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PREVALENCE OF *CHLAMYDIACEAE* AND TETRACYCLINE RESISTANCE GENES IN WILD BOARS OF CENTRAL EUROPE

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ABSTRACT: Our aim was to investigate the occurrence and distribution of *Chlamydia suis* and other *Chlamydiaceae* in the wild boar (*Sus scrofa*) population of Switzerland and Northern Italy and the detection of tetracycline resistance genes by PCR. We collected a total of 471 conjunctival swabs (n=292), rectal swabs (n=147), and lung tissue samples (n=32) belonging to 292 wild boars. The prevalence of *Chlamydiaceae* in the investigated wild boar populations was very low (1.4%, 4/292). We found *C. suis* in rectal or conjunctival swabs but not in lung samples. The low chlamydial prevalence might be attributed to limited contacts between wild boars and outdoor domestic pigs due to strict biosecurity measures or limited numbers of rural pig herds. The *tet*A(C) gene fragment was detected in six samples, which were all negative for *Chlamydiaceae*, and was probably not of chlamydial origin but more likely from other bacteria. The low tetracycline resistance rate in wild boar might be explained by the lack of selective pressure. However, transmission of resistance genes from domestic pigs to wild boar or selective pressure in the environment could lead to the development and spread of tetracycline-resistant *C. suis* strains in wild boars.

Key words: *Chlamydiaceae*, PCR, tetracycline resistance, wild boars.

INTRODUCTION

Chlamydial species of the family *Chlamydiaceae* belong to the Gram-negative obligate intracellular bacteria and infect a broad range of animals and humans including wild animals, e.g., wild boar (Borel et al. 2018). The Eurasian wild boar (*Sus scrofa*) is highly prevalent in most parts of Europe (Cahill et al. 2003; Jansen et al. 2007). In 2012 and 2013, the number of hunted wild boar in countries such as Spain, Poland, France, Italy, and Germany ranged between 200,000 and 640,000 wild boar per year (Massei et al. 2015). The numbers of hunted wild boar have massively increased over the past 50 yr with a current annual continental hunting of over 2.2 million (Massei et al. 2015). In Switzerland, there are two distinct populations of wild boar. The first population is found in the north of the country, covering the region from Geneva to St. Gallen including most parts of the Jura Mountains and other regions of the Swiss Midlands, and is connected to the wild boar population in neighboring Germany and France. The second population is distributed in the southern parts of the canton Ticino and is mixing with the wild boar population in Northern Italy. An increase of the wild boar population in Switzerland has been observed (Meier 2015), identical to what has been notified in other regions of Europe (Massei et al. 2015). However, the relative size of the Swiss wild boar population is currently one of the smallest in Europe (Meier 2015) compared to the wild boar population estimates of other countries.

It has been reported that there is a high probability of interaction between domestic pigs having outdoor access and wild boar in certain geographic regions, such as the junction between the Jura Mountains where the wild boar population has the highest density and the Swiss Midlands where most

outdoor piggeries are found (Wu et al. 2011). Close contact between domestic pigs and wild boars, which is considered a risk factor for transmission of pathogens, was assessed by a recent questionnaire (Wu et al. 2012): 31% of the participating game wardens and 25% of the pig owners have observed or documented such interactions. Contacts were reported in all 17 Swiss cantons in which wild boars were present at time of study. Boar-pig hybrids are the hybridized offspring of a cross between the Eurasian wild boar (*Sus scrofa*) and any domestic pig (*Sus scrofa domesticus*). Hybridization, which is also the type of contact carrying the highest risk of pathogen transmission, was registered in 5% of the piggeries included in the latter study (Wu et al. 2012). The following risk factors according high contact were identified: pigs in enclosures separated from the piggery building (>5 m), a large distance between pig enclosures and other houses (>500 m), piggeries near the forest (<500 m), and the presence of fences and electric fences <60 cm high. Generally, the risk was higher for piggeries with pasture than for those with concrete ground, and the risk of hybridization was highest for the Mangalitza breed (Wu et al. 2012). In a similar survey in Corsica (Jori et al. 2017), 44% of the questionnaire responders reported interactions linked with sexual attraction of wild boar by domestic sows (including sexual interactions and fights between wild and domestic boar). Studies on pathogen occurrence in various countries showed a growing evidence that hybridization, both in pig and wild boar populations, poses an increased health risk (Rossi et al. 2008; Goedbloed et al. 2015; Jori et al. 2016).

Previous surveys (Hotzel et al. 2004; Di Francesco et al. 2013) suggested the role of wild boar as a wildlife reservoir for the same *Chlamydiaceae* species known to infect domestic pigs including *Chlamydia suis*, *Chlamydia pecorum*, *Chlamydia abortus*, and *Chlamydia psittaci* (Schautteet and Vanrompay 2011). *Chlamydia suis* is the most common chlamydial species reported in pigs (Longbottom 2004; Schautteet and Vanrompay 2011), in which it has been associated with respiratory disease, diarrhea, and conjunctivitis (Pospischil et al. 2010). Moreover, subclinical chlamydial infections due to *C. suis* are highly prevalent among domestic pigs and make them more susceptible to other infections (Schautteet and Vanrompay 2011). Conjunctivitis associated with *C. suis* was reported in German, Estonian, and Swiss pigs that were kept under intensive husbandry conditions (Becker et al. 2007; Schautteet et al. 2010).

Chlamydial infections in animals are commonly treated with tetracycline and its derivatives (Chopra and Roberts 2001). The genomic island (Tet-island) found in *C. suis* confers tetracycline resistance and is thought to have been obtained through horizontal gene transfer (Lenart et al. 2001; Sandoz and Rockey 2010). The first detection of tetracycline-resistant *C. suis* strains present on a Swiss pig farm was in 2011 (Borel et al. 2012), and the widespread presence of such strains was further corroborated in Swiss fattening pigs (Wanninger et al. 2016). Our aim was to investigate the occurrence and distribution of *C. suis* and other *Chlamydiaceae* in the wild boar population of Switzerland and Northern Italy, including the detection of tetracycline resistance genes by PCR.

MATERIALS AND METHODS

Sample collection

We collected a total number of 471 samples belonging to 292 wild boars (Table 1). In Switzerland, hunters and butchers collected 169 conjunctival and 61 rectal swabs as well as lung samples (n=32) from 169 wild boars originating from 20 hunting grounds in three cantons (Zurich, Aargau, and Solothurn). From the canton Ticino, 73 eye and 73 rectum swabs (total 146 samples) from 73 animals were submitted. Additionally, three eye swabs (from three animals) from the Principality of Liechtenstein and two eye swabs (from two animals) from Germany were assessed. A total of 58 swab samples (45 conjunctival and 13 rectal) of 45 animals from Northern Italy were examined.

Flocked swabs (FLOQSwabs, Copan Flock Technologies, Brescia, Italy) were harvested from freshly killed wild boars. For the eye samples, the swabs were first introduced into the conjunctival sac of one eye, rotated several times under light

TABLE 1. Numbers of wild boars (*Sus scrofa*) sampled from four Swiss cantons (Zurich, Aargau, Solothurn, Ticino), the Principality of Liechenstein, Germany, and Italy. Samples consisted of eye and rectum swabs and lung samples. We investigated a total number of 471 samples from 292 wild boars for the occurrence of *Chlamydiaceae* and tetracycline resistance genes by PCR.

Origin	No. animals	Type of samples	No. samples
Switzerland			
Zurich	77	Eye swabs	77
		Rectum swabs	55
Solothurn	6	Eye swabs	6
		Rectum swabs	6
Aargau	86	Eye swabs	86
		Lung samples	32
Ticino	73	Eye swabs	73
		Rectal swabs	73
Principality of Liechtenstein	3	Eye swabs	3
Germany	2	Eye swabs	2
Italy	45	Eye swabs	45
		Rectal swabs	13

pressure and then the same procedure was repeated on the other eye using the same swab. For the rectum swabs, the swab was introduced into the rectum, rotated several times, retracted, and the tubes were closed with a cap. Only lung samples were taken from eviscerated pigs (n=32).

The samples taken by the hunters or a butcher were sent by priority mail to the laboratory, where they were immediately frozen at –20 C until further processing. On average, it took 3–4 d between sampling and freezing, as many animals were shot during the weekend or late at night.

DNA extraction and determination of DNA concentration

Swab samples were extracted according to the manufacturer's instructions using the Maxwell instrument (Maxwell 16 AS1000, Promega, Fitchburg, Wisconsin, USA) as previously described (Hoffmann et al. 2015). The only modification from the recommended protocol was that the samples were not vortexed after addition of the lysis buffer and proteinase K. Each swab was eluted in 330 µL elution buffer.

The DNA concentration was measured using the Nanodrop-1000 (Witec AG, Lucerne, Switzerland). All samples with a concentration of >120 ng/µL were also diluted 10-fold for later real-time quantitative PCR (qPCR) in order to avoid PCR inhibition. Subsequently, the samples were stored at +4 C until further processing.

PCR analysis for *Chlamydiaceae* DNA

The workflow and methods used in this study are presented in Figure 1. Primers and probes, PCR reactions mixes, and cycling conditions used in the present study are summarized in Table 2 and in the Supplementary Material Table 1. A total of 471 DNA samples were screened for the presence of *Chlamydiaceae* DNA by using a real-time PCR targeting the 23S rRNA gene (*Chlamydiaceae* family-specific).

The *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA gene (Chlam23S-qPCR; Everett et al. 1999) included primers Ch23S-F and Ch23S-R, and probe Ch23S-p (Microsynth, Balgach, Switzerland) described by Ehricht et al. (2006). The internal amplification control eGFP amplified with primers eGFP-1-F and eGFP-10-R, and probe eGFP-Hex (Microsynth) was added to each reaction. The PCR was conducted on a Thermocycler 7500-Fast ABI (Thermo Fisher Scientific, Waltham, Massachusetts, USA). All samples were tested in duplicate, and samples with a cycle threshold of <38 in duplicate PCR reactions were considered positive or questionable positive if the mean cycle threshold value was >38. Quantitation was performed using 10-fold dilutions (10^7 copies to 10 copies/µL) of the *C. abortus* genomic DNA positive control (standard curve).

Presence of *tet*A(C) via PCR

To test for the presence of the *C. suis* Tet-island, which carries the tetracycline efflux pump-encoding gene *tet*A(C), all samples ($n = 471$) were tested for the presence of *tet*A(C) by conventional PCR (Dugan et al. 2004), including a positive control (SWA-107) and a negative control (SWA-86; Wanninger et al. 2016). After amplification of the DNA, the samples were loaded onto a gel (1.5% agarose) for 45 min at 100V and 400mA. Thereafter, the gel was visualized using UVP BioDoc-It 220 UV Imaging System (Ultra-Violet Products Limited, Cambridge, UK).

Other Tet-island-specific PCRs: *tet*R(C), *tet*R(C)-*tet*A(C), and invasin-like

Three additional Tet-island–specific PCRs were applied. Specifically, a PCR targeting the *tet*R(C) regulator amplifying a 608-base pair (bp) fragment of the *tet*R(C) region (Dugan et al. 2004; Donati et al. 2016), another PCR targeting a 457-bp fragment that included both the *tet*R(C) and the *tet*A(C) region (Donati et al. 2016), and a 900-

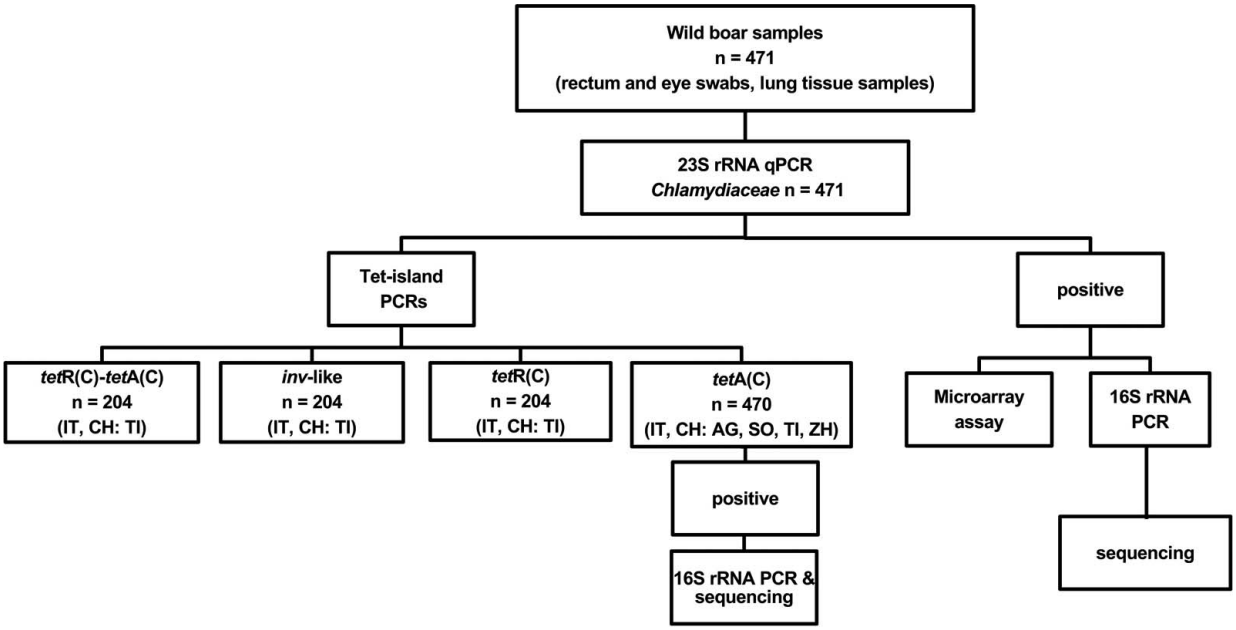

FIGURE 1. A total of 471 samples of 292 wild boars (*Sus scrofa*) from Switzerland, Northern Italy, Germany, and Liechtenstein were collected during hunting. All samples (n=471, rectum and eye swabs, lung tissues samples) were screened for the presence of *Chlamydiaceae* by a 23S rRNA real-time quantitative PCR (qPCR). To determine the chlamydial species, positive samples (n=4) were tested by the Microarray assay and the 16S rRNA PCR, followed by sequencing. Three PCR methods detecting different tetracycline resistance gene determinants, such as *tet*R(C)-*tet*A(C), invasin-like (*inv*-like) region, and *tet*R(C), were applied to 204 samples from Italy and the canton of Ticino (Switzerland). A PCR detecting *tet*A(C) was performed on 407 samples from Italy and Switzerland (cantons AG, SO, TI, ZH). Positive samples (n=6) with the latter PCR were also investigated by the 16S rRNA PCR, followed by sequencing. IT=Italy; CH=Switzerland (including samples of Fürstentum Liechtenstein and Germany); TI=canton of Ticino; AG=canton of Aargau; SO=canton of Solothurn; ZH=canton of Zurich.

bp fragment targeting the intact invasin-like region (*inv*-like; Dugan et al. 2004) were performed on 204 samples (Table 2). A tetracycline-resistant strain (SWA-141; Wanninger et al. 2016) and a tetracycline-sensitive strain (SWA-94; Wanninger et al. 2016) were used as positive and negative controls for *tet*R(C) and *tet*R(C)-*tet*A(C), respectively. In contrast, SWA-94 was the positive control of the *inv*-like PCR while SWA-141 served as a negative control. After amplification of the DNA, the samples were loaded onto a gel (1.5% agarose) together with their controls for 60 min at 100V and 400mA. Thereafter, the gel was visualized as described earlier.

Identification of the chlamydial species by microarray assay

All samples that were positive or questionable positive by the 23S rRNA qPCR were further analyzed by microarray techniques to identify the chlamydial species (Borel et al. 2008). This is a species-specific 23S rRNA Arraymate microarray assay (Alere, Jena, Germany). The test was used as described (Hoffmann et al. 2015; Wanninger et al. 2016).

16S rRNA PCR and sequencing

All samples positive or questionable positive by the 23S rRNA qPCR or the *tet*A(C)-PCR were examined by a PCR targeting a partial sequence of the 16S rRNA gene, as described in Blumer et al. (2007) with primers modified from Everett et al. (1999; Table 2). The final PCR products were purified by QIAquick PCR Purification Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions. Finally, the purified sample was eluted in 30 µL elution buffer and the purified DNA product was prepared for Sanger sequencing at Microsynth (Balgach, Switzerland).

The quality of the sequences was assessed by 4Peaks (Nucleobytes, Aalsmeer, the Netherlands). Subsequently, if possible, a consensus sequence was created from the sequences of the forward and reverse primers using Geneious Prime software version 2019.2.1 (Biomatters, Auckland, New Zealand). For species identification, the partial or

TABLE 2. Wild boars (*Sus scrofa*) were sampled from four Swiss cantons (Zurich, Aargau, Solothurn, Ticino), the Principality of Liechenstein, Germany, and Italy. Samples consisted of eye and rectum swabs and lung samples. We investigated a total number of 471 samples from 292 wild boars for the occurrence of *Chlamydiaceae* and tetracycline resistance genes by PCR. All samples of this study were screened for *Chlamydiaceae* by the Chlam-23S-qPCR, including an internal amplification control eGFP. Positive samples were further classified by the 16S rRNA PCR. The detection of tetracycline resistance genes *tet*A(C), *tet*R(C), and *tet*R(C)-*tet*A(C) was performed by PCR. The presence of an intact invasin-like (*inv*-like) region was determined by PCR. All PCR methods have been previously described and used published primer sets.

PCR method[a]	Gene target and amplicon size[b]	Name	Sequence (5′→3′)	Reference
Chlam-23S-qPCR	23S rRNA, 111 bp	Ch23S-F	CTGAAACCAGTAGCTTATAAGCGGT	Ehricht et al. 2006
		Ch23S-R	ACCTCGCCGTTTAACTTAACTCC	
		Ch23S-P	FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA	
eGFP-qPCR	eGFP, 177 bp	eGFP-1-F	GACCACTACCAGCAGAACAC	Hoffmann et al. 2006
		eGFP-10-R	CTTGTACAGCTCGTCCATGC	
		eGFP-Hex	VIC-AGCACCCAGTCCGCCCTGAGCA-none	
*tet*A(C) PCR	*tet*A(C), 525 bp	CS43	AGCACTGTCCGACCGCTTTG	Dugan et al. 2004
		CS47	TCCTCGCCGAAAATGACCC	
*tet*R(C) PCR	*tet*R(C), 608 bp	tetR-F	TTGGGGCAACCATTTCTGGT	Dugan et al. 2004; Donati et al. 2016
		CS38	CCAAGGGATGACGACGACTG	
*tet*R(C)-*tet*A(C) PCR	*tet*R(C)-*tet*A(C), 457 bp	tetRC-F	TGCGTCGAGCAACGCACGCT	Dugan et al. 2004; Donati et al. 2016
		CS43rev	CAAAGCGGTCGGACAGTGCT	
inv-like region	Intact *inv* gene, 900 bp	CS02	CGTTTCAGGAATACCCACTTCG	Dugan et al. 2004
		CS106	ACACTTCAGGTTTTCGCCGTAG	
16S rRNA PCR (partial)	16S rRNA, 278 bp	16S IGF	GATGAGGCATGCAAGTCGAACG CCA	Blumer et al. 2007
		16S IGR	GTGTTGGCGGTCAATCTCTC	

[a] PCR = conventional PCR; qPCR = real-time quantitative PCR.

[b] bp = base pairs.

consensus sequences were then searched against the database of the National Center for Biotechnology Information using BLASTn (National Center for Biotechnology Information 2019).

RESULTS

Determination of DNA concentration

The total DNA concentration of all 471 extracted samples varied between 0.6 and 1452.02 ng/μL. In general, the DNA concentrations were higher in the rectum samples compared to the lung and eye samples.

Chlamydiaceae-specific qPCR

A total of 471 samples from 292 animals were examined by *Chlamydiaceae*-specific real-time PCR. Of these, 0.9% (4/471) were positive or questionable positive. Specifically, three of 147 (20%) rectum swabs (33-R, 57-R, 664-R) and one of 292 (0.3%) eye swabs (165-A) were positive, all originating from Swiss wild boars <2 yr old. For rectal swabs 33-R and 57-R, corresponding eye swabs were available, both of which were negative. The corresponding lung tissue of sample 165-A also tested negative. The chlamydial load was low for all four samples. The detailed results are listed in Table 3.

Testing of *tet*A(C) via PCR

All 471 samples were assessed by the *tet*A(C) PCR, resulting in six positive samples (1.3%), but they were negative in the qPCR for *Chlamydiaceae* (Table 3). These specimens included three eye swabs (112-A, 123-A, 161-A), two rectum swabs (54-R, 60-R), and one lung sample (150-L) originating from six individual animals from Switzerland (Table 3). Corresponding eye swabs from animals 54-R, 60-R, and 150-L and corresponding rectal swabs from animals 112-A and 123-A were *tet*A(C)-negative and *Chlamydiaceae*-negative.

PCRs targeting *tet*R(C), *tet*R(C)-tetA(C), and *inv*-like region

The PCRs targeting *tet*R(C), *tet*R(C)-*tet*A(C), and the intact *inv*-like region were performed from 204 wild boar samples from 118 animals. All were negative.

Identification of *Chlamydiaceae* species by the microarray assay

All four qPCR-positive samples (33-R, 57-R, 664-R, 165-A) were tested by the microarray. Samples 33-R, 57-R, and 664-R neither hybridized to a family-specific nor a species-specific probe. Therefore, these three samples could not be typed by the microarray assay. The sample 165-A (eye swab) hybridized to the Chlamydia_1 probe and to the species-specific probe *C. suis*, confirming the presence of *C. suis* DNA in this sample (Table 3).

The 16S rRNA PCR and sequencing

Of the four samples positive in the *Chlamydiaceae* qPCR, we obtained sequences from two samples (33-R, 57-R) following 16S rRNA PCR and Sanger sequencing. We performed BLASTn searches on 33-R (219 bp, GenBank accession no. MN519473) and 57-R (208 bp, MN519474) revealing a nucleotide identity of 99% with *C. suis* for both samples. In one sample (664-R), no DNA remained that could be used to perform the 16S rRNA PCR and the remaining sample (165-A) was already characterized by microarray assay.

DISCUSSION

We examined samples consisting of eye swabs, rectum swabs, and lung tissues of 292 wild boars from the Swiss cantons of Zurich, Aargau, Solothurn, and Ticino as well as from Germany, the Principality of Liechtenstein, and Northern Italy for *Chlamydiaceae* using 23S rRNA real-time PCR. In addition, all samples were assessed by a conventional *tet*A(C) PCR to detect the tetracycline resistance gene known to occur in *C. suis* (Dugan et al. 2004). Moreover, 204 samples were examined by PCRs targeting other sequences within the *C. suis* genomic island containing tetracycline resistance genes.

The *Chlamydiaceae*-specific real-time PCR was positive in only 0.9% (4/471) samples, corresponding to 1.4% (4/292) animals tested. Three positive samples were from rectal swabs from the canton of Zurich and Ticino and one

TABLE 3. Details of wild boar (*Sus scrofa*) samples that were either positive for *Chlamydiaceae* real-time quantitative PCR (qPCR) or *tet*A(C) by PCR. Wild boars were sampled from four Swiss cantons (Zurich [ZH], Aargau [AG], Solothurn [SO], Ticino [TI]), the Principality of Liechenstein, Germany, and Italy. Samples consisted of eye and rectum swabs and lung samples. We investigated a total of 471 samples from 292 wild boars for the occurrence of *Chlamydiaceae* and tetracycline resistance genes by PCR.

Animal no.	Origin	Age (yr)	Sex	Type of sample	qPCR	Cycle threshold value (mean)	Number of copies/µL	Microarray[a]	16S rRNA and sequencing[a]	*tet*A(C) PCR[b]
33-R	ZH	<2	Female	Rectum	Positive	42.1	2.8	Negative	*Chlamydia suis*	Negative
54-R	ZH	<2	Male	Rectum	Negative	Undetermined	Undetermined	ND	ND	Positive
57-R[c]	ZH	<2	Male	Rectum	Positive	38.5	34.1	Negative	*Chlamydia suis*	Negative
60-R	ZH	<2	Male	Rectum	Negative	Undetermined	Undetermined	ND	ND	Positive
112-A	SO	>2	Female	Eye	Negative	Undetermined	Undetermined	ND	ND	Positive
123-A	ZH	>2	Female	Eye	Negative	Undetermined	Undetermined	ND	ND	Positive
150-L	AG	<2	Male	Lung	Negative	Undetermined	Undetermined	ND	ND	Positive
161-A	ZH	<2	Male	Eye	Negative	Undetermined	Undetermined	ND	ND	Positive
165-A	AG	<2	Male	Eye	Positive	36.8	30.7	*Chlamydia suis*	ND	Negative
664-R	TI	<2	Male	Rectum	Positive	37.6	1.5	Negative	Insufficient sample	Negative

[a] ND = not done.

[b] Primer CS43, CS37 (*tet*A(C) PCR).

[c] Extracted DNA was diluted 1:10 prior to PCR analysis.

was from an eye swab from the canton of Aargau. Of these, three samples could be assigned to the species *C. suis* by 16S rRNA sequencing or Arraymate microarray.

The results showed a *C. suis* infection prevalence lower than that previously shown in wild boars from countries included in this study, such as Germany and Italy. In a German study (Hotzel et al. 2004), tissue samples (lung, pulmonary lymph node, large intestine, and uterus) from 14 hunted wild boars were examined. *Chlamydiaceae* were detected in 57% (8/14) of the samples by PCR. Sequencing of the PCR products revealed mainly *C. psittaci* (10 positive samples out of four animals), but also *C. abortus* (4/2) and *C. suis* (3/2). Among all organs, the lung was most frequently found to be infected. In Italy, wild boar blood samples were tested for antibodies against *Chlamydiaceae* (Di Francesco et al. 2011). Antibody titers to chlamydiae were detected in 63.6% (110/173) of the samples tested, with a specific reactivity to *C. suis* in 25% (44/173) serum samples. In a further study (Di Francesco et al. 2013), 50% (22/44) of wild boars were positive for *Chlamydiaceae* and *Parachlamydiaceae* by PCR. Sequencing of the amplicons identified *C. suis* and *C. pecorum* in 12 and 5 samples, respectively. In order to evaluate the systemic distribution of *Chlamydiaceae*, tissue samples collected from four wild boars were examined using PCR, showing the presence of *C. suis* in lung, pulmonary lymph node, and large intestine, *C. psittaci* in lung and small intestine, and *C. pecorum* in the pulmonary lymph node and small intestine.

Contrary to these previous studies, the prevalence of *Chlamydiaceae* in the investigated wild boar populations was very low (1.4%, 4/292). *Chlamydia suis* was only found in rectal or conjunctival swabs, with no evidence of *C. suis* in the lung samples. The lower prevalence in our study could be, in part, explained by differences in methods (molecular detection versus antibody detection by serology). However, previous studies (Hotzel et al. 2004; Di Francesco et al. 2013) also used molecular methods resulting in higher prevalences (14% and 27%, respectively) as observed in our study. We think that the lower *C. suis* prevalence observed in our study might be attributed to limited contact between wild boars and domestic pigs in the investigated areas.

The *tet*A(C) gene is the only known antimicrobial resistance gene present in *Chlamydiaceae* (Dugan et al. 2004). The first tetracycline-resistant *C. suis* strains were reported in US pig farms in the early 1990s (Lenart et al. 2001). Subsequently, *C. suis*–tetracycline resistant strains have been documented in pigs from various countries. A preliminary study in Switzerland (Borel et al. 2012) could identify *Chlamydiaceae* from 12 eye swabs and 3 pooled fecal samples from 12, 6-wk-old domestic pigs by real-time PCR. *Chlamydia suis* was subsequently identified in all positive samples (eye swabs and pooled fecal sample). The *tet*A(C) gene-coding region was detected in one pooled fecal sample and one eye swab. In a recent Swiss study investigating 59 *C. suis* isolates from fattening pigs, 32 isolates were positive for *tet*A(C) while 27 were negative (Wanninger et al. 2016). In another study of 39 sows from Belgian, Cypriote, and Israeli herds, 83 % (19/23) of *C. suis*-positive sows were infected with tetracycline-resistant *C. suis* strains (Schautteet et al. 2013). During a study in Italy (Di Francesco et al. 2008), 14 chlamydial isolates were collected from domestic pigs with conjunctival and/or reproductive disorders reared in four farms in Northern and Southern Italy. All *C. suis* isolates carried the *tet*A(C) gene associated with tetracycline resistance. Similary, Donati et al. (2016) detected *tet*A(C) and *tet*R(C) transcripts in 11 *C. suis* isolates from Italian pigs cultured in the absence and presence of tetracycline. Despite the widespread presence of *C. suis* tetracycline-resistant strains in pigs, no data are currently available about the occurrence of tetracycline-resistant *C. suis* in wild boar.

In our study, the tetracycline resistance gene *tet*A(C) was detected in six samples, but all six samples were negative for *Chlamydiaceae* by PCR. In addition, PCRs targeting *tet*R(C), *tet*R(C)-*tet*A(C), and *inv*-like gene

fragments were negative. These results suggested that the *tet*A(C) gene fragment detected in the six samples was probably not of chlamydial origin but more likely from other bacteria such as *Escherichia coli* (Wasyl et al. 2018) or *Streptococcus suis* (Chen et al. 2013).

In general, development of tetracycline resistance in *C. suis* after tetracycline administration is very common in pig farms, and it has been shown that resistance occurs more frequently and rapidly under the selective pressure of treatment with tetracycline (Wanninger et al. 2016; Seth-Smith et al. 2017). Due to a lack of selective pressure, it is less likely that increased resistance rates can be detected in wild boar. However, transmission of the resistance genes from domestic pigs to wild boar or selective pressure in the environment could lead to the presence of tetracycline-resistant *C. suis* strains in wild boars. In theory, tetracycline-resistant *C. suis* fecal shedding from fattening pigs could contaminate crops via liquid manure, waste water, and sludge.

We detected *C. suis* in wild boar in Switzerland but to a much lesser extent than in domestic pigs. In fattening pigs from Switzerland, 2,461 samples were investigated for the prevalence of *Chlamydiaceae* by sampling conjunctival and fecal swabs from 29 pig herds at the beginning and end of the fattening period (Hoffmann et al. 2015). They found that 94.3% of fecal swabs in the first sampling and 92.0% in the second sampling were positive for *Chlamydiaceae*, as well as 45.9% and 32.6% of the conjunctival swabs, respectively. *Chlamydia suis* was identified as the most common chlamydial species in these samples (Hoffmann et al. 2015).

The low chlamydial prevalence observed in our study of a wild boar population could be attributed to limited contacts between wild boars and outdoor domestic pigs due to strict biosecurity measures or limited numbers of rural pig herds. Furthermore, tetracycline-resistant *C. suis* strains do not appear to be widespread in Swiss wild boar. In order to obtain more comprehensive epidemiologic data on the chlamydial prevalence in wild boar in Switzerland, additional animals from other cantons would need to be studied. Of particular importance would be the regions with many domestic pigs, such as the cantons of Lucerne and St. Gallen. Furthermore, it would be of interest to investigate internal organs for the presence of *Chlamydiaceae* to elaborate if systemic infections in wild boar are present, as suggested by previous studies (Hotzel et al. 2004; Di Francesco et al. 2013).

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SUPPLEMENTARY MATERIAL

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