Development of a rapid and specific method to diagnose pathogenic Dichelobacter nodosus for control of fotroot in Switzerland

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Key words
Footrot; surveillance; diagnostic method; virulence gene marker of D. nodosus

Aim of the study
The present project intended to develop a robust diagnostic method for the detection of *D. nodosus* from clinical specimens and for determination of virulent subtypes of *D. nodosus* to be applied in control of footrot. This method should fulfil the logistic requirements of field examinations (ease of sample-taking from the animals, simple transport of non-cooled samples).

Material and methods
Clinical and epidemiological data and corresponding samples for bacteriological evaluation of *D. nodosus* were collected from 600 sheep of affected herds (clinically affected and clinically healthy sheep) and from 150 sheep of herds that had currently a negative footrot status and had no history of footrot for >2 years. The main target genes were $AprV_2$ and $AprB_2$.

Results and significance
The data revealed the two proteases known as virulent AprV2 and benign AprB2 to correlate fully to the clinical status of the individuals or the footrot history of the herd. In samples taken from affected herds, the aprV2 gene was found as a single allele whereas in samples from unaffected herds several alleles with minor modifications of the aprB2 gene were detected. The different alleles of aprB2 were related to the herds. A competitive real-time PCR based on allelic discrimination of the protease genes aprV2 and aprB2 was successfully developed. The new method allowed direct detection and differentiation of virulent and benign *D. nodosus* from interdigital skin swabs in a single test: Clinically affected sheep harbored high loads of only virulent strains, whereas healthy sheep had lower loads of predominantly benign strains.

This assay will help to elucidate the epidemiology of and support efforts to combat ovine footrot. It has the great potential to serve as the basis for a relevant improvement of the efficacy of the BGK footrot control program, currently adopted by the Canton of Grison. Furthermore, this new tool represents the prerequisite for the initiation of a pilot study for a CH-nation wide footrot control program that might potentially be implemented in the future.

Publications, posters and presentations
Anna Stäuble, Adrian Steiner, Joachim Frey and Peter Kuhnert (2014) Simultaneous Detection and Discrimination of Virulent and Benign Dichelobacter nodosus in Sheep of Flocks Affected by Foot Rot and in Clinically Healthy Flocks by Competitive Real-Time PCR). Journal of Clinical Microbiology 52: 1228–1231.

Anna Stäuble, Adrian Steiner, Lea Normand, Peter Kuhnert, Joachim Frey (2014) Molecular genetic analysis of Dichelobacter nodosus proteases AprV2/B2, AprV5/B5 and BprV/B in clinical material from European sheep flocks. Veterinary Microbiology 168: 177-184.

Anna Stäuble, Adrian Steiner, Joachim Frey (2014) Genetik von Dichelobacter nodosus und Weiterentwicklung der Moderhinke-Diagnostik. Forum 5:6-11.

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