An Official Journal of Bangladesh Livestock Research Institute (BLRI)

Bangladesh Journal of Livestock Research

Journal Homepage: https://www.banglajol.info/index.php/BJLR

ISSN 1022-3851

Isolation and molecular identification of Lumpy Skin Disease (LSD) virus from infected cattle in Bangladesh

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Abstract

Lumpy Skin Disease (LSD) is a new disease of cattle in Bangladesh. It is endemic in Africa but through the last few years disease beings to spread to other countries of the world. The disease was widely spreaded in the many other countries in Asia and some parts of Europe. In Bangladesh, the disease was first time detected in April 2019, in southern part and then continued to spread all over the country.The disease caused enormous economic losses causing cutaneous and internal lesions, affecting milk production, hide quality and in some cases death of infected animal. LSD suspected samples were collected from different areas of the country during the period from July 2019 to January 2020. In this study, a total of 36 clinically suspected LSD samples of skin crustnodules, pus and ocular discharge were collected. Samples were examined by the published PCR protocol for LSD virus, GPV and SPV. Around 78% samples were found positive for LSD virus in PCR test. LSD virus was also identified from pus and ocular discharge of infected cattle. The virus can grow in the lamb testicular cell and clinically the disease is characterized by distinctive nodular lesions mostly on the skin of the affected animals.The results indicated that the LSD virus is circulating in the outbreak are as and is an emerging transboundary cattle disease in Bangladesh.

(**Keywords:** cattle, lumpy skin disease, PCR, isolation, molecular identification.)

Bang. J. Livs. Res. Vol. 26 (1&2), 2019: P. 15-20 https://doi.org/10.3329/bjlr.v26i1-2.49933

Introduction

Lumpy skin disease (LSD) is a disease of cattle caused by Lumpy Skin disease Virus (LSDV). It is a DNA virus belonging to the genus Capripoxvirus within the family Poxviridae (Buller *et al.*, 2005; Douglass *et al.*, 2019). Goat pox and Sheep pox viruses are two other species in this genus. The LSDV was first reported in Zambia in 1929 from which it spread south to southern African countries and north to Sudan (Umberto *et al.*, 2018). This disease has a significant economic impact in the cattle industry in Africa which is circulating for a long time in that region. The disease has currently been spreading aggressively in many parts of Asia including Bangladesh

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(Beard, 2016). In April 2019, Bangladesh first experienced LSD and after that within a short time it spread all over the country. The active movement of blood sucking vectors like fly can be a route for LSD transmission in a short distance (Dogan *et. al.*, 2016; Douglass *et al.*, 2019). It is observed enormous fly nuisance in outbreak areas where the flies are sitting and biting the infected skin wound and may spread the virus more quickly.

All types of cattle breeds, ages and sexes are found susceptible although the disease is more severe in cross breed and young calves (Aboelkhair *et al.*, 2019). Lactating cows cause severe production losses due to LSDV with decrease skin quality. Morbidity rate of the virus is high (up to~45%) but the mortality is generally rare (less than 10%) (Coetzer, 2004; OIE, 2017). However, milk yield drop, abortion, infertility and weight loss along with decreased skin quality due to this disease was reported from all affected countries. The disease is highly host specific and mainly cattle and water buffalo are more susceptible to this virus. Holstein Friesian and its crossbreed cattle are exhibiting higher morbidity and mortality due to this disease outbreak, when compared to local cattle (OIE, 2017). The wild ruminant species in Africa have not identified during extensive serological surveys which appears to be highly host specific (OIE, 2017). Vaccination is the only effective way to control the spread of LSDV in endemic countries. Stamping-out, culling infected animals and restriction of animal movement are recommended to control this virus (OIE, 2017; Bary *et al*., 2018; Ali *et al.*, 2019). Bangladesh first reported LSD outbreak to OIE in August

Table 1. Specific primer and thermal cycler profile for LSD virus detection by PCR

Primer		Amplicon size	Reference
Forward	5′-TCCGAGCTCTTTCCTGATTTTTCTTACTAT-3′	192 bp	Ireland and Binepal, 1998
Reverse	5′-TATGGTACCTAAATTATATACGTAAATAAC-3′		
Thermal profile: 2 minutes at 95°C; then 45 seconds at 95°C, 50 seconds at 50°C and 1 minute at 72°C (34 cycles); 2 minutes at 72°C and hold at 4°C until analysis.			
E10R-f	CCGCTCGAGGCCACCATGAATCCTAAACACTGGGGAAGAGC	285	Zhao *et al*., 2017
E10R-r	CGCGGATCCCGAAGCGGTAATACCTTATTTAAATTG		
RPO132-f	CCGCTCGAGGCCACCATGAATAGGTTCAAGGAAAAGCAT		
RPO132-r	CGCGGATCCCGCATTATTTTTTCATACGAT	746	

2019 (OIE, 2019). Therefore, the objective of the study was to isolate and identify of LSDV in outbreak areas of Bangladesh.

Materials and Methods

Sample collection

The study was conducted during the period of July 2019 to January 2020. A total of thirty six (n=36) LSD affected cattle samples (skin nodules and crusts), ocular discharge and infected pus were collected from Jessore, Jhenaidah, Chattogram, Rajshahi, Pabna, Dhaka, and Rangpur districts of Bangladesh and stored at -80°C in Bangladesh Livestock Research Institute.

Molecular identification of LSDV

The collected samples were processed and extracted individually for viral DNA by using the commercial kits of Monarch® Genomic DNA Purification Kit, UK following its manufacturer's guidelines. DNA extracts were then evaluated by the PCR test described by Ireland and Binepal (1998). PCR test kits (DreamTaq Green PCR Master Mix, ThermoFisher, USA) were used for the PCR assay. Primers and thermal profile along with description of positive and negative controlsare presented in Table 1. The generating PCR products of 192bp and run through 1.5% of agarosegel which was stained with ethidiumbromide and visualized in UV trans-illuminator after electrophoresis (Figure 1). The presence of other capripox viruses like goat pox virus (GTPV) and sheep pox virus (SPPV) were also verified by PCR test with specific primers and thermal profile described by Zhao *et al.* (2017) in same sample.

Culture of LSDV in lamb testicular cells

Lamb testicular cell was prepared as described by Ferris and Plowright (1958). PCR confirmed LSDVwas inoculated inlamb testicular cell for the isolation of virus.

Results and Discussion

Around 78% samples (N=36) were found LSDV positive by PCR test and detailed results are presented in Table 2. Samples of pus, and ocular discharges were found to be more sensitive for LSDV diagnosis. The findings are supported by Dogan *et al.* (2016).

Majority of the samples (64%) were PCR positive both for LSDV and Goat pox virus (Table 2). However, no samples were found positive for sheep pox virus. The LSDV were grown into the lamb testicular cells and typical cytopathic effects (CPE) was shown in continuous 5th passages on cell culture and reconfirmed LSDV by PCR test.

LSD is endemic in many African and Asian countries. It is rapidly spreading throughout the Middle East, including Turkey (Dogan *et al.*, 2016). In the year 2019, this virus started spreading to other parts of Asia including China, India and Pakistan (OIE, 2019). Animal movement from neighboring infected countries is an important source of transmission of LSDV in Bangladesh.

The LSDV became rapidly speeded from initial outbreak areas to most of the districts of Bangladesh in a short time due to large number of animal movement inside the country, targeting the marketing opportunity of holy Eid–ul-Azha where live animal sacrifice is an important part (Khokon *et al.*, 2017; Ali *et al.*, 2019; Ali, 2018). Similar findings also reported by Ireland and Binepal (1998), where they reported outbreaks that

Table 2. Description of PCR test results

Sl. No.	Location	Sample	Results		
			LSDV	GTPV	SPPV
1	Dhamrai, Dhaka	Skin nodule	+	-	-
2	Dhamrai, Dhaka	Skin nodule	+	-	-
3	Dhamrai, Dhaka	Skin nodule	-	-	-
4	Dhamrai, Dhaka	Ocular swab	+	-	-
5	Sadar, Jashore	Skin nodule	+	+	-
6	Sadar, Jashore	Skin nodule	+	+	-
7	Sadar, Jashore	Skin nodule	+	+	-
8	Sadar, Jashore	Skin nodule	+	+	-
9	Sadar, Jashore	Skin nodule	+	+	-
10	Sadar, Jashore	Skin nodule	+	+	-
11	Sadar, Jashore	Pus	+	+	-
12	Sadar, Jhenaidah	Pus	+	+	-
13	Sadar, Jhenaidah	Pus	+	+	-
14	Sadar, Jhenaidah	Ocular swab	+	+	-
15	Sadar, Jhenaidah	Skin nodule	+	+	-
16	Sadar, Jhenaidah	Skin nodule	+	+	-
17	Sadar, Jhenaidah	Skin nodule	-	-	-
18	Anwara, Chattogram	Skin nodule	+	+	-
19	Anwara, Chattogram	Skin nodule	+	+	-
20	Anwara, Chattogram	Skin nodule	+	+	-
21	Anwara, Chattogram	Skin nodule	+	+	-
22	Anwara, Chattogram	Skin nodule	+	+	-
23	Anwara, Chattogram	Skin nodule	+	+	-
24	Godagari, Rajshahi	Skin nodule	-	-	-
25	Godagari, Rajshahi	Skin nodule	+	+	-
26	Godagari, Rajshahi	Skin nodule	+	+	-
27	Godagari, Rajshahi	Skin nodule	+	+	-
28	Sadar, Pabna	Skin nodule	-	-	-
29	Sadar, Pabna	Skin nodule	+	+	-
30	Sadar, Pabna	Skin nodule	+	+	-
31	Sadar, Pabna	Skin nodule	-	-	-
32	Badarganj, Rangpur	Skin nodule	+		-
33	Badarganj, Rangpur	Skin nodule	+	-	-
34	Badarganj, Rangpur	Skin nodule	-	-	-
35	Badarganj, Rangpur	Skin nodule	-	-	-
36	Badarganj, Rangpur	Skin nodule	-	-	-

+: Positive result, -: Negative result

have occurred in Turkey, most likely originated by the introduction of LSDV from neighboring countries including Syria. Turkey also reported that large number of cattle movement from one province to another providence has triggered the rapid spread of LSDV in their country. The introduction of infected animals is the major way to introduce LSDV into a country, in particular by spreading to long-distances (OIE, 2017).

The outbreak areas in Bangladesh like Rajshahi, Jessore and Jhenidah is very close to the international border with neighboring countries. The spread of LSDV is usually limited to a short distance when infected animals are not moved to non-affected areas (OIE, 2017; Douglass *et al*., 2019). The speeding of LSDV started from April 2019 in Bangladesh and it is observed till January 2020 with increased frequency of outbreak.

Conclusion

The result indicates that the lumpy skin disease virus is widely circulating in cattle of studied areas in Bangladesh. The lumpy skin disease virus can easily grow in the lamb testicular cell. Clinically the disease is characterized by distinctive nodular lesions mostly on the skin of the affected animals.

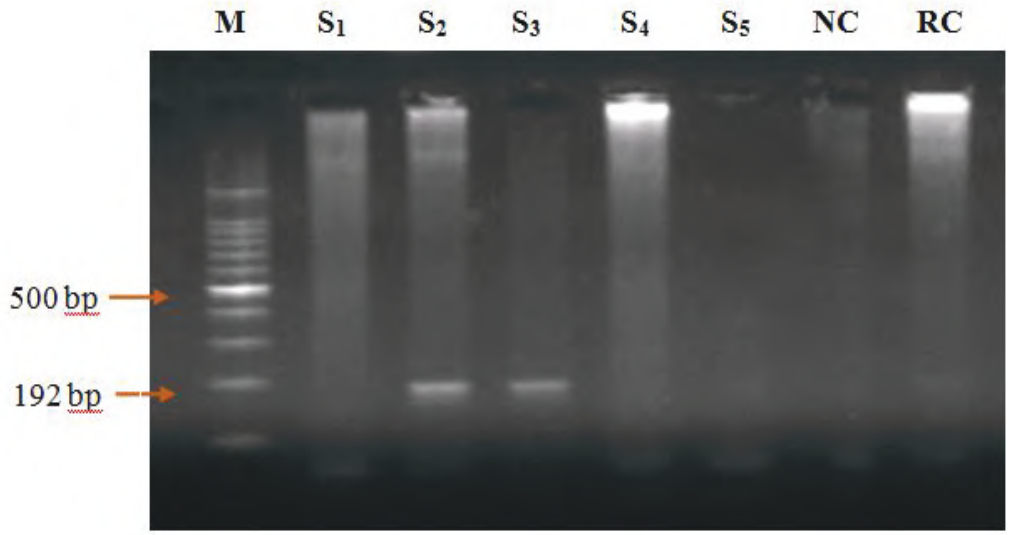

Figure1. Agarose gel electrophoresis of PCR products showing the specific band of 192bp amplified with LSD specific primers. Lane M: 100bp DNA molecular weight marker; Lane S1-S5: LSD field samples, Lane NC: Negative control; Lane RC: Reagent control.

Acknowledgements

The study was funded by the research project "Study on Lumpy Skin Disease (LSD) in Bangladesh" under Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Bangladesh. The authors also acknowledged the Department of Livestock Services (DLS) for the interactive cooperation during the outbreak investigations.

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