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Differentiation of sheep poxvirus vaccines from field isolates and other Capripoxvirus species

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Background
The genus Capripoxvirus (CaPV) within the family Poxviridae comprises three closely related viruses, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV) causing sheep pox (SPP), goat pox (GTP) and lumpy skin disease (LSD) in small ruminants and cattle respectively. LSD has emerged in Europe in 2015 and first incursions of SPP in the European Union were reported in Bulgaria and Greece in 2013. Live attenuated SPPV vaccines are widely used in many countries to control SPP and GTP. With the increasing number of reports on SPP in previously vaccinated sheep herds, it is imperative to develop new diagnostic tools for differentiation of SPPV field strains from attenuated vaccine strains.

Objective
This work aimed at identifying appropriate diagnostic targets to develop assays for the rapid and accurate differentiation SPPV vaccine strains from SPPV field isolates and other CaPVs.

Methodology
To identify a suitable molecular target for the development of these assays, the full genomes of several SPPV vaccines strains and SPPV field isolates were compared. A unique 84-base pair nucleotide deletion located between the DNA ligase and the B22R gene was exploited to develop a gel-based PCR, and a region containing a 48bp deletion within the B22R gene of SPPV vaccines strains only, as well as species-specific nucleotide difference between SPPV field isolates, GTPV and LSDV, was targeted to develop a HRM assay.

Results
The gel-based assay was readily able to differentiate SPPV vaccines from field isolates. However, this method alone could not differentiate SPPV field isolates from GTPV and LSDV. In contrast, the HRM based method allowed the differentiation of SPPV vaccines from field isolates and further enabled the genotyping of capripoxviruses isolates. Out of 61 samples tested, we identified 4 SPPV vaccines, 14 SPPV field isolates, 11 GTPVs and 32 LSDVs. The two assays were both sensitive and specific and in agreement with the sequencing data of the tested samples.

Conclusion
The assays described herein are reliable and rapid methods for the differentiation of SPPV Vaccines from SPPV field isolates. While the gel based assay needs to be combined with capripoxvirus species-specific assays, the HRM assay stands alone as a tool to differentiate SPPV vaccines from field isolates and simultaneously genotype SPPVs, GTPVs and LSDVs. The methods are suited for routine use during outbreak investigations in both capripoxvirus enzootic and disease-free countries.

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Evaluation of Capripoxvirus surface proteins as antigens for the detection of antibodies using indirect ELISA

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Background
Lumpy Skin Disease (LSD), Sheeppox (SPP) and Goatpox (GTP) are contagious diseases of ruminants with a devastating impact on the livestock industry and trade, also affecting the living conditions of poor rural and small farmers. LSD, SPP, GTP were mainly confined to Africa, the Middle East and Asia, with some sporadic incursions of SPP in Greece and Bulgaria. However, in 2015 the first incursions of LSD occurred in the European Union. Due to their potential for rapid spreading, a highly sensitive and specific serological method for active and passive surveillance of SPP, GTP and LSD is needed. In addition, such a tool could serve for post-vaccination monitoring.

Objective
The aim of this work was to evaluate recombinants proteins of the capripoxvirus virion for use in an indirect ELISA (iELISA) to detect anti-capripox antibodies in vaccinated and naturally infected small ruminants and cattle sera.

Method
We have identified, characterized, expressed and purified capripoxvirus virion surface protein (CVSP) that react to positive SPP, GTP and LSD sera samples by Western Blot and iELISA. The iELISA was further optimised and evaluated using sera samples from vaccinated, experimentally and naturally infected sheep, goat and cattle.

Results
Twenty experimentally infected positive and 130 negative LSD, SPP and GTP sera samples were tested and correctly identified using the CVSP iELISA. We tested sera collected at multiple time points from several LSD experimentally infected animals. We observed a time dependant increased in anti-LSD antibody production after day 14 post infection. For specificity, five ORF positive sera were tested. None of the ORF positive sera was positive in the CVSP iELISA. A number of field sera collected during capripoxvirus outbreaks and vaccination campaigns from animals with known infection/vaccination status were tested by the CVSP iELISA and compared to the virus neutralization assay. The complete dataset and results of the field test evaluation will be presented.

Conclusion
As capripox diseases spread, and to strengthen disease surveillance and control programmes, there is an ever-increasing need for rapid and effective antibody detection assays. The CVSP tested in the present study demonstrated to be good antigen candidates for the development of sensitive and specific serological assays. The prototype iELISA developed has the potential to be used as an effective and rapid method for capripoxvirus antibody detection in vaccinated, experimentally and naturally infected sheep, goat and cattle sera.