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Molecular epidemiology of *Brachyspira hyodysenteriae* in the Swiss pig production

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Key words
Brachyspira hyodysenteriae; pig; sanitation; antibiotics; molecular epidemiology; genotyping; MLST; WGS

Aim of the study
The objectives of this project are i) to establish a molecular-based surveillance system identifying *B. hyodysenteriae* clones in pig herds with swine dysentery (SD) in Switzerland; ii) to trace back sources of the different BH clones using information associated with the life path of the pigs that developed SD such as TVD number (Tierverkehrsdatenbank) and trade information from the positive herd (e.g. origin of purchased pigs, trader and transportation); iii) to identify new emerging clones which may be resistant to pleuromutilins; iv) to identify the different potential origins of pigs which are subclinically infected with BH along the entire pig production. The methodology developed for this project and the results should serve as a basis for the introduction of a future control program for SD in Switzerland.

Material and methods
Envelopes containing 5 swabs each as well as the project flyer and submission form in both French and German were distributed to veterinarians. Feces samples were analyzed for the presence of *B. hyodysenteriae*, *B. pilosicoli* and apathogenic *Brachyspira* spp. (*B. intermedia, B. innocens, B. murdochii*) using selective plates for enrichment and selection, and specific real-time PCR assays for species identification. *B. hyodysenteriae* and *B. pilosicoli* were characterized using a newly developed species specific PCR for multilocus sequence typing (MLST). Presence of the pleuromutilin resistance genes *tva*(A) and *tva*(B) was determined by PCR. Whole genome sequences were obtained using both long read Oxford nanopore technology (ONT) and short read Illumina technology.

Results and significance
From October 2019 to December 2020, feces samples of 597 pigs with diarrhea from 136 herds have been analyzed for the presence of *Brachyspira* spp. Three different herds were found positive for *B. hyodysenteriae* in October 2019, July 2020 and October 2020 (1.7% of the total number of pigs tested; 2.2% of the total number of herds tested). The *B. hyodysenteriae* isolates of the two herds positive in October 2019 and 2020 belong to the Swiss clonal lineage ST196, and the isolate of the herd positive in July 2020 belongs to ST66. Seventy-three herds and 203 animals were positive for *B. pilosicoli* (34.0% of the pig tested; 53.7 % of the herds), which belong to 43 ST indicating a large diversity among isolates between the different infected herds. *B. intermedia*, *B. innocens*, or *B. murdochii* were detected in 277 animals (46.4%) from 88 herds (64.7%) either associated with only *B. pilosicoli* (in 93 samples), *B. hyodysenteriae* (3 samples), with both (1 sample) or alone (180 samples). None of the *B. hyodysenteriae* strains contained the pleuromutilin resistance gene *tva*(A), whereas *tva*(B) was detected in 26 genetically diverse *B. pilosicoli* isolates from 19 herds (9.4% of the herds). WGS was obtained for *B. hyodysenteriae* strains of ST196 and ST66 confirming genome heterogeneity among isolates of the same ST. Data including TVD number, herd size, weight of the animal, type of diarrhea and location of the

herds were obtained for each *B. hyodysenteriae* positive sample. TVD documents of two of the three herds which were positive for *B. hyodysenteriae* have so far been obtained. Epidemiological analyses will determine whether herds with the same ST have an epidemiological link such as the same supplier or trader. Characteristics of positive herds will also be described.
The number of herds with *B. hyodysenteriae* recovered from feces samples of pigs having clinical signs of SD is low (2.2%). *B. hyodysenteriae* isolates belong to the ST196 and ST66, which were already detected in Switzerland the past years. The only two herds with the same ST were found positive with a time interval of one year, making a direct common source of both infections unlikely. This confirms that there are few common sources of *B. hyodysenteriae* within the pig production in Switzerland. Most likely, this fact is due to the efforts of the industry to control this disease. However, 53.7% of the tested herds harbored pigs with a heterogeneous population of *B. pilosicoli*. The presence of *B. pilosicoli* in half of the pig herds with signs of diarrhea as well as the presence of the pleuromutilin resistance gene *tva*(B) in genetically diverse *B. pilosicoli* isolates indicates that this species has potential for adaptation and may play an underestimated role as a cause of pig diarrhea in Switzerland.

Publications, posters and presentations

Zeeh F and Nathues H. *Brachyspira*-Projekt. "Schweizerische Vereinigung für Schweinemedizin" (SVSM) Newsletter Nr. 17 / October 2019.

Zeeh F. Schweinedysenterie: Neue Erkenntnisse und ein neues Angebot. Swisseporcs Information 11/2019, S. 20-21.

Zeeh F. Durchfall bei Absetzferkeln, Jagern und Mastschweinen: Kostenfreie Untersuchung von Kotproben. Suisseporcs Information 7/2020, S. 25.

García-Martín AB, Schwendener S, Perreten V. The *tva*(A) gene of *Brachyspira hyodysenteriae* confers resistance to pleuromutillin and streptogramin A in *Escherichia coli*. Annual Swiss Society for Microbiology Meeting, Zurich, Switzerland, 3 – 4 September 2019.

García-Martín AB, Schmitt S, Zeeh F, Perreten V. Whole-genome sequence of the predominant *Brachyspira hyodysenteriae* causing swine dysentery in pigs in Switzerland. GCB Students' Symposium, University of Bern, Bern, Switzerland, 30 January 2020.

García-Martín AB, Schmitt S, Zeeh F, Perreten V. Whole-genome sequencing reveals the integration of a new prophage into the genome of *Brachyspira hyodysenteriae*. FEMS Online Conference on Microbiology 2020, 28 – 31 October 2020.

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