

Identification and Differentiation by PCR and MALDI-TOF-MS of *Brachyspira* spp. Isolated from Dogs

C. Bode*¹, S. Ziegler*¹, J. Hauptmann², T. Heydel², K. Klimke², C.G. Baums²
¹ Students of the Faculty of Veterinary Medicine, University of Leipzig, Germany
² Institute for Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany
* Contributed equally

Introduction

Brachyspira are known to cause severe symptomatic infection in poultry and pigs, characterized by diarrhea, weight loss and reduced egg production. Porcine and dog isolates of *Brachyspira pilosicoli* are very similar to those of humans. Therefore a possible role as zoonotic agent is under discussion. Presently insufficiently studied is the prevalence, distribution and pathogenic potential in dogs. The aim of this study was the characterization of *Brachyspira* subpopulations in asymptomatic, clinically healthy dogs by PCR and MALDI-TOF-MS.

Materials and Methods

Rectal swab samples (n = 6) were taken in a first screening round. In a second screening round 10 samples were taken from 10 dogs living in one kennel. In a third screening round 2 samples were taken from 2 dogs living without contact to the other experimental animals. All samples were cultured on trypticase-soja agar (TSA) enhanced with antibiotics (A) in addition to blood agar. The putative *Brachyspira* isolates in pure culture were differentiated using MALDI-TOF-MS analysis (B) and Multiplex-PCR (C).

Experimental Approach

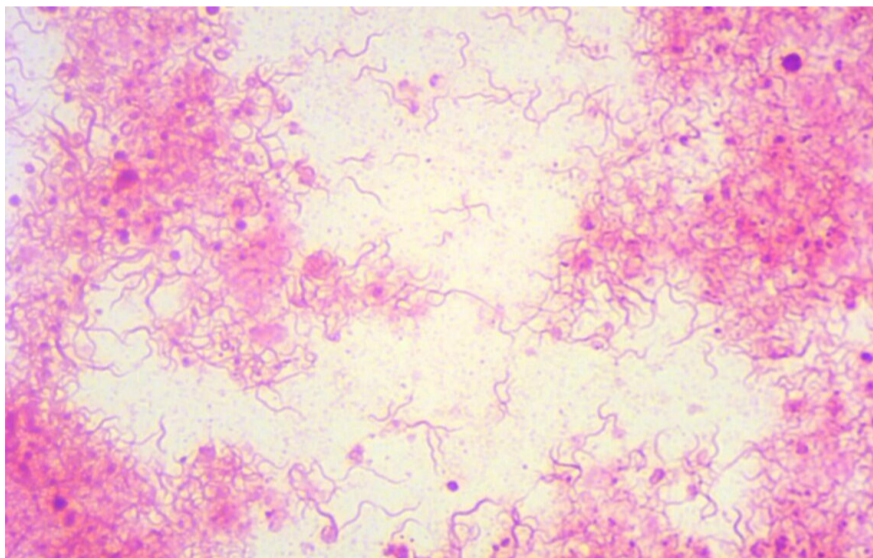
A Agars were Created with the Following Antibiotics

TSA 3 :

Colistin
Rifampicin
Spectinomycin

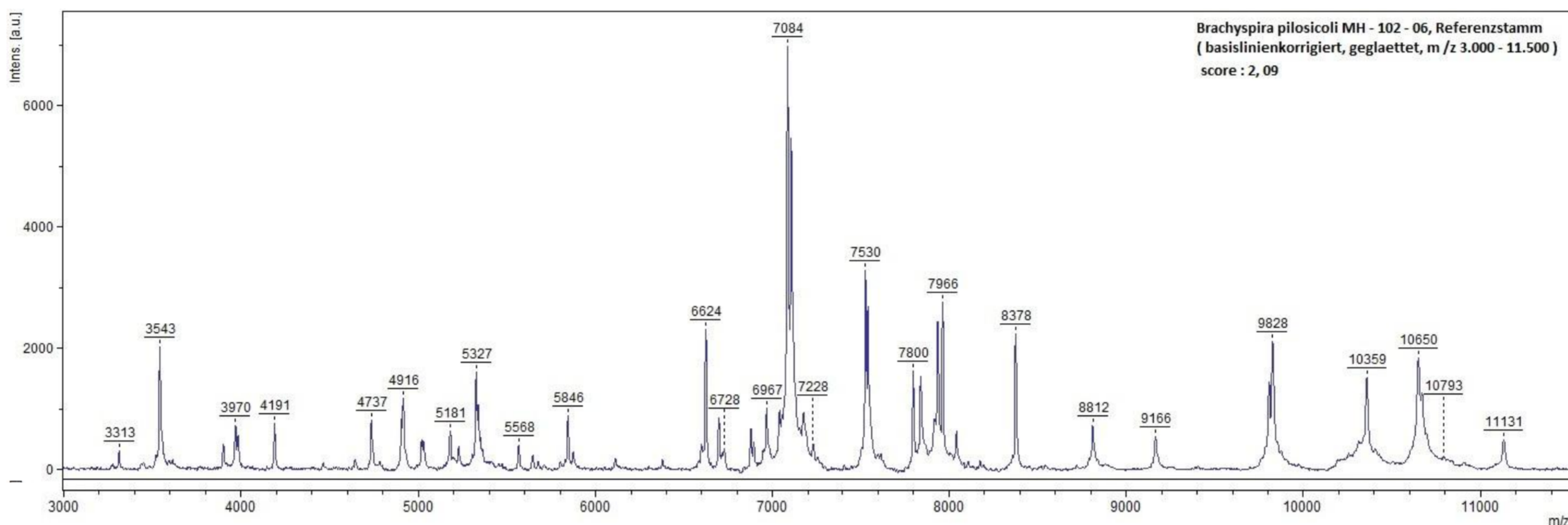
TSA 5 :

Colistin
Rifampicin
Spectinomycin
Spiramycin
Vancomycin



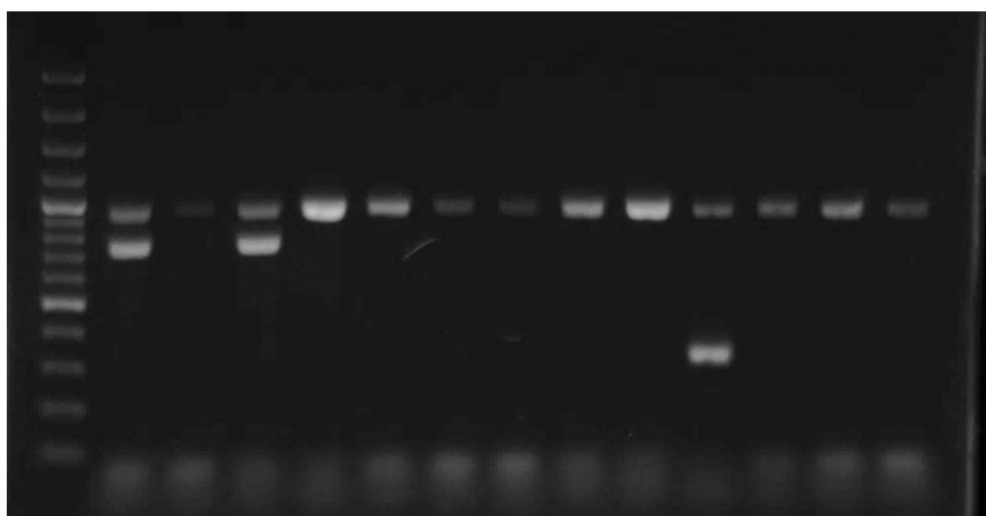
Gram stain 07.06.17 from first screening round

B MALDI-TOF-MS Spectrum (Matrix-Associated-Laser-Desorption-Ionisation Time of Flight Massspectrometry)



Representitive spectrum of *Brachyspira pilosicoli*

C MP-PCR Differentiation of Pathogenic *Brachyspira* (K.K. 06.01.17)



- 1) **nox - PCR**
 - nox gene ➤ *Brachyspira* sp.
 - 939 bp amplicon
- 2) **abgB - PCR**
 - hippurat-hydrolase gene ➤ *B. pilosicoli*
 - 744 bp amplicon
- 3) **tnaA - PCR**
 - tryptophanase gene ➤ *B. intermedia*
 - 325 bp amplicon ➤ *B. hyodysenteriae*

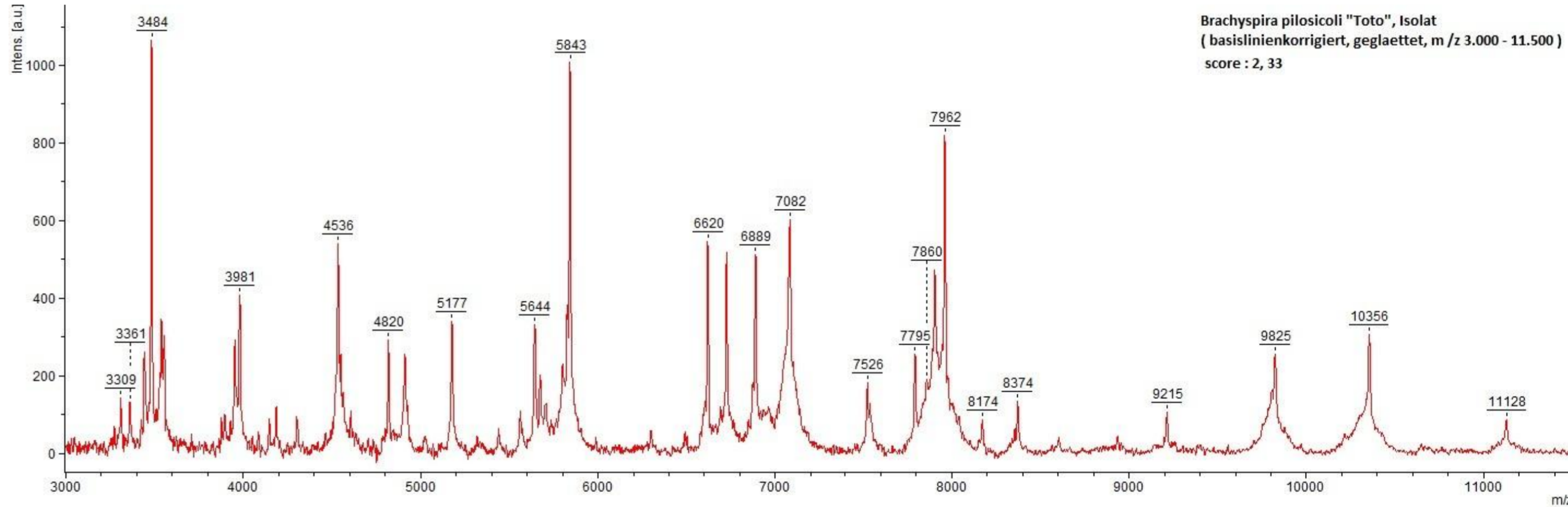
Results

A Comperative Growth on TSA 3 and TSA 5 Representative cultures 07.06.17 taken from dog ,Lola‘.



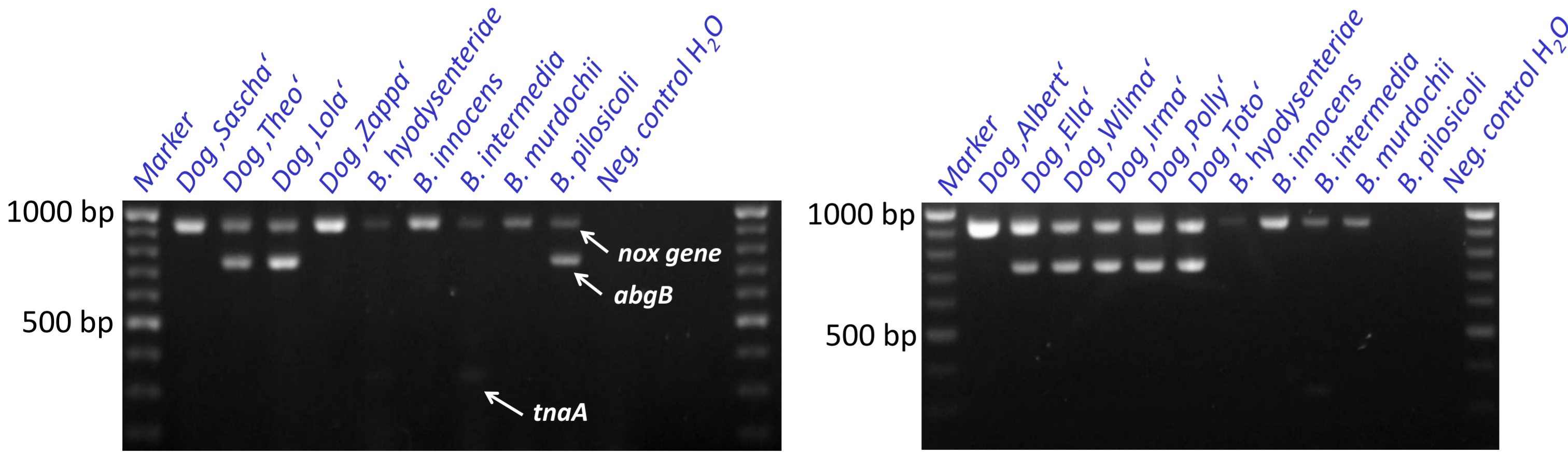
Brachyspira growth was much enhanced on TSA 3. Semiquantative analysis showed 3,5 cm for TSA 3 vs. 0,5 cm for TSA 5 from central starting point.

B MALDI-TOF-MS Single spectrum of *B. pilosicoli*



B. pilosicoli could be identified in 6 isolates.

C Multiplex PCR



Brachyspira could be proven to be present in all isolates. The abgB-PCR (744 bp), specific for *B. pilosicoli*, could be identified in 7 isolates.

Isolat	Dog Name	MALDI-TOF-MS Result	MALDI-TOF-MS Score	MP-PCR			Remark
				nox-Gen	abgB	tnaA	
VA II _{1,2,3}	Toto	<i>B. pilosicoli</i>	2.33	+	+	-	
VA II _{2,3}	Zappa	<i>B. species</i>		+	-	-	
VA II _{1,2}	Sascha	<i>B. species</i>		+	-	-	
VA II _{1,3}	Theo	<i>B. species</i>		+	+	-	
VA II _{1,2}	Albert	<i>B. species</i>		+	-	-	
VA _{7,8,10}	Irma	<i>B. pilosicoli</i>	2.06	+	+	-	
VA _{8,9,10}	Polly	<i>B. pilosicoli</i>	2.15	+	+	-	
VA II	Lola	<i>B. pilosicoli</i>	2.13	+	+	-	
VA II _{2,3}	Ella	<i>B. pilosicoli</i>	1.53	+	+	-	
VA II _{1,2,3}	Wilma	<i>B. pilosicoli</i>	2.01	+	+	-	
-	Gina	-	-	-	-	-	neg. culture
-	Eila	-	-	-	-	-	neg. culture

Conclusions

Culturing of *Brachyspira* species was achieved in all dogs on TSA 3. PCR analyses of the nox- and abgB -PCR showed presence of *Brachyspira* in all isolates (nox +) and *B. pilosicoli* in 7 isolates (abgB +). Interestingly, two dogs, with no contact to the kennel dogs, were tested negative already in culture, suggesting spread among the kennel dogs. The clinical implications for asymptomatic, apparently healthy dogs with positive *Brachyspira* culture are presently unknown. Whether *Brachyspira* carriers need treatment for prevention of public health problems also warrants further research.