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Rapid and simple colorimetric loop-mediated isothermal amplification (LAMP) assay for the detection of bovine alphaherpesvirus 1

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Key words
colorimetric LAMP; isothermal amplification; bovine alphaherpesvirus 1; infectious bovine rhinotracheitis; rapid detection; resource-limited application

Aim of the study
As the causative agent of Infectious Bovine Rhinotracheitis (IBR) and Infectious Pustular Vulvovaginitis/Balanoposthitis (IPV/IPB), bovine alphaherpesvirus 1 (BoHV-1) is responsible for high economic losses in the cattle industry worldwide. This study aimed to establish a fast, colorimetric loop-mediated isothermal amplification (LAMP) assay for the detection of viral DNA in nasal swabs that may be a cost-efficient and fast alternative to real-time PCR in case of an acute IBR outbreak.

Material and methods
Four published and three newly designed primer sets for IBR-LAMP were compared using cell culture grown virus spiked into nasal swabs. The newly designed primer set targeting the coding region of the tegument protein V67 provided fastest and most reliable results. Subsequently, these primers were used to test different LAMP reagents and conditions. Furthermore, analytical sensitivity and specificity were evaluated using dilution series of a BoHV-1 bacterial artificial chromosome (BAC) to determine the copy number detection limit and other ruminant alphaherpesviruses such as BoHV-2 and -5, CvHV, BuHV and CpHV, respectively. Once the t protocol was established, it had to be tested with “real” samples. Since Switzerland is free of IBR, 26 positive swab samples from Scotland were imported and used to evaluate test performance. These samples were also positive for other viral and bacterial pathogens causing respiratory disease, such as bovine parainfluenzavirus-3 or Pasteurella. Finally, to test if the test could also be used as a pen-side test, e.g. under resource-limited conditions, a simplified DNA precipitation in combination with a makeshift water bath heated by a gastronomic immersion heater was used and tested with the “real” samples.
For the further evaluation of the test, a field study in collaboration with Prof. Sergio Rosati (University of Turin) was planned which included collecting nasal swabs from acute IBR outbreaks and reactivating latently infected animals with corticosteroids.

Results and significance
The Colorimetric WarmStart LAMP kit (NEB) using phenol red as read-out was chosen as reagent because it allowed visual end-point read-out (color change from pink to yellow in case of a positive reaction – visual LAMP) as well as real-time amplification measurement using a real-time PCR machine if Syto 9 Green Fluorescent Nucleic Acid Stain was added to the reaction mix (real-time LAMP). In combination with the V67 primers, amplification was visible after only 8 minutes while the other primer sets used up to 44 minutes and the real-time (q) PCR 90 minutes. The best incubation temperature was shown to be 65°C. The detection limit (analytical sensitivity) was with 14 copies per ul very low but somewhat worse than in qPCR where it was only 1.4 copies. In contrast, analytical specificity of the LAMP assay was better than the qPCR as it showed only

weak cross-reaction with CvHV-2 and BuHV-1 while qPCR reacted stronger, particularly with BoHV-5. When extracting DNA from the 26 clinical samples from Scotland using the QIAmp DNA Mini Kit, the results of the qPCR, of the visual and concurrent real-time LAMP were identical. All nine positive samples were recognised within 30 minutes incubation time by the LAMP assay independent of sample quality or CT-value and no false-positive with any negative sample was observed. Using the nasal swab medium directly in the LAMP assay did not provide reliable results. However, a simplified DNA precipitation method, requiring only an easily prepared buffer and a cheap micro-centrifuge worked well after 1:5 dilution of the DNA prior to testing. While being a little less sensitive (two weak-positive samples became indeterminate), this DNA precipitation is fast and does not require sophisticated laboratory equipment. Since LAMP does not require changes of temperature like PCR does it can be performed in a simple water bath. Combining the simplified DNA precipitation with a makeshift water bath heated by a gastronomic immersion heater (sous-vide stick) allowed successful application of the colorimetric LAMP assay.
Unfortunately it was not possible to perform the planned field study which included the stay of the doctoral student at the University of Turin for several weeks to sample and test nasal swabs from acute IBR cases due to the outbreak of the SARS CoV-2 virus and the subsequent global pandemic.

In summary, this LAMP assay may be flexibly applied in different settings. Using the real-time readout option and extracted DNA it represents a fast (30 min run time) and cost-efficient alternative to qPCR in routine veterinary diagnostic labs. On the other hand, while sensitivity is slightly reduced, the use of a simple and fast DNA precipitation, portable equipment and the visual readout option enables application of the assay as an affordable and practicable diagnostic tool in resource-limited settings.

Publications, posters and presentations

A manuscript describing the method was submitted to the Journal of Virological Methods on 9th of September 2020 and was accepted with minor revisions on the 25th of October.

Poster presentation, 3.-4.February 2020, Paris Maison-Alfort:
IBR and BVD Control and Eradication in the EU: Towards the harmonisation of diagnostics in the context of EU recognition of control programmes. A CoVetLab workshop.

Oral presentation: Meeting of Master- and Doctoral Students in Virology, Münchenwiler 31st October – 1st November 2019.

Project *Entwicklung und Evaluation einer diagnostischen Methode zum Nachweis von BoHV-1 während der Akutphase. Development and evaluation of a diagnostic tool for the detection of BoHV-1 during the acute phase. 1.18.03*

Project duration *1.10.2018-30.09.2020, 24 months.*