Antimicrobial susceptibility of *Brachyspira hyodysenteriae* in Switzerland

C. Kirchgässner, S. Schmitt, A. Borgström, M. M. Wittenbrink

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland

DOI 10.17236/sat00066

Received: 31.03.2016
Accepted: 25.04.2016

Abstract

Brachyspira (*B.*) *hyodysenteriae* is the causative agent of swine dysentery (SD), a severe mucohaemorrhagic diarrheal disease in pigs worldwide. So far, the antimicrobial susceptibility patterns of *B. hyodysenteriae* in Switzerland have not been investigated. Therefore, a panel of 30 porcine *B. hyodysenteriae* isolates were tested against 6 antimicrobial agents by using the VetMIC Brachy panel, a broth microdilution test. Tiamulin and valnemulin showed high antimicrobial activity inhibiting all isolates at low concentrations. The susceptibility testing of doxycycline revealed values from ≤0.25 µg/ml (47%) to 2 µg/ml (10%). The MIC values of lincomycin ranged between ≤0.5 µg/ml (30%) and 32 µg/ml (43%). For tylosin, 57% of the isolates could not be inhibited at the highest concentration of ≥128 µg/ml. The MIC values for tylvalosin were between ≤0.25 µg/ml (10%) and 8 µg/ml (20%). These findings reveal Switzerland's favourable situation compared to other European countries. Above all, tiamulin and valnemulin are still effective antimicrobial agents and can be further used for the treatment of SD.

Keywords: *Brachyspira hyodysenteriae*, antimicrobial susceptibility testing, resistance, swine dysentery, Switzerland

Antimikrobielle Empfindlichkeit von *Brachyspira hyodysenteriae* in der Schweiz

Brachyspira (*B.*) *hyodysenteriae* ist der Erreger der Schweinedysenterie (SD), einer schweren mukohämorrhagischen Durchfallerkrankung bei Schweinen weltweit. Bisher ist das Empfindlichkeitsmuster von *B. hyodysenteriae* gegenüber Antibiotika in der Schweiz nicht untersucht worden. Daher wurde eine Auswahl von 30 porzinen *B. hyodysenteriae* Isolaten auf ihre Empfindlichkeit gegenüber 6 antimikrobiellen Wirkstoffen mittels VetMIC Brachy panel, einem Mikrodilutionstest, getestet. Tiamulin und Valnemulin zeigten eine starke antimikrobielle Aktivität und hemmten alle Isolate bei niedrigen Konzentrationen. Die Empfindlichkeitsprüfung von Doxycyclin ergab Werte von ≤0.25 µg/ml (47%) bis 2 µg/ml (10%). Die MHK-Werte von Lincomycin reichten von ≤0.5 µg/ml (30%) bis 32 µg/ml (43%). Für Tylosin konnten 57% der Isolate nicht bei der höchsten Konzentration von ≥128 µg/ml gehemmt werden. Die MHK-Werte für Tylvalosin lagen zwischen ≤0.25 µg/ml (10%) und 8 µg/ml (20%). Diese Befunde zeigen die günstige Situation der Schweiz verglichen mit anderen europäischen Ländern. Besonders Tiamulin und Valnemulin sind noch wirksame antimikrobielle Substanzen, die weiterhin für die Behandlung von SD eingesetzt werden können.

Schlüsselwörter: *Brachyspira hyodysenteriae*, Empfindlichkeitsprüfung, Resistenz, Schweinedysenterie, Schweiz

Introduction

Brachyspira (*B.*) *hyodysenteriae* is an anaerobic intestinal spirochaete causing swine dysentery (SD), a disease associated with mucohaemorrhagic diarrhea in pigs (Taylor and Alexander, 1971; Glock and Harris, 1972; Harris et al., 1972b). It is one of the most important diseases in pig production worldwide. In Switzerland, SD was etiologically confirmed for the first time in 2008 (Speiser, 2008). Since then, the pathogen has been spreading throughout the country (Prohaska et al., 2012). Currently, *B. hyodysenteriae* is detected in nearly 2% of all pig herds served by the Swiss Pig Health Service (Figi et al., 2014). Up to now, antimicrobial therapy remains the only treatment available. The selection of antimicrobial agents admitted for the treatment of SD in Switzerland is restricted to 3 antimicrobial agents (tiamulin, valnemulin and lincomycin). Since culturing of *B. hyodysenteriae* is difficult and time-consuming, antimicrobial susceptibility testing is not performed routinely in diagnostics. Therapy of clinical SD is mostly started without a final diagnosis and information about the susceptibility of the isolate. An increase in resistance is observed all over Europe (Rohde et al., 2004; Hidalgo et al., 2009; Pringle et al., 2012). Until now, no data of antimicrobial susceptibility of *B. hyodysenteriae* exist for Switzerland. The testing in this study was performed with a broth microdilution test developed for *Brachyspira* spp. This is the first investigation of the antimicrobial susceptibility of Swiss *B. hyodysenteriae* isolates.

Material and Methods

Isolation and differentiation

A panel of 30 porcine *B. hyodysenteriae* isolates from 8 different Swiss cantons collected from 30 different farms between 2009 and 2015 were tested (Fig. 1). The isolation and differentiation of the strains is described elsewhere (Prohaska et al., 2014). In short, faecal swabs were streaked on a selective agar (Dünser et al., 1997) and incubated anaerobically (TRILAB, bioMérieux, Switzerland) for 2 to 7days at 42°C. Samples were investigated using native microscopy. If positive, they were subcultured on Columbia blood agar (Oxoid, Switzerland) and analysed by a duplex PCR targeting the *nox* gene for *B. hyodysenteriae* and the 16S rRNA gene for *B. pilosicoli* (La et al., 2003). *B. hyodysenteriae* isolates were stored at −80°C in brain heart infusion with glycerine and fetal calve serum. The frozen isolates were restreaked on Columbia blood agar, controlled for contamination by native microscopy, and all isolates were additionally analysed by a multiplex real-time PCR targeting the 23S rRNA gene to avoid culture of different *Brachyspira* spp.

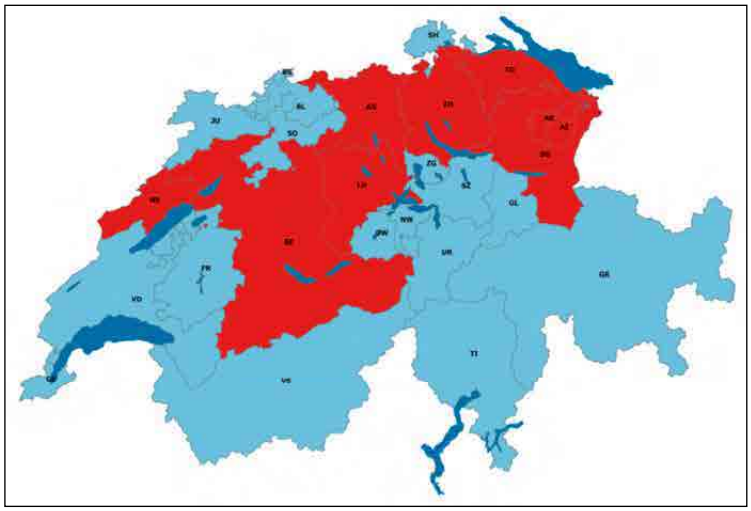

Figure 1: *B. hyodysenteriae* isolates investigated in this study are sampled from the red-coloured cantons of Switzerland.

Susceptibility testing

Antimicrobial susceptibility testing was performed using the VetMIC Brachy panel (Statens veterinärmedicinska anstalt, Sweden), a broth microdilution test including the pleuromutilins tiamulin and valnemulin, the tetracycline doxycycline, the macrolide tylosin and its derivate tylvalosin (aivlosin) as well as lincomycin, a lincosamide. The VetMIC Brachy panel provides a 48-well plate coated with the 6 different antimicrobials in arithmetically decreasing concentrations. The inoculation of the VetMIC Brachy panel was done with modifications to the manufacturer's instructions and is described as follows: The pure isolates were incubated anaerobically at 42 °C for 3 days. Cultures were suspended in brain heart infusion with 10% fetal calve serum (BHI + FCS) and diluted up to an OD_{600} of 0.6 to 0.8 E (BioPhotometer, eppendorf, Switzerland). The suspension was diluted 1:1000 in BHI + FCS, corresponding to 10^7 bacterial cells per ml. The VetMIC Brachy plates were inoculated with 500 µl of the diluted bacterial suspension per well. Additionally, a control plate without antimicrobials and a negative control with BHI + FCS only was included. The plates were incubated in an anaerobic jar (AnaeroGen 2.5 l, Thermo Scientific, Switzerland) shaking at 37 °C for 4 days. All cultures were controlled twice for contamination by native microscopy and by streaking on a Columbia blood agar plate (aerobic and anaerobic at 37°C) before and after the incubation of the VetMIC Brachy panel. For control purposes, *B. hyodysenteriae* ATCC strain 27164 was tested in parallel at every approach. The evaluation was done by controlling the wells for growth with a mirror. A well was considered positive for growth if a macroscopically visible turbidity could be detected. The minimal inhibitory concentration (MIC) was the lowest concentration at which no visible growth in a well could be detected.

Results

The antimicrobial susceptibility testing of the control strain *B. hyodysenteriae* ATCC 27164 revealed a good reproducibility of the assay. The distribution of the MICs for all isolates and agents as well as the concentrations used for the different antimicrobial agents is shown in Figure 2. The pleuromutilins, tiamulin and valnemulin, were able to inhibit growth at very low concentrations. For tiamulin, all isolates could be inhibited at a concentration of 0.125 µg/ml and 70% already at a concentration of ≤0.063 µg/ml. Therefore, the MIC inhibiting 90% of the isolates (MIC_{90}) was 0.125 µg/ml. Valnemulin suppressed growth at concentrations of ≤0.031 µg/ml with the exception of 2 isolates which had higher MICs of 0.063 µg/ml and 0.125 µg/ml, respectively. For both agents, a clear unimodal distribution of the isolates can be observed (Fig. 2A/B). In the case of doxycycline, 10% of the isolates showed growth up to concentrations of 2 µg/ml. Nevertheless, the MIC inhibiting 50% of the isolates (MIC_{50}) was 0.25 µg/ml and MIC_{90} was 1 µg/ml. Nearly half of the isolates (47%) were inhibited at a low concentration of ≤0.125 µg/ml. A bimodal distribution is seen in Figure 2C, parting the isolates into two populations with the major part being inhibited at lower concentrations. A wider range of MICs can be observed for the lincosamides and macrolides. For lincomycin, isolates grew up to a concentration of 32 µg/ml (43% of the tested strains). One third of the isolates was already inhibited at a concentration of ≤0.5 µg/ml, but MIC_{50} was 16 µg/ml and MIC_{90} was 32 µg/ml. Isolates can be separated into 2 populations (Fig. 2D). For tylosin, nearly 60% of the isolates were able to grow at the highest concentration tested (128 µg/ml, Fig. 2E). Thus, tylosin had the highest MIC_{90} (>128 µg/ml) and the widest distribution of MICs ranging from ≤2 µg/ml to >128 µg/ml compared to the other antimicrobial agents tested. Isolates with increased MICs for tylosin also showed high MICs for lincomycin. For tylvalosin, a tylosin derivate, the MIC values ranged from ≤0.25 µg/ml to 8 µg/ml. Since 37% of the isolates had a MIC of 4 µg/ml, MIC_{50} could be set at this concentration and MIC_{90} was 8 µg/ml. No growth was detected at the 2 highest concentrations (16 µg/ml and 32 µg/ml) tested (Fig. 2F). Both macrolides showed a bimodal distribution of isolates (Fig. 2E/F).

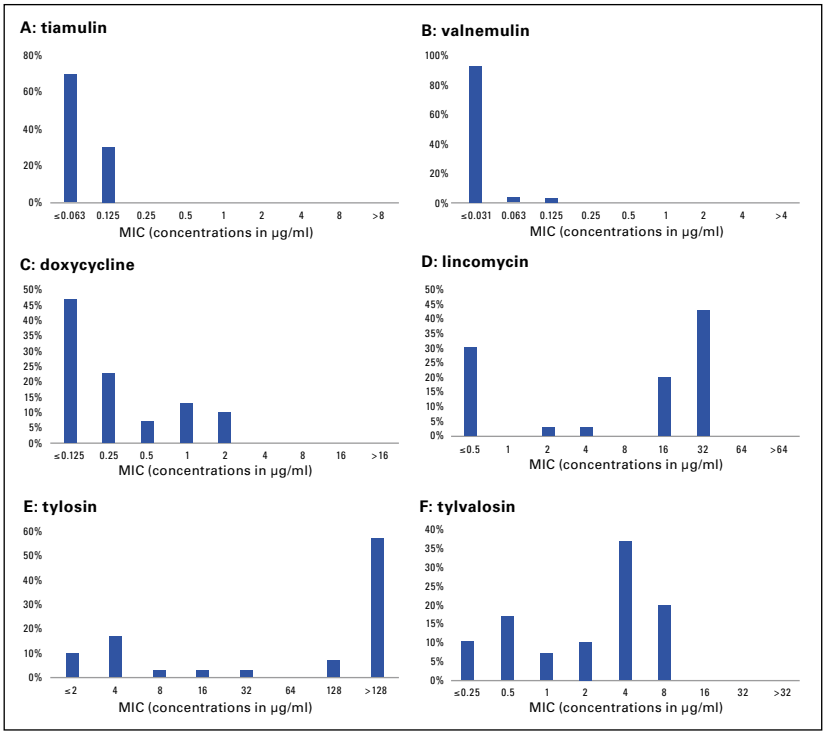

Figure 2: Distribution of MICs of 30 Swiss field isolates for the 6 antimicrobial agents.

Discussion

This is the first study on the antimicrobial susceptibility of Swiss *B. hyodysenteriae* isolates. Antimicrobial therapy remains the only treatment option for SD. Facing progressive resistance in *B. hyodysenteriae* isolates all over Europe (Rohde et al., 2004; Sperling et al., 2011; Hidalgo et al., 2011) and due to the restricted selection of therapeutics admitted for the treatment of SD, an evaluation of the situation in Switzerland is more than necessary. The antimicrobial agents currently licensed for treatment of SD in Switzerland are the pleuromutilins tiamulin and valnemulin and the lincosamide lincomycin. Hence, all these antimicrobial agents were included in the study. The remaining antimicrobial agents additionally tested in our study are the tetracycline doxycycline and the macrolides tylosin and its derivate tylvalosin. At the moment, there is no product containing tylvalosin available in Switzerland. Products containing doxycycline or tylosin have a Swiss license for the treatment in swine. For the therapy of SD the use of these products is not allowed except for cases where it has to be assumed that authorised products are ineffective, for example because of resistance. Therefore, knowledge about antimicrobial susceptibility patterns of the pathogen is required.

A major restriction of antimicrobial susceptibility testing of *B. hyodysenteriae* is the difficulty of interpreting the results due to a lack of standardised established breakpoints. An overview of the classification of the isolates according to the established breakpoints is given in Table 1. For tiamulin, an initially proposed clinical breakpoint is >4 µg/ml (Rønne and Szancer, 1990). However, the susceptible wild-type population had MIC values below 0.5 µg/ml (Karlsson et al., 2003). Thus, a revision of the tiamulin breakpoint to <0.5 µg/ml and <1 µg/ml, respectively, is postulated (Karlsson et al., 2003; Lobová et al., 2004; Burch, 2005; Vyt and Hom-

mez, 2006). Irrespective of the breakpoint used, all Swiss isolates tested in this study were susceptible to tiamulin. For valnemulin, a breakpoint of >0.125 µg/ml is the only one available (Burch, 2005), and all isolates investigated in this study were fully susceptible to valnemulin. Less is known about the resistance mechanism of pleuromutilins, but it develops slowly and stepwise (Karlsson et al., 2001; Lobová et al., 2004) indicating the involvement of several genes. Alterations of the ribosomal binding sites induced by mutations of the L3 and 23S rRNA genes are connected to reduced susceptibility to tiamulin and valnemulin (Pringle et al., 2004; Hidalgo et al., 2011; Hillen et al., 2014). For doxycycline, an investigation of German and Swedish isolates parted population into a more susceptible group with MICs from 0.125 µg/ml to 0.25 µg/ml and a group showing decreased susceptibility with MICs between 1 µg/ml to 4 µg/ml. A bimodal distribution of isolates is an indication for an acquired resistance mechanism and genetic investigation of the less susceptible strains revealed a mutation in the 16S rRNA gene (Pringle et al., 2007). Due to the lack of clinical breakpoints for doxycycline, Zmudzki et al. (2012) used the MIC of ≥1 µg/ml as a cut-off value. The tested Swiss isolates showed the same bimodal distribution and applying the cut-off value, the major group of nearly 80% of the isolates can be considered as susceptible to doxycycline and about 20% of the isolates showed decreased susceptibility. The results for the lincosamides and macrolides revealed a broader range of concentrations but also a bimodal distribution of the isolates. According to the breakpoints of Rønne and Szancer (1990), 37% of the isolates were in the susceptible range (≤4 µg/ml) for lincomycin, and the remaining strains can be classified as intermediate (>4 µg/ml to ≤16 µg/ml). None of the isolates was found to be resistant. However, therapy outcome by using lincomycin is not always in accordance with the *in-vitro* susceptibility testing in *B. hyodysenteriae*, since treatment was efficient for strains classified as resistant (Smith, 1990; Vyt and Hommez, 2006). Therefore, Burch (2005) suggests a clinical breakpoint of >50 µg/ml. In this case, all isolates can be classified as susceptible to lincomycin. For tylosin, nearly two thirds of the isolates are resistant to tylosin (Rønne and Szancer, 1990; Burch, 2005). According to Rønne and Szancer (1990), 17% of the isolates are categorised as intermediate. Three isolates had a MIC of ≤2 µg/ml and cannot be classified. Applying the breakpoint proposed by Burch (2005), the remaining third of the isolates is susceptible to tylosin. For tylvalosin, the tylosin derivate, there is only one breakpoint of >16 µg/ml available (Burch, 2005), and all tested isolates of the study were susceptible. The mechanism of macrolide and lincosamide resistance is well known. In general, both antimicrobial classes have the same binding site at the 50S subunit of the ribosome and cross-resistance is quite common (Vester and Douthwaite, 2001). For *B. hyodysenteriae*, a point mutation in the 23S rRNA gene is responsible for decreased susceptibility (Karlsson et al., 1999). Additionally, resistance development has been facilitated by the former use of tylosin as a growth promoter and the ongoing preventive administration for respiratory diseases in countries with less rigidly regulated antimicrobial application. Thus, high MIC values for lincomycin and tylosin were already observed at the beginning of antimicrobial susceptibility testing of *B. hyodysenteriae* (Kitai et al., 1979; Messier et al., 1990; Rønne and Szancer, 1990) and is still common all over the world (Karlsson et al., 1999; Clothier et al., 2011; Zmudzki et al., 2012). Referring to the situation in Switzerland, the high rate of resistance against tylosin is not surprising. Crossresistance between tylosin and lincomycin was observed as well, since isolates with high MICs to tylosin showed also increased MICs to lincomycin. In addition to the present reduced susceptibility, further development of resistance can be anticipated. In Poland, a rapid increase of lincomycin-resistant isolates was observed within 10 years (Binek, 1994). Trends towards lower susceptibility are also seen in the pleuromutilins (Lobová et al., 2004; Rohde et al., 2004; Hidalgo et al., 2011). In Hungary, tiamulin-resistant *B. hyodysenteriae* isolates occurred in the early 1990s after an observation period in which no resistant isolate was found (Molnár, 1996). Up to now, tiamulin resistance is present in many countries worldwide (Novotna and Skardova, 2002; Rohde et al., 2004; Clothier et al.,

Table 1: Distribution and classification of the isolates according to clinical breakpoints. The range of the measured concentrations is coloured light grey.

antimicrobial agent	MIC in µg/ml (number of isolates)													interpretation (isolates in %)					
														Rønne and Szancer, 1990				Burch, 2005	
	≤0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	not classifiable	S	I	R	S	R
tiamulin		21	9	0	0	0	0	0	0					0	100	0	0	100	0
valnemulin	28	1	1	0	0	0	0	0						/	/	/	/	100	0
doxycycline			14	7	2	4	3	0	0	0				/	/	/	/	/	/
lincomycin					9	0	1	1	0	6	13	0		0	37	63	0	100	0
tylosin							3	5	1	1	1	0	19	10	0	17	73	33	67
tylvalosin				3	5	2	3	11	6	0	0			/	/	/	/	100	0

2011) with rates of up to 17.6% of the isolates in Spain (Hidalgo et al., 2009). Moreover, multiresistant isolates occur in Spain and the Czech Republic (Sperling et al., 2011; Hidalgo et al., 2011). The confrontation with progressive resistance leads to another problem. Culture of the slow-growing *B. hyodysenteriae* is fastidious and takes a long time. Therapy of SD with clinical symptoms must be started before the agent is diagnosed and results of antimicrobial susceptibility testing are present. Consequently, an optimised and accelerated diagnosis of SD as well as a genetic screening method for resistance is highly required and needs further research. At the moment, the situation in Switzerland is still favourable. Nevertheless, to maintain the status quo, a responsible use of antimicrobial agents and a continuous monitoring are essential.

Sensibilité antimicrobienne de *Brachyspira hyodysenteriae* en Suisse

Brachyspira (*B.*) *hyodysenteriae* est l'agent de la dysenterie porcine, une affection diarrhéique muco-hémorragique grave des porcs connue dans le monde entier. Jusqu'à ce jour, la sensibilité aux antibiotiques *B. hyodysenteriae* n'avait pas été étudiée en Suisse. C'est pour cela qu'on a examiné, au moyen du test de micro dilution VetMIC Brachy panel, un choix de 30 isolats porcins de *B. hyodysenteriae* quant à leur sensibilité face à 6 substances antimicrobiennes. La Tiamuline et la Valnémuline ont montré une activité antimicrobienne élevée, bloquant tous les isolats à de faibles concentrations. Les tests de sensibilité vis-à-vis de la Doxycilline ont donné des valeurs comprises entre ≤0.25 µg/ml (47%) et 2 µg/ml (10%). Les valeurs de CMI de la Lincomycine variaient entre ≤0.5 µg/ml (30%) et 32 µg/ml (43%). Avec la Tylosine, 57% des isolats n'ont pas pu être bloqués avec la concentration la plus élevée de ≥128 µg/ml. Les valeurs de CMI pour la Tylvalosine se situaient entre ≤0.25 µg/ml (10%) et 8 µg/ml (20%). Ces résultats montrent que la situation suisse est favorable en regard d'autres pays européens. La Tiamuline et la Valnémuline en particulier restent des substances antimicrobiennes efficaces qui peuvent continuer à être utiliser pour lutter contre la dysenterie porcine.

Sensibilità antimicrobica della *Brachyspira hyodysenteriae* in Svizzera

La *Brachyspira* (*B.*) *hyodysenteriae* è l'agente eziologico della dissenteria dei suini (SD), una grave malattia diarroica muco-emorragica nei suini in tutto il mondo. Finora, il modello di sensibilità di *B. hyodysenteriae* verso gli antibiotici non è ancora stato analizzato in Svizzera. Pertanto, una selezione di 30 suini con isolati di *B. hyodysenteriae* sono stati testati per la loro sensibilità a 6 principi attivi antimicrobici dal pannello VetMIC Brachy, un test di microdiluizione. Tiamulina e valnemulina erano caratterizzate da una forte attività antimicrobica e hanno inibito tutti gli isolati a basse concentrazioni. Il test di sensibilità della doxiciclina ha dato valori da ≤0.25 g/ml (47%) a 2 ug/ml (10%). I valori di MIC della lincomicina si situavano da ≤0.5 g/ml (30%) a 32 ug/ml (43%). Per la tilosina non si è potuto inibire il 57% degli isolati alla più alta concentrazione di ≥128 ug/ml. I valori di MIC della tilvalosina si situavano da ≤0.25 g/ml (10%) a 8 ug/ml (20%). Questi risultati dimostrano la situazione favorevole della Svizzera rispetto ad altri Paesi europei. Particolarmente la tiamulina e la valnemulina sono ancora sostanze antimicrobiche efficaci che possono continuare ad essere utilizzate per il trattamento della SD.

References

Binek M.: Dynamics of susceptibility of *Serpulina hyodysenteriae* to different chemotherapeutics in-vitro. Proc.13th Int. Pig Vet. Soc. Cong., Bangkok, Thailand, 1994, 203.

Burch D. G. S.: Pharmacokinetic, pharmacodynamic and clinical correlations relating to the therapy of colonic infections in the pig and breakpoint determinations. Pig J.2005, 56: 8-24.

Clothier K. A., Kinyon J. M., Frana T. S., Naberhaus N., Bower L., Strait E. L., Schwartz K.: Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. J. Vet. Diagn. Invest. 2011, 23: 1140–1145.

Dünser M., Schweighardt H., Pangerl R., Awad-Masalmeh M., Schuh M.: Swine dysentery and spirochetal diarrhea – A comparative study of enteritis cases caused by *Serpulina*. Wien. Tierärztl. Mschr. 1997, 84: 151–161.

Figi R., Goldinger F, Fuschini E., Hartnack S., Sidler X.: Eradication of swine dysentery as modified partial depopulation in a nucleus sow breeding farm. Schweiz. Arch. Tierheilk. 2014, 156: 373–380.

Glock R. D., Harris D. L.: Swine Dysentery II. Characterization of Lesions in Pigs Inoculated with *Treponema hyodysenteriae* in Pure and Mixed Culture. Vet. Med. Small Anim. Clin. 1972, 67: 65–68.

Harris D. L., Glock R. D., Christensen C. R., Kinyon, J. M.: Swine Dysentery I, Inoculation of Pigs with *Treponema hyo-*

dysenteriae (New Species) and Reproduction of Disease. Vet. Med. Small Anim. Clin. 1972b, 67: 61–64.

Hidalgo À., Carvajal A., García-Feliz C., Osorio J., Rubio P.: Antimicrobial susceptibility testing of Spanish field isolates of *Brachyspira hyodysenteriae*. Res. Vet. Sci. 2009, 87: 7–12.

Hidalgo À., Carvajal A., Vester B., Pringle M., Naharro G., Rubio P.: Trends towards Lower Antimicrobial Susceptibility and Characterization of Acquired Resistance among Clinical Isolates of *Brachyspira hyodysenteriae* in Spain. Antimicrob. Agents Chemother. 2011, 55: 3330–3337.

Hillen S., Willems H., Herbst W., Rohde J., Reiner G.: Mutations in the 50S ribosomal subunit of *Brachyspira hyodysenteriae* associated with altered minimum inhibitory concentrations of pleuromutilins. Vet. Microbiol. 2014, 172: 223–229.

Karlsson M., Fellström C., Gunnarsson A., Landén A., Franklin A.: Antimicrobial Susceptibility Testing of Porcine *Brachyspira* (*Serpulina*) Species Isolates. J. Clin. Microbiol. 2003, 41: 2596–2604.

Karlsson M., Fellström C., Heldtander M. U. K., Johansson K.-E., Franklin A.: Genetic basis of macrolide and lincosamide resistance in *Brachyspira* (*Serpulina*) *hyodysenteriae*. FEMS Microbiol. Lett. 1999, 172: 255–260.

Karlsson M., Gunnarsson A., Franklin A.: Susceptibility to pleuromutilins in *Brachyspira* (*Serpulina*) *hyodysenteriae*. Anim. Health Res. Rev. 2001, 2: 59–66.

Kitai K., Kashiwazaki M., Adachi Y., Kume T., Arakawa A.: In Vitro Activity of 39 Antimicrobial Agents Against *Treponema hyodysenteriae*. Antimicrob. Agents Chemother. 1979, 15: 392–395.

La T., Phillips N. D., Hampson D. J.: Development of a Duplex PCR Assay for Detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in Pig Feces. J. Clin. Microbiol. 2003, 41: 3372–3375.

Lobová D., Smola J., Čížek A.: Decreased susceptibility to tiamulin and valnemulin among Czech isolates of *Brachyspira hyodysenteriae*. J. Med. Microbiol. 2004, 53: 287–291.

Messier S., Higgins R., Moore C.: Minimal inhibitory concentrations of five antimicrobials against *Treponema hyodysenteriae* and *Treponema innocens*. J. Vet. Diagn. Invest. 1990, 2: 330–333.

Molnár L.: Sensitivity of strains of *Serpulina hyodysenteriae* isolated in Hungary to chemotherapeutic drugs. Vet. Rec. 1996, 138: 158–160.

Novotná M., Škardová O.: Brachyspira hyodysenteriae: detection, identification and antibiotic susceptibility. Vet. Med.-Czech 2002, 47: 104–109.

Pringle M., Fellström C., Johansson K.-E.: Decreased susceptibility to doxycycline associated with a 16S rRNA gene mutation in *Brachyspira hyodysenteriae*. Vet. Microbiol. 2007, 123: 245–248.

Pringle M., Landén A., Unnerstad H. E., Molander B., Bengtsson B.: Antimicrobial susceptibility of porcine *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* isolated in Sweden between 1990 and 2010. Acta Vet. Scand. 2012, 54.

Pringle M., Poehlsgaard J., Vester B., Long K. S.: Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in *Brachyspira* spp. isolates. Mol. Microbiol. 2004, 54: 1295–1306.

Prohaska S., Huber H., Sydler T., Wittenbrink M. M.: Combination of culture and PCR for identification of pathogenic *Brachyspira* spp. in Switzerland from 2009–2011. Tagungsband der Fachgruppe Bakteriologie und Mykologie, 2012, Leipzig.

Prohaska S., Pflüger V., Ziegler D., Scherrer S., Frei D., Lehmann A., Wittenbrink, M. M., Huber H.: MALDI-TOF MS for identification of porcine *Brachyspira* species. Lett. Appl. Microbiol. 2013, 58: 292–298.

Rohde J., Kessler M., Baums C. G., Amtsberg G.: Comparison of methods for antimicrobial susceptibility testing and MIC values for pleuromutilin drugs for *Brachyspira hyodysenteriae* isolated in Germany. Vet. Microbiol. 2004, 102: 25–32.

Rønne H., Szancer J.: In vitro susceptibility of Danish field isolates of *Treponema hyodysenteriae* to chemotherapeutics in swine dysentery (SD) therapy. Interpretation of MIC results based on the pharmacokinetic properties of the antibacterial agents. Proc. 17th Int. Pig Vet. Soc. Cong., 1990, Lausanne, Switzerland, 126.

Smith F.: In vivo efficacy of lincomycin against an apparantly resistant strain of *Treponema hyodysenteriae*. Proc. 17th Int. Pig Vet. Soc. Cong., Lausanne, Switzerland, 1990, 134.

Speiser S. A.: Untersuchung zu *Lawsonia intracellularis-*, *Brachyspira hyodysenteriae-* und *Brachyspira pilosicoli-* Infektionen bei Absetz- und Mastschweinen in der Schweiz. Dissertation, Universität Bern, 2008.

Šperling D., Smola, J., Čížek A.: Characterisation of multiresistant *Brachyspira hyodysenteriae* isolates from Czech pig farms. Vet. Rec. 2011, 168: 15.

Taylor D. J., Alexander T. J. L.: The production of dysentery in swine by feeding cultures containing a spirochaete. Br. Vet. J. 1971, 127: 58–61.

Vester B., Douthwaite S.: Macrolide Resistance Conferred by Base Substitutions in 23S rRNA. Antimicrob. Agents Chemother, 2001, 45: 1–12.

Vyt P., Hommez J.: Antimicrobial susceptibility of *Brachyspira hyodysenteriae* isolates compared with the clinical effect of therapy. Vlaams Diergeneeskd Tijdschr. 2006, 75: 279–285.

Zmudzki J., Szczotka A., Nowak A., Strzelecka H., Grzesiak A., Pejsak Z.: Antimicrobial susceptibility of *Brachyspira hyodysenteriae isolated* from 21 Polish farms. Pol. J. Vet. Sci. 2012, 15: 259–265.

Corresponding author

Constanze Kirchgässner
Institute of Veterinary Bacteriology
Vetsuisse Faculty, University Zurich
Winterthurerstrasse 270
8057 Zurich, Switzerland
Phone: 0041 44 635 86 10
Fax: 0041 44 635 96 12
E-Mail: constanze.kirchgaessner@vetbakt.uzh.ch