poxviridae*. The present study was conducted in state of Karnataka to understand the disease lesions and viral distribution in the internal organs. A total of 19 cattle were subjected for detailed post-mortem was conducted on animals died due to suspected LSD. Various internal organs were collected for confirmation of LSDV. The typical pox lesions were observed on all the internal organs and the LSDV was detected in all the internal organs of affected animals by conventional PCR for targeting P32 gene. In conclusion, based on the postmortem lesions and PCR the disease was confirmed as LSD. There is an urgent need for further controlled and large-scale field studies on pathological and epidemiological aspects of LSD in India for better design of prevention and control strategies such as mass vaccination and vector control.

Keywords: Cattle, lumpy skin disease, pm examination, PCR and P32 gene

Introduction
Lumpy skin disease is a transboundary, emerging disease of cattle. It is OIE listed "list-A" infectious viral disease due to its rapid spread and having severe economic impact in terms of losses such as decrease in milk production, damage to hide, mastitis, infertility in males and females, decreased semen quality, and death (Irons *et al*., 2005) [11]. It is caused by lumpy skin disease virus, which is host specific belongs to the genus capripox virus. Other members of the genus include sheep pox virus causes sheep pox disease and goat pox virus causes goat pox disease in sheep and goat respectively. LSDV comes under subfamily Chordopoxvirinae of the family Poxviridae (Brenner *et al*., 2006) [6]. LSD causes high morbidity and low-to-moderate mortality, and the severity of the disease may vary from subclinical infection to death which is based on the virus strain, vector prevalence, age and immune status of the host (OIE, 2008) [14]. Recent outbreaks created havoc in terms of heavy morbidity and mortality. The first outbreak of LSD was reported from Odisha state with 7.1% morbidity rate ranged from 0.75% to 14.04% in different villages and no mortality (Sudhakar *et al*. 2020) [16]. Disease is characterized by rapid eruption of multiple circumscribed skin nodules, generalized lymphadenopathy and high fever (Coetzer, 2004) [7]. In addition, LSDV can cause mastitis and orchitis as well as necrotic plaques on the membranes of the upper respiratory tract, the oral cavity and lungs. In the present study, we conducted postmortem examination of cattle died due to suspected LSD and confirmed the virus by PCR in different internal organs.

Materials and Methods
Collection of samples from post-mortem
In the present study, postmortem of 19 animals suspected for LSD was conducted in different districts of Karnataka and detailed gross lesions in various internal organs were recorded. Different morbid samples such as scab, skin, representative samples of lung, liver, kidney, intestine, lymph node and heart were collected in sterile condition and were transported to laboratory on cold chain for further studies.

DNA extraction from tissue samples
DNA extraction from the scabs, skin biopsy samples and tissues collected at post mortem was carried out as per the manufacturer's protocol. DNA was isolated using DNeasy blood and

tissue kit (Qiagen Pvt. Ltd) and final elution of DNA was done in 30 µl of elution buffer and stored at – 20 °C until further use.

PCR amplification of P32 gene

The PCR was carried out first to amplify the partial fragment of P32 gene amplification for diagnosis of *Capripoxvirus* (Reddy *et al*., 2013) [15] followed by LSDV specific primers (Ireland and Binepal, 1998) [17]. Polymerase chain reaction was carried out in a final reaction mixture of 25 µl using 0.2ml capacity thin wall PCR tube using Dream Taq PCR Master Mix 2X (Thermo Fisher Scientific Inc.). The polymerase chain reaction tubes containing the mixture were tapped gently, spun briefly and the components were transferred to the thermal cycler [Thermal cycler S1000 (Bio-Rad, California, USA)]. The final PCR protocol used for primers was standardized for the amplification conditions. The non-template control consisted of sterile water instead of DNA template, while positive control consisted of DNA from reference LSD virus obtained from ICAR-NIVEDI Bengaluru.

Results

Post-mortem investigation

In the present study detailed post mortem investigation of 19 animals was carried out and all the animals showed externally nodular skin lesions throughout the body. The size of the nodules was varied from 7 mm to 3 cm. The numbers of nodules also varied, with only few nodules in some animals and in few animals very high number distributed all over the body. The nodular lesions were observed on various parts of the body, randomly distributed on head, neck, limbs, lateral aspect of the body, tail, limbs, udder, external genitalia. In few animals, lesions were also observed on muzzle, nasal cavity, hard palate, scrotum and vulval lips. In rare cases maggot infestation of the wounds was also noticed.

Gross pathology in necropsied LSD cases

The skin lesions varied in size and extended throughout the thickness of the skin. The inner aspect of the skin where the nodules were present, appeared congested and haemorrhagic with necrotic areas. There was varying amounts of serous atrophy of fat subcutaneously and affected areas appeared yellowish with gelatinous appearance of the depleted fat tissues. Superficial lymph nodes, especially prescapular and prefemoral were enlarged and oedematous with multifocal haemorrhages. The necrotic lesions were observed on tongue, tracheal mucosa, larynx, serosal surface of rumen, reticulum, abomasum and gallbladder. The lesions on rumen, reticulum and abomasum appeared erythematic, either circumscribed or irregular with a central paler necrotic area and were also surrounded by a line of hyperaemia. Lungs in necropsied cases showed multifocal patches of consolidation which appeared congested and dark in colour. Such areas were firm and hard to palpate. Liver and kidneys in some of the cases appeared slightly enlarged and revealed discrete, multifocal, necrotic foci (Fig. 1).

Confirmation of LSD by PCR

Representative tissue samples were collected during necropsy such as scab/skin, trachea, lung, liver, kidney, heart, intestine, rumen, abomasum, reticulum, spleen, tongue and lymph node were subjected for confirmation of infection by PCR for P32 gene of lumpy skin disease virus (Fig. 2).

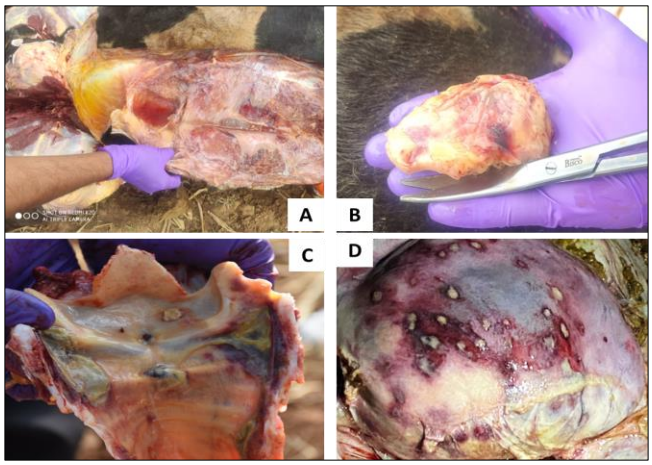

Fig 1: The photographs showing various PM lesions: The subcutaneous fat gelatinization (A), Lymph node enlargement, oedema and haemorrhages (B), Trachea with gunshot wounds and reticulum serosal surface showing necrotic lesions (D).

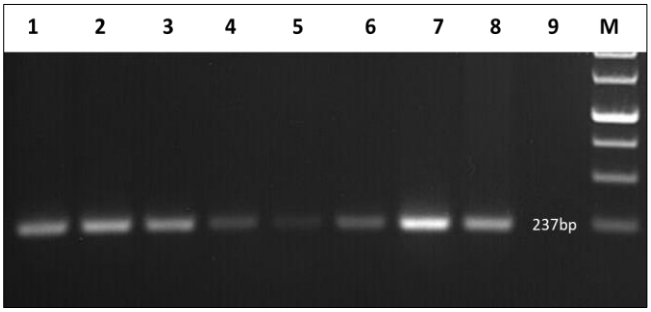

Fig 2: The agarose gel image showing specific amplification of 237 bp targeting P32 gene of LSD, Lane M: 100bp ladder, Lane 1: Skin, Lane 2: Heart, Lane 3: Trachea, Lane 4: Lung, Lane 5: Tongue, Lane 6: Rumen, Lane 7: Reticulum, Lane 8: Abomasum and Lane 9: Negative control.

Discussion

Grossly the cutaneous lesions in necropsied cases were similar to those found in field outbreak cases. The skin nodules were found extending throughout the thickness of the skin, subcutaneous tissue and in some cases in to the muscle and the inner aspect of the skin where the nodules were present, appeared congested and haemorrhagic with necrotic areas. Similar observations were also reported by Ahmed and Dessouki (2013) [2]; Abera *et al.* (2015) [1] and Gharban *et al.* (2019) [9]. There was varying amounts of serous atrophy of fat subcutaneously and affected areas appeared yellowish, moist with gelatinous appearance of the depleted fat tissues which indicated infection associated emaciation with depletion of fat depots. Necropsy of affected animals revealed systemic infection with affection of various organs with presence of mostly necrotic lesions. Multiple circumscribed slightly raised necrotic lesions were observed on tongue, tracheal mucosa, larynx, rumen, reticulum, abomasum and gallbladder in most of the cases. The lesions in tongue, trachea and larynx varied in size and consisted of central slightly depressed ulcerative area covered by fibrino-necrotic material surrounded by a line of hyperaemia. Grossly in all the necropsied cases lymph nodes were enlarged, oedematous and congested. Similar affection of various organs in LSD cases on post mortem were observed by Ahmed and Dessouki (2013) [2], Mulatu and Feyisa (2018) [13], Babiuk *et al.* (2008b) [13], Abera *et al.* (2015) [1], Gharban *et al.* (2019) [9] and Kononov *et al.* (2019)

[12]. The affection of various organs could be attributed to the dissemination of virus following leukocyte associated viraemia. Gharban *et al.* (2019) [9] expressed that the exact pathogenesis for the development of lesions in multiple organs systems is not well understood however, there are similarities in the lesions between LSD and other capripox virus induced infections such as goat pox and sheep pox.

In the present study the lesions were consistently observed in larynx, trachea, lungs and rumen. Gharban *et al.* (2019) [9] also reported that the mucous membranes of upper digestive and respiratory tracts were often affected with development of multiple discrete ulcerative lesions causing difficulty in swallowing, severe dyspnoea and asphyxia. They also observed lesions in rare cases involving kidney, udder, uterus, testes, urinary bladder, gall bladder, liver similar to that reported by Abera *et al.* (2015) [1] which were also the finding in the current study. The fibrinopurulent necrotic lesions observed in the mucous membranes of larynx and trachea and bronchopneumonia could be attributed to the secondary bacterial infection as also reported by Mulatu and Feyisa (2018) [13]. Lungs lesions in LSD cases were also reported by House *et al.* (1990) [1], Barnard *et al.* (1994) [5], Awadin *et al.* (2011) [3], FAO manual, (2017) [18] and Mulatu and Feyisa (2018) [13] which were characterized by patchy areas of consolidation, congestion and haemorrhage. In the present study, six animals showed necrotic lesions on dorsum of tongue and on the upper palate also. Other organs collected did not reveal any lesions but showed PCR positivity for P32 gene.

To assess the involvement of internal organs and also to determine systemic infection in LSD, representative tissue samples collected from scab/skin, liver, lungs, trachea, kidney, spleen, lymph node, heart, rumen, reticulum, abomasum and intestine were subjected for PCR for P32 gene LSD virus. PCR on samples from internal organs such as lung, liver, kidney, heart, lymph node and intestine gave positive reaction for P32 gene of Capripox virus which indicated the systemic spread of virus in the body during infection. Awadin *et al.* (2011) [3] found presence of LSDV DNA in lymph node by PCR.

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