Sero-Epidemiology of Lumpy Skin Disease Virus, Swat Valley Switzerland, Pakistan

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ABSTRACT
Lumpy Skin Disease (LSD) is a viral illness caused by the LSD virus. It ranks among the most economically impactful transboundary and emerging diseases affecting cattle. In Swat district, Khyber Pakhtunkhwa (KPK), an outbreak investigation was conducted from January 2023 to July 2023. In the latter part of 2022, an outbreak of Lumpy Skin Disease (LSD) affected all seven tehsils of Swat district, prompting a comprehensive investigation. Numerous animals underwent examination, and blood samples were collected from those actively infected. The Veterinary Research and Disease Investigation Center Balogram (VRDIC Balogram) played a central role in characterizing the virus using various molecular techniques and conventional PCR. Clinical examinations were conducted on both infected and in-contact animals, accompanied by a questionnaire survey designed to pinpoint potential risk factors associated with the disease. The findings indicated that LSD was present in 27.94% (443/1585) of the examined animals, with blood samples collected from 443 clinically positive cases for further laboratory analysis. Among different age groups, morbidity rates were notably higher in mixed breeds compared to indigenous breeds, detailed as 30% (52/170), 24% (38/160), 28% (34/120), 33% (20/60), and 53% (29/55). Mortality rates and case fatality were significantly elevated in young animals compared to other age groups. Conventional PCR confirmed that DNA extracts from blood samples collected from higher number of animals virus isolates were positive for LSDV The questionnaire survey highlighted communal points, such as markets, watering, and grazing areas, as common sources of infection, along with the introduction of sick animals to the herd. These findings provide valuable insights into the dynamics and risk factors associated with the LSD outbreak in the Swat district. In conclusion, the economic losses resulting from the LSD outbreak were substantial. Recommendations include enhancing diagnostic facilities, implementing strategic control measures, and raising awareness within communities for early detection and reporting.

INTRODUCTION

Lumpy skin disease (LSD) serves as a prominent illustration of an emerging infectious disease due to its recent swift dissemination and geographical expansion in previously disease-free Asian nations (Woah 2022). The causative agent of LSD is the Lumpy skin disease virus (LSDV), a member of the Poxviridae family and Capripox genus. Its emergence poses a significant threat, resulting in considerable economic losses (Şevik & Doğan, 2017).LSDV is transmitted through various means, including blood, nasal secretions, saliva, milk, and semen (Abutarbush et al., 2015). The spread is facilitated by direct transmission through arthropods, particularly bloodsucking insects, and contaminated feed and water (Şevik & Doğan, 2017). While cattle are the primary hosts, water buffaloes have also shown susceptibility to the virus (Kiplagat et al., 2020). Clinical manifestations in cattle and water buffaloes include anorexia, skin nodules, reduced body weight, decreased milk production, and, in some cases, complications such as mastitis and myiasis (Abutarbush et al., 2015).

LSD has been reported in various regions, with its initial case documented in Zambia in 1929 (Fick & Viljoen, 1999). Subsequently, it spread to Sub-Saharan Africa, South-Eastern Europe, and Asian countries (Givens, 2018). In Asia, Bangladesh reported the first case, leading to the infection's proliferation in neighboring countries like India, Bhutan, Nepal, Hong Kong, Vietnam, Myanmar, the Middle East, Europe, West Asia, and Thailand (Black et al., 1986). Pakistan encountered its first case in November 2021, specifically in the Jamshoro district of Sindh. The emergence of LSD in Pakistan had severe repercussions for the livestock sector (Abutarbush & Tuppurainen, 2018).

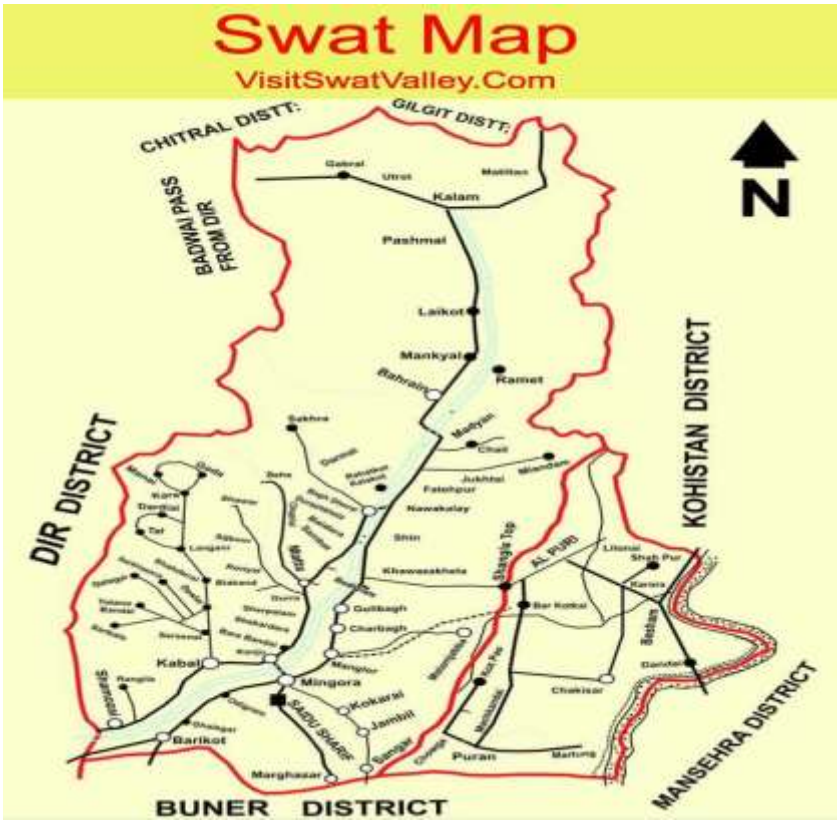
By April 2022, approximately 36,000 cattle were infected in Pakistan, resulting in a death rate of 0.8% (Şevik & Doğan, 2017). This epidemic significantly impacted five million dairy farmers and meat sellers, leading to substantial economic

fallout (Awadin et al., 2011). Furthermore, concerns arose about the virus potentially infecting humans through the consumption of milk and meat from affected animals (Namazi & Khodakaram Tafti, 2021).Livestock sectors in industrialized nations, like Pakistan, struggle with new viruses due to limited resources and inadequate measures (Abutarbush et al., 2015). Insufficient diagnostic capabilities and delayed viral identification can lead to rapid disease spread and increased infection rates Şevik & Doğan, 2017). Economic losses from LSD are significant, affecting herds and preventing access to lucrative export markets. The livestock industry in tropical Asia also faces challenges, including infectious diseases (Abutarbush et al., 2015). economic impact of LSD outbreaks in the livestock industry is substantial, affecting milk production, cattle reproduction, and hide quality, which leads to financial losses for farmers. Additionally, trade restrictions on live animals and animal products exacerbate national economic losses. Tropical Asia's favorable environmental conditions facilitate the spread of LSD through blood-sucking insects like mosquitoes, flies, and ticks. Large-scale farms often experience higher incidences of the disease compared to small backyard farms (Gari et al., 2010).Egypt has faced significant LSD outbreaks since 1988, with notable events in 1989 and 2006. Similar outbreaks occurred in Pakistan's Swat district, facilitated by bloodsucking insects and uncontrolled livestock transport, leading to severe economic losses in both regions (Namazi & Khodakaram Tafti, 2021). Effectively combat LSD in tropical Asia, it is crucial to understand the molecular characteristics of the LSDV virus efforts (Badhy et al., 2021). By targeting specific genes such as p32, fusion, RPO30, GPCR, and ANK, molecular techniques like real-time PCR and DNA sequencing can be utilized to confirm the presence of LSDV and gain insights into its genetic makeup (Şevik & Dogan, 2017).

MATERIALS AND METHODS

Study Area

The outbreak investigation took place from January 2023 to July 2023 in Swat district of KPK, which is situated 200 km away from the capital city, Islamabad. Swat spans 5337 square kilometers and comprises seven tehsils: Babuzai, Barikot, Charbagh, Bahrain, Matta, Khwazakhela, and Kabal. This area was chosen for the study in response to a report of a Lumpy Skin Disease (LSD) outbreak at VRDIC Balogram (Figure 1).



Shows colors of different Tehsil of District Swat (Bahrain=black, Matta=purple, Kabal=red, Barikot=yellow, Babuzai=light green, Charbagh=blue, Khwaza-khela=green).

Study Population

An active outbreak investigation was conducted in an extensive management system within the local cattle population. All age and sex groups of animals reared under two different systems—production and management—were involved in the outbreak investigation in the Swat district of KPK for data collection.

Study Design

The study areas were selected based on reports of LSD outbreaks to VRDIC Balogram. The study was carried out at the Health and Diagnostic Investigation Center (VRDIC) in Balogram, Swat. Active outbreaks were assessed in collaboration with the Regional Veterinary Laboratory, zonal veterinary offices, and district veterinary clinics. Professionals working in these facilities recorded clinical and epidemiological data, collected samples for isolation and characterization.

Questionnaire Survey and Epidemiological Data Collection

Questionnaires were used to interview 20 pastoralists on LSD occurrence and its associated impacts. The data consisted of records of sex, mortality, and the source of the disease within the cattle population. The study area expressed their views and shared their practical knowledge on the current exposure of LSD, using their native languages (Ghundaan or Draparri). The questions included awareness about the clinical signs of LSD, the age and sex of affected cattle, seasons of LSD outbreak, livestock movement, product status, abortion in pregnant cows, presence of death due to the disease, vaccination status, and water sources for the livestock. The questionnaire was used to interview individual cattle owners at each outbreak or study site. Relevant data was gathered by observing clinically sick animals and interviewing cattle owners and animal health workers in the field. Information was carefully recorded in a designed format.

Sample Size and Sampling Technique

During the study period, a total of 1585 animals were examined. A field investigation was conducted at the specific site of the Swat district outbreak. Animals showing clear signs, symptoms, and suspected to be diseased with LSD were selected for sample collection. However, some animals with no symptoms were also included in the sample collection.

Data Management and Analysis

The gathered information was coded, recorded, and saved in a Microsoft Excel worksheet 2010. Before statistical analysis, the data underwent rigorous inspection and appropriate coding. Subsequently, the data was imported from Microsoft Excel and analyzed using the Statistical Package for Social Sciences (SPSS) version 20 software. Morbidity and mortality ratios were calculated according to gender and age category.

DNA Extraction

The Qiagen kit was utilized for DNA extraction and the manufacturer's instructions Supernatant from centrifuged samples was collected into fresh micro centrifuge tubes Proteinase K was added, vortexed, and incubated at 56°C for 10 minutes Ethanol (96-100%) was mixed in, and the sample was placed onto a QIAamp mini spin column Buffers AW1 and AW2 were used for further purification Buffer AE was added for final elution.

Conventional PCR

Nucleic acid was extracted from CAMs (pock lesions) using the QIAamp DNA mini kit Conventional PCR was performed to amplify the LSDV envelope protein gene (P32) and ORF 103 gene Emerald Amp GT PCR mastermix kit (Takara) was used according to the manufacturer's instructions PCR products were electrophoresed in 1.5% agarose gel with ethidium bromide DNA bands were visualized using ultraviolet light at 192 bp and 570 bp for P32 and ORF 103 genes, respectively.

Table 1 Cycling conditions of conventional PCR

Gene	**Primary denaturation**	**Amplification**				**Final extension**
		Secondary denaturation	Annealing	Extension	No of cycles	
P32	94˚C 5 min	94˚C 30 sec	50˚C 45 sec	72˚C 45 sec	30	72˚C 7 min
ORF 103	94˚C 5 min.	94˚C 30 sec	50˚C 45 sec	72˚C 45 sec	30	72˚C 7 min

Polymerase Chain Reaction (PCR)

In the detection of the virus, a real-time polymerase chain reaction (RT-PCR) test was employed, utilizing Capri poxvirus-specific primers.

Forward: 5″ GGTGTAGTACGTATAAGATTATCGTATAGAAACAAGCCTTTA-3″ Reverse: 5″-AATTTCT-TTCTCTGTTCCATTTG-3″The amplification process occurred in a final volume of 20 µl, containing the following components:10 µl of EvaGreen Super Mix 2 µl of forward and reverse primers (total 4 µl)4 µl of RNase-free water 2 µl of template DNAFor melting curve analysis, the following amplification program was utilized Initial denaturation at 95°C for 3 minutes 45 cycles at 95°C for 15 seconds, followed by 58°C for 80 seconds Final cycles at 95°C for 1 minute, 40°C for 1 minute, and 40-85°C for 5-10 secondsPositive samples were identified based on amplified fluorescence curves, melting curves at 73°C, and cycle threshold (Ct) values from the test. Samples with Ct values exceeding 40 were considered negative, indicating the absence of the virus in the tissue specimens and nasal swabs.

Agarose Gel Electrophoresis

Amplified products were loaded onto a 2% agarose gel in TAE buffer with 10 mg/ml ETDM-bromide stain, 20 µl of the PCR product was combined with 4 µl loading buffer and loaded into wells on the gel. Electrophoresis was conducted at 100 volts for approximately 60 minutes. A DNA molecular weight marker was used to determine the migration distance of the DNA samples Positive outcomes were identified based on the size of the bands observed on the agarose gel.172 bp band: Indicates positive results for LSDV and GTPV DNA,151 bp band: Signifies a positive outcome for SPPV. This method reliably detects specific DNA strands and confirms the success of PCR for the targeted viruses.

RESULTS

Prevalence of Lumpy Skin Disease

A total of 1585 animals were examination. Out of 443 clinical sign (positive). The selected sample collection of 443 animal which contribute 27.94% of the total examined animals. However the samples collected was from the half of the total animal means the total samples collected were recorded 305 and obtained 68% of examined animal and clinical signs in Figure 2.

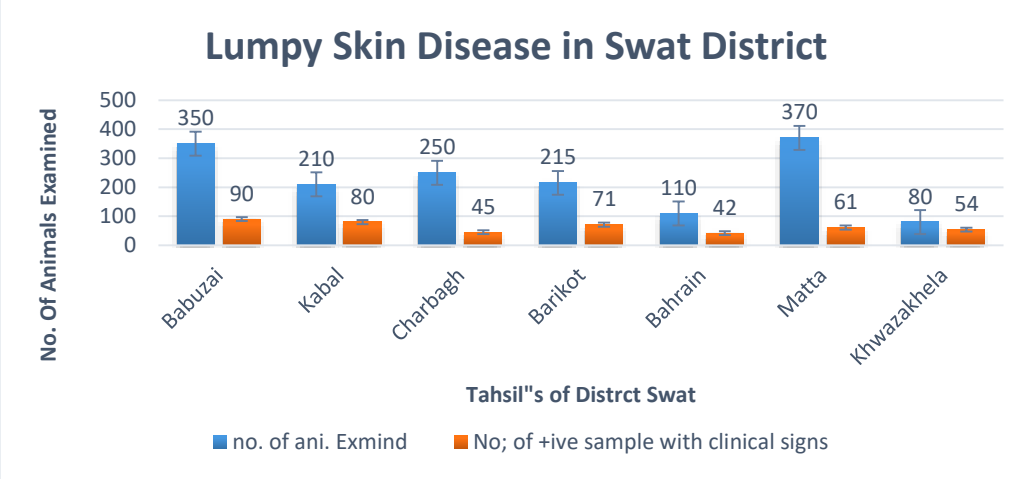


Figure 2. Prevalence of Lumpy Skin Disease (LSD) in tehsil of swat district

Total Samples collected showing positive samples in all tehsils of district of Swat

The common clinical signs were observed in affected cattle by LSD, were fever, development of different sizes of circumscribed nodules on the skin, necrotic nodules, deep scab formation, swelling of dewlap, and enlargement of superficial lymph nodes. Lacrimation, dewlap and superficial lymph nodules enlargement were very prominent. Burst necrotic wounds were often complicated with secondary infection.

Morbidity rate in overall Swat district

The total sample were collected from each tehsil of swat district which 443 obtained which was about 63.28% of the total Postive animals' with clinical signs and symptom. The number of positive PCR samples from all district were recorded 305 with 68.84% of the total collected samples.

Morbidity rate of LSD in Tehsil Babuzai different age and breed of animals

A different age groups sample were collected from total calves was 10 out of 100 examined animals, while from heifers and adults were counted 32 and 48 out of 110 and 140 checked animals. The total sample were collected from local breeds were 08, from mix breed sample collected 52 and from the indigenous breeds was collected 170 number. The morbidity rate in mix breeds were relatively higher as in indigenous breeds.

Table 2 Age Wise Prevalence of Lumpy Skin Disease in Babuzai Tehsil of Swat district animals

Age groups	**No: of examined animals**	**No of +ve Samples**	**Prevalence %**
Calves	100	10	10%
Heifers	110	32	29.09%
Adults	140	48	34.28%
Total	350	90	

Table 3 Breed Wise Prevalence of Lumpy Skin Disease in Babuzai Tehsil of Swat district

Breeds	**No of examined animals**	**No of +ve Samples**	**Prevalence %**
Local breeds	45	8	17.77%
Mix breeds	135	30	22.22%
Indigenous breed	170	52	30.58%
Total	350	90	

Morbidity rate of LSD in tehsil matta in different age and breed of animals

A total number of samples collected from calves were 08, while from the heifer and adults' animals, the number of samples were collected different age group of each category animal 23 and 30. The total samples were collected from local breeds 09, and from mix breed indigenous breeds, the number of samples were examined 14 and 38 and respectively. The morbidity rates in mix breeds was relatively higher as compared in indigenous breeds.

Table 4 Age Wise Prevalence of Lumpy Skin Disease in Matta Tehsil of Swat district animals

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	120	08	6.66%
Heifers	110	23	20.90%
Adults	140	30	21.42%
Total	370	61	

Table 5 Breeds Age Wise Prevalence of Lumpy Skin Disease in Matta Tehsil of Swat district animals

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	65	09	13.84%
Mix breeds	145	14	9.65%
Indigenous breed	160	38	23.75%
Total	370	61	

Table Morbidity rate of LSD in Tehsil Kabal in different age and breed of animals

The total number of samples were collected from different age group of animals which heifer 29and adult 44 which indicated that calves were 07, The result shows a significant p value of 0.001 and the morbidity rate were high observed in mature cows tehsil kabal.. The total number samples collected from local breeds which counter number were 11, from mix and indigenous breeds, the number of samples were 24 and 45 observed. The morbidity rates in mix breeds as compared higher in indigenous breeds. Shows that the rate of morbidity among different group of ages in cattles.

Table 6 Age Wise Prevalence of Lumpy Skin Disease in Kabal Tehsil of Swat district animals

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	35	07	20%
Heifers	75	29	38.66%
Adults	100	44	44%
Total	210	80	

Table 7 Breeds Wise Prevalence of Lumpy Skin Disease in Kabal Tehsil of Swat district animals

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	30	11	36.66%
Mix breeds	65	24	36.92%
Indigenous breed	115	45	39.13%
Total	210	80	

Morbidity rates of LSD in Tehsil Barikot in different age and breed of animals

The morbidity rate among different age group in cattle heifer and adults it was examined that the heifer and adult ratio 00.6, 23 and 42 samples were pointed out and different age group of animals. The result shows a significant p value of 0.027 and the morbidity rate examined high in mature cows in tehsil Barikot. The total number of sample collected from local breeds 11, from mix breed and indigenous breeds 36 and 34 respectively.

Table 8 Age Wise Prevalence of Lumpy Skin Disease in Barikot Tehsil of Swat district

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	40	06	15%
Heifers	75	23	30.66%
Adults	100	42	42%
Total	215	71	

Table 9 Breeds Wise Prevalence of Lumpy Skin Disease in Barikot Tehsil of Swat district

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	30	11	36.66%
Mix breeds	65	26	40%
Indigenous breed	120	34	28.33%
Total	215	71	

Morbidity rate of LSD in Tehsil Bahrain in different age and breed of animals

A total number of samples were collected from calves, heifers and adults, 12 and 26. The total sample collected from local breeds were 07, from mix breed and that from indigenous breeds, the number of samples collected were 15 and 20. The morbidity rates in mix breeds was relatively higher and high in indigenous breeds.

Table 10 Age Wise Prevalence of Lumpy Skin Disease in Bahrain Tehsil of Swat district

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	25	04	16%
Heifers	35	12	42.28%
Adults	50	26	52%
Total	110	42	

Table 11 Breeds Wise Prevalence of Lumpy Skin Disease in Bahrain Tehsil of Swat district

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	15	07	46.66%
Mix breeds	35	15	42.85%
Indigenous breed	60	20	33.33%
Total	110	42	

Morbidity rates of LSD in Tehsil Charbagh in different age and breed of animals

A total number of sample were collected from different group of animals calves were 06, heifers and adults, 23 and 42 from each. The total sample collected from local breeds were 05, from mix breed and that from indigenous breeds, the number of samples collected were 15 and 25. The morbidity rates in mix breeds was relatively higher and high in indigenous breeds.

Table 12 Age Wise Prevalence of Lumpy Skin Disease in Charbagh Tehsil of Swat district

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	40	06	15%
Heifers	80	17	21.25%
Adults	130	22	16.92%
Total	250	45	

Table 13 Breed Wise Prevalence of Lumpy Skin Disease in Charbagh Tehsil of Swat district

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	30	05	16.66%
Mix breeds	60	15	25%
Indigenous breed	160	25	15.62%
Total	250	45	

Morbidity rates of LSD in Tehsil Khwaza Khela in different age and breed of animals

From different age groups total number of sample were collected from calves were 03, while heifers and adults 21 and 30 examined animals from each group. The total sample collected from local breeds were 08, from mix breed and that fro indigenous breeds, the 17 and 29 noted. The morbidity rates was confirmed from mix breed higher as indigenous breeds.

Table 14 Age Wise Prevalence of Lumpy Skin Disease in Khwaza Khela Tehsil of Swat district

Age groups	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Calves	10	03	30%
Heifers	30	21	70%
Adults	40	30	75%

Total	80	54	

Table 15 Breed Wise Prevalence of Lumpy Skin Disease in Khwaza Khela Tehsil of Swat district

Breeds	**No: of examined animals**	**No: of +ve Samples**	**Prevalence %**
Local breeds	20	08	40%
Mix breeds	25	17	68%
Indigenous breed	55	29	52.72%
Total	80	54	

DISCUSSION

The study was conducted in District swat to investigate the outbreak of lumpy skin disease in cattle. The present study reported an outbreak of lumpy skin disease occurred at the end of late 2022. As far as the objective is to characterize LSDV from an outbreak cases, the occurrence of LSD was examined using clinical diagnosis, virus isolation and PCR. Generation of sequence data were analyzed and release up to completion. in present study samples (20) typical clinical cases was for tested and confirmed as positive for LSD using PCR. It is observed from clinical manifestations, finding, such as fever, circumscribed nodules on the skin, necrotic nodules, enlargement of superficial lymph nodes and lacrimation are in agreement examined by Gari et al. (2010) in different areas of the country. Host susceptibility, age, immunological status of the animal, dose and route of virus inoculation affects the severity of disease identified by (Knopvelsiekte, 2008).

Present study, observed morbidity rate (18%) is slightly higher than that reported by Alemayehu et al., (2013), who recorded 6.1% and 13.61%, respectively in different parts of the country. Other authors reported that wide ranges of morbidity rates ranged from 3% up to 85% (Tuppurainen et al., 2012). Moreover, it is far higher than reported by Davies (1991), who indicated that the usual morbidity rate measured up to 1 to 5%. The outbreaks of the disease, the morbidity rate varies depending on host susceptibility and the abundance of mechanical arthropod vectors. present findings observed (1.34%) is slightly lower than reported by Leliso, et al. (2021), who reported 4.97% morbidity rate. The current finding agreed with the study of Alemayehu et al. (2013) recorded 1.8% in feedlot. Woods (1988) also examined that higher mortality rates above 5%. The present study, matched fatality rate (7.44%) is also lower than noted by Leliso (2021) and Alemayehu et al. (2013), who find out 36.48% and 30%, fatality rate measured in different parts of the country. However, the latter was conducted on feedlot cattle and result based on clinical diagnosis, which may not be suitable for direct comparison.

In the present observation it was isolated that LSDV samples collected from naturally infected cattle by inoculation on Vero cell. Characteristic pock lesions were observed after 1st passage and become clear after 3rd passage, this finding agreed with House et al (1990), who successfully cultivated LSDV to detected the characteristic pock lesions. The CPEs characterized by rounding of cells, aggregation of dead cells and destruction of monolayers is in line with the observation of Leliso et al. (2021).

The PCR technique is highly suggested by different authors as a means the confirmation of LSD clinical specimens (Leliso et al., 2021; Zeynalova et al., 2016). the present findings, Zeynalova et al. (2016) indicated that, on average, skin nodule samples exhibited higher concentration of virus than other samples, as evidenced by the lower average Ct values observed in PCR testing. From PCR test it is examined that detection and identification of the LSD outbreak measured by causative agent. The PCR assay used in this work showed that high specificity as a unique band of the expected size (172bp) was obtained for DNA samples derived from skin biopsies, nasal swab and Neethling reference strain of LSDV. The present finding state that outbreak was reported at the end of November 2016 after the end of the main rainy season in most parts of the lowland and some highland agroecological zones. The seasonality of the outbreaks was also substantiated by questionnaire respondents who provided information on active disease surveillance. This agreement with the report of Leliso (2021) who indicated that, the disease is higher during rainy season and decreases in the dry season. Others environmental risk factors associated with spread of LSD were found to be in worm humid agro-climate, communal grazing/watering and introduction of new animals in a herd. The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it decreases during the dry season (Gari et al., 2010).

This findings associated with LSD mortality and veterinary expenses for treating sick animals are suggestive of heavy losses in the sector. In line with current finding, various studies indicated that, lumpy skin disease causes severe economic losses as a result of the prolonged debilitating clinical course of the illness, reduced weight gain, temporary or permanent loss of milk production, infertility problems or even sterility in bulls, abortions in pregnant cows and permanent damage to hides (Leliso., 2021).

CONCLUSIONS

LSD was found to be the major cattle health problem causes severe economic loss due to permanent damage to hides, a prolonged debilitating clinical course, reduced weight gain, temporary or permanent loss of milk production, temporary or permanent infertility or even sterility in bulls, and abortion of pregnant cows. The present study LSD cause significant effect from morbidity and mortality of animals that leads to economic loss due to abortion of pregnant cows, milk yield reduction, cost of dead animals, and cost of treatment. From outbreak investigated of this present study all age group of animals were infected with significantly higher morbidity and mortality rate in young animals than other group. Extracted DNA from skin biopsies and nasal swabs tested by using PCR revealed that all tested samples were LSDV. This implies failure of LSD vaccines, due to studied group of animals were annually vaccinated against the disease.

Lumpy skin disease is considered as transboundary and trade band disease which has significant impendent on livestock market and animal products.

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Statement of conflict of interest

The mentioned authors have declared no conflict of interest.

CONTRIBUTION

IK, ALB, SRS, conceived and designed the experiments. IK performed the experiments. AK analyzed the data. AK revised the manuscript. AK, wrote the manuscript.

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