First Molecular Characterization of Poxviruses in Cattle, Sheep, and Goats in Botswana

Boitumelo Magret Modise (✉ boikensmod@gmail.com)

NVL: National Veterinary Laboratory <https://orcid.org/0000-0002-6651-3373>

Tirumala Bharani Kumar Settypalli

Animal Production and Health laboratory. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Tebogo Kgotlele

NVL: National Veterinary Laboratory

Dingrong Xue

Academy of National food and Strategic Reserves and Academicsadministration: Livsmedelsverket

Kebonyemodisa Ntesang

NVL: National Veterinary Laboratory

Kago Kumile

NVL: National Veterinary Laboratory

Ivancho Naletoski

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

John Frederick Nyange

NVL: National Veterinary Laboratory

Carter Thanda

NVL: National Veterinary Laboratory

Kenny Nametso Macheng

NVL: National Veterinary Laboratory

Chandapiwa Marobela-Raborokgwe

NVL: National Veterinary Laboratory

Gerrit Johannes Viljoen

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Giovanni Cattoli

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Charles Euloge Lamien

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Keywords: Lumpy skin disease virus, Pseudocowpox virus, Orf virus, RPO30, GPCR, B2L gene, Botswana

Posted Date: April 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-419694/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Virology Journal on August 14th, 2021. See the published version at <https://doi.org/10.1186/s12985-021-01634-9>.

Abstract

Background

Poxviruses within the Capripoxvirus, Orthopoxvirus, and Parapoxvirus genera can infect livestock, with the two former presenting also zoonotic importance. In addition, they induce similar clinical symptoms in common host species, creating a challenge for diagnosis. Although endemic in the country, poxvirus infections of small ruminants and cattle have received little attention in Botswana, and there was no prior attempt of using molecular tools to diagnose the diseases and characterize the pathogens.

Methods

A high-resolution melting (HRM) assay was used to detect and differentiate poxviruses in samples from four cattle (from Mahalapye, Kasane, and Molepolole), one sheep (from Jwaneng), and one goat (from Kasane). Molecular characterization of capripoxviruses and parapoxviruses was undertaken by sequence analysis of RPO30 and GPCR genes.

Results

The HRM assay revealed lumpy skin disease virus (LSDV) in three cattle samples, pseudocowpox virus (PCPV) in one cattle sample, and orf virus (ORFV) in one goat and one sheep sample. The phylogenetic analyses, based on the RPO30 and GPCR, and the inspection of the multiple sequence alignments showed that the LSDV sequences of Botswana were more like those of common LSDV field isolates encountered in Africa, Asia, and Europe. The Botswana PCPV presented unique features and clustered between camel isolates and cattle isolates of the PCPV group. The Botswana ORFV from goat differed from the ORFV collected in sheep.

Conclusions

This study is the first report on the genetic characterization of pox virus diseases circulating in cattle, goats, and sheep in Botswana. It shows the importance of molecular methods to differentially diagnose pox virus diseases of ruminants.

Background

Poxviruses exist throughout the world, and are responsible for several economically significant and zoonotic diseases affecting human beings as well as wildlife and domestic animals(1, 2). Generally, poxviruses are epitheliotropic and may cause localized cutaneous lesions or generalized lesions involving the skin as well as many organs and tissues (mucosal part of the respiratory and digestive tracts, lungs and kidney). Lesions on the skin of the affected animals can be clinically confused with other cutaneous diseases. Economically, pox virus diseases cause losses due to damage to the skin, decreased milk and meat production and trade restriction in addition to morbidity and mortality (3, 4).

The poxviruses are complex, linear, enveloped, double-stranded DNA viruses with large genomes of 130 – 360 kb in length (5, 6). They belong to the *Poxviridae* family, which is divided into two subfamilies: *Entomopoxvirinae*, which infect insects, and *Chordopoxvirinae*, which infect vertebrates (7). The Chordopoxvirus subfamily comprises ten genera: Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, Cervidylipoxviruses, Crocodylipoxviruses, and Yatapoxvirus (8).

The genera among the *Chordopoxvirinae* that affect livestock (especially the ruminants) are Orthopoxvirus -cowpox virus (CPXV) and vaccinia viruses (VACV)-, Capripoxvirus -goatpox virus (GTPV), sheeppox virus (SPPV), and lumpy skin disease virus (LSDV)-, and Parapoxvirus – Orf virus (ORFV), Pseudocowpox virus (PCPV), and bovine papular stomatitis virus (BPSV)-. Some of these poxviruses have similar geographical distribution. They also can infect the same animal species (i.e., ORFV, SPPV, and GTPV cause generalized or localized cutaneous lesions in both sheep and goats), creating a challenge for the clinical diagnosis of these diseases.

Poxviruses are transmitted through several routes such as small abrasions of the skin (e.g., orf), or directly or indirectly from a contaminated environment by aerosol infection through the respiratory tract (e.g., sheeppox and goatpox), or possibly by mechanical transmission by biting arthropods (e.g., lumpy skin disease and sheeppox).

In Botswana, pox virus diseases in ruminants seem to be receiving little or no attention. The first publication on pox virus diseases in Botswana was on orf virus in goats (9), without molecularly characterizing the virus. Lumpy skin disease was first observed in 1943 following an outbreak in Ngamiland in northern Botswana. With years, the disease has spread virtually to all the districts in the country (10). In Botswana, LSD is controlled through vaccination with live attenuated vaccines; LSDV Neethling strain (Onderstepoort Biological Products; OBP, South Africa) and attenuated South African LSDV field isolate (Lumpyvax, MSD Animal Health-Intervet, South Africa). Even though LSD has been endemic and widespread in Botswana for many years, there is no genetic information available on LSDVs circulating in Botswana.

This study reports the first confirmed case of pseudocowpox in Botswana, and the first molecular characterization of LSDV, ORFV, and PCPV in the country.

Methods

Study areas and sample collection

The study was conducted using four archived clinical samples collected from 2010, 2016, 2017 outbreaks, and two samples from a recent outbreak in 2019. These samples were from different regions of Botswana (Figure 1). The samples included three skin biopsies, one skin crust, one lip skin scab as well as one wart. Epidemiological information on the clinical signs and attempted clinical diagnosis were

collected from sample submission forms that accompanied the samples when they were submitted to Botswana National Veterinary Laboratory (BNVL).

Sample preparation and DNA extraction

The samples were cut with a scalpel blade into small pieces and homogenized (10 % w/v) in sterile phosphate buffered saline (PBS), then centrifuged at 2500 rpm for 10 min to collect the supernatant. DNA was extracted from 200 µl of the supernatant using DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions. The DNA was eluted in 80 µl elution buffer, then kept at -20 °C until further use.

Molecular detection and genotyping

The extracted DNA was tested using an HRM multiplex real-time assay for the simultaneous detection and differentiation of eight poxviruses of medical and veterinary importance (11). The method can detect and differentiate members of three different genera of poxviruses *Orthopoxvirus*, *Capripoxvirus*, and *Parapoxvirus* and provides additional genotyping of the viruses within each of the three genera: CPXV and CMLV in the *Orthopoxvirus* genus; GTPV, SPPV, and LSDV in the *Capripoxvirus* genus; ORFV, PCPV, and BPSV in the *Parapoxvirus* genus.

The PCR was set up in a 20 µl reaction volume containing 1x SsoFast™ EvaGreen® Supermix (Bio-Rad), equal concentration (200 nM) of each of the forward and reverse primers (Table 1), and 2 µl of sample DNA. Each run included positive control plasmids representing each of the eight pathogens, and a negative control comprising nuclease-free water. The PCR reactions and melting curve analysis were performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories), as earlier described with slight modifications (11). Briefly, an initial denaturation step at 95 °C for 4 min, followed by 40 cycles at 95 °C for 1 s, 59 °C for 5 s and 70 °C for 5 s. The PCR products were then denatured at 95 °C for 30 s, cooled down at 65 °C for 60 s, and melted from 65 °C to 85 °C with an increment of 0.2 °C every ten seconds and a continuous data acquisition. Data was analyzed using the CFX Maestro Software (Bio-Rad) and the Precision Melt Analysis Software (Bio-Rad).

Sequencing

The G-protein-coupled chemokine receptor (GPCR) (12), and the 30 kDa DNA-dependent RNA polymerase subunit (RPO30) (13) genes of the *Capripoxviruses* were amplified using primers (14) in Table 1. The PCR reaction was performed in a reaction volume of 20 µl containing 500 nM of each of the forward and reverse primers, 0.2 mM of dNTPs, 1x buffer (Qiagen), 2.5 U of Taq DNA polymerase (Qiagen), and 2 µl template DNA. The amplification consisted of an initial denaturation at 95 °C for 4 min followed by 35 cycles of 95 °C for 40 s, 55 °C for RPO and 56 °C for GPCR for 30 s, and 72 °C for 1 min, and a final extension step at 72 °C for 7 min.

For the *Parapoxviruses*, the partial B2L gene was amplified by PCR in a reaction volume of 25 µl containing 500 nM of each of the primers (15) in Table 1, two mM dNTPs, 1X PCR Buffer (Qiagen), 2.5 U

Taq polymerase (Qiagen) and five µl template DNA. The cycling conditions were: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 50 s, 52 °C for 60 s and 72 °C for 90 s, and a final extension at 72 °C for 7 min.

All PCR products were separated by electrophoresis on a 1.5 % agarose gel at 100 V for 1 hr. The positive PCR products were purified using the Wizard SV Gel and PCR clean-up system kit (Promega) according to the manufacturer's instructions, then sequenced commercially by LGC Genomics (Germany). The sequences were edited and assembled using Vector NTI Advance™11.5 software (Invitrogen, Carlsbad, CA, USA). All sequences were submitted to GenBank.

Phylogenetic analysis

For phylogenetic reconstructions of the RPO30, GPCR, and B2L gene trees, multiple sequence alignments of the nucleotides sequences were performed separately for each gene using the muscle algorithm and the codon option implemented in MEGA7 (16). Additional sequences of the RPO30 and GPCR gene for CaPVs (LSDVs, GTPVs, and SPPVs) and the B2L gene for parapoxviruses (ORFV, PCPV, and BPSV), were retrieved from GenBank and included for comparative analyses.

The sequence alignment FASTA file was converted into a Nexus format file using Seaview programme version 4.7 (17). The Bayesian phylogenetic inference was performed with BEAST. First, the BEAUti module was used to generate BEAST files using the TN93 +G nucleotide substitution and a UPGMA starting tree. The Markov Chain Monte Carlo method was run with BEAST for 10,000,000 generations with a sample taken each 10,000 generations. The TRACER program was used to inspect the log files and determine the optimum number of burn-in based on the Effective Sample Sizes (ESS > 200). TreeAnnotator was used to generate the Maximum Clade Credibility (MCC) after discarding the 2% burn-in. The tree was visualized with the associated meta-data using the ggtree package in R version 3.5.2 (18). Additionally, for the GPCR tree, the multiple sequence alignment file of the nucleotide sequences was imported and a slice of the alignment, between positions 80 and 120, was visualized together with the tree (18).

Results

Epidemiological information on the clinical signs and attempted clinical diagnosis

It appeared that most animals had lesions suggestive either of poxvirus infections, and sometimes, papilloma virus infections (Table 2).

Molecular detection

As summarised in table 2, PCPV DNA was detected in one sample (BOT_BOV/2010/6389) and LSDV DNA in three samples (BOT_BOV/2016/172, BOT_BOV/2017/1657, and BOT_BOV/2019/246) from cattle using the HRM real-time PCR assay. ORFV DNA was detected in sheep and goat samples, respectively

(BOT_OV/2017/158 and BOT_CAP/2019/74), and Orthopoxviruses DNA was absent in the samples. Figure 2 shows the melting peaks corresponding to PCPV, LSDV, and ORFV in the Botswana samples.

Sequencing

Two fragments for the RPO30 gene (554 bp and 520 bp) and three fragments for the GPCR (617 bp, 603 bp, and 684 bp) were successfully amplified and sequenced in all the three LSDV positive samples (BOT_BOV/2016/172, BOT_BOV/2017/1657, and BOT_BOV/2019/246). Similarly, approximately 1210 bp fragment of the B2L gene was amplified and sequenced in the remaining three samples that were positive for PCPV (BOT_BOV/2010/6389) and ORFV (BOT_OV/2017/158, and BOT_CAP/2019/74). The complete RPO30 and GPCR genes and partial B2L gene sequences were submitted to the GenBank database under accession numbers MW748471 to MW748479.

Molecular characterization and phylogenetic analysis

In both GPCR and RPO30 gene trees, all three cattle samples from Botswana (BOT_BOV/2016/172, BOT_BOV/2017/1657, and BOT_BOV/2019/246) clustered within the LSDV group (Figures 3 and 4). On the GPCR gene tree, the LSDV group was further subdivided into two subgroups: the first subgroup included the LSDVs from Botswana, and field LSDVs from Europe, the Middle East and Africa, and LSDV KS1 derived vaccinal viruses. The second subgroup consisted of LSDV Neethling-like viruses, and the historical LSDV Haden 1959 from South Africa (Figure 3).

The multiple sequence alignments of the GPCR gene showed that all the Botswana LSDVs had sequences identical to each other, and presented a 12-nucleotide deletion found in common field LSDVs (Figure 3).

On the RPO30 gene tree (Figure 4), LSDV isolates produced 3 sub-groups: the first one consisted of the Botswana LSDVs and common field LSDVs encountered in Africa, the Middle East and Europe. The second subgroup contained LSDV KS1-like viruses and LSDV NI-2490, while the third consisted of LSDV Neethling like viruses and the historical LSDV Haden 1959 (Figure 4).

The RPO30 gene sequences were identical for all the Botswana LSDVs and identical to common field isolates, but different from the the vaccinal strains derived from KS1 and Neethling viruses. The Botswana isolates differed from LSDVs from Sudan (GU119938) and (GU119944) by a single non synonymous nucleotide difference leading to amino acid substitutions at one position (T¹⁴®N and D¹⁰²®G) respectively.

On the phylogenetic tree of the B2L gene, the sequence of sample BOT_BOV/2010/6389 clustered with PCPVs (Figure 5). Within the PCPV group, BOT_BOV/2010/6389 clutered between the B2L sequences of camel isolates and those of cattle/reindeer isolates. The B2L sequences of the two samples BOT_OV/2017/158 and BOT_CAP/2019/74, collected from small ruminants, clustered within the ORFV group (Figure 5).

The BLAST results showed that the nucleotide identities of the Botswana PCPV, BOT_BOV/2010/6389, against published parapoxviruses ranged from 98.63 %-98.97 % with the highest identity (98.97 %) to PCPV strains strain 3/07 (KF478804) isolated from cattle in Germany, and strain VR634 (GQ329670) collected from man infected by cattle in New Zealand.

The B2L sequence of ORFV BOT_CAP/2019/74 was 100 % identical to that of strain OV-SA00 (AY386264) isolated from a goat in the USA. The multiple sequence alignments of the B2L gene revealed 24 nucleotide changes between the B2L gene of BOT_CAP/2019/74 and BOT_OV/2017/158.

Discussion

This study presents the first confirmed case of PCPV in Botswana, and the first molecular characterization of poxviruses affecting sheep, goats and cattle in the country, from animals presenting clinical lesions consistent with poxvirus infections. The DNAs from Lumpy skin disease virus (3 samples), PCPV (1 sample), and ORFV (2 samples) were detected in archived clinical samples collected in 2010, 2016, 2017 outbreaks, and samples from two recent outbreaks in 2019 using an HRM assay for the differential diagnosis of poxvirus infections (11).

It is worth noting that the status of the 2010 sample was previously undetermined, until the availability of this HRM assay which showed the presence of PCPV. Hence, the HRM assay has enabled the correct identification of LSDV and PCPV in cattle and ORFV in small ruminants in a single test as previously stated (11).

The attempted clinical diagnosis of the pox virus diseases were mostly consistent with the results of the HRM assay for LSDV and ORFV. The LSD suspected cattle had typical clinical signs that included skin nodules/bumps/lesions (19, 20) and orf suspected small ruminants had papilloma warts on the udder and/ or lesions on and around the mouth and buccal cavity as described earlier (21, 22). However, in this study, the lesions described for the PCPV positive animal were mostly suggestive for papillomavirus infections, though, the lesions on the udder of cattle are also common in pseudocowpox infections (23, 24).

For all samples, the sequencing data were in full agreement with the HRM data. There was no sequence difference in the RPO30 and GPCR genes of Botswana LSDV samples collected in three separate outbreaks, in 2016, 2017 and 2019, at 3 different geographic locations (Mahalapye, Kasane, and Molepolole), suggesting that the virus is well conserved and that the same strain is responsible for all these outbreaks.

Moreover, the Botswana LSDVs sequences presented similar features to common field isolates of LSDV encountered in Africa, Europe and Asia, including the presence of the 12-nucleotide deletion in their GPCR gene (12, 14, 25-27).

The existence of PCPV in cattle has been reported worldwide, including Japan (28, 29), USA (30), Brazil (31) and Germany (32). In Africa, the disease has recently been reported in Zambia (33) and now in Botswana (this study). Interestingly, the phylogenetic analysis revealed that the newly sequenced PCPV from Botswana was distinct from common cattle PCPVs encountered elsewhere, behaving like an intermediate isolate between cattle isolates of PCPV and camel isolates of PCPV (known as CCEV). This finding is uncommon, as most isolates recovered from cattle usually clustered with the cattle/reindeer isolates. For instance, PCPVs that were recently reported in Zambia presented substantial sequence variation within the same herd and during the same outbreak events in 2017 and 2018, but were all clustering with the cattle isolates of PCPVs (33). The unique genetic feature of the Botswana PCPV suggests that by the time the outbreak occurred in 2010, PCPV was already well established in the country.

The path of introduction of the PCPV strain to Botswana is unknown and needs to be further investigated. Further genetic analysis of the PCPV strain is required to determine if it is genetically distinct enough to be classified as cattle and reindeer isolate or camel variant of PCPV virus.

The phylogenetic tree based on the B2L gene sequence, and the scrutiny of the multiple sequence alignment showed that the sample collected in sheep in 2017 in northern Botswana differed from that of the sample collected in goat in 2019 in the southern part of the country, suggesting that at least two different strains of ORFV are circulating in the country.

The caprine ORFV B2L sequence was fully like a sequence from an ORFV OV-SA00 (AY386264) collected from a goat in the USA, in 2000. The ovine ORFV sequence differed from most ovine sequences, but displayed 99.6 % amino acid similarity level to isolate FJ-ZX (KC568400) collected from a goat in China.

Conclusion

This paper reports the first molecular detection and characterization of pox virus diseases circulating in sheep, goat and cattle in Botswana. It shows the importance of molecular methods for the differential diagnosis of pox virus diseases in ruminants (the presence of LSDV and PCPV in cattle and ORFV in small ruminants). As PCPV is a well-known zoonotic disease transmissible through direct contact (e.g. milkers nodules), the information provided here is also of public health relevance.

Abbreviations

BNVL – Botswana National Veterinary Laboratory

BPSV – Bovine papular stomatitis virus

CMLV – Camelpox virus

CPXV – Cowpox virus

DNA – Deoxyribonucleic acid

GTPV – Goatpox virus

HRM – High resolution melting

LSDV – Lumpy skin disease virus

MCC – Maximum Clade Credibility

ORFV – Orf virus
PBS – Phosphate buffered saline

PCR – Polymerase chain reaction

PCPV – Pseudocowpox virus

PPV – Parapoxvirus

SPPV – Sheeppox virus

Declarations

Ethics approval and consent to participate

Samples used were for routine diagnosis at the Botswana National Veterinary Laboratory.

Consent for publication

The study was approved for publication by the Ministry of Agricultural Development and Food Security, Botswana.

Availability of data and materials

DNA sequences generated and analyzed under the current study are available in GenBank under accession numbers MW748471, MW748472, and MW748473 (B2L gene), MW748474, MW748475, and MW748476 (RPO30 gene), MW748477, MW748478, and MW748479 (GPCR gene).

Competing interests

The authors declared no conflicts of interest.

Funding

This study was supported by VETLAB network initiative of the Joint FAO/IAEA Division, funded through the African Renaissance and International Cooperation fund of South Africa and the Peaceful Uses Initiatives (PUI) by Japan and the United States of America.

Authors' contributions

Conceived and designed the study: BMM, and CEL.

Performed the experiments: BMM, TBKS, TK, DX, KN, KK, JFN, CT.

Analyzed the data: BMM, DX, CEL.

Contributed reagents/materials/analysis tools: IN, GJV, GC, CEL.

Wrote the paper: BMM.

Supervised the study: KNM, CMR, GJV, GC, CEL.

Edited the final manuscript: TBKS, JFN, CMR, GJV, GC, CEL.

All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the Veterinary field personnel, and the Disease Control Unit for the collection of samples from the field.

References

1. Brettschneider H, Voster JH, Lane EP, Van Wilpe E, Biden P, Dalton DL, et al. Molecular detection of Yaba monkey tumour virus from a vervet monkey. *J S Afr Vet Assoc.* 2013;84(1):1-5.
2. Moss B. Poxviridae. In: Knipe DM, Howley PM, Cohen JI, Griffin DE LR, Martin MA, Rancaniello VR, et al., editors. *Fields virology*. 2. 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. p. p 2129–59.
3. Gambo P, Maguda A, Adole J, Dyek D, Ifende V, Bot C, et al. A survey of viral diseases of livestock characterized by skin lesions in Kanam Local Government Area of Plateau State, Nigeria. *Nig Vet J.* 2018;39(3):250-62.
4. Babiuk S, Bowden TR, Boyle DB, Wallace DB, Kitching RP. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. *Transbound Emerg Dis.* 2008;55(7):263-72.
5. Stanford MM, McFadden G, Karupiah G, Chaudhri G. Immunopathogenesis of poxvirus infections: forecasting the impending storm. *Immunol Cell Biol.* 2007;85(2):93-102.
6. Essbauer S, Pfeiffer M, Meyer H. Zoonotic poxviruses. *Vet Microbiol.* 2010;140(3-4):229-36.
7. Maclachlan NJ, Dubovi EJ. *Fenner's veterinary virology*. 4th ed. New York: Academic press; 2010.
8. Haller SL, Peng C, McFadden G, Rothenburg S. Poxviruses and the evolution of host range and virulence. *Infect Genet Evol.* 2014;21:15-40.

9. Baipoledi E, Nyange J, Hyera J. A severe case of contagious ecthyma in Tswana goats: case report. *J S Afr Vet Assoc.* 2002;73(2):86-7.
10. OIE Handistatus II, Multiannual animal disease status, computer database [Internet]. 2016 [cited (accessed 01 August 2020)].
11. Gelaye E, Mach L, Kolodziejek J, Grabherr R, Loitsch A, Achenbach JE, et al. A novel HRM assay for the simultaneous detection and differentiation of eight poxviruses of medical and veterinary importance. *Sci Rep.* 2017;7:42892.
12. Le Goff C, Lamien CE, Fakhfakh E, Chadeyras A, Aba-Adulugba E, Libeau G, et al. Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. *J Gen Virol.* 2009;90(Pt 8):1967-77.
13. Lamien CE, Le Goff C, Silber R, Wallace DB, Gulyaz V, Tuppurainen E, et al. Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. *Vet Microbiol.* 2011;149(1-2):30-9.
14. Gelaye E, Belay A, Ayelet G, Jenberie S, Yami M, Loitsch A, et al. Capripox disease in Ethiopia: genetic differences between field isolates and vaccine strain, and implications for vaccination failure. *Antiviral Res.* 2015;119:28-35.
15. Gelaye E, Achenbach JE, Jenberie S, Ayelet G, Belay A, Yami M, et al. Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008–2013. *Virology journal.* 2016;13(1):1-12.
16. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol.* 2016;33(7):1870-4.
17. Gouy M, Guindon S, Gascuel O. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol.* 2010;27(2):221-4.
18. Yu G, Smith DK, Zhu H, Guan Y, Lam TTY. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol Evol.* 2017;8(1):28-36.
19. Wainwright S, El Idrissi A, Mattioli R, Tibbo M, Njeumi F, Raizman E. Emergence of lumpy skin disease in the eastern mediterranean basin countries. *FAO.* 2013. 2016.
20. Tageldin MH, Wallace DB, Gerdes GH, Putterill JF, Greyling RR, Phosiwa MN, et al. Lumpy skin disease of cattle: an emerging problem in the Sultanate of Oman. *Trop Anim Health Prod.* 2014;46(1):241-6.
21. Smith GW, Scherba G, Constable PD, Hsiao V, Behr MJ, Morin DE. Atypical parapoxvirus infection in sheep. *J Vet Intern Med.* 2002;16(3):287-92.
22. Nandi S, De UK, Chowdhury S. Current status of contagious ecthyma orf disease in goat and sheep – A global perspective. *Small Rumin Res.* 2011;96(2-3):73-82.
23. Büttner M, RZIHA HJ. Parapoxviruses: from the lesion to the viral genome. *J Vet Med.* 2002;49(1):7-16.

24. Sant'Ana FJd, Leal FA, Rabelo RE, Vulcani VA, Moreira Jr CA, Cargnelutti JF, et al. Coinfection by Vaccinia virus and an Orf virus–like parapoxvirus in an outbreak of vesicular disease in dairy cows in midwestern Brazil. *J Vet Diagn Invest.* 2013;25(2):267-72.
25. Ochwo S, VanderWaal K, Ndekezi C, Nkamwesiga J, Munsey A, Witto SG, et al. Molecular detection and phylogenetic analysis of lumpy skin disease virus from outbreaks in Uganda 2017–2018. *BMC Vet Res.* 2020;16(1):1-10.
26. Sprygin A, Babin Y, Pestova Y, Kononova S, Wallace DB, Van Schalkwyk A, et al. Analysis and insights into recombination signals in lumpy skin disease virus recovered in the field. *PLoS One.* 2018;13(12):e0207480.
27. Agianniotaki EI, Tasioudi KE, Chaintoutis SC, Iliadou P, Mangana-Vougiouka O, Kirtzalidou A, et al. Lumpy skin disease outbreaks in Greece during 2015–16, implementation of emergency immunization and genetic differentiation between field isolates and vaccine virus strains. *Vet Microbiol.* 2017;201:78-84.
28. Ohtani A, Yokoyama A, Narushige H, Inoshima Y. First isolation and genetic characterization of pseudocowpox virus from cattle in Japan. *Virol J.* 2017;14(1):172.
29. Matsumoto H, Setoyama H, Matsuura Y, Ohtani A, Shimizu K, Okada A, et al. Sequential detection of pseudocowpox virus and bovine papular stomatitis virus in a same calf in Japan. *J Vet Med Sci.* 2019;81(3):440-3.
30. Black W, Walburger MT, Baker R, Claire O-H, Aimee R, Margo W, et al. Identification of Pseudocowpox Virus in Angus Bull with Failure to Breed. *Austin Virol Retrovirol.* 2014;1(1):05.
31. Cargnelutti JF, Flores MM, Teixeira FR, Weiblen R, Flores EF. An outbreak of pseudocowpox in fattening calves in southern Brazil. *J Vet Diagn Invest.* 2012;24(2):437-41.
32. Friederichs S, Krebs S, Blum H, Wolf E, Lang H, von Buttlar H, et al. Comparative and retrospective molecular analysis of Parapoxvirus (PPV) isolates. *Virus Res.* 2014;181:11-21.
33. Ziba MW, Chitala C, Settypalli TBK, Mumba M, Cattoli G, Fandamu P, et al. First detection and molecular characterisation of pseudocowpox virus in a cattle herd in Zambia. *Virol J.* 2020;17(1):152.

Tables

Table 1. Primers used in this study for the HRM assay and sequencing.

Method	Primer name	Primer sequence	Amplicon size	Target & References
HRM	CaPV-HRM-For	TCCTGGCATTTTAAGTAATGGT	100	Capripoxviruses (1)
	CaPV-HRM-Rev	GTCAGATATAAACCCGGCAAGTG		
	PPV-HRM-For	TCGAAGATCTTGTCCAGGAAG	112	Parapoxviruses (1)
	PPV-HRM-Rev	CCGAGAAGATCAACGAGGTC		
	OPV-HRM-For	AGGACTAGCCGCGGTAAC TTT	56	Orthopoxviruses (1)
	OPV-HRM-Rev	ACAAGATAGAAGCGATGGATACTT		
Sequencing	CpGPCR-OL1F	TGAAAAATTAATCCATTCTTCTAAACA	617	Capripoxviruses (2)
	CpGPCR-OL1R	TCATGTATTTTATAACGATAATGCAAA		
	CpGPCR-OL2F	TTAGCGGTATAATCATTCCAAATA	603	
	CpGPCR-OL2R	GCGATGATTATGATGATTATGAAGTG		
	CpGPCR-OL3F	CACAATTATATTTCCAAATAATCCAA	684	
	CpGPCR-OL3R	TGTACATGTGTAATTTTAATGTTTCGTA		
	CpRPO30-OL1F	CAGCTGTTTGTTTACATTTGATTTTTT	554	
	CpRPO30-OL1R	TCGTATAGAAACAAGCCTTTAATAGA		
	CpRPO30-OL2F	TTTGAACACATTTTATTCCAAAAAG	520	
	CpRPO30-OL2R	AACCTACATGCATAAACAGAAGC		
	ORFV-B2Lf-For	GACCTTCCGCGCTTTAATTT	1210	Parapoxviruses (3)

Table 2. Summary of clinical signs, attempted clinical diagnosis and molecular findings of the different poxviruses.

Sample ID	District	Type of samples	Case history (clinical signs)	Attempted clinical diagnosis	Molecular findings
BOT_BOV/2010/6389	Mahalapye	Skin crust, serum and w/blood	-Recumbent -Bleeding wounds on the udder, mouth, anus and legs	Papillomiosis Mineral deficiency	Pseudocowpox positive
BOT_BOV/2016/172	Kasane (Chobe)	Skin biopsy	Cattle developing skin nodules all over the body	LSD	Lumpy skin disease positive
BOT_OV/2017/158	Jwaneng	Lip skin scab, w/blood and sera	Lesions on and around mouth and buccal cavity	Orf Goitre Blue tongue	Orf positive
BOT_BOV/2017/1657	Molepolole	Skin biopsy	Cow with skin bumps and lameness	LSD	Lumpy skin disease positive
BOT_BOV/2019/246	Mahalapye	Skin biopsy, nasal swab, w/blood and serum	Lesions on the skin, nasal discharge and lacrimation	LSD	Lumpy skin disease positive
BOT_CAP/2019/74	Kasane	Udder with warts	Udder of a dead goat with papilloma warts like/ orf infection on udder	Orf Papilloma warts	Orf positive

*Animal species: Bov: bovine; Cap: caprine and Ov: ovine

*Date samples received: 2010, 2016, 2017 and 2019

Figures

Figure 1

Map of Botswana showing the location of where samples were collected (red stars). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

HRM detection of pox viral diseases in cattle, goats, and sheep samples from Botswana. The positive control for each of the eight poxviruses displayed a unique melting peak, shown in green color. One cattle and three cattle samples clustering with PCPV and LSDV, respectively, and one goat and one sheep samples clustering with ORFV are shown in purple colour.

Figure 3

Maximum clade credibility (MCC) tree based on the complete GPCR gene sequences of Capripoxviruses. Only the portion of the alignment between positions 80 and 120 is shown. The posterior probabilities are plotted as respective nodes labels. LSDVs from Botswana are highlighted in red and reference sequences are represented with their accession numbers.

Figure 4

Maximum clade credibility tree based on the complete RPO30 complete gene sequences of capripoxviruses. The posterior probabilities are plotted as respective nodes labels. LSDVs from Botswana are highlighted in red and reference sequences are represented with their accession numbers.

Figure 5

Maximum clade credibility tree based on the partial B2L gene sequences of parapoxviruses. The posterior probabilities are plotted as respective nodes labels. The cattle PCPV sequence and sheep and goat ORFV sequences from Botswana, are highlighted in blue.