Shiga toxin-producing *Escherichia coli* isolated from hunted wild boar *(Sus scrofa)* in Switzerland

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Shigatoxin produzierende *Escherichia coli* aus in der Schweiz erlegten Wildschweinen *(Sus scrofa)*

Shigtoxin produzierende *Escherichia (E.) coli* (STEC) sind zoonotische, lebensmittelbedingte Krankheitserreger von grosser Bedeutung für die öffentliche Gesundheit. Während Wiederkäuer als Hauptreservoir gelten, gelten Wildtiere zunehmend als Überträger und potenzielle Reservoire von STEC. Ziel dieser Studie war es, das Vorkommen von STEC in insgesamt 59 Kotproben von gejagten Wildschweinen (*Sus scrofa*) aus zwei verschiedenen Regionen der Schweiz (Kanton Thurgau in der Nordschweiz und Kanton Tessin in der Südschweiz) zu bestimmen und die Isolate mittels vollständiger Genomsequenzierung zu charakterisieren. Nach einem Anreicherungsschritt wurden Shigatoxin-kodierende Gene (*stx*) durch eine Echtzeit-PCR in 41 % (95 %-Konfidenzintervall (95 %-KI) 0,29 – 0,53) der Proben nachgewiesen, wovon aus 22 % (95 % CI 0,13 – 0,34) derselben Proben STEC gewonnen wurden. Es wurden sieben verschiedene Serotypen und sechs verschiedene Sequenztypen (STs) gefunden, wobei hauptsächlich O146:H28 ST738 (n = 4) und O100:H20 ST2514 (n = 4) vorkamen. Die *stx*-Subtypen *stx1c/stx2b* (n = 1), *stx2a* (n = 1), *stx2b* (n = 6) und *stx2e* (n = 6) wurden identifiziert. Kein Isolat enthielt das *eae*-Gen, aber alle enthielten zusätzliche Virulenzgene, am häufigsten *astA* (n = 10), *hlyE* (n = 9) und *hra* (n = 9). STEC O11:H5, O21:H21 und O146:H28 enthielten Virulenzfaktoren, die mit extraintestinalen pathogenen *E. coli* (ExPEC) assoziiert sind, und STEC O100:H20 und O155:H26 besassen *sta1* und/oder *stb* und waren STEC/enterotoxigene *E. coli* (ETEC)-Hybridpathotypen.

Unsere Ergebnisse zeigen, dass Wildschweine Träger von STEC sind und diese somit in der Umwelt verbreitet werden können, was möglicherweise auch zur Kontamination landwirtschaftlicher Nutzpflanzen und Wasserquellen führt. Zu den Serogruppen gehörte STEC O146, einer der häufigsten nicht-O157-Serogruppen, welche europaweit von kranken Menschen isoliert wird und ein Risiko für die öffentliche Gesundheit darstellt. Da bei Schweinen häufig über Stx2e-produzierende STEC berichtet wurde, kann angenommen werden, dass Wildschweine ein potenzielles Risi-

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Shiga toxin-producing *Escherichia* (*E.*) *coli* (STEC) are zoonotic foodborne pathogens of significant public health importance. While ruminants are considered the main reservoir, wild animals are increasingly acknowledged as carriers and potential reservoirs of STEC. The aim of this study was to determine the occurrence of STEC in a total of 59 faecal samples of hunted wild boars (*Sus scrofa*) from two different regions in Switzerland (canton Thurgau in northern Switzerland and canton Ticino in southern Switzerland), and to characterise the isolates using a whole genome sequencing approach. After an enrichment step, Shiga-toxin encoding genes (*stx*) were detected by real-time PCR in 41 % (95 % confidence interval (95 %CI) 0,29 – 0,53) of the samples, and STEC were subsequently recovered from 22 % (95 %CI 0,13 – 0,34) of the same samples. Seven different serotypes and six different sequence types (STs) were found, with O146:H28 ST738 (n = 4) and O100:H20 ST2514 (n = 4) predominating. Subtyping of *stx* identified isolates with *stx1c/stx2b* (n = 1), *stx2a* (n = 1), *stx2b* (n = 6), and *stx2e* (n = 6). No isolate contained the *eae* gene, but all harboured additional virulence genes, most commonly *astA* (n = 10), *hlyE* (n = 9), and *hra* (n = 9). STEC O11:H5, O21:H21, and O146:H28 harboured virulence factors associated with extra-intestinal pathogenic *E. coli* (ExPEC), and STEC O100:H20 and O155:H26 possessed *sta1* and/or *stb* and were STEC/enterotoxigenic *E. coli* (ETEC) hybrid pathotypes.

Our results show that wild boars are carriers of STEC which may be distributed in the environment, possibly leading to the contamination of agricultural crops and water sources. The serogroups included STEC O146 which belongs to the most common non-O157 serogroups associated with human illness in Europe, with implications for public health. Since Stx2e-producing STEC have frequently been reported in swine and pork, STEC O100:H20 harbouring *stx2e* in faeces of wild boars may be relevant to free-range systems of pig farming because of the potential risk of transmission events at the wildlife–livestock interface.

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ko zur Übertragung von STEC O100:H20, die *stx2e* enthalten, auf Schweine in Freilandhaltungssysteme darstellen könnten.

Schlüsselwörter: Jagd, Wildschweine, Shigatoxin, *Escherichia coli,* Serotypen, Virulenzgene, hybride Pathotypen

Keywords: Hunting, wild boars, Shiga-toxin, *E. coli*, serotypes, virulence genes, hybrid pathotypes

Introduction

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are zoonotic pathogens that can spread to humans and cause gastrointestinal illnesses which may include severe non-bloody or bloody diarrhea (BD), haemorrhagic colitis (HC), and occasionally the potentially life-threatening haemolytic uremic syndrome (HUS).[20] STEC are characterized by their ability to produce one or two different types of Shiga toxin which are encoded by the *stx1* and *stx2* genes.[25,48] Many STEC strains also feature additional virulence genes encoding toxins and adherence factors have been shown to be important for the pathogenicity of STEC, such as *astA* (enteroaggregative *E. coli* heat-stable toxin 1), *eae* (adherence factor intimin encoded within the Locus of Enterocyte Effacement (LEE) pathogenicity island), *ehxA* (enterohemolysin), *iha* (IrgA homolog adhesin), among others.[19] STEC associated with toxin subtypes *stx2a* or *stx2d* and *eae* are linked to the risk of severe clinical manifestations including HC and HUS.[25] The overall most frequently reported STEC serogroups related to severe infections in the EU in 2021 were O157 (15,2 % of the human cases) and O26 (14,8 %).[11] The most common virulence gene combination in strains isolated from severe cases was *stx2*/*eae*, followed by *stx1*/*stx2*/*eae*, with the *stx* subtypes *stx2a*, *stx1a*, *stx2c* and *stx2d* reported most often.[11]

Although linked to foodborne outbreaks, the majority of STEC infections remain sporadic and are associated with the consumption of undercooked or raw meat and raw milk, person-to-person transmission, and contact with animals or their environment.[3,18,24] While bovine ruminants undoubtably represent the principal STEC reservoir, an increasing number of other animals have been identified as carriers and potential reservoirs of STEC, including wild animals.[8,22]

The Eurasian wild boar (*Sus scrofa*) is widely distributed in most parts of Europe, including Switzerland.[28] In Switzerland, there are two wild boar populations represented by a northern population ranging from Geneva to St. Gallen, and a southern population distributed throughout the canton Ticino.[28] The two populations are geographically separated by the Alps which act as a natural barrier between northern and southern Switzerland.[28] During the past decades there has been an increase and spatial expansion of the wild boar population in Switzerland, similar to the situation in other European countries.[12] This development may come with an increased threat to animal and human health, especially in areas densely populated with wild boars, such as in rural and agricultural regions.[13,31] Close contact between wild boars and free-range livestock may promote pathogen transmission to food-producing animals.[12] Further, scavenging wild boars invading livestock grazing areas and agricultural lands in search of feed can contaminate pastures, crops, and water with faecal pathogens including STEC.[2] The aim of this study was to assess the occurrence of STEC in faecal samples of hunted wild boar in Switzerland, and to analyse the STEC isolates for serotypes, multilocus sequence types, and virulence gene content, using a whole genome sequencing approach.

Materials and Methods

Sampling

Sampling took place between December 2022 and February 2023. All animals were legally hunted for human consumption. No animal was killed for the purpose of providing samples. Ethical approval was not required for this study. Faecal samples from 59 animals were collected by the hunters in the field immediately after shooting and evisceration. From each sampled animal the location of hunting was noted. After opening of the large intestine, faecal matter was collected from the colon, transferred to sterile tubes, placed in cooler boxes for transport and stored at -20°C until processing.

Samples originated from 12 wild boars shot in seven municipal areas in the canton Thurgau located in northern Switzerland, and from 47 wild boars shot in 21 municipal areas in the canton Ticino located in southern Switzerland (Table 1). Overall, 59 samples were available for analysis.

Screening

Prior to microbiological analysis, the faecal samples were thawed at 4°C overnight. A sterile cotton swab of each sample was placed in a sterile blender bag (Seward, Worthing, UK), homogenized at a 1:10 ratio in Enterobacteriaceae Enrichment (EE) broth (BD, Franklin Lakes, USA), and incubated at 37°C for 24 h. Cultures were streaked onto sheep blood agar (Difco™ Columbia Blood Agar Base EH; Becton Dickinson) and incubated again overnight at 37°C, as described previously.[33] Colonies were washed off with 2 mL NaCl (0,85 %) and 100 µL thereof combined with 200

µL Gram-negative lysis buffer. Samples were heated for 50 min at 60°C followed by 10 min at 99°C, and centrifuged (11,000 rpm, 2 min). Samples were then screened for *stx1* and *stx2* by real-time PCR (LightCycler R 2.0 Instrument, Roche Diagnostics Corporation, Indianapolis, IN, USA) using the QuantiNova Multiplex PCR Kit (Qiagen, Hombrechtikon, Switzerland) according to the guidelines of the European Union Reference Laboratory.[10]

Table 1: Municipal areas of shooting locations, and the results of PCR screening of faecal samples for *stx*, of 59 hunted wild boars shot during December 2022 and February 2023.

Sample ID	Canton	Postal code	Municipal area	PCR *stx* result[a]
WS63	Thurgau	8500	Frauenfeld	–
WS82	Thurgau	8500	Frauenfeld	*stx1, stx2*
WS64	Thurgau	9216	Hohentannen	*stx2*
WS89	Thurgau	8553	Hüttlingen	–
WS32	Thurgau	8553	Hüttlingen	*stx1*
WS71, WS85	Thurgau	8505	Pfyn	–
WS88, WS90	Thurgau	8266	Steckborn	–
WS33	Thurgau	8266	Steckborn	*stx1*
WS84	Thurgau	8512	Thundorf	–
WS34	Thurgau	8583	Weinmoos	–
WS74, WS75	Ticino	6661	Auressio	–
WS78	Ticino	6670	Avegno	–
WS72	Ticino	6954	Bigorio	–
WS79	Ticino	6934	Bioggio	*stx2*
WS54, WS73	Ticino	6835	Breggia	–
WS55, WS56, WS57	Ticino	6835	Breggia	*stx2*
WS50, WS58, WS60, WS62	Ticino	6614	Brissago	–
WS49, WS52, WS61	Ticino	6614	Brissago	*stx2*
WS51	Ticino	6614	Brissago	*stx1, stx2*
WS47	Ticino	6837	Bruzella	–
WS45	Ticino	6655	Centovalli	*stx2*
WS53	Ticino	6678	Coglio	*stx2*
WS81	Ticino	6655	Cremaso	–
WS36, WS38	Ticino	6656	Golino	–
WS37	Ticino	6656	Golino	*stx2*
WS40, WS69	Ticino	6672	Gordevio	–
WS42, WS43, WS44	Ticino	6655	Intragna	–
WS41	Ticino	6655	Intragna	*stx2*
WS70	Ticino	6814	Lamone	–
WS66	Ticino	6814	Lamone	*stx2*
WS68	Ticino	6605	Locarno Monti	*stx2*
WS46	Ticino	6834	Morbio Inferiore	–
WS39	Ticino	6832	Pedrinate	–
WS77	Ticino	6832	Pedrinate	*stx2*
WS25, WS31, WS35	Ticino	6622	Ronco	–
WS26, WS27, WS29, WS30	Ticino	6622	Ronco	*stx2*
WS59	Ticino	6867	Serpiano	–
WS28	Ticino	6832	Seseglio	*stx2*
WS67	Ticino	6807	Taverne	–

[a] PCR positive samples were further cultured and a total of 13 STEC were isolated by growth on CHROM agar™ or RAPID' *E. coli* agar™. For details see text.

Recovery of STEC

In the event of a *stx* positive PCR result, one loopful of suspension was streaked onto STEC Chromagar plates (CHROMagar, Paris, FR), and RAPID' *E. coli* agar (Bio-Rad, Hercules CA, USA) to obtain single colonies. The plates were incubated at 37°C overnight. From each plate, up to 30 individual colonies suspicious for *E. coli* were picked (mauve colonies on STEC Chromagar plates; violet colonies on Rapid' *E. coli* plates) and suspended in 0,5 ml 0,85 % NaCl. The suspensions were pooled in groups of material from ten colonies and screened for *stx1* and *stx2* genes by real-time PCR (LightCycler R 2.0 Instrument, Roche Diagnostics Corporation, Indianapolis, IN, USA) using the QuantiNova Multiplex PCR Kit (Qiagen, Hombrechtikon, Switzerland) according to the guidelines of the European Union Reference Laboratory.[10] In the case of a positive PCR result for *stx1* or *stx2*, the pool was taken apart and the ten colonies were tested again individually. From plates yielding more than one *stx1* and/or *stx2* positive colony, one presumptive STEC isolate was randomly chosen for subsequent characterisation by whole genome sequencing (WGS) analysis.

DNA extraction and whole genome sequencing

STEC isolates were grown on sheep blood agar at 37°C overnight prior to DNA isolation using the DNA blood and tissue kit (Qiagen, Hombrechtikon, Switzerland). The DNA libraries were prepared using a Nextera DNA Flex Sample Preparation Kit (Illumina, San Diego, CA, USA). Whole genome sequencing was performed on an Illumina MiniSeq Sequencer (Illumina, San Diego, CA, USA). The Illumina-reads files passed the standard quality checks using the software package FastQC 0.11.9 (Babraham Bioinformatics, Cambridge, UK) and were assembled using the Spades 3.14.1 based software Shovill 1.0.4,[41] using default settings. The assembly was filtered, retaining contigs > 500 bp and annotated using the NCBI prokaryotic genome annotation pipeline.[46] The O and H-types were identified using SerotypeFinder 2.0.1[14] and the most recent version of the database from 18 May 2022. The sequence type (ST) of each strain was determined based on seven housekeeping genes with the tool «MLST»[42] using PubMLST as database (https://pubmlst.org/).[17] Stx types were determined by an in silico PCR using the perl script in_silico_pcr (https://github.com/egonozer/in_silico_pcr) with the option «-m, allow one mismatch» and primer sets described in the European Union Reference Laboratory for *E. coli* manual for *stx* genes detection.[9] The virulence gene profiles were determined using VirulenceFinder 2.0.3[15] and the database version from 12 December 2022. Core genome (cg) MLST was performed with assembled genomes sequences using Ridom Seqsphere+ 8.5.1 (Ridom GmbH, Münster, Germany) with standard settings.

Table 2: GenBank accession numbers for 13 STEC isolated from wild boar faeces.

Strain ID(s)	Accession no.
WS26a	JATAQP000000000
WS27	JATAQQ000000000
WS28	JATAQR000000000
WS29a	JATAQS000000000
WS49b	JATAQT000000000
WS51	JATAQU000000000
WS53a	JATAQV000000000
WS57	JATAQW000000000
WS64	JATAQX000000000
WS66	JATAQY000000000
WS68b	JATAQZ000000000
WS77	JATARA000000000
WS79	JATARB000000000

Statistical analysis

Comparisons of the proportions of *stx*-positive samples and of samples containing STEC from wild boar from different groups (the canton Thurgau or the canton Ticino) were performed by Fisher's exact test. The significance criterion was set at $p \leq 0,05$. Calculations were performed using GraphPad (https://www.graphpad.com).

Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the BioProject accession number PRJNA984448. Accession numbers for the individual isolates from this study are listed in Table 2.

Results

Real-time PCR screening for *stx* genes and isolation of STEC

Real-time PCR screening showed *stx1* and/or *stx2* in 24 (41 %, 95 %CI 0,29 – 0,534) of the 59 faecal samples. Of the 24 positive samples, 22 (92 %, 95 %CI 0,73 – 0,988) contained *stx2*, either alone or in combination with *stx1*. The proportions of faecal samples from the canton Thurgau and from the canton Ticino that tested positive for *stx* were 4/12 (33 %, 95 %CI 0,136 – 0,61) and 20/47 (43 %, 95 %CI 0,295 – 0,567), respectively (Table 3), and the difference was not statistically significant (p = 0,7447). In each canton, positive samples were linked to different municipal areas (Table 1).

STEC were isolated from 13 of the 24 *stx*-positive samples, corresponding to a recovery rate of 54 % (95 %CI 0,35 – 0,721) and an overall STEC prevalence of 22 % of the 59 samples (95 %CI 0,132 – 0,343) (Table 3). The proportions of samples containing cultured STEC were 1/12 (8 %,

95 %CI 0 – 0,375) of those from the canton Thurgau and 12/47 (26 %, 95 %CI 0,151 – 0,396) from the canton Ticino, respectively, and the difference was not significant (p = 0,2679).

Serotypes, multilocus sequence types (MLST) and phylogenetic relationship

Overall, seven different serotypes were identified including O8:H19 (n = 1), O11:H5 (n = 1), O21:H21 (n = 1), O100:H20 (n = 4), O146:H28 (n = 4), O155:H26 (n = 1), O166:H28 (n = 1) (Table 4). Six sequence types (STs) were assigned among the STEC, consisting of ST56 (n = 1), ST201 (n = 1), ST683 (n = 1), ST738 (n = 4), ST1104, and ST2514 (n = 4) (Table 4). STEC O166:H28 (isolate ID WS-51) was assigned to a new ST, ST15443 (Table 4). Isolates with the same serotype (i.e., O100:H20, and O146:H28) were assigned to the same sequence types (ST2514, and ST738, respectively) (Table 4).

The population structure of the isolates was visualized with a cgMLST-based phylogenetic tree. The isolates were phylogenetically clearly distinct, with ≥15 different alleles between each pair of neighbouring isolates, except for the four STEC O100:H20, all recovered from wild boars of the canton Ticino, which grouped in a tight cluster within their respective serotype and ST (Figure 1).

Shiga toxin subtypes and additional virulence determinants

Subtyping of the *stx* genes revealed that 12 of 13 (92 %, 95 %CI 0,65 – 0,99) carried *stx2*g enes only; i. e. *stx2a* (n = 1), *stx2b* (n = 5), and *stx2e* (n = 6) (Table 3 and Table 4). One of 13 (8 %, 95 %CI 0 – 0,35) harboured the combination of *stx1c* and *stx2b* (Table 3 and Table 4). The *stx2a* gene, which is associated with severe disease was identified in the O11:H5 isolate WS-64 recovered from wild boar from the canton Thurgau (Table 4).

In addition to *stx* genes, a number of additional virulence genes were identified among the strains, including genes encoding toxins *astA* (n = 10), *ehxA* (n = 3), *eilA* (n = 2), *hlyE* (n = 9), *sta1* (n = 5), *stb (n = 4),* and *subA* (n = 6), *usp* (n = 4), and adhesins *afaAB* (n = 1), *air* (n = 1), *hra* (n = 9), *iha* (n = 4), *lpfA* (n = 7), and *ompT* (n = 12) (Table 4).

Further virulence genes included those associated with the ability to survive bactericidal serum activity (*iss,* n = 7), with acidic tolerance (*gad,* n = 7), a gene encoding the capsule polysaccharide export protein (*kpsE,* n = 2), an outer membrane protein complement resistance gene (*traT,* n = 12), and the tellurite resistance gene *terC* (n = 13). Notably, none of the isolates in this study carried the *eae* gene, which is associated with highly pathogenic STEC.[21] Additionally, some virulence genes found among the isolates are associ-

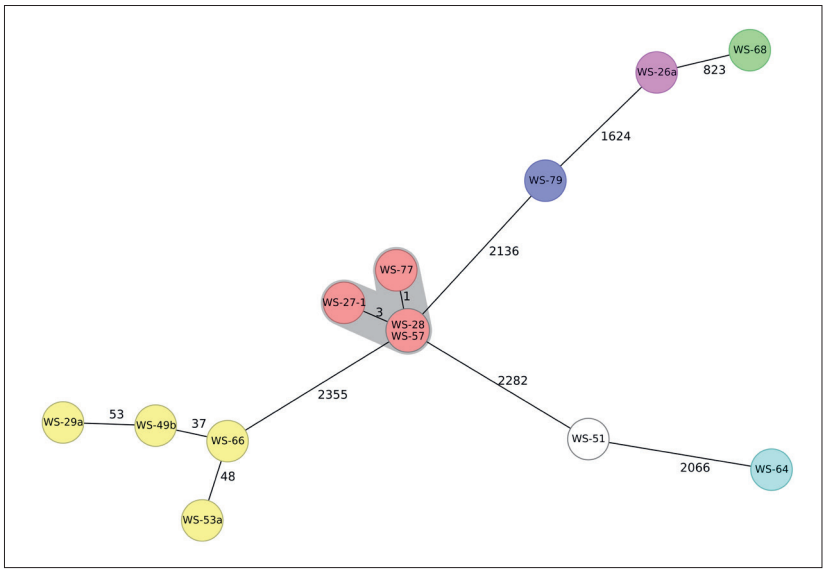

Figure 1: Phylogenetic relationship of 13 Shiga toxin producing *Escherichia coli* (STEC) isolated from faecal samples from hunted wild boar based on their core genome multilocus sequence type (cgMLST) allelic profiles. The minimum spanning tree was generated using SeqSphere+ (Ridom GmbH). The colours of the circles represent sequence types (STs) according to in silico MLST. Numbers on connecting lines indicate the number of allelic differences between two isolates. Each circle contains the isolate ID, as listed in Table 4. The STEC O100:H20 cluster is displayed with a grey background colour.

Table 3: Detection of *stx* genes by PCR and isolation of Shiga toxin-producing *Escherichia coli* (STEC) from 59 faecal samples from hunted wild boars from two different cantons in Switzerland.

Canton	No. animals	No. (%, 95 %CI) of PCR *stx*-positive samples[a]	No. (%, 95 %CI) of PCR *stx1*-positive samples	No. (%, 95 %CI) of PCR *stx2*-positive samples	No. (%, 95 %CI) of PCR *stx1/stx2*-positive samples	No. (%, 95 %CI) of STEC-positive samples	*stx* subtypes (no.) among cultured STEC[b]
Thurgau	12	4 (33 %, 0,136–0,61)	2 (17 %, 0,035–0,46)	1 (8 %, 0–0,375)	1 (8 %, 0–0,375)	1 (8 %, 0–0,375)	*stx2a* (1)
Ticino	47	20 (43 %, 0,295–0,567)	0 (0 %, 0–0,09)	19 (40 %, 0,276–547)	1 (2 %, 0- 0,121)	12 (26 %, 0,151–0,396)	*stx1c/stx2b* (1), *stx2b* (5), *stx2e* (6)
Total	59	24 (41 %, 0,29–0,534)	2 (3 %, 0,003–0,122)	20 (34 %, 0,231–0,467)	2 (3 %, 0,003–0,122)	13 (22 %, 0,132–0,343)	*stx2a* (1), *stx1c/stx2b* (1), *stx2b* (5), *stx2e* (6)

[a]PCR positive samples were further cultured and STEC were isolated by growth on CHROM agar™ or RAPID' *E. coli* agarTM. For details see text.
[b]The *stx* subtypes were determined using whole genome sequencing based methods. For details see text.

ated with extra-intestinal pathogenic *E. coli* (ExPEC) including c*huA* (n = 6), *fyuA* (n = 1), *ireA* (n = 6), *irp2* (n = 1), *papC* (n = 1), and *usp* (n = 4) (Table 4).

Discussion

The increase in the population of wild boar currently observed in European countries may enhance the risk of exposure of humans and domestic animals to zoonotic pathogens, including STEC.[39] Although only representative for the cantons Thurgau and Ticino, the present study nevertheless shows that wild boars in Switzerland are carriers of STEC and confirms the importance of wildlife as disseminators of these pathogens.[8,22]

With an overall prevalence of 22 % (95 %CI 0,13 – 0,34), the occurrence of STEC in the present study is higher than what was found in previous investigations from other European countries including Germany with 6,9 % (37 of 536 samples, 95 %CI 0,05 – 0,094) in 2016,[35] Spain with 3,3 % (3 of 90 samples, 95 %CI 0,007 – 0,098) during 2013 – 2015,[1] and Portugal with 14 % (8 of 56 samples, 95 %CI 0,071 – 0,26), in 2019,[7] By contrast, our results are more similar to those from a study from Italy in which 21,7 % (38 of 175 samples, 95 %CI 0,162 – 0,284) contained STEC during 2018 – 2019,[6] and lower that what was recently reported from a study from Poland with 28,3 % (43 of 152 samples, 95 %CI 0,213 – 0,362) in 2017 – 2018.[45] Notably, variations between countries should be interpreted with utmost caution due to the differences in study designs and testing methodologies. Further, the sampling number in our study was small compared to most of the studies mentioned above and consequently, the results should be interpreted with care.

The absence of the *eae* gene in all STEC and the predominance of *stx2b* and *stx2e* genes indicate that wild boar in Switzerland appear to mainly carry LEE-negative STEC of moderate pathogenic potential, which is in agreement with previous investigations from Spain and Portugal.[1,7] However, our results are in contrast those from studies from Germany, Italy, and Poland,[6,35,45] which identified wild boar as carriers of *eae*-negative and *eae*-positive STEC including STEC O157:H7, which is the serotype most commonly associated with serious illness in humans.[25] Taken together, these data suggest possible regional variations in the distribution of STEC serotypes and genotypes in wild boar in different European countries.

A high proportion (four isolates out of 13) of the STEC isolated from wild boar faeces in this study belonged to serotype O146:H28 (ST738) carrying *stx2b*. STEC belonging to the O146 serogroup have emerged as one of the most frequent non-O157 STEC associated with sporadic human illness in Europe, with STEC O146:H28 harbouring *stx2b* accounting for 4 % of non-O157 STEC infections in Switzerland in 2017.[25,32] STEC O146:H28 harbouring *stx2b* were identified previously in wild boar faeces from Portugal and Spain,[1,7] and from wild boar meat in Switzerland and

Table 4: Characteristics of 13 Shiga toxin-producing *Escherichia coli* (STEC) isolated from faecal samples from hunted wild boar in two cantons in Switzerland.

Sample ID(s)	Strain ID(s)	Canton	Serotype	ST[a]	*stx1*	*stx2*	Other virulence genes
WS79	WS-79	Ticino	O8:H19	201	–	*stx2e*	*gad, hlyE, iss, lpfA, ompT, papC, terC, traT*
WS64	WS-64	Thurgau	O11:H5	1104	–	*stx2a*	*astA, chuA, ehxA, eilA, fyuA, gad, hlyE, irp2, ompT, terC, traT*
WS26	WS-26a	Ticino	O21:H21	56	–	*stx2b*	*astA, ehxA, gad, hlyE, ireA, iss, lpfA, ompT, subA, terC, traT*
WS27, WS57	WS-27–1, WS-57	Ticino	O100:H20	2514	–	*stx2e*	*astA, gad, hlyE, hra, ompT, sta1, stb, terC, traT*
WS28	WS-28	Ticino	O100:H20	2514	–	*stx2e*	*astA, hlyE, hra, ompT, sta1, stb, terC, traT*
WS77	WS-77	Ticino	O100:H20	2514	–	*stx2e*	*astA, hlyE, hra, ompT, sta1, stb, terC, traT*
WS29	WS-29a	Ticino	O146:H28	738	–	*stx2b*	*astA, chuA, ehxA, hra, iha, ireA, iss, lpfA, ompT, subA, terC, traT, usp*
WS49, WS53	WS-49b, WS-53a	Ticino	O146:H28	738	–	*stx2b*	*astA, chuA, hra, iha, ireA, iss, lpfA, ompT, subA, terC, traT, usp*
WS66	WS-66	Ticino	O146:H28	738	–	*stx2b*	*astA, chuA, hra, iha, ireA, iss, kpsE, lpfA, ompT, subA, terC, traT, usp*
WS68	WS-68b	Ticino	O155:H26	683	–	*stx2e*	*gad, hlyE, hra, lpfA, sta1, terC, traT*
WS51	WS-51	Ticino	O166:H28	15443	*stx1c*	*stx2b*	*afaA, afaB, air, chuA, eilA, gad, hlyE, ireA, iss, kpsE, ompT, subA, terC*

ST, sequence type; -, absence of gene.
[a]STs were determined based on the sequenced of seven housekeeping genes *adk, fumC, gyrB, icd, mdh, purA, recA* using the PubMLST database (https://pubmlst.org/).

Germany.[30,33] Moreover, *stx2b*-harbouring STEC O146:H28 has repeatedly been found in wheat flour samples in Germany, Sweden, and Switzerland.[23,36,43] STEC O146:H28 from German flour shared the same sequence type (ST738) and had several virulence characteristics in common with the STEC O146:H28 from this study (i.e., *astA*, *chuA*, *iha*, *ireA*, *iss*, *lpfA*, and *subA*).[36] Similarly, STEC O146:H28 identified in Swedish flour was typed ST738 and had a very similar combination of virulence traits (*astA, hra, iha, ireA, iss, lpfA, ompT, subA, terC, usp)* compared to the STEC O146:H28 from this study (for the STEC O146:H28 from Swiss flour no WGS data was available for comparison)[43]. Our findings suggest that faecal shedding of roaming wild boars may in part contribute to crop contamination with this STEC serotype. This is of public health interest since wheat flour has recently emerged as a potential source of foodborne STEC disease cases linked to the consumption of raw flour or unsafe handling of flour-based food products.[37] Therefore, although wild boar probably play a minor role as a source of human STEC infection, these observations indicate that at least a proportion of STEC O146:H28 infections may be attributable to these animals. Notably, all STEC O146:H28 in this study contained one or more virulence factors which are characteristic of ExPEC.[16,26,34,44] The STEC/ExPEC pathotype was also found in STEC O11:H5 (isolate WS-64), STEC O21:H21 (isolate WS-26a) and STEC O166:H28 (isolate WS-51). Thus, although infections with the STEC in this study are not very likely to cause severe gastrointestinal symptoms, their potential to cause extra-intestinal disease such as urinary tract infections or bacteraemia should be considered.[40]

A further frequently observed serotype in this study was STEC O100:H20 (ST2514) harbouring *stx2e*. While rarely implicated in human illness,[27] STEC O100:H20 carrying *stx2e* has been identified in healthy pigs and pig carcasses in China and Poland, [4,29,47] and is thought to be swine-specific.[4] Phylogenetic analysis showed that the STEC O100:H20 in this study were closely related by cgMLST, which is, despite the limited sampling number in this study, suggestive of a common origin (all were recovered from wild boars within the canton Ticino), or indicative of a specific lineage of this serotype associated with wild boar in this region of Switzerland. Of note, all STEC O100:H20 in this study possessed *sta1* encoding the heat-stable enterotoxin type Ia, and *stb,* encoding the heat-stable enterotoxin type II, thus exhibiting a STEC/enterotoxigenic *E. coli* (ETEC) hybrid pathotype. This hybrid pathotype was also identified in one STEC O155:H26 carrying *stx2e* and *sta1* (isolate WS-68B). Enterotoxins are among the key virulence determinants of ETEC in pig neonatal diarrhea and post-weaning diarrhea.[38] Thus, although none of the STEC/ETEC in this study carried additional genes for fimbrial adhesins (e.g., F4 or F18) which are typically found in STEC/ETEC linked to disease in pigs,[38] the occurrence of STEC/ETEC in wild boar may be relevant to animal husbandry, especially to free-range systems of pig farming, because of the potential risk of transmission events and disease emergence at the wildlife–livestock interface.[5,12]

This study has several limitations. First, it should be considered that sample numbers were quite low, and that sampling was restricted to wild boar faeces obtained from only two cantons in Switzerland. Therefore, the results cannot be generalised to other geographical regions. Second, there were unequal sample sizes among the two areas from which faeces samples were obtained. Thus, there remains the possibility of unintended overrepresentation of the proportion of positive samples from the southern part of Switzerland (the canton Ticino). Finally, the restriction of WGS analysis to only one isolated colony per sample may have led to underestimate the variability of the STEC occurring in the wild boar populations in the study areas.

Conclusions

This study confirms wild boar as faecal carriers of STEC. Although the STEC serotypes identified in this study suggest a limited zoonotic infection risk to humans, wild boar as a game species may pose the risk of transmitting STEC to hunters, food handlers and consumers. Moreover, this study underlines the possible role of roaming wild boar in the dissemination of different STEC serotypes throughout the environment. Crops and water may be contaminated through wild boar faeces, and close contact between wild boars and domestic livestock with free-range farming systems could lead to pathogen transmission events. Some of the STEC carried by wild boar including hybrid STEC/ExPEC O146:H28 and STEC/ETEC O100:H20 may have implications for public and veterinary health.

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Shiga toxin-producing *Escherichia coli* isolated from hunted wild boar (*Sus scrofa*) in Switzerland

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Escherichia coli producteur de Shiga toxine isolé chez des sangliers *(Sus scrofa)* chassés en Suisse

Les *Escherichia (E.) coli* producteurs de shiga-toxine (STEC) sont des agents pathogènes zoonotiques d'origine alimentaire qui revêtent une grande importance pour la santé publique. Alors que les ruminants sont considérés comme le principal réservoir, les animaux sauvages sont de plus en plus souvent reconnus comme porteurs et réservoirs potentiels de STEC. L'objectif de cette étude était de déterminer la présence de STEC dans un total de 59 échantillons fécaux de sangliers (*Sus scrofa*) chassés provenant de deux régions différentes de Suisse (canton de Thurgovie dans le nord de la Suisse et canton du Tessin dans le sud de la Suisse) et de caractériser les isolats en utilisant une approche de séquençage du génome entier. Après une étape d'enrichissement, les gènes codant pour la Shiga-toxine (*stx*) ont été détectés par PCR en temps réel dans 41% (intervalle de confiance à 95% (95%CI) 0,29 - 0,53) des échantillons, et les STEC ont ensuite été récupérés dans 22% (95%CI 0,13 - 0,34) des mêmes échantillons. Sept sérotypes différents et six types de séquence (ST) différents ont été trouvés, avec une prédominance de O146:H28 ST738 (n = 4) et O100:H20 ST2514 (n = 4). Le sous-typage des *stx* a permis d'identifier des isolats avec *stx1c*/*stx2b* (n = 1), *stx2a* (n = 1), *stx2b* (n = 6) et *stx2e* (n = 6). Aucun isolat ne contenait le gène *eae*, mais tous hébergeaient d'autres gènes de virulence, le plus souvent *astA* (n = 10), *hlyE* (n = 9) et *hra* (n = 9). Les STEC O11:H5, O21:H21 et O146:H28 présentaient des facteurs de virulence associés à des *E. coli* pathogènes extra-intestinaux (ExPEC), et les STEC O100:H20 et O155:H26 possédaient *sta1* et/ou *stb* et étaient des pathotypes hybrides STEC/E. coli entérotoxinogène (ETEC).

Nos résultats montrent que les sangliers sont porteurs de STEC qui peuvent être disséminés dans l'environnement, conduisant éventuellement à la contamination des cultures agricoles et des sources d'eau. Les sérogroupes comprenaient le STEC O146 qui appartient aux sérogroupes non-O157 les plus courants associés à des maladies humaines en Europe, avec des implications pour la santé publique. Étant donné que des STEC producteurs de Stx2e ont souvent été signalés chez les porcs, les STEC O100:H20 hébergeant *stx2e* dans les fèces de sangliers peuvent être pertinents pour les systèmes d'élevage de porcs en plein air en raison du risque potentiel de transmission à l'interface entre les animaux sauvages et le bétail.

Mots clés: Chasse, sangliers, Shiga-toxine, *E. coli*, sérotypes, gènes de virulence, pathotypes hybrides

Escherichia coli produttore di tossina Shiga nei cinghiali selvatici *(Sus scrofa)* cacciati in Svizzera

Gli *Escherichia coli* (*E. coli)* che producono la tossina Shiga (STEC) sono patogeni di origine alimentare di notevole importanza per la salute pubblica. Anche se i ruminanti sono considerati come il serbatoio principale, gli animali selvatici sono sempre più spesso identificati come portatori e potenziali serbatoi della STEC. Lo scopo di questo studio è di determinare la presenza della STEC in 59 campioni fecali di cinghiali selvatici cacciati (*Sus scrofa*) provenienti da due regioni differenti della Svizzera, il canton Turgovia nella Svizzera settentrionale e il canton Ticino nella Svizzera meridionale, e di caratterizzare gli isolati mediante un sequenziamento del genoma completo. Dopo una fase di arricchimento, i geni che codificano la tossina Shiga (*stx*) sono stati rilevati mediante PCR in tempo reale nel 41% (intervallo di confidenza al 95% (IC 95%) 0,29 - 0,53) dei campioni, e le STEC sono state successivamente recuperate nel 22% (IC 95% 0,13 - 0,34) degli stessi campioni. Sono stati identificati sette diversi sierotipi e sei diversi tipi di sequenza (ST), con O146:H28 ST738 (n = 4) e O100:H20 ST2514 (n = 4) predominanti. La sottotipizzazione di *stx* ha identificato isolati con *stx1c*/*stx2b* (n = 1), *stx2a* (n = 1), *stx2b* (n = 6) e *stx2e* (n = 6). Nessun isolato conteneva il gene *eae*, ma tutti contenevano geni di virulenza aggiuntivi, di cui i più comuni che sono stati rilevato sono *astA* (n = 10), *hlyE* (n = 9) e *hra* (n = 9). Le STEC O11:H5, O21:H21 e O146:H28 possedevano fattori di virulenza associati patogeni extra-intestinali di *E. coli* (ExPEC), e le STEC O100:H20 e O155:H26 possedevano *sta1* e/o *stb* e appartenevano a patotipi ibridi STEC/*E. coli* enterotossigenici (ETEC).

Questi risultati dimostrano che i cinghiali selvatici sono portatori di STEC che possono essere diffusi nell'ambiente, portando potenzialmente alla contaminazione di colture agricole e fonti d'acqua. I sierogruppi includevano le STEC O146, che appartengono ai sierogruppi non-O157 più comuni associati alle malattie umane in Europa, con implicazioni per la salute pubblica. Dato che le STEC produttrici di Stx2e sono state frequentemente segnalate nei suini e nella carne di maiale, le STEC O100:H20 che ospitano *stx2e* nelle feci dei cinghiali selvatici possono essere rilevanti per i sistemi di allevamento all'aperto di suini a causa del potenziale rischio di eventi di trasmissione all'interfaccia fauna selvatica-bestiame.

Parole chiave: caccia, cinghiali selvatici, tossina Shiga, *E. coli*; sierotipi, geni di virulenza; patotipi ibridi

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