Food and Agriculture Organization
of the United Nations

Lumpy skin disease symposium

How science can support evidence-based disease management and control

Hybrid: Virtual and in HQ Rome

14–16 March 2023

Book of abstracts

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2023

Foreword

The lumpy skin disease symposium team is pleased to welcome you all.

The European Commission for the Control of Foot-and-Mouth Disease (EuFMD) and FAO's Emergency Prevention System for Animal Health (EMPRES-AH) have worked together to provide a wide platform to present and discuss their most updated findings on a wide range of lumpy skin disease (LSD) and lumpy skin disease virus-related topics.

In these three days of meetings, we aim to see how science can support disease management and control, with a great panel of speakers.

We are certain there will be excellent discussions, and we look forward to learning the latest findings on lumpy skin disease.

We would like to thank all the speakers, our panel of experts, those working behind the scenes in logistic support and FAO, for providing a splendid venue.

The LSD team

The lumpy skin disease symposium organizing committee does not publish corrections for typographical errors or other minor issues that do not substantively impact the article's scientific understanding or integrity. It is authors' responsability to ensure that the manuscript presents a grammatically correct copy.

Contents

Acronyms

CPE	Cytopathic effect
DIVA	Differentiate infected from vaccinated animals
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
KLH ELISA	Keyhole limpet hemocyanin
FMD	Foot-and-mouth disease
GPV	Goatpox virus
GTPV	Goatpox virus
IFAT	Indirect immunofluorescence antibody test
IFNg	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPMA	Immunoperoxidase monolayer assay
LAV	Live attenuated vaccine
LSD	Lumpy skin disease
LSDV	LSD virus
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
rtPCR	Real-time polymerase chain reaction
pv	Post vaccination
RNA	Ribonucleic acid
mRNA	Messenger RNA
SPPV	Sheeppox virus
$TCID_{50}$	Median tissue culture infectious dose
VNT	Virus neutralization test

Epidemiological features of the lumpy skin disease outbreaks in Nigeria

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Lumpy skin disease (LSD) is an economically important disease of cattle caused by the LSD virus (LSDV). LSDV was introduced into Nigeria in 1974 through the Niger Republic and subsequently from the Cameroon Republic in 1976. Since the first report of the disease, it is now endemic in the country with yearly outbreaks in intensive farms and transhumance herds resulting in economic losses and a threat to the developing dairy industry. At first, the disease was restricted to the northern parts of Nigeria, but presently LSD has been reported in most parts of the country. Based on available data, LSD was initially a seasonal disease in Nigeria occurring during the rainy season. However, the current epidemiologic status confirms that the disease is being reported throughout the year. Another important epidemiological feature is the repeated outbreaks of the disease in livestock farming communities for 3-4 years in the same herds/farms leading to economic losses. Risk factors associated with LSD outbreaks in Nigeria include restocking from livestock markets, location of sedentary herds along transhumance migration routes, and sharing of watering points. Recently, co-infections of LSDV with Pseudo-cowpox are also becoming common in both intensive farms and transhumance herds resulting in the severity of LSD in affected animal populations. Available data shows that LSD outbreaks morbidity and mortality rates in Nigeria are 3%-49% and 1%-6% respectively. Understanding the epidemiological features of LSD outbreaks in Nigeria is critical to sustainable control measures in the country.

Keywords:
Lumpy skin disease, epidemiology, co-infection, seasonality, Nigeria

Lumpy skin disease transmission risks connected to cattle movements in southeast Asia

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Introduction
The movement of cattle across Southeast Asia has no doubt played a part in the spread of LSD in recent years but the rapid nature of the spread over vast distances which have generally been against the direction of traditional cattle trade flows suggests that other means of spread have been primarily responsible for long distance transmission during the period from 2013 to the present.

Materials and Methods
A simple subjective analysis of publicly available information and personal experience.

Results
The general direction of the flow of LSD from the Middle East to India and China then south to Indonesia is in almost all cases opposite to the usual direction of the commercial live cattle trade across these regions.
Government policies also appear powerless to exert any significant restriction on the spread of the virus within the borders of countries affected since 2013. In some case government policies may well promote the spread of LSD.

Discussion
While there is no hard evidence, it appears reasonable to assume that the transmission of the LSD virus across such vast distances against the flow of normal cattle movements is likely to be driven by the movement of infected insect vectors on prevailing winds. Within national borders, government policies may also be playing a significant role by promoting local, short and long-distance transmission. Where threats of quarantine and emergency slaughter are combined with insufficient levels of government support and compensation to small farmers, the incentive for cattle owners to move infected animals away from government attention is usually strong enough to ensure that many infected cattle are transported to non-restricted (non-infected) areas where sales can be completed to salvage some value from the infected or suspect animals. This type of illicit movement is also likely to be occurring near national borders where government restrictions and cattle prices are significantly different on either side of the border.
In local terms it appears that the means of transmission of LSD are reasonably well understood but more research needs to be conducted to confirm the means of spread of the virus over vast distances.

Development of a mRNA vaccine for lumpy skin disease and companion diagnostic tests for DIVA

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Lumpy skin disease continues to spread into new regions and is difficult to control. Although existing live attenuated lumpy skin disease vaccines are effective and have been demonstrated to be able to eradicate the disease, they have some limitations. Because the predominant vaccines are live attenuated viruses, it is not possible to differentiate infected from vaccinated animals (DIVA). These limitations prevent the pre-emptive use of vaccination in countries that are at high risk of lumpy skin disease virus outbreaks prior to an incursion. An mRNA lumpy skin disease vaccine, which has DIVA capabilities with companion diagnostic ELISAs, is being developed as part of a collaborative RNA vaccine development pipeline for emergency animal diseases led by the New South Wales (NSW) Department of Primary Industries (DPI) and Tiba Biotech, based in Boston, MA. This pipeline brings together a collaborative network including UNSW/NSW RNA Pilot facility, CFIA, ACDP, and international partners. Tiba Biotech provides access to next-generation RNA vaccines exploiting self-amplifying mRNA technology and their proprietary RNABL® polyester dendrimer delivery (US patent, 2020). Six potentially protective antigens from lumpy skin disease virus have been identified by CFIA researchers and are currently being evaluated in mRNA vaccine constructs in mice prior to their evaluation in sheep to protect against sheep pox as monovalent vaccines. The results of these trials, with respect to protection from disease and the induction of capripoxvirus neutralizing antibodies, will be used to identify the optimal combination of vaccine antigens. The optimal mRNA vaccine will subsequently be evaluated for the ability to protect sheep against sheep pox and then in cattle to protect against lumpy skin disease virus challenge. Companion ELISA diagnostics using these antigens expressed using baculovirus are being developed in parallel to allow differentiation of infected from vaccinated animals.

The different strategies of managing Lumpy skin disease in Israel in five different outbreaks, between 1989 and 2019. What have we learned?

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Dr. Tamir Goshen, Director of the Israeli Veterinary Services
Dr. Boris Even Tov, Director of Galil-Golan Veterinary District
Dr. Yuval Hadani, Director of Western-Galilee Veterinary District

Since 1989, five incursions of lumpy skin disease (LSD) were recorded in Israel. These outbreaks have been conducted by four different strategies as follow.
The first outbreak of LSD in Israel occurred in 1989 in one village containing 17 dairy herds. As the disease was confined to this village only, and was established in all herds, the strategy of the Israeli Veterinary Services was to cull all the cattle (800 dairy cows) in the village.

The second outbreak in Israel occurred in three different locations in 2006. During this outbreak, due to the spread to several villages, it was decided to cull only the sick cows (a total of 205 dairy cows have been culled).

The third outbreak occurred in 2007. The disease affected 9 farms in different locations (five dairy herds and four free ranging beef cattle herds). In this episode too, only the sick cows have been culled (a total of 508 heads- 141 from dairy herds and 367 from free ranging cattle herds).

It is assumed that these three outbreaks were introduced into the country from the southwest.

The fourth and most important outbreak occurred during 2012-2013, with a total number of 284 affected herds (135 milking herds, 140 free ranging beef cattle herds and 9 feedlots), with a total of 5,466 animals reported as showing clinical symptoms. 629 animals have died from the disease and 357 have been euthanized. This massive outbreak started in the north of the country and spread all over. The spread of the disease shows a very interesting pattern with persistent fluctuation in the distance from most far affected new herd, to its closest previous affected herd.
In this episode, where vaccination has been applied a few months after the emergence of the disease, the economic losses were huge and included decreased fertility performance, decreased milk yield, losses due to quarantine measures, death and euthanasia losses, treatment costs (including vaccination and labor), decrease in animals' value etc. Animal welfare issues have also been seen and taken into consideration. During this outbreak, two different types of vaccines have been administered, and their efficacy has been evaluated.

The fifth outbreak, introduced from the northeast of the country, occurred in 2019 in which 17 herds were affected by the disease. The Israeli Veterinary Services managed this outbreak by implementing immediate vaccination of the affected herds in the surrounding control area, followed by a mandatory vaccination of all the cattle in Israel, as the spread of the disease could not be controlled by local measures implemented.

During the last outbreak, the Israeli Veterinary Services developed new aid tools for disease control, surveillance and prevention of the disease: A new application for LSD vaccine reporting and surveillance by drone of remote grazing herds in unreachable areas.

Systematic review and expert ranking of potential risk factors for lumpy skin disease

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Lumpy Skin Disease (LSD) is an internationally spreading disease of cattle caused by a capripox virus. LSD has a profound impact on animal welfare and causes substantial economic losses. Risk assessments are critical for the efficient control and management of complex infectious livestock diseases, such as LSD, and require knowledge of underlying risk factors. Here potential LSD risk factors were identified that have previously been considered in the literature and ranked their importance. Search strings were conducted with 'LSD' and disease 'risk' keywords that were used to systematically query eight literature databases, including PubMed (MEDLINE), Web of Science and Scopus, EFSA and FAO databases, as well as thesis and dissertation networks without specifying historic time limits. Following deduplication, two independent reviewers screened 721 records against pre-defined selection and exclusion criteria. During this process, all potential risk factors mentioned in the screening materials were recorded and 218 records identified that elicited comments with suggestions for 505 potential risk factors. These risk factors were then grouped and ranked through a participatory tool for expert opinion-elicitation that included participant experiences as a weighting factor. It was found that the rankings were largely consistent among participants. When taking expert experiences into account, LSD risks associated with the immune status of cattle, environmental factors, surveillance and disease control were deemed most important for LSD occurrence. Systematic literature searching for potential LSD risk factors created a comprehensive portfolio of possible drivers for transboundary LSD occurrence, disease-related problem-solving approaches and LSD risk assessments.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773701.

Key words

Lumpy Skin Disease, LSD, cattle diseases, systematic review, risk factors, participatory epidemiology, ranking, Rapid Automatic Keyword Extraction (RAKE), co-occurrence network.

Lumpy skin disease - a double antigen elisa for a triple objective: population screening, disease free status recovering and post-vaccination monitoring

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Introduction
Lumpy skin disease virus (LSDV), the etiologic agent of LSD, was initially located in northern and central Africa, and merged into Europe in 2017. Serology can be used for the detection and the surveillance of LSD, for post vaccination monitoring and to recover disease-free status. The ID Screen® CPV Double Antigen ELISA is a pan-Capripox ELISA which can be used for those purposes.

Material and Methods
The ID Screen® ELISA was performed as per manufacturer's instructions. It can be used with serum or plasma of domestic and wild ruminants and any other susceptible species. To determine samples status, either immunoperoxidase monolayer assay (IPMA), viral neutralization (VNT) or Indirect ImmunoFluorescence Antibody Test (IFAT) were performed (at Sciensano, the FLI or TPI).

Results and Discussion
Specificity was evaluated through the analysis of 1 050 disease-free sera from cattle sampled in non-endemic and non-vaccinated areas (France, Germany, Belgium). Measured specificity : 99.7% [99.2% - 99.9%]. - 11 field cattle samples vaccinated with a commercial live attenuated LSD Neethling vaccine, sampled 5 months post-vaccination were tested with ELISA, IPMA and VNT: eight samples were found positive with the ELISA, 2 by VNT and all were negative by IPMA. - 75 field cattle samples vaccinated with a commercial live attenuated LSD Neethling vaccine (OBP), sampled about five months post-vaccination were tested: the ELISA picks up 44 samples, the IPMA 40. 71% gave similar result. - 48 field cattle samples vaccinated with a commercial live attenuated LSD Neethling strain vaccine (Lumpyvax®, MSD), sampled two months post-vaccination were tested: the ELISA picks up 36 samples, the IPMA 12.- 5 animals showing clinical signs of Parapox virus infection in the mouth region, IFAT positive, were tested.
No cross-reactivity could be ascertained with the ELISA.

Conclusions
The ID Screen® ELISA is easy to handle, with ready-to-use reagents, and allows for high throughput screening. Its specificity is excellent, ensuring the best Positive Preditive Value even at low prevalences. The kit successfully detects antibodies against LSDV either in infected or vaccinated animals, and have at least equivalent sensitivity compared to other techniques. Seroconversion is generally observed between 25 to 30 days post vaccination with Neetheling based vaccines. Seroconversion rate after vaccination can vary upon the vaccine strain used (Neetheling, SPPV or GTPV), the vaccine quality, and also the number of injections received by the animals. Preliminary data indicated that 40 to 70% seroconversion could be measured after a primo vaccination. However, this was measured on a limited number of samples. To address this question, a study is currently being conducted within a EU funded project (DEFEND*). The aim of this study is to assess the persistence of antibodies post vaccination for LSDV among individual cattle vaccinated under programme conditions in selected countries within the Balkans.

Investigation of suspected outbreaks of Lumpy Skin Disease in southern Punjab, Pakistan

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Introduction

Lumpy skin disease (LSD) is an emerging infectious disease affecting cattle and water buffaloes. Being transboundary in nature, LSD hits animals in many southeastern countries in the recent past, and this is also the case in Pakistan. On 28 October 2021, the FAO-FMD project, Pakistan received information from livestock department, South Punjab, that there were some animals at two dairy farms showing symptoms and clinical picture like, fever, nodular skin lesions, nasal and lachrymal discharge, swollen lymph nodes, and reduced milk production. The field veterinary staff suspected these symptoms similar to that of Lumpy skin disease. After having discussion with livestock department authorities Bahawalpur (South Punjab), in order to carry out necessary investigations and for recommending the appropriate control measures, the project immediately arranged a team to visit the affected area on 3rd November 2021. A questionnaire was designed to be used to investigate this suspected outbreak along with sample collection kit.

Materials and Methods

A cross-sectional study was carried out by visiting the affected villages along with local veterinary staff and active cases findings were recorded. Keeping in view the vector transmission nature, important epidemiological information like environmental conditions, presence of mosquitoes, flies, and ticks, etc. existence were also monitored in affected area. Semi-structured interview was done on two farms and all the information was collected on a predesigned questionnaire. The team also walked around the farms and interviewed the owners, helpers and close relatives to collect other epidemiological information linked to the possible incursion and spread of disease.

Samples were collected from animals that meet the case definition for suspected LSD. The working case definition for suspected LSD was any animal having at least two symptoms: fever, nodular skin lesions, nasal and lachrymal discharge, swollen lymph nodes, and reduced milk production. A total of 70 samples including serum, blood, skin scabs, and swabs (ocular, nasal. oral) were collected randomly from all clinically affected eight animals representing cattle and calves. The collected samples were handed over to the National Veterinary Laboratory, Islamabad for further investigation.

Results the samples collected were tested through real-time PCR for the detection of LSDV using ID Gene LSD DIVA Triplex real-time PCR kit. The percentage of positive sample was 70% which showed high prevalence of LSD in the area.

Discussion

It was evident thorough epidemiological investigations of suspected farms that LSD had never been seen before in that area. Laboratory confirmation revealed that Lumpy skin disease was first time identified in Pakistan, thereby strict precautionary measures including strategic vaccination, well-designed surveillance and movement control can limit the spread of virus.

The role of Stomoxys calcitrans flies in LSDV transmission from clinical infected cattle

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Several haematophagous arthropod species have been put forward to play a role in the transmission of LSDV. However, conclusive data is limited on the importance of biting flies as potential vectors in LSDV transmission. In order to investigate the role of *Stomoxys calcitrans* an in vivo transmission model was optimized and subsequently used in several animal studies. Following feeding upon experimentally infected clinical viremic donor bulls, Stomoxys flies were transferred to naïve acceptors animals. These acceptors animals were clinically examined on a daily basis and sampled at regular intervals for virological (PCR, virus isolation), serological (ELISA, IPMA, VNT) and immunological (IFNg release assay) analysis. The exposure to the flies varied from hundreds of flies during multiple days and batches (= mass exposure) and a single event with only 20 flies (=minimal exposure) representing conditions that could be encountered in the field as well as during transport or in stables with vector control.

Four independent trials were conducted using mass-exposure conditions with 4 to 5 acceptor animals per trial. In each trial transmission was observed as 1 to 3 acceptor animals became clinically ill, including the development of the typical nodules. The clinical data was confirmed by laboratory analyses as these animals developed a viremia and seroconverted. No significant difference was found in the genomic load of the flies which were placed on acceptors which became infected and those which did not. The exact incubation period in the acceptors could not be exactly determined due to the experimental set-up and varied from 6 to potentially 27 days. In the minimal exposure trial whereby only 20 flies were allowed to feed only once on the acceptor animals, LSDV transmission was similarly observed in 5 out of 10 acceptors. The transmission was evidenced by the development of nodules, viremia and seroconversion. The time between the one-time feeding and the appearance of nodules was in general between 10 and 19 days (n=4) but went up to 35 days (n=1).

Our findings on transmission of LSDV by *S. calcitrans* has important implications for the control measures against LSD. Animal movement restrictions should be implemented during active vector periods and infected animals should be removed or separated from susceptible animals. This separation or quarantine should last sufficiently long as the incubation period can be long. It also shows the need for an integrated vector management in combatting the LSD. However, the observation that even a limited exposure to infected flies can result in LSDV transmission demonstrates that even then vigilance is warranted. The above-mentioned possibility of long incubation period has also implications for the setup of transmission studies as sufficiently long observation / sampling period need to be implemented.

Evaluation of the safety, immunogenicity and efficacy of a new live-attenuated lumpy skin disease vaccine in India

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Lumpy skin disease (LSD) was reported for the first time in India in 2019 and since then, it has become endemic. Since a homologous (LSD-virus based) vaccine was not available in the country, goatpox virus (GPV)-based heterologous vaccine was authorized for mass immunization to induce protection against LSD in cattle. This study describes the evaluation of safety, immunogenicity and efficacy of a new live-attenuated LSD vaccine developed using an Indian field strain (LSDV/India/2019/Ranchi). The virus was attenuated by continuous passage (P=50) in Vero cells. The vaccine (50th LSDV passage in Vero cells, named as Lumpi-ProVac^{Ind}) did not induce any local or systemic reaction upon its experimental inoculation in calves (n=10). At day 30 post-vaccination (pv), the vaccinated animals were shown to develop antibody- and cell-mediated immune responses and exhibited complete protection upon virulent LSDV challenge. A minimum Neethling response (0.018% animals; 5 out of 26 940 animals) of the vaccine was observed in the field trials conducted in 26 940 animals. There was no significant reduction in the milk yield in lactating animals (n=10 108), besides there was no abortion or any other reproductive disorder in the pregnant animals (n=2 889). Sero-conversion was observed in 85.18% animals in the field by day 30 pv.

Seroprevalance and associated risk factors of lumpy skin disease in Lesotho

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Lumpy skin disease (LSD) is a viral disease of economic importance and is endemic in most African countries including Lesotho. This study was conducted to assess prevalence and transmission risk factors associated with LSD in cattle populations from 2019 to 2021 in Lesotho. Bovine serum samples (2 250) from ten districts of Lesotho were screened for antibodies against Capripox virus using ELISA kit. Data were recorded and analyzed on excel based formula for *scotopic photopic ratio*, prevalence, single factor analysis of variance and Tukey Kramel method to determine differences of means by years, districts and agro-ecological zones. A questionnaire survey was conducted to assess farmer knowledge and awareness on LSD, cattle vaccination, animal interaction, access to veterinary services, sharing grazing and water points, animal movement and regulation and different aspects of management regimes. The antibodies against lumpy skin disease virus (LSDV) were detected in 10.5 % (236 samples) overall in present study. Highest mean prevalence was recorded at lowlands with 26% Senqu River Valley and mountains zones with 33%and 5% respectively. District wise comparison resulted in 50 (22.2%) positive samples from Maseru followed by 38 (16.9%) and 30 (??%) from Mafeteng and Leribe districts respectively. Qachas' Nek district was the lowest with 9 positive (9%) samples. Identified lumpy skin disease associated risk factors include no vaccination record, low farmer awareness and knowledge, low regulation, communal grazing, sharing of water points, and sharing points, high movement within the country and cross borders. In conclusion, prevalence of LSDV varied among districts and between ecological zones. The differences may be attributed to changes in climatic conditions, variance in husbandry practices and socio-economic factors. These findings can be used to review present protocols and develop new tailor-made strategies for effective management of lumpy skin disease in Lesotho.

Evaluation of the efficacy of live attenuated heterologous vaccines for the control of lumpy skin disease in cattle

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Lumpy skin disease (LSD) is a bovine viral disease with fever and the formation of nodules as main symptoms. It belongs to the genus of Capripoxviruses, together with sheeppox (SPPV) and goatpox virus (GTPV). The virus is mainly transmitted mechanically via arthropod vectors. Vaccination has proven to be a very effective tool to prevent and/or control the disease. Previous studies have shown that homologous (LSDV-based) live attenuated vaccines (LAV) provide good protection against LSDV challenge. Since cross-protection is known to occur between Capripox viruses, several countries however use heterologous (sheep- or goatpox-based) vaccines.

In this study, the efficacy of four sheeppox based LAVs (LAV1-4), based on different strains, and 1 goatpox based LAV (LAV5) to protect against an LSDV challenge were evaluated. The vaccination scheme and dose prescribed by the manufacturer was followed. The dose for vaccination of cattle with an SPPV vaccine is between 3X and 10X higher than the dose for the vaccination of sheep. For each vaccine, 7 cows were vaccinated and infected 21 days post vaccination. The results were compared with 5 non-vaccinated challenge control animals.

For the animals vaccinated with the sheeppox LAVs, seroconversion was not detected in 5, 3, 2 and 5 animals of the four groups, respectively, in the period after vaccination. Furthermore, those that did seroconverted scored only weakly positive in the immunoperoxidase monolayer assay. The induced interferon gamma response was variable between the different vaccines. Based on this response, none of the vaccines was able to generate a cell mediated immune response in all of the vaccinated animals. The indications that LAV1-4 might not induce a protective immune response in all animals was confirmed by the LSDV challenge. For LAV 1 to 4, respectively 6, 2, 4 and 3 animals developed clinical signs upon challenge which were comparable to those observed in clinically infected animals in the control group. Besides the typical clinical lesions, also viremia, viral secretion and spread to different organs was found in the vaccinated animals showing clinical signs. The results with the sheeppox LAVs were in contrast with those obtained with the goatpox vaccine. All goatpox LAV vaccinated animals had seroconverted by the time of challenge and a strong IFN gamma response was detected after vaccination. All animals showed an increase in body temperature and a small local reaction upon challenge. These clinical signs are also seen with homologous LAVs. No nodule formation or enlargement of lymph nodes was observed. The clinical data were supported by the virological analyses. No viremia was detected and viral DNA was only found in one organ in two different animals.

None of the SPPV based LAVs were suitable for vaccination to prevent LSDV infection at the vaccination dose used. On the other hand, the protection induced by the GTPV based LAV was comparable to the results obtained by homologues LAV vaccines and could therefore be used for vaccination against LSDV infection. It should however be emphasized that this cannot be extrapolated to other goatpox virus based vaccines without extensive validation.

Experience on combatting lumpy skin disease in Albania

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Introduction
Lumpy Skin Disease (LSD) was clinically diagnosed for the first time on 28 June 2016 in the village of Vlashaj in Northeastern Albania. The authors review the literature on the experience of the surveillance and control of LSD in Albania since its first appearance.

Materials and methods
A national emergency control plan was immediately prepared to manage human and financial resources of the veterinary service. The National Veterinary Service, EFSA and FAO collaborated in the descriptive and analytic data elaboration which assisted in disease surveillance and control.

Results
In 2016, the overall morbidity and mortality were recorded at 42 percent and 12 percent, respectively. In 2017, the overall morbidity and mortality were recorded at 22 percent and 6 percent, respectively. Small-scale farms (e.g., 2-3 animals) were predominantly affected. Real-time Polymerase Chain Reaction (rtPCR) diagnosis was performed in most collected samples from not vaccinated animals. In 2018, seven suspected cases tested negative. The last outbreak was, in 2019, identified in Northwestern part of the country.
The force of infection was modelled, and the fat-tailed kernel was the best fit to data and predicted the risk of transmission reduced by 95% at a distance of 4.1 km. Therefore, proximity to affected farms can be considered a risk factor for LSD spread. Evidence of seasonality in the transmission of LSDV between herds associated with temperature was found. Modeled relative vector abundance (*Stomoxys calcitrans*) and a significant decline in the Basic Reproduction Number (R0) during the epidemic suggest that the seasonality in the LSDV transmission and the abundance of potential LSD vectors are the major risk factors contributing to LSD spread. No significant differences in the probability of LSD infection were found between different types of production (dairy, beef, or mixed production).
Mass vaccination with the live attenuated homologous Neethling vaccine started on 26 July 2016. The estimated vaccine effectiveness at the farm level obtained from transmission modelling was 76.5% (95% CI: 71.8–80.6%) and was higher than that derived using survival analysis, i.e., 62.5% (95% CI: 54.1–69.5). The total cost of disease and control measures in 2016 was around 4.06 million euros, and in 2017 were 1.48 million euros. It was decided to end mass vaccination in 2022.

Discussion
The commitment, collaboration, and assistance of national, regional, and international stakeholders (EFSA, European Commission, FAO, and WOAH) were the main elements for successful LSD surveillance and control. Achieving the highest vaccination coverage with the live attenuated homologous Neethling vaccine in the shortest period of time is the cornerstone to rapidly control LSD outbreaks.

Emergence of lumpy skin disease virus in Pakistan and its isolation in lamb kidney cells

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Introduction

Lumpy skin Disease (LSD) is a viral disease affecting cattle that was first reported in Pakistan in year 2020-21. Since then, the disease has become endemic in the country, with frequent outbreaks reported in different parts of the country, including Sindh and Punjab provinces. Population of animals in Pakistan is approximately 50 million cattle. The spread of the disease is facilitated by the movement of infected cattle while lack of effective disease control measures. LSD has significant economic consequences for the livestock industry in Pakistan, as it causes significant losses in milk and meat production. Transmission is due to arthropod vectors, including biting flies, mosquitoes and hard ticks. Lumpy skin is characterized by cutaneous nodules on different body locations. The government has implemented various measures to control the spread of the disease, including vaccination campaigns and movement restrictions, but LSD continues to be a major challenge for the livestock industry in Pakistan.

Objective

The molecular identification and isolation of the LSD virus for disease prevention and control in Pakistan.

Methods

This study represents a significant step forward in our understanding of the LSD virus and its replication in vitro. The isolation of lumpy skin disease virus from an outbreak sample was performed using lamb kidney cells as a primary cell culture system, allowing us to detect the virus in suspected samples with a high degree of accuracy. The extraction of viral DNA from outbreak sample and its ability to isolate and grow in vitro, will enable us to study its biology and develop strategies for controlling its spread and pathogenesis.

Results

Clinical observations of the affected cattle indicated typical LSD symptoms. The scabs were analyzed by PCR amplification of extracted DNA using primers of known sequence. The presence of a disease characterized by bumps on the skin was confirmed by amplification of a 199-bp DNA fragments.

RT-PCR revealed three positive samples. When inoculated on lamb kidney cells, specific cytopathic effects (Fig: 3) was observed on the 18th day after infection. Cell rounding and clumping, as well as vacuolation, were cytotoxic effects. When 70-80% of cells developed CPEs, viruses were harvested. Local virus isolates were freeze-thawed, centrifuged, and kept at -80 °C until further study.

Positive sample showing characteristic CPE.

Discussion

PCR is a valuable tool for the detection of the LSD virus. This study provides the foundation for the future research for the virus and its genetic diversity in order to develop effective preventions. The successful isolation of the LSD virus in lamb kidney cells is a crucial step towards the development of effective vaccine and control for this virus, in Pakistan. Further studies are needed to fully characterize the virus and understand its epidemiology and transmission.

Simulating the spread and control of lumpy skin disease in Australia

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Introduction
Although Australia is currently free from lumpy skin disease (LSD), the disease has spread through southeast Asia in recent years, with outbreaks reported by near-neighbours Indonesia and Singapore in 2022. Northern Australia is considered to be the region most vulnerable to an incursion of LSD via wind-borne or otherwise transported infected insect vectors, or via the illegal movement of infected animals or animal products. An outbreak of LSD would cause serious socioeconomic consequences due to impacts on exports, particularly of live cattle and dairy products. The disease would also be difficult and costly to control and eradicate. In response to the escalating threat of LSD, Australian governments in partnership with industry and stakeholder groups, developed a National Lumpy Skin Disease Action Plan outlining priorities to strengthen Australia's preparedness for an LSD incursion, which includes the development of epidemiological modelling systems.

Methods
The Australian Animal Disease Spread model (AADIS), originally developed to support foot-and-mouth disease preparedness and response, is being adapted to provide a national-scale modelling capability to simulate spread and control of LSD within Australia. AADIS uses a hybrid modelling approach to simulate within- and between-herd transmission of infectious diseases and includes a range of control options such as movement restrictions, surveillance, tracing, stamping out, and vaccination. Parameterisation of control measures and resources are guided by the Australian Veterinary Emergency Plan (AUSVETPLAN) with inputs from state and territory animal health authorities. Modelling the spread and control of insect vector-borne livestock diseases such as LSD poses significant challenges, particularly for remote, extensive cattle production systems like those in northern Australia. Novel approaches are required to address these challenges in a way that realistically represents LSD epidemiology whilst being computationally efficient.

Discussion
In the absence of prior experience of LSD within Australia, epidemiological models can provide an evidence base to support LSD preparedness. Modern epidemiological models are sophisticated tools requiring multi-disciplinary approaches. This presentation will discuss our approach to adapting the AADIS model to LSD and overcoming challenges posed by the complexities of vector-borne spread and the management and control of LSD in the context of Australia's unique cattle production systems. These include representing the populations at risk, addressing vector distributions and ecology, disease transmission within and between herds, and designing and implementing control measures. Collaboration between governments (federal, states and territories) and universities has provided the foundation for development of this model, which will be used to support LSD preparedness action planning in Australia and enable veterinary authorities to test a range of outbreak scenarios and mitigation strategies.

Development of a new diva inactivated vaccine against lumpy skin disease in calves

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Introduction
Lumpy skin disease virus (Poxviridae family - Capripoxvirus genus) is the aetiological agent of LSD, a disease primarily transmitted by hematophagous biting affecting principally cattle. Currently, only live attenuated vaccines are commercially available but their use is limited in the endemic areas thus, there is a need for safer vaccines, especially in LSD-free countries.
The research aims to develop a safe and efficacious inactivated vaccine. Moreover, in this study keyhole limpet hemocyanin (KLH) was used as a marker to distinguish infected from vaccinated animals (DIVA). The vaccine safety and efficacy and its effects on milk production in cows were evaluated.

Materials and Methods
LSDV was amplified on MDBK cells, an inactivated vaccine and a placebo with KLH were produced. To evaluate the safety and
immunogenicity and efficacy, the vaccines were tested on two groups of 6 male calves. The animals were inoculated with a dose of 2mL vaccine or placebo at T0 and T28. LSD/ϒ-interferon test, KLH (IgM-IgG) ELISA, LSD ELISA and seroneutralization (SN) were performed every 7 days on the blood samples collected.
At T60 the animals were subjected to the challenge test using a viral field strain (10 6.62 $TCID_{50}$/mL). Moreover, the vaccine effects on lactation were studied on 2 groups of 10 Friesian breed cows. A statistical analysis was performed.

Results
In all vaccinated animals a seroconversion was observed starting from T35 by ELISA and from T42 by SN test. The antibody titres (IgM-IgG) against the KLH were revealed starting from T7.
The variations of γ-interferon concentrations were observed in vaccinated animals, unlike the control group, starting from T7 and in all the animals from T28. After challenge, at T10, 4 out of the 6 control animals were euthanized, because of the high clinical score. Instead, none of the vaccinated animals except one showed any severe symptoms.
No significant statistical differences ($p<0.05$) between the two groups have been recorded about the vaccine effects on qualitative and quantitative milk parameters.

Conclusions
The safety and efficacy studies of the inactivated vaccine demonstrated that the product developed was able to protect animals from severe clinical signs of LSD. The immune response was achieved starting seven days after the booster dose (T28), demonstrating the correctness of the used vaccination schedule as proved by the evolution of antibody response.
The analyses of the milk quantity and quality showed that the use of the inactivated vaccine does not affect the milk production, unlike the negative side effects caused by the attenuated vaccines, as reported in literature.
Moreover, the use of KLH allowed us to discriminate vaccinated from unvaccinated animals and infected animals. This vaccine would enable to correctly identify vaccinated animals during outbreaks, making the disease containment easier throughout the application of DIVA strategy.

Summary of lumpy skin disease surveillance programme in 2020-2022

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Introduction

Lumpy Skin Disease (LSD) first occurrence in the country was in April 2016, while in 2017 only three additional outbreaks were confirmed. In total 1 535 cattle farms were affected and 4 327 bovine animals culled and safely disposed. Compulsory vaccination of all cattle older than six months, using live attenuated vaccines, was in place from 2016 until 2019. This paper aims to describe the methodology and the results of the LSD Surveillance Program (SP) implemented in the post vaccination period 2020-2022.

Materials and Methods

In addition to passive surveillance, the major activities of the Food and Veterinary Agency (FVA), for regaining the LSD-free status, were directed towards active surveillance. Two main components of this methodology were clinical examinations (animal health visits - AHVs) and serological screening. A stratified random selection method was used to select the farms (high and low-risk areas) and the cattle (age and vaccination status) included in the surveys.

The AHVs was designed as a cohort study and selected farms that were not visited (in each phase of the LSD-SP) or didn't have animals during the visits were excluded from the subsequent visits. Annually, four AHVs were foreseen on each selected farms, with a minimum of 5 weeks between the visits. All animals present on the farms were subjected to clinical examination and the results were recorded into the designated LSD module in the electronic database.

The serological survey aimed at the detection of the presence of antibodies against LSDV in the serum of non-vaccinated animals, older than 6 months. All seropositive animals, as well as all the animals on the farm fulfilling the criteria for testing were sampled (serum, whole blood and nasal swab) and tested/re-tested using the ELISA and qPCR methods.

Results

The data for the foreseen and conducted AHVs for LSD-SP (2020-2022) are presented in Table 1. Out of 194 100 examinations performed during the AHVs, only eight animals (one in 2020, and seven in 2022) presented clinical symptoms resembling LSD. All eight animals were sampled and tested with Real-Time PCR method, but the LSDV proviral DNA was not detected.

Detailed results of the serological surveillance are presented in tables: 2 and 3. Shortly, the results revealed the presence of seropositive animals in all three years of the LSD-SP. Highest seroprevalence was observed in 2020 (18, 3%), while the lowest in 2021 (4,7%) (Table 2). However, follow up testing of the seropositive animals, using the Real-Time PCR method didn't confirm the presence of LSDV (Table 4).

Discussion and Conclusion

Misinterpretations of sampling instructions, resulting in a sampling of vaccinated animals, during the first year of the LSD-SP, led to an upgrade of the LSD module in 2021 to prevent similar events. The reduced number of AHVs in 2021 was a result of the movement restrictions imposed due to the COVID-19 protocols. The results of the LSD-SP conducted in the past three years provide reliable evidence that LSDV is not circulating in the cattle population. However, the results of the serological surveys, indicate the necessity of further research to determine the possible reasons for seropositivity.

The epidemiological and economic impact of a potential lumpy skin disease outbreak in India: a regional analysis of Punjab

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Introduction
The outbreak of animal diseases has both explicit and implicit economic repercussions. In the present study, a simple mathematical model has been developed to estimate the economic impact of Lumpy Skin Disease (LSD) in India along with epidemiological metrics.

Materials and Methods
The econometrics is based on all the direct and indirect losses and expenditures incurred during the entire course of disease in a dairy farm specifically considering production losses, reproduction losses, treatment costs, preventive costs and other costs. Primary data were collected from 100 dairy farms located in Indian state of Punjab in which LSD outbreak occurred. Based on the results, the economic impact of the disease was extrapolated for the total bovines effected due to the disease in the LSD outbreak occurred in India from July 2022 to October 2022.

Results
Results revealed 45.25 percent morbidity in cattle, 0.19 percent in buffaloes and 27.04 in pooled sample of bovines with 12.40 percent mortality in cattle. The morbidity and mortality were far too high in cattle as compared to buffaloes and crossbred cattle was found to be highly susceptible for LSD. Moreover, it was found that there is loss of about 45.10 percent milk yields of the milch animals. Among the expenses and losses, maximum expenses were made on treatment in case of cattle and maximum losses were under production head in case of buffaloes. A net economic loss of INR 1135.26 crores (USD 137.26 million) in Punjab and INR 18337.76 crores (USD 2217.26 million) in India was estimated due to LSD outbreak in bovines.

Discussion
The mathematical modelling approach provided an evidence of coherence among the prevailing factors and aided to estimate economic impact to near precision. The developed methodology can be used for calculating health economics of other diseases affecting bovines. The practices pursued at the dairy farms along with their costs can aid in devising suitable prevention plans and policies against LSD for the time to come.

Recombinant vaccine-like lumpy skin disease strain Udmurtiya/2019 causes infection via indirect contact in an experimental study

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Introduction

Lumpy skin disease virus (LSDV) is an important capripoxvirus of cattle, buffalos and some wild life species. The disease was previously restricted to sub-Sahara Africa, but since the 1990's it had an aggressive expansion North direction towards the Middle East, Europe, Balkans, Russia*n Federation* and recently into Asia. The unprecedented expansion in Russia*n Federation*, Kazakhstan and South-East Asia, has been associated with multiple incursions of not only classical field isolates, but also recombinant vaccine-like strains (RVLSs). The latter contains genomes constructed from recombination events between the Neethling and the KSGPO vaccine strains. The RVLSs emerged under novel seasonality patterns, with RVLSs capable of spreading without arthropod activity and overwintering during freezing and snowy conditions.

Materials and methods

The RVLS Udmurtiya/2019 caused an outbreak during the freezing winter of Russia*n Federation* in 2019. In order to investigate the capability of this strain to transmit via indirect contact between animals, the following experimental study was conducted. Bulls were housed in an insect proof animal biosafety level 3 facility, where half the animals were infected intravenously with Udmurtiya/2019, whilst the remaining five animals were mock-inoculated but kept in contact with the inoculated group. Both the infected / inoculated group (IN) and uninfected / in-contact group (IC), were monitored for 41 days with continuous registration of body temperature, observations for clinical signs and collection of blood samples and nasal swabs for testing of LSDV presence using real-time PCR.

Results

Results indicated that cohabitation of animals from both groups was sufficient to transmit the virus from the IN to the IC-group, with the onset of clinical signs including pyrexia (~41°C) and classical LSD nodular skin lesions starting at 10 dpi for the IN group and 16 dpi for the IC-group. Additionally, the presence of LSDV genomes as well as anti-LSDV antibodies were detected in swabs, blood and serum samples from animals belonging to both groups. These results provides another proof for RVLS transmission in a controlled environment without direct contact between diseased and healthy animals, in the absence of insect vectors.

Discussion

Similar clinical symptoms were observed during a study involving a second novel recombinant LSDV strain, Saratov/2017. In this work, Udmurtiya/2019 exhibited prolonged virus shedding in blood and nasal secretions of more than 30 days, whilst Saratov/2017 was detected in blood for the same period, but nasal shedding lasted only for 27 days. In both these cases, the observed shedding patterns of recombinant strains were three times longer compared to classical LSDV isolates, using both blood and nasal swabs. This study raises concerns regarding the transmission of Capripoxviruses, with special emphasis on the novel recombinant strains circulating in SE-Asia. The subclinical infection attributed to contagious RVLSs poses a serious risk of transmission, since virus shedding occurs in the absence of clinical symptoms.

Emergence and distribution of lumpy skin disease in cattle in several districts in Sri Lanka from 2020 to 2023

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Introduction
Lumpy skin disease (LSD) is a viral disease that affects cattle and buffalo caused by lumpy skin disease virus (LSDV) belonging to the *Capripoxvirus* genus of the family *Poxviridae*. Disease is transmitted by blood feeding insects such as certain species of flies, mosquitoes and ticks. It causes high fever and multiple nodular skin lesions on head, neck, udder, scrotum, vulva and perineum, and enlargement of superficial lymph nodes. LSD causes high morbidity but low mortality, however, death rates are higher among calves. The disease can lead to significant economic losses due to loss of milk and beef production, abortion in females and sterility in males. This study intends to discuss the first reported LSD in Sri Lanka and spreading of the disease in LSD free districts.

Materials and methods
Conventional PCR was performed on the samples from skin nodules for confirmation. Total DNA was extracted using Qiagen DNeasy blood and tissue kit according to manufacturer's protocol. The coding region of viral attachment protein gene of LSDV was detected following the conventional PCR methods described by WOAH.

Results
There were total of nine reported outbreaks that were confirmed by PCR since 2020 to 2023. The condition was first reported in 2020 in Sri Lanka and observed lesions in cattle in districts of Eastern province. Ampara district in Eastern province (Addalachchenai and Mahaoya area) was reported with this unusual cases in October 2020. The disease spread rapidly throughout the district from October 2020 to March 2021. Then it further spread to Trincomalee district (Thambalagamuwa, Muthur, Mahadiwulwewa, Kuchchaveli) in 2021. The third district affected in Eastern province was Batticaloa (Chenkalady) in 2021. New cases again have reported in Badulla district (Etampitiya) in 2022 that is geographically adjacent district to Ampara. The most recent outbreaks were reported in Jaffna (Thellipallai, Kayts island) and Kilinochchi (Kilinochchi, Palai) districts in 2023 (Fig 1).

Discussion
LSD has not been reported in Sri Lanka before 2020, the conducted laboratory investigations confirmed the occurrence of LSD among the cattle in Ampara district and further spreading nearby districts in northern and eastern provinces of Sri Lanka. Case history has provided information on milk yield reduction during the disease outbreaks and that was low in comparison to total milk production before the outbreaks in affected districts.

Phylogenomic characterization of historic and recent lumpy skin disease virus isolates from South Africa, including the prototype strain Neethling

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Introduction
The poxvirus, lumpy skin disease virus (LSDV), has been endemic to South Africa since the 1940s, and in 1960 a live-attenuated vaccine (LAV) was commercially released for use in the country to mitigate the spread of this disease. This vaccine (Neethling/vaccine/LW-1959) was generated from serial passages of the prototype LSDV strain, Neethling-WC/RSA/1957. The latter was isolated in 1957 from an outbreak in the Western Cape province of South Africa and was subsequently used to prove the infectious nature of the virus and the causative agent of the resulting disease in cattle. Complete genomes of the prototype strain and various LSDV isolates from outbreaks in South Africa were determined to identify genetic differences between the virulent wild type and vaccine strains.

Materials and methods
Complete genome sequences of the prototype LSDV strain, Neethling-WC/RSA/1957, and other wild-type isolates from the 1950s, 1970s and post-2010 were generated. The newly-constructed consensus sequences and data available from GenBank were included in an alignment using CLC Genomics vs.9. The alignment was used to assess the phylogenomic relatedness using Bayesian and Maximum Likelihood algorithms, whilst single nucleotide polymorphisms (SNPs) were identified in CLC Genomics.

Results
Phylogenomic analysis classified all sequences before 2000 into cluster 1.1, along with previous sequences of the vaccine strain (Neethling/vaccine/LW-1959), the oldest known isolate (LSDV/Haden/RSA/1954) and virulent viruses isolated in the 1990s from South Africa. On the contrary, all the recent isolates grouped within cluster 1.2, along with LSDVs from other parts of Africa, the Middle East, Europe and Asia (Figure 1). These results improve our current understanding of the evolution of LSDV and demonstrate that the population dynamics of circulating isolates are not constant, with LSDVs associated with different genetic clusters dominating the landscape over time. In this study, seven single nucleotide polymorphisms were identified between the Neethling-WC/RSA/1957 prototype strain and the live-attenuated vaccine strain (LW-1959) derived from it. These SNPs were either non-synonymous or resulted in the early termination of the affected open reading frame. None of the SNPs were identified in other high-passage attenuated strains, providing new insights into virus attenuation and possible markers for DIVA assays.

Discussion
Differences between clusters 1.1 and 1.2 were incorrectly suggested to play a role in virulence, since the assumptions were based on the concept that cluster 1.1 only contains vaccine strains and that all differences with virulent viruses in cluster 1.2 were due to attenuation. This study is the first to describe the complete genome of the LSDV type-strain, LSDV/Neethling-WC/RSA/1957 and additional wild-type viruses isolated under constant circulation in South Africa. Analysis of the newly-sequenced viruses demonstrates the differences between the wild-type virulent viruses and the derivative LAV strains, and the genetic drift exerted on viruses in cluster 1.1 over a 40-year period.

Estimating lumpy skin disease vaccine dose demand using a vaccine demand estimation model

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Vaccine demand estimation model (VADEMOS) is a decision-support tool with a web-based interface developed by the EuFMD that aims to quantify number of vaccine doses needed in endemic countries over a given period of time combing estimates of livestock demographics, outbreak data, expected vaccine coverage and vaccine schedule. VADEMOS has been initially developed for Foot-and-mouth disease and it is currently being adapted to calculate the expected demand of lumpy skin disease doses in those countries that intend to conduct a control and eradication programme. Initially, the application predicts the population growth of different susceptible species and different countries using an ARIMA model, a type of time series forecasting model that takes into account past observations to predict future values of a time series. The dataset used in this analysis derives from FAO database which contains the population of different species in the countries included for the period of 1994 to 2021. A subset of the data is created for each country and each species, and the model is fit to test the predictions. The output is subsequently combined with additional data like the envisaged vaccination coverage of the population, vaccination schedule adopted during the campaign and vaccine cost (set by the user). The initial results of this analysis for LSD, which are provided through median and credible interval, will be presented in the conference. These results will be useful for planning LSD control and eradication strategies using vaccination campaigns and for informing the budget needed at country and regional level.

The lumpy skin disease symposium is organized in collaboration with the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) and the Emergency Prevention System for Animal Health (EMPRES-AH) of FAO.