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Retrovirus-induced lung lesions in the Swiss sheep population: detailed *in situ* study to improve the diagnostics of notifiable animal diseases

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Key words
MVV, CAEV, JRSV, ovine pulmonary adenocarcinoma (OPA), diagnostic, co-infections, cell tropism

Aim of the study
This study was performed to: (i) optimise the diagnostic tools for the detection of notifiable ovine lung diseases caused by the small ruminant retroviruses, namely Jaagsiekte retrovirus (JRSV), Maedi-Visna virus (MVV) and Caprine arthritis encephalitis virus (CAEV) by developing new immunohistological protocols; (ii) investigate the viral cell tropism and pathogenic processes, including co-infections and their potential interference of lesion development; (iii) identify the target cell(s) for viral infection in the lung; (iv) gain data on prevalence of ovine pulmonary viral diseases and their co-infection in Switzerland.

Material and methods
Lung and pulmonary lymph node were sampled from suspected OPA cases, inflammatory lung lesions and control lungs. Tissues were a) processed for histology and immunohistology (IH), and b) underwent DNA extraction and real time PCR. Conserved protein regions for the *in silico* prediction of B-cell epitopes were selected, followed by testing the antigenicity of peptides for JSRV, MVV and CAEV. Peptide sequences were used for customised production of polyclonal antibodies. PCR-positive OPA cases and formalin-fixed and paraffin embedded MVV- and CAEV-infected synovial cell cultures served as positive controls. Additionally, IH of the inflammatory lesions were performed with the following relevant cell markers: CD3 and CD20 for T-and B-cells respectively and Iba1 for macrophages. Pan-cytokeratin (PCK 26) as an epithelial marker, surfactant protein C (SP-C) as a marker for type II-pneumocytes, and synaptophysin as a marker for neuroendocrine cells were used in selected JSRV-positive cases. Double immunolabelling for virus antigens and relevant cell markers was performed as following: MVV and Iba1; JRSV and one of the subsequent markers: Iba1, PCK26 or synaptophysin. A morphometrical analysis considering the overall positive cell number for MVV and percentage of positive area for JRSV was performed on selected cases positive for the aforementioned viruses.

Results and significance
Approximately 50% of the investigated lungs were histologically diagnosed as OPA. Histological signs of JSRV/MVV/CAEV co-infection were detected in 27% of the lungs. JSRV was detected by qPCR in 37.5 % of the cases, 2 thereof coinfected with MVV. 3.8% of the lungs were tested positive for CAEV infection by qPCR, 3 thereof showed an MVV/CAEV co-infection. MVV was detected by qPCR in 12.5 % of the samples.
JRSV IH revealed virus antigen in the cytoplasm of neoplastic bronchial epithelial cells and in few macrophages located in the tumour stroma. JRSV infection was confirmed by IH in almost all of the lungs showing histological features of OPA and most of these animals were also tested positive by qPCR.
MVV IH revealed the presence of cytoplasmic viral antigen in interstitial macrophages and hypertrophic Type II pneumocytes. In CAEV IH, scattered bronchial epithelial cells and inflammatory cells within the alveolar septae showed the presence of cytoplasmic viral antigen.

In the investigated lymph nodes of the OPA cases, 2 animals showed epithelial cell populations (interpreted as tumour metastasis) which were PCK26 positive. Besides a parenchymal nodal detection, tumour cell emboli could also be shown in a perinodal lymph vessel, underlining the importance of lymph node assessment in this neoplastic disease.
Double immunolabelling of JSRV with PCK26 demonstrated that neoplastic bronchial epithelial cells were infected. A colocalisation of JRSV antigen within macrophages was demonstrated by double immunolabeling of JSRV/Iba1. The JRSV/synaptophysin immunolabeling did not reveal any colocalisation in neuroendocrine cells. MVV antigen was colocalized within macrophages demonstrated by MVV/Iba1 immunolabelling.
This study confirms the presence of JSRV, CAEV and MVV in single infected as well as co-infected sheep in Switzerland. These newly developed and established immunohistological protocols provide a useful tool for the detection of retroviral antigen in routinely formalin-fixed and paraffin-embedded ovine tissue. Furthermore, this technique is beneficial to investigate viral cell tropism and pathogenetic events in co-morbidities, including their interdependency.

Publications, posters and presentations
A manuscript of this study is in preparation and will be submitted to the journal “Viruses”. Furthermore, this study will be presented at the annual ESVP/ECVP meeting in Athens in September. An abstract has already been accepted by the scientific committee of this congress (Title of presentation: Evaluation of customised peptide antibodies for detection of small ruminant retroviruses).

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