



## Antimicrobial susceptibility and genotyping of *Brachyspira hyodysenteriae* in Swiss pig herds

Vincent Perreten<sup>1</sup>, Ana B. García-Martín<sup>1</sup>, Sarah Schmitt<sup>2</sup>, Heiko Nathues<sup>3</sup>, Friederike Zeeh<sup>3</sup>

<sup>1</sup>Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, CH-3012 Bern; <sup>2</sup>Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich; <sup>3</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Department for Clinical Veterinary Medicine, CH-3012 Bern

### Key words

Antibiotic resistance, MLST, WGS, pleuromutillin, *tva*(A), mobile genetic elements, swine dysentery

### Aim of the study

This study aimed to (i) determine antimicrobial susceptibility of *B. hyodysenteriae* isolates from Switzerland; (ii) determine the resistance mechanisms associated to decreased susceptibility to antibiotics used for treatment of swine dysentery (SD); (iii) determine genetic relatedness between isolates of different origins and isolation dates; (iv) determine the complete genome sequence of strains circulating in Switzerland; (v) identify markers for PCR detection of antibiotic-resistant *B. hyodysenteriae*.

### Material and methods

*B. hyodysenteriae* strains were isolated on selective agar plates and identified by MALDI TOF-MS, *Bnox*-PCR and real-time PCR. Purity of the cultures was tested by microscopic visualisation of Gram-stained smears. Antimicrobial susceptibility to tiamulin, valnemulin, doxycycline, tylvalosin, lincomycin and tylosin was determined by the broth dilution method using the VetMIC Brachy panel (National Veterinary Institute, SVA, Sweden). Presence of point mutations and genes known to be associated with antimicrobial resistance in *B. hyodysenteriae* was assessed by PCR and sequence analysis. Genetic relatedness was determined by multilocus sequence typing (MLST) analysis of seven conserved genes according to a previously described scheme (<https://pubmlst.org/brachyspira/>). Whole genome sequence (WGS) was performed using Pacific Biosciences R.S. (PacBio) sequencer (Genome Technology Facility, University of Lausanne) and/or Oxford Nanopore technology (Minlon) (Institute of Veterinary Bacteriology, University of Bern) coupled with Illumina MiSeq sequencing.

### Results and significance

Fifty-one *B. hyodysenteriae* isolated over an 8-year period at the Institutes of Veterinary Bacteriology of the University of Zurich and of the University of Bern were available for further molecular characterization by MLST and antibiotic resistance profile and mechanisms. The isolates originated from 27 different Swiss pig herds situated in different geographical regions from Switzerland. They belonged to only four sequence types (ST) such as ST6 ( $n = 1$ ), ST66 ( $n = 9$ ), ST196 ( $n = 37$ ) and ST197 ( $n = 4$ ) indicating that very few clones are circulating in Switzerland. ST196, which is the most frequent ST, represents a new clonal lineage identified in Switzerland. Forty-one isolates exhibited decreased susceptibility to macrolides and lincomycin associated with an A2058 T/G mutation in the 23S rRNA gene. Six isolates exhibited decreased susceptibility to doxycycline associated with a G1058C mutation in the 16S rRNA gene. The strains were susceptible to the pleuromutillins tiamulin and valnemulin (García-Martín et al, 2018).

WGS was performed for one representative of each ST detected in Switzerland (ST6, ST66, ST196 and ST197) and for one multidrug-resistant strain (ST83) obtained from Italy. Genome analysis of the ST83 strain revealed for the first time the presence of a lincosamide resistance gene *lnc*(C) gene identified on a transposon in *B. hyodysenteriae* (De Luca et al., 2017). This strain also contains the recently described gene *tva*(A) which has been suspected to confer resistance to pleuromutillins in *B. hyodysenteriae*. To confirm functionality, we cloned the gene into *Escherichia coli* and *Staphylococcus aureus*. Expression of the cloned *tva*(A) gene was shown to confer

decreased susceptibility to pleuromutilin and streptogramin A antibiotics in *E. coli* providing evidence of the direct association of *tva(A)* with the pleuromutilin and streptogramin A resistance phenotype (García-Martín et al., 2019). Screening Italian strains as well as Swiss strains for the presence of *Inu(C)* and *tva(A)* revealed that these genes were only present in the Italian strains and not in the Swiss strains. The *tva(A)* gene could serve as a good PCR marker for the identification of *B. hyodysenteriae* with higher propensity for resistance development to pleuromutilins. The complete genome of strains of ST6, ST66, ST197, ST196, and ST83 will serve as scaffold for further comparative genome analysis of additional Swiss and International strains.

The Swiss *B. hyodysenteriae* population is characterized by a low genetic diversity, with macrolide-lincosamide-resistant isolates of ST196 being predominant. This study also demonstrated that *B. hyodysenteriae* may acquire antibiotic resistance genes associated with clinically important antibiotics against SD like the pleuromutilins. The low diversity among *B. hyodysenteriae* in Switzerland suggest that the isolates may originate from a few common sources.

### **Publications, posters and presentations**

García-Martín AB, Perreten V, Rossano A, Schmitt S, Nathues H, Zeeh F. Predominance of a macrolide-lincosamide-resistant *Brachyspira hyodysenteriae* of sequence type 196 in Swiss pig herds. *Vet Microbiol.* 2018 Nov;226:97-102. doi: 10.1016/j.vetmic.2018.10.007.

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García-Martín AB, Zeeh F, Schmitt S, Rossano A, Nathues H, Perreten V. Genetic diversity and antimicrobial susceptibility of *Brachyspira hyodysenteriae* in Switzerland. Annual Swiss Society for Microbiology Meeting 2018. Lausanne, Switzerland. 28 – 30 August 2018.

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

Zeeh F, Schmitt S, García-Martín AB, Nathues H, Perreten V. Low diversity among *Brachyspira hyodysenteriae* isolates in Switzerland. 10<sup>th</sup> European Symposium of Porcine Health Management (ESPHM), Barcelona, Spain, 9 – 11 May 2018.

García-Martín AB. New insights into molecular mechanisms of antibiotic resistance in *Brachyspira hyodysenteriae*. HoPa seminar, Institute of Veterinary Bacteriology, Bern, Switzerland, 21 June 2019.

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