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Atypical porcine pestivirus (APPV): It's presence in Switzerland and its role in the diagnosis of ruminant and porcine pestiviruses

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Key words
Pestivirus, atypical porcine pestivirus (APPV), congenital tremor, Switzerland, piglet, molecular epidemiology, nucleotide sequence, phylogenetic analysis, RT-PCR, diagnostics.

Aim of the study
The aims of this study are to (i) establish a method to detect APPV viral RNA by quantitative, real-time RT-PCR (qRT-PCR), (ii) to determine the prevalence of APPV in domestic pigs in Switzerland, iii) to determine the cross-reactivity of currently used methods to diagnose ruminant pestiviruses and CSFV, and (iv) to attempt to develop an ELISA to detect serum antibodies to the APPV strains currently circulating in Switzerland.

Material and methods
Based on APPV sequences publicly available and on a few sequences that were at our disposal at the start of the project, we designed new primers and probes in a conserved region of the 5'-UTR of the viral genome. Positive samples were partially sequenced, which later confirmed that the primer region chosen was indeed correct. Using this newly developed method, various pig sera from the Vetsuisse Faculty in Zurich and Bern and from sera stored in the BLV serum-bank stored at the IVI were analysed for the presence of APPV genome. In parallel, we developed a similar method for the detection of viral RNA of Linda virus, another porcine pestivirus recently detected in Austria. As only one sequence is available for this virus to date, primers were designed in a region conserved for this Linda virus and the related Bungowannah viruses.
Possible cross-reactivities with currently used tests for ruminant pestiviruses and CSFV were tested using *in vitro* transcribed RNA of APPV, BVDV, and CSFV that were analyzed using the newly developed assay for APPV, the currently used RT-PCR assay for CSFV and the pan-pesti RT-PCR used in the diagnostic division for ruminant pestiviruses.
Finally, for establishing a serological assay, two strategies were envisioned. On the one hand, we will develop a LIPS (luciferase immunoprecipitation system) assay based on NS3 as antigen as described [Burbelo et al. 2012, J. Virol. 86:11, 6171-6178] for equine hepaciviruses. Alternatively, we investigate a more classical format of an ELISA based on tagged-E^{rns} as antigen expressed in HEK cells as recently described by us [Lussi et al., 2018, Sci. Rep. 8, 8226]. By means of the tag, we can purify the antigen and coat it to ELISA plates for detection of antibodies to APPV-E^{rns}.

Results and significance
Results: Using the 5'-UTR region, we were able to develop a quantitative real-time RT-PCR assay. The sensitivity of the assay was high as tested using *in vitro*-transcribed RNA as template. This test was already used as diagnostic test on request for clinical cases on an informal basis, and we were able to detect a number of positive serum samples in the field in recent years. Retrospectively, we analysed several hundreds of serum samples from the years 1986, 2006, 2011, and 2015 obtained from the Vetsuisse Faculty in Zurich and the BLV serum databank. The prevalence for virus positive samples (viral RNA) in these years was between 7 and 18%, and virus positive samples were detected in farms throughout Switzerland. In 2018, samples were collected from breeding farms (in contrast to the previous years with sampling at the slaughterhouse). In this random sample, the prevalence for viral RNA was less than 1 %. Using *in vitro*-transcribed RNA of APPV, CSFV and BVDV, no significant cross-reactivities at concentrations that are relevant in the field were detected using the currently applied RT-PCR assay for APPV and CSFV. By contrast, occasional interference yielding only Ct values >34 of

APPV RNA at higher concentrations were observed in the pan-pesti qRT-PCR used in the diagnostics for ruminant pestiviruses. Conversely, no positive result of BVD viral RNA was found in the newly established APPV qRT-PCR.

Sequencing of a number of virus samples obtained from different years revealed that the virus strains circulating in Switzerland differ considerably from the isolates described to date from Asia, Europa and South and North America, further confirming our strategy to develop diagnostic tests that include the strains present in Switzerland.

Due to technical difficulties, a serological assay could not yet be developed. For a proof-of-principle, we use BVDV-antigens to establish these new methods. Currently, the expression plasmid encoding for the NS3 antigen linked to the luciferase to be used in the LIPS assay could finally be cloned. In parallel, tagged E^{rns} of BVDV was similarly expressed and purified, which will enable to perform these proof-of-principle experiments using bovine sera and commercially available serological tests for BVDV as positive controls. In addition, we already successfully expressed and purified tagged APPV E^{rns} in adequate quantities.

Significance: Our qRT-PCR assay is able to efficiently detect the APPV strains found in Switzerland and, based on sequence similarities, most likely also the majority of strains reported worldwide to date. Importantly, there is no significant cross-reactivity between the different RT-PCR assays currently used for diagnosis of ruminant pestiviruses or CSFV. Using this new assay, we showed that APPV is widely present in Switzerland already for many years (even prior to the first description of the virus in the literature 2015), and the virus prevalence is surprisingly high in young animals, whereas surviving animals obviously overcome the infection. By contrast, Linda virus could not be detected in any of the samples. Obviously, APPV has been circulating within the Swiss pig population for many decades without any entry of virus strains from abroad. As the mechanisms of virus transmission, e.g. by semen, is not yet known, testing and/or quarantining any imported animals or material is highly recommended. Despite the rather high prevalence, the true economic impact might have been neglected to date, and possible strategies for its reduction needs to be investigated.

Publications, posters and presentations

- Kaufmann C. et al. (2018) “Atypical porcine pestivirus (APPV) in Switzerland: An emerging virus?” Annual Meeting of the Swiss Society for Microbiology (SGM/SSM) in Lausanne, August 28-30, 2018 (Poster).
- Kaufmann C. et al. (2019) Long-term circulation of atypical porcine pestivirus (APPV) within Switzerland. Viruses-Basel 11:7, 653 [https://www.mdpi.com/1999-4915/11/7/653].

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