Sheep and Goat Pox Viral Diagnostics and Vaccine Development Collaboration

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Etiology
The Capripoxvirus Group

LUMPY SKIN DISEASE

Limited Host Range

No Known Wildlife Reservoir

SHEEP POX

GOAT POX

97% similar at the genetic level
Capripoxvirus have no serotypes
History of capripox

- Sheep pox and goat pox are old diseases being observed in ancient times
- In 1929 a disease, presumably LSD, termed pseudo-urticaria was encountered in Zambia
- LSD recognized as an infectious disease in 1943 and rapidly spread throughout Africa despite enforced control measures. Measures were unsuccessful do vector spread.
- Sheep pox, goat pox and lumpy skin disease are foreign animal diseases in Canada
- Current OIE status
  - OIE listed diseases
Epidemiology
sheep pox and goat pox

- **Transmission**
  - Contact with infected animals
  - Aerosol transmission
  - Contact with infected wool or bedding
  - Insect vectors biting flies mosquitoes likely can act as vectors but it is not proven
  - Virus is stable in the environment for weeks

- **Carrier state**
  - There is no carrier state with sheep and goat pox
Epidemiology of lumpy skin disease

- **Transmission**
  - Mode of transmission has not been established fully but biting insects are believed to play a major role
  - Not infectious without the vector

- **Influences affecting transmission**
  - Spread along watercourses and during the wet season
  - Periodic epidemics occur in most African countries
Geographic range of sheep and goat pox
Geographic range of lumpy skin disease
Diagnostic tests for capripoxvirus

- Tools for diagnostics were not well developed
- Primary LK or LT cells are currently used for diagnostic tests
- Disadvantages of primary cells are
  - variations between cell lots
  - experimental cell artifacts that can be misinterpreted as CPE
Characterization of cells for capripoxvirus isolation

![Graph showing TCID\textsubscript{50}/50\mu l (Log\textsubscript{10}) over OA3.Ts passage number]

- OA3Ts
- LKS
Virus isolation of poxviruses

A Uninfected OA3.Ts immunostained using anti-capripox sheep sera.

B Capripox-infected OA3.Ts immunostained using anti-capripox sheep sera.

C Orf virus–infected OA3.Ts immunostained with polyclonal anti-orf sheep sera.
Species specificity of sheep and goat pox

- Different isolates can behave differently in sheep and goats, with most isolates more virulent in either sheep or goats, while others are pathogenic in both species.
- This issue of naming strains SPPV, GTPV or sheep and goatpox virus remains problematic since historically the virus nomenclature has been based on field observation of the host species affected.
- There is little else to support the species strain designation except when the viruses are used to experimentally infect both hosts under controlled conditions.
Species specificity of sheep and goat pox

Clinical disease varies between the virus isolate and the host
Disease can range from high mortality to very mild disease
Viral shedding of Yemen isolate in sheep and goats

Viral shedding occurs at the time of clinical disease and can last until disease resolution. Depends on the virus isolate and host.
Viral shedding of Vietnam isolate in sheep and goats
Viraemia in capripoxvirus-infected sheep and goats

Viraemia occurs in sheep pox and goat pox at the onset of clinical disease. Depends on the virus isolate and host.
Conclusions

- Higher viral shedding is associated with more severe disease
- More tissues are positive for virus in animals with severe disease
- Different capripoxviruses have a different host preferences
- Pathogenesis varies depending on the host and virus
Gross lesions following experimental infection in sheep and goats

(A) Primary lesion on the lateral thorax of a sheep at DPI 4.
(B) Multifocal papular exanthem on the lateral chest of a goat at DPI 8.
(C) Multifocal ulcerative enanthem in the soft palate of a goat at DPI 15.
(D) Multifocal nodular pneumonia in a goat at DPI 11.
(E) Multifocal nodular enanthem in the rumen mucosa of a goat at DPI 13.
(F) Multifocal nodular lesions in the liver of a goat at DPI 13.
Goat skin lesion 6 DPI

Immunohistochemical staining for capripoxviral antigen shows positive cytoplasmic staining in epidermal epithelial cells with cytoplasmic inclusion bodies (arrow) as well as in SPC’s throughout the dermis (arrowhead).
Positive immunolabelling for capripoxviral antigen is observed in the cytoplasm of large histiocytic-like cells (SPC’s) located within alveolar septa (arrows). Bar = 20 µm.
LSD in experimentally infected cattle

Gross pathology of tissues from lumpy skin disease virus-infected cattle. Skin nodules presented at DPI 14 (a) and DPI 15 (b and c). Gross pathology of the rumen collected at DPI 9 (d). DPI, days post-inoculation.
Current serological tests

- Virus neutralization testing is the only accepted test
- This test is laborious and requires six days for results
- Hence there is a need to develop an ELISA for capripoxvirus
Antibody detection
Cloning & expression of recombinant antigens

- **Background**

Eight full-length capripoxvirus genome sequences were available in GenBank

42 of 156 possible ORFs selected

- 36 of 147 common to SPPV, GTPV & LSDV
- 6 of 9 specific to LSDV
- Priority given to major structural & surface antigens
- All 42 ORFs inserted into prokaryotic vectors (Gateway)
- Expressed in *E coli* as N terminal 6xHis tag constructs

Preliminary screening identified 4 candidate antigens, 2 of which were readily purified by affinity chromatography
Purification of recombinant proteins

Enzyme immunoassay

- TMB substrate
- Rec protein G-HRP
- Ab with specificity to coating Ag
- S095 and S103
Preliminary estimates of DS\textsubscript{n} and DS\textsubscript{p}

Sheep experimental sera (1:400)

Vaccinated
DS\textsubscript{n}: 14.3\% (4.9-30.3\%)
DS\textsubscript{p}: 94.7\% (92.1-96.6\%)

Infected
DS\textsubscript{n}: 97.1\% (85.0-99.5\%)
DS\textsubscript{p}: 94.7\% (92.1-96.6\%)

Preliminary estimates of DSn and DSp
Goat experimental sera (1:400)

Vaccinated
DSn: 11.4% (3.3-26.8%)
DSp: 95.1% (91.6-97.5%)

Infected
DSn: 94.7% (73.9-99.1%)
DSp: 95.1% (91.6-97.5%)
Preliminary estimates of DSn and DSp
Sheep field sera (Mongolia); IAH (1:400)

DSn: 100.0
DSp: 88.9

Percent Positivity

VNT Negative (n = 9)  VNT Positive (n = 11)

>11.6
DSn: 100.0
DSp: 88.9
Preliminary estimates of DSn and DSp
Goat field sera (Mongolia); IAH (1:400)

DSn: 100.0
DSP: 90.9

>19.1

Percent Positivity

VNT Negative (n = 11)  VNT Positive (n = 19)
Cattle experimental sera
IAH (1:200)

Percent Positivity vs. DPI

- Calf 83
- Calf 84
Preliminary estimates of DS\(n\) and DS\(p\)

Cattle field sera (Ethiopia); NCFAD (1:400)

\[ \text{Sn: 4.6} \quad \text{Sp: 96.5} \]

> 52.7

VNT Negative (n = 286)  VNT Positive (n = 1111)
Summary
Serology based on recombinant antigens

- Analytical sensitivity and specificity appear acceptable in sheep and goats following experimental infection with virulent wild type viruses, but is poor following vaccination.
- Although the analytical sensitivity in experimentally infected cattle appears acceptable, the diagnostic sensitivity is particularly low in naturally infected cattle.
- The reason for the marked difference in the performance characteristics observed for sheep and goat versus cattle sera is not known.
  - Are antibodies against 095 and 103 relatively short lived?
  - Are these antigens less immunogenic in cattle?
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