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Prevalence of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in wild boars in Switzerland

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ABSTRACT

Between October 2007 and March 2008, 153 wild boars shot in the Canton of Geneva in Switzerland were sampled. Fifty-one percent of the animals were males and 49% were females. The age of most (81%) animals varied between 6 months and 2 years. Prevalence of enteropathogenic *Yersinia* in tonsils and faeces was studied using culture and PCR methods and in tissue fluid of tonsils using an ELISA system. Prevalence of anti-*Yersinia* antibodies in tissue fluid was 65%. Detection rate of enteropathogenic *Yersinia* in tonsils of 153 wild boars by real-time PCR was 44%. *Ail*-positive *Yersinia enterocolitica* and *inv*-positive *Yersinia pseudotuberculosis* were detected in 35 and 20% of the animals, respectively. Both species were detected in 10% of the animals. Isolation rate of enteropathogenic *Yersinia* was low; *ail*-positive *Y. enterocolitica* and *inv*-positive *Y. pseudotuberculosis* were found in 9 and 3% of the animals, respectively. Prevalence was shown to be significantly higher in tonsils than in faeces. Furthermore, females were more commonly positive than males. This study shows that the prevalence of enteropathogenic *Yersinia* is high and both enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* are common findings in tonsils of wild boars in Switzerland.

1. Introduction

Yersiniosis is a disease that affects wild and domestic animals as well as humans. Enteric yersiniosis is caused by pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. However, human yersiniosis, which is very common in Europe, is mostly caused by *Y. enterocolitica* (EFSA, 2007). The disease is transmitted by the faecal–oral route and typical symptoms are fever, abdominal pain and diarrhoea (Bottone, 1997; Jalava et al., 2006). Acute yersiniosis in animals, which is more frequently caused by *Y. pseudotuberculosis*, is characterised as enteritis and enlargement of lymph nodes and spleen whereas chronic infections may cause granulomatous nodules and localised abscesses affecting various organs, typically liver and lungs (Brügmann et al., 2001; Zhang et al., 2008).

Y. enterocolitica and *Y. pseudotuberculosis* have been recovered from diverse animal sources ranging from farm animals and domestic pets to free-living and captive wild animals (Bottone, 1997; Fukushima and Gomyoda, 1991). However, human pathogenic strains of *Y. enterocolitica* have frequently been isolated only from asymptomatic pigs at slaughter (Fredriksson-Ahomaa et al., 2006). In Switzerland, the prevalence of pathogenic *Y. enterocolitica* has shown to be high in the tonsils of pigs at slaughter: 85 and 34% with PCR and culturing, respectively (Fredriksson-Ahomaa et al., 2007). *Y. pseudotuberculosis* has also sporadically been isolated from tonsils of slaughter pigs (Niskanen et al., 2002; Ortiz Martinez et al., 2009). Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* can easily be detected using PCR targeting the chromosomally encoded virulence genes *ail* and *inv*, respectively (Fredriksson-Ahomaa et al., 2007; Niskanen et al., 2008).

In the past years, the wild boar population has increased considerably in Europe including Switzerland (Köppel et al., 2007). In the same time also out-door farming of domestic pigs is getting more popular, which may raise the risk of contact between wild boars and domestic pigs and in this way also raise the risk of transmission of pathogenic *Yersinia* between the animals. Until today, very little information is available about wild boars as carriers of this pathogen. Thus, the goal of this study was to provide data on the prevalence of enteropathogenic *Yersinia*, including both pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in male and female wild boars of different weights using different detection methods.

2. Materials and methods

2.1. Sampling and sample preparation

In total, 153 wild boars shot in the Canton of Geneva in Switzerland were studied. Of 151 animals with known gender, 49% (73) were females and 51% (78) were males (Table 1). The weight of most animals (120/148) varied between 20 and 60 kg (Table 2). Tonsil

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Table 1
Prevalence of enteropathogenic *Yersinia* in the 153 wild boars shot in Switzerland.

Species	Sex (no. of animals)	Number of positive animals		
		PCR (%)	Culture (%)	ELISA[a] (%)
Enteropathogenic *Yersinia*	Female (73)	36 (49)	10 (14)	50 (70)
	Male (78)	31 (40)	7 (9)	47 (60)
	Not known (2)	1	0	2
	All animals (153)	68[b] (44)	17[c] (11)	99 (65)
ail-positive *Y. enterocolitica*	Female (73)	28 (38)	9 (12)	
	Male (78)	24 (31)	5 (6)	
	Not known (2)	1	0	
	All animals (153)	53 (35)	14 (9)	
inv-positive *Y. pseudotuberculosis*	Female (73)	17 (23)	2 (3)	
	Male (78)	13 (17)	2 (3)	
	Not known (2)	0	0	
	All animals (153)	30 (20)	4 (3)	

[a] OD > 0.2.
[b] In 15 animals both pathogens were detected.
[c] From one animal both pathogens were isolated.

and faeces samples were collected between October 2007 and March 2008. Of 146 animals both tonsils were studied and from seven animals only one tonsil was available for testing. Faecal samples were obtained from 73 animals. In total, 299 tonsil and 73 faecal samples were studied. The tonsil and faeces samples were collected immediately after evisceration and placed in sterile plastic bags, which were stored at −20 °C until examination. About a 10-g tonsil and a 1-g faeces sample were homogenised in 90 ml of tryptic soy broth (CASO, Merck, Darmstadt, Germany). About 100–500 µl tissue fluid of tonsils of 153 wild boars were collected from the plastic bags and stored at −20 °C until further examination by ELISA.

2.2. Detection of ail-positive Y. enterocolitica and inv-positive Y. pseudotuberculosis using real-time PCR and culture methods

Real-time PCR was used to detect *ail*-positive *Y. enterocolitica* and *inv*-positive *Y. pseudotuberculosis* directly from the overnight enrichment (37 °C, 16–18 h) in CASO broth. The DNA was extracted from 100 µl of overnight enrichment using InstaGene (BioRad, Hercules, CA) based on the chelating properties of Chelex resin. After centrifugation, the supernatant was removed and the pellet was resuspended in 50 µl of InstaGene, which was incubated at 56 °C for 15 min and then at 100 °C for 10 min. After centrifugation, the supernatant was used as template. A real-time PCR protocol based on SYBRGreen was used for both pathogens according to Fredriksson-Ahomaa et al. (2007). A 170 bp-fragment of *ail* gene according to Nakajima et al. (1992) and a 183 bp-fragment of *inv* gene according to Thoerner et al. (2003) from *Y. enterocolitica* and *Y. pseudotuberculosis*, respectively, were amplified. Briefly, 2 µl of the template was added to 23 µl of the master mix, which contained 1× ready-to-use mix (iQ™SYBRGreen Supermix, BioRad) and 200 nM of primers. A 3-step protocol (denaturation at 95 °C for 10 s, annealing at 56 °C for 20 s and elongation at 72 °C for 10 s) with 40 cycles followed by a melting curve analysis was performed. The PCR fluorescence was detected using the iQ™5 Multicolour Real-Time PCR Detection System (BioRad). A threshold cycle (Ct) under 38 and a specific melting temperature (Tm) indicated a positive result. The Tm for *ail*-positive *Y. enterocolitica* and *inv*-positive *Y. pseudotuberculosis* was 79 °C and 81 °C, respectively.

Y. enterocolitica and *Y. pseudotuberculosis* were isolated using direct plating, non-selective and selective enrichment. Hundred microliters was directly plated on a selective CIN (cefsulodin–irgasan–novobiosin) agar plate (Merck) after homogenisation. For non-selective enrichment, the samples were incubated in CASO broth (Merck) at 25 °C for 16–18 h and then one loop (20 µl) was plated on a CIN agar plate. For selective enrichment, 1 ml of the homogenate was transferred into 9 ml of irgasan–ticarcillin–potassium chlorate (ITC broth) (Merck), incubated at 25 °C for 2 d and finally one loop was streaked on a CIN agar plate. CIN agar plates were incubated at 30 °C for 18–20 h and further 24 h at room temperature. Urease-positive isolates were identified using API 20E. The presence of the chromosomally encoded *ail* gene of *Y. enterocolitica* and *inv* gene of *Y. pseudotuberculois* were studied by real-time PCR. The *ail*-positive *Y. enterocolitica* isolates were bio- and serotyped (Fredriksson-Ahomaa et al., 2007). Biotyping of *Y. enterocolitica* was performed according to ISO 10273 (Anonymous, 2003) using pyrazinamidase and tween activity, esculin hydrolysis, indole production, and salicin, xylose and trehalose fermentation tests (Wauters et al., 1987). *Y. pseudotuberculosis* was biotyped using raffinose and melibiose fermentation, and citrate utilisation tests (Tsubokura and Aleksic, 1995). Serotyping was performed with the slide agglutination using commercial *Y. enterocolitica* O:3, O:5, O:9 and O:27 antisera (Sifin, Berlin, Germany) and *Y. pseudotuberculosis* O:1 to O:4 antisera (MastGroup, Bootle, UK).

2.3. Yersinia ELISA

Anti-*Yersinia* antibodies were determined in juice extracted from tonsils of 153 animals using a microtitre plate based enzyme immunoassay (PIGTYPE® YOPSCREEN, Labor Diagnostic, Leipzig, Germany) according to manufacturer's instructions. The antigens used in the test are *Yersinia* Outer Proteins (Yops), which are expressed only by pathogenic *Yersinia* strains. This ELISA kit is suitable for quantification of *Yersinia* antibodies in meat juice samples. The optical density (OD) was measured in a spectrophotometer (680 Microplate Reader, BioRad) and an OD value of 0.2 was used as cut-off value.

3. Results

The prevalence of enteropathogenic *Yersinia* in the tonsils of wild boars was 44% and 11% using PCR and culture methods, respectively (Table 1). The prevalence of anti-*Yersinia* antibodies was 65% using an OD value of 0.2. Pathogenic *Y. enterocolitica* was detected in 35% and *Y. pseudotuberculosis* in 20% of the animals using PCR. Fifteen wild boars (10%) were carrying both species.

Pathogenic *Y. enterocolitica* strains were isolated from 9% and *Y. pseudotuberculosis* from 3% of the animals. From one animal both species were found. The bioserotypes 2/O:5,27, 2/O:9 and 4/O:3 were identified in *Y. enterocolitica* strains isolated from 3, 4 and 5 animals, respectively. Two of the *Y. pseudotuberculosis* strains belonged to bioserotype 1/O:1, one to 2/O:1 and one to 1/O:2.

The prevalence of enteropathogenic *Yersinia* was higher in females than in males with all three methods (Table 1), however, without a statistical difference (Fisher's test, $p > 0.05$). The prevalence of anti-*Yersinia* antibodies in wild boars was increasing when the animals were getting older but, using PCR and culture methods, the prevalence of enteropathogenic *Yersinia* was decreasing with age (Table 2). The prevalence of enteropathogenic *Yersinia* was significantly (Fisher's test, $p < 0.05$) higher among young animals (<20 kg) than among

Table 2
Prevalence of enteropathogenic *Yersinia* among the 153 wild boars of different weights.

Weight	Age[a] (month)	No. of animals	Number of positive animals		
			PCR (%)	Culture (%)	ELISA[b] (%)
<20	<6	10	7 (70)	4 (40)	5 (50)
20–40	6–12	53	23 (43)	7 (13)	29 (55)
40–60	12–24	67	31 (46)	5 (7)	43 (64)
>60	>24	18	5 (28)	0 (0)	17 (94)
Not known		5	2	1	5
All animals		153	68 (44)	17 (11)	99 (65)

[a] Estimated from the weight according to Brooks (http://texnat.tamu.edu/symposia/feral/feral-16.htm) and Hebeisen et al. (2008).
[b] OD > 0.2.