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## LSD epidemiology and diagnosis (clinical & laboratory)

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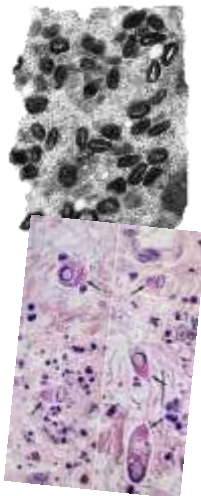
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### Introduction

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- ✓ Lumpy skin disease virus belongs to the *Capripoxvirus*-genus within the *Poxviridae*-family
- ✓ Other members of the genus are *Sheeppox virus* and *Goatpox virus*
- ✓ LSD is characterized by fever, nodules on the skin, and it may lead to severe economic losses, especially when introduced into naive population
- ✓ Vector-borne LSD is notoriously difficult to eradicate without vaccination
- ✓ Large double-stranded DNA virus with or without an envelope
- ✓ Stable virus, survives well in the environment such as wintertime and drought
- ✓ Most disinfectants are effective but disinfection of the environment is difficult as the virus remains well protected inside scabs shed by infected animals

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- Balkan Slavic dialects**  
 (Balkan Slavic dialects)  
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- Legend:**  
 From within the dialect area  
 Inhabited areas  
 1:10 scale

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## Epidemiology in nutshell

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- ✓ Morbidity rate varies between 5 to 45% and mortality rate usually remains below 10%
- ✓ The first (index) case is usually associated with cattle movements
- ✓ Particularly in Europe, the outbreaks are seasonal - more common (but not limited to) warm and wet seasons with abundance of blood-feeding arthropods
- ✓ Severe cases are highly characteristic and easy to recognize
- ✓ Early stages and mild cases difficult to recognize even for the most experienced vets
- ✓ In dairy cattle versus free-ranging beef cattle
- ✓ By the time severe cases are detected in the free-ranging herds the virus has already been circulating for weeks
- ✓ After a quiet winter period outbreaks may start again in spring time when skin lesions are well hidden under a long winter coat – difficult to detect without palpating the skin

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## Host range (1)


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- ✓ **Domestic cattle** and Asian water buffalo are susceptible
- ✓ There is no epidemiological evidence/reports on susceptibility of small ruminants for LSD
- ✓ Wild ruminants as reservoir or susceptibility in Europe or Caucasus is not known
- ✓ Some evidence from Africa (Springbok, impala and giraffe can show clinical disease. Seropositive African buffaloes, blue wildebeest, eland, giraffe, impala and greater kudu.



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## Host range (2)

- ✓ No studies if LSDV affects European wildlife
- ✓ LSD detected in saliva from affected cattle with generalized form of the disease, while blood was already negative
- ✓ Non-invasive sampling with salt lick type of baits was performed to collect saliva from red deer and follow deer and to be tested for LSD
- ✓ The NI sampling was performed in area where the disease was present in domestic cattle
- ✓ **Results: no evidence that LSDV affects red and follow deer. Non invasive surveillance for LSD works well for cattle**



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## Transmission

- ✓ blood-feeding insects, such as certain species of flies and mosquitoes, or ticks.
- ✓ direct contact
- ✓ contaminated feed, water and equipment
- ✓ trans-placental transmission are reported - calves are known to be born covered by skin lesions
- ✓ sucking calves may get infected via milk or from skin lesions in the teats (rare due to maternal antibodies)
- ✓ iatrogenic transmission - by contaminated needles during veterinary treatments or vaccination campaigns
- ✓ Seminal transmission via natural mating or artificial insemination – real importance in the field needs to be investigated

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## Mechanical transmission of LSDV by vectors

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- ✓ Vectors are likely to vary between affected regions
- ✓ Vector must bite/feed frequently and change the host between feedings
- ✓ Finding PCR positive vectors from the environment indicates that they have been feeding on infected animal



### Blood feeding insect vectors

- ✓ Transmission has been demonstrated by mosquito (*Aedes aegypti*) (Chihota *et al* 2001)
- ✓ Suspected transmission by stable fly (*Stomoxys calcitrans*) – transmission of sheepox/goatpox virus was demonstrated by Kitching *et al* 1986
- ✓ *Tabanus spodopterus* – females (Bulgaria 2016)



**The Big Question: Does biological transmission occur?**

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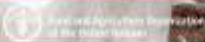
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Movie: LSD affected herd

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## Transmission by tick vectors

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- ✓ Mechanical transmission has been experimentally demonstrated
- ✓ African tick species: male *Rhipicephalus appendiculatus* (and *Amblyomma hebraeum*) ticks
- ✓ *Rhipicephalus* (*Boophilus*) *decoloratus* – venereal transmission during the copulation process – more evidence required to demonstrate biological transmissions



### Bulgaria 2016: LSDV found in

- ✓ *Hyalomma marginatum* - females
- Rhipicephalus bursa* - males + females



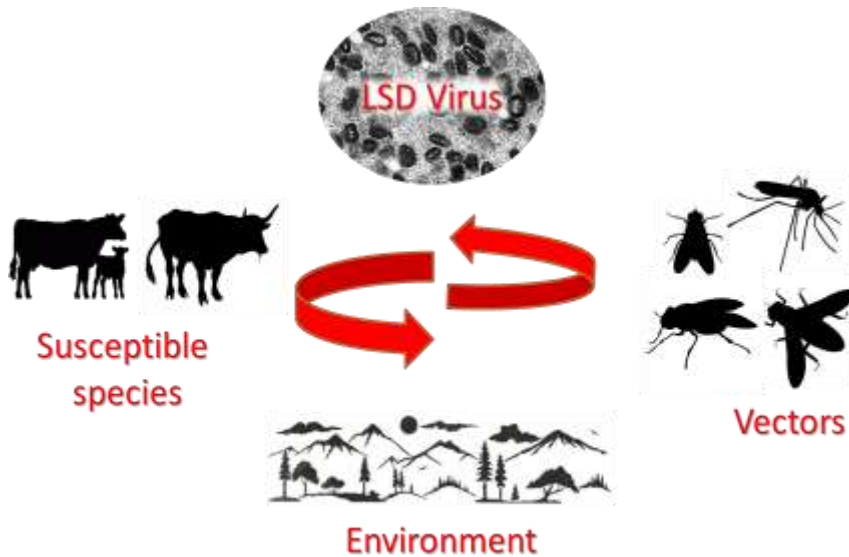
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## What is needed for LSD?

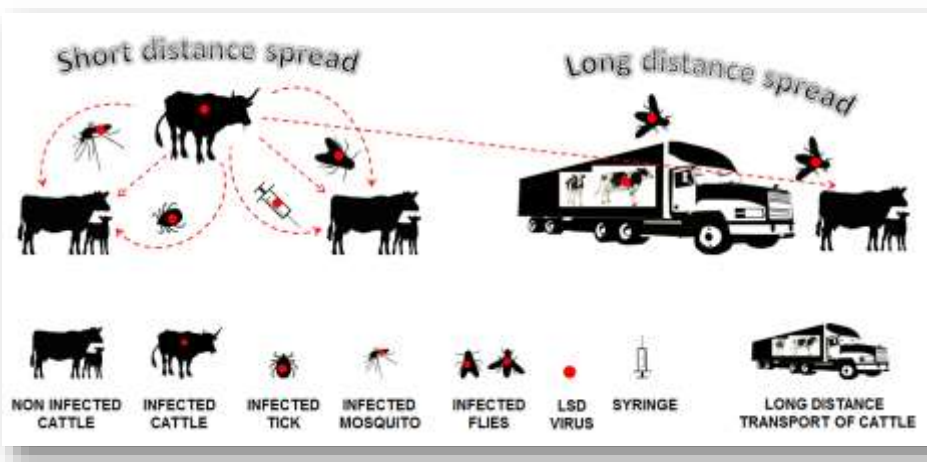
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## Clinical signs (1)

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- ✓ Incubation time varies from 4-7 days up to 5 weeks
- ✓ High fever (40-41°C), stop eating and giving milk – start of viraemic stage
- ✓ Easily noticed in dairy cattle – not noticed in free-ranging beef cattle
- ✓ Markedly enlarged lymph nodes (particularly prescapular and precrural)
- ✓ Skin lesions start to develop following days - often in many animals at the same time
- ✓ Excessive salivation, eye and nasal discharge due to the ulcerative lesions inside the mouth, also in nasal and ocular mucous membranes
- ✓ Later swellings in the leg and lameness may be detected
- ✓ Oedema of the dewlap



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## Clinical signs (2)

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- ✓ Notice that not all affected animals show clinical signs although majority of them develop at least short-lasting viraemia
- ✓ Circular skin lesions of 1 to 5 cm in diameter (sometimes larger)
- ✓ Mild cases may show only a few lesions or lesions may cover the entire body in severely infected animals
- ✓ Within one to two weeks the top of the lesion forms a scab which then sloughs off, leaving a raw ulcer, prone to fly strike
- ✓ In some cases, the lesions remain for long (sitfasts)



Train-of-trainer program f













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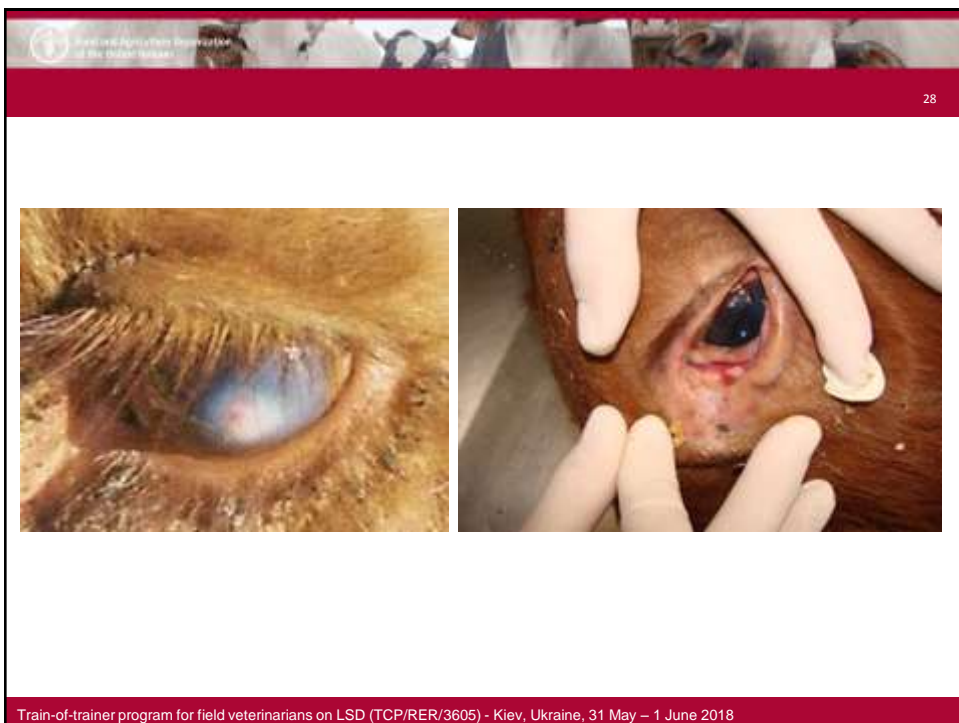


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## Differential diagnosis

- ✓ Pseudo lumpy skin disease; BHV-2 (Bovine herpes virus) - more superficial lesions and shorter course of the disease
- ✓ Parapox lesions (bovine papular stomatitis) in the mucous membranes of the mouth
- ✓ Insect bites and allergic reactions (urticaria)
- ✓ Early ringworm lesions – often ringworm gets worse during LSD infection
- ✓ Demodicosis
- ✓ Besnoitiosis (widely distributed in Africa, recently also in central and western Europe)
- ✓ Onchocerciosis

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## Active virological and serological surveillance

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- ✓ Monitoring susceptible cattle population at high- or low-risk regions to detect evidence of infection with LSDV
- ✓ Requires sufficient laboratory capacities to carry out basic diagnostic tests that can identify LSD antigen and antibodies
- ✓ Following molecular methods are required
  - General CaP viral DNA detecting real-time or conventional PCR
  - Differentiation between sheeppox, goatpox and LSD virus
  - Differentiation between virulent and vaccine strains
- ✓ Serological tools include serum and virus neutralisation assays, ELISA, IPMA

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## Virological testing

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- ✓ Viremia lasts approximately 4 to 12 days but low levels
- ✓ EDTA blood samples
- ✓ Saliva samples (swabs) are excellent sample material
- ✓ Only 40 to 50% of experimentally infected animals develop generalised skin lesions
- ✓ Molecular tests can differentiate the vaccine from the field strain

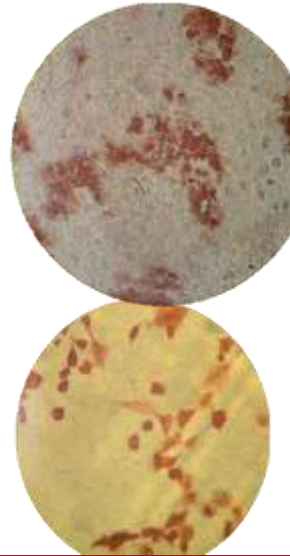
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## Serological tests available

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- ✓ Serum/virus neutralisation assays – gold standard test
- ✓ Immunoperoxidase monolayer assay (IPMA)
  - OA3.Ts cells (Babiuk et al., 2007) + IPMA staining
  - In-house assay used by Coda Cerva (EU reference laboratory)
- ✓ Good specificity and reasonable sensitivity
- ✓ Too labour- and time-consuming and slow assays
- ✓ High biosecurity level laboratory facilities are required as working with a live virus and cell cultures is involved
- ✓ New ELISA now available



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### ID Screen® Capripox Double Antigen Multi-species

Multi-species Double Antigen ELISA for detection of antibodies against capripoxviruses including Lumpy skin disease virus (LSDV), sheepox virus (SPPV) and goatpox virus (GTPV) in serum or plasma from cattle, sheep, goats or other susceptible species

- ✓ Major improvement for sero-surveillance at high-risk regions
- ✓ Limited to non-vaccinated population
- ✓ Support demonstration of the disease-free status
- ✓ Allows large-scale testing
- ✓ Does not require high-containment laboratory facilities nor working with infectious virus and cell cultures
- ✓ Better sensitivity than neutralization test
- ✓ Test was validated by CODA CERVA and FLI in Germany

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## Sero-surveillance

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- ✓ Immunity against LSDV is humoral and cell-based
- ✓ Infected animals develop antibodies mainly in the early stages of infection
- ✓ Antibodies against LSDV can usually be detected approximately 6 months post-outbreak after which the levels will gradually decrease below the detectable levels
- ✓ Critical points:
  - It is currently not possible to differentiate LSDV-infected from vaccinated animals serologically
  - Not possible to differentiate between members of CaPV group
  - Neither neutralization tests nor IPMA are suited to high-throughput antibody detection
  - The humoral serological response is highly variable at individual level
  - Exact duration of the humoral response is not known

## In presence of clinical signs

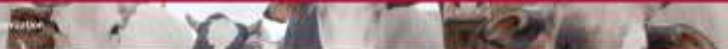
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- ✓ Sampling for virological testing
  - Blood samples - EDTA blood
  - skin lesion (nodules) (39 days pi)
  - saliva (12-18 days pi)
  - nasal and ocular fluids/swabs (12-21 days pi)
  - milk (unknown)
  - semen (42 days pi)
- ✓ Serum for serological testing





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# Thank you for the attention!

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