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Pathogenic potential to humans of Shiga toxin-producing *Escherichia coli* isolated from wild boars in Poland

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ABSTRACT

The aim of the study was to investigate the presence of Shiga toxin-producing *Escherichia coli* (STEC) in the wild boar population of north-eastern Poland, and to evaluate the potential health risk associated with wild boars carrying STEC/AE-STEC strains. In Poland, the African Swine Fever (ASF) virus has been a growing problem in domestic pigs and wild boars, one of the main reservoirs of the virus, because of this hunters, veterinary practitioners and foresters thus face a greater risk of coming into contact with animals.

Rectal swabs samples were obtained from 152 wild boars hunter-harvested in the 2017/2018 season (autumn-winter) in north-eastern Poland. The samples were enrichment in modified buffered peptone water. Polymerase chain reaction (PCR) assays were conducted to determine the virulence profile of *stx1*, *stx2* and *eae* and *aggR* genes, identify subtypes of *stx1* and *stx2* genes, and perform O and H serotyping.

STEC/AE-STEC virulence genes were detected in 43 isolates (28.29%): STEC in 17 isolates (11.18%) and AE-STEC in 26 isolates (17.11%), respectively. None of the tested isolates carried the *aggR* gene. The most dangerous AE-STEC virulence profile associated with HUS was found in 2 isolates (1.32%): *stx1NS/stx2a/d/eae* serotype ONT:H7 and *stx2a/eae* serotype O146:H7. Six of the 152 tested samples belonged to serogroup O157 (3.95%), including one AE-STEC isolate with virulence profile *stx2g/eae* and five EPEC isolates.

The results of this study suggest that wild boars in north-eastern Poland can carry STEC/AE-STEC strains that are potentially pathogenic for humans. This is the first report documenting the virulence of STEC and AE-STEC isolates from wild boars in Poland.

1. Introduction

Shiga toxin-producing *Escherichia* (*E.*) *coli* (STEC) are enteric pathogens responsible for serious diseases in humans, including diarrhoea (D), bloody diarrhoea (BD) and the haemolytic uraemic syndrome (HUS). The pathogenic capacity of STEC is determined by the number of virulence genes, including *shiga toxin 1* (*stx1*), *shiga toxin 2* (*stx2*), *Escherichia coli* attaching and effacing (*eae*) or transcriptional activator of aggregative adherence fimbria I (*aggR*) genes (FAO/WHO, 2018). Stx-encoding genes (*stx* genes) are carried by mobile genetic elements which can be exchanged between bacteria through horizontal gene transfer. *Stx* genes encode a large family of Shiga toxins divided in two major subfamilies, Stx1 and Stx2, both of which include many subtypes and variants. Variants for Stx1 and Stx2 are grouped in three (Stx1a, Stx1c and Stx1d) and seven Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) subtypes, respectively (Scheutz et al., 2012). The *eae* gene encodes intimin, and it is located on the locus of enterocyte effacement (LEE) which is a pathogenicity island. Attaching-effacing AE-STEC strains (AE-STEC) are classified as enterohaemorrhagic *E. coli* (EHEC). STEC strains that do not harbour the *eae* gene, but carry a plasmid encoding the *aggR* gene have been recently associated with EHEC and HUS (FAO/WHO, 2018). Although O157:H7 *E. coli* has been the serotype most frequently implicated in human disease, > 400 O:H types of STEC (mostly O26, O45, O103, O104, O111, O121 and O145) have been associated with infections (Alonso et al., 2017; Bielaszewska et al., 2011). According to the most recent FAO/WHO report (2018), the health risks associated with STEC infections in humans cannot be reliably assessed based on serotype data alone. The potential threats

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can be more effectively identified by screening STEC strains for the presence of genes encoding virulence factors: *stx1*, *stx2*, *eae* and *aggR*. Recent research indicates that STEC strains belonging to subtype *stx2a* and harbouring adherence genes *eae* or *aggR* pose the greatest health risk for humans and have the greatest potential to cause HUS (FAO/WHO, 2018; Asakura et al., 2019; Haddad et al., 2018). According to the strategy for testing STEC to discern the level of health risk based on virulence genes, strains harbouring *stx2a* or *stx2d* and *eae* or *aggR* genes are the most dangerous for humans. These strains have the greatest potential to cause D, BD and HUS in humans. Strains with the virulence profile *stx2c/eae* or *stx1a/eae* have been detected in patients with D and BD. Other *stx* subtypes have been identified in D patients (FAO/WHO, 2018). The progression of infection to HUS depends on numerous parameters, including host factors, pathogen load and antibiotic treatment. There are various routes of STEC transmission to humans, including ingestion of contaminated meat or water, contact with animal faeces, or person-to-person contact. In Poland, the African Swine Fever (ASF) virus has been a growing problem in domestic pigs and wild boars, one of the main reservoirs of the virus, ever since the first case of ASF was reported in February 2014. The epidemic continues to spread to other regions of the country (https://bip.wetgiw.gov.pl/asf/mapa). The wild boar population is Poland is estimated at 229,000 (http://wiescirolnicze.pl/ile-jest-dzikow-w-twoim-wojewodztwie/06.2018). Hunters and foresters thus face a greater risk of coming into contact with infected animals, which are often found in urban and suburban areas, and their excrements lead to environmental contamination. In view of the above, the aim of this study was to investigate the presence of STEC/AE-STEC strains in wild boars, and to evaluate the potential health risk associated with the transmission of pathogenic strains to humans.

2. Materials and methods

2.1. Sample collection

The experimental material consisted of 152 rectal swabs obtained from 152 wild boars that were hunter-harvested in the 2017/2018 autumn-winter season in north-eastern Poland. The samples were collected with the voluntary support of hunters. The health status of the animals was not ascertained. Samples for analysis were collected from each animal before evisceration, they were immediately placed in tubes and transported to the laboratory within 48 h. All samples were collected during routine examinations; therefore, ethical approval for animal experimentation was not required.

2.2. Detection of STEC strains and PCR virulotyping

Samples were enriched in 5 ml of buffered peptone water (BPW) (BTL, Łódź, Poland) for 16–24 h at 37 °C. Overnight cultures of 1 ml were centrifuged at 13000 ×*g* for 5 min, and the pellet was used for DNA preparation. Genomic DNA isolation was performed with the Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's instructions. All samples were tested for the presence of *stx1*, *stx2*, *eae* and *aggR* genes (Schmidt et al., 1999; Schmidt et al., 1994a, 1994b; Schmidt et al., 1994b). Primer sequences and annealing temperatures are specified in Table 1. The HotStartTaq *Plus* DNA Polymerase Kit (Qiagen) and the HotStartTaq *Plus* Master Mix Kit (Qiagen) were used in the PCR assay. A loopful of each PCR-positive culture was plated onto MacConkey agar (Merck) and incubated at 37 °C for 18 to 24 h. In each PCR-positive culture, 50 *E. coli* suspect colonies were tested for *stx*1, *stx*2 and *eae* or *aggR* genes to obtain STEC isolates for future analysis and to characterise strains positive for *stx* and/or *eae/aggR*. All isolates that tested positive for *stx* and/or *eae* and/or *aggR* were stored for further analyses at −70 °C in BPW (BTL, Łódź, Poland) containing 30% glycerol.

2.3. Shiga toxin subtyping

The *stx1* and *stx2* subtypes (without *vtx2f*) were identified in STEC isolates according to the subtyping nomenclature proposed by the 8th VTEC 2012 Symposium in Amsterdam, Netherlands. Stx subtypes were identified by the multiplex PCR-based method recommended by the European Reference Laboratory for VTEC (EU-RL VTEC) and the European Centre for Disease Prevention and Control External Quality Assurance (ECDC EQA, 2010 to 2011) (Feng et al., 2011; Persson et al., 2007; Scheutz et al., 2012).

2.4. Molecular O and H serotyping

The PCR assay recommended by the European Reference Laboratory for VTEC (EU-RL VTEC) - EU-RL VTEC_Method_003_Rev1 available at http://old.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_03_Rev_1.pdf was used to detect O antigen-encoding genes (*wzx*, *wbgN*, *wzy*, *rfb*) specific to the thirteen (O26, O45, O55, O91, O103, O104, O111, O113, O121, O128, O145, O146 and O157) most frequent and pathogenic serogroups in humans and animals (Bielaszewska et al., 2011; DebRoy et al., 2005; Li et al., 2006; Liu et al., 2007; Monday et al., 2007; Paton and Paton, 1999; Perelle et al., 2004). The presence of the flagellar genes (*fliC*) H7, H8, H11, H21 and H28 was also detected by PCR (Durso et al., 2005; Gannon et al., 1997; Mora et al., 2012). Primer sequences and annealing temperatures are specified in Table 2. The HotStartTaq *Plus* DNA Polymerase Kit (Qiagen) and the HotStartTaq *Plus* Master Mix Kit (Qiagen) were used in all PCR assays. The amplified fragments were separated by electrophoresis on 2% agarose gel with the Midori Green Advanced DNA Stain (Nippon Genetics Europe GmbH, Germany) in 1× TAE buffer for 30–45 min. The products were visualised under UV light. PCR results were analysed and archived with the use of the GelDoc gel documentation system (Bio-Rad).

2.5. Statistical analysis

The prevalence and CI (95%) of *E. coli* O157 strains and non-O157 STEC and AE-STEC strains were calculated with the Binomial (Clopper-Pearson) 'exact' method based on the beta distribution, at a significance level of $\alpha = 0.05$. Statistical analyses were performed with free EpiTools epidemiological calculators (http://epitools.ausvet.com.au) (Brown et al., 2001).

2.6. Sequencing

The chosen amplicon was purified with the Clean-up purification kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's recommendations and sequenced to confirm their specificity (Genomed S.A., Warszawa, Poland). Sequence data was compared with the nucleotide sequence of the previously identified *vtx2g* genes in *E. coli* using BLASTN v. 2.2.18 (Altschul et al., 1997). The sequence described in this paper has the following GenBank Acc. No.: MH320768.

3. Results

In the group of 152 tested samples, STEC/AE-STEC virulence genes were detected in 64 samples (42.11%, 95% CI = 34.15–50.37). Positive samples were further processed to obtain STEC isolates, and we were able to isolate STEC/AE-STEC from 43 (28.29%, 95% CI = 21.29–36.16) of them: STEC were detected in 17 isolates (11.18%, 95% CI = 6.65–17.30), AE-STEC in 26 isolates (17.11%, 95% CI = 11.49–24.05), respectively. EPEC virulence genes were detected in 60 samples (39.47%, 95% CI = 32.05–47.41) and we were able to isolate 47 isolates (30.92%, 95% CI = 23.68–38.92) of them. None of the analysed isolates carried the *aggR* gene (Table 3). In the group of 43 STEC/AE-STEC isolates characterised in this study, *stx2* was the predominant *stx* gene which was detected in 27 isolates (62.79%, 95%

Table 1
Primers and annealing temperature used in this study for PCR.

Target gene	Primer	Oligonucleotide sequences (5′ – 3′)	Amplicon size (bp)	Annealing temp. (°C)
stx$_1$	KS7	CCCGGATCCATGAAAAAAACATTATTAATAGC	285	56
	KS8	CCCGAATCCAGCTATTCTGAGTCAACG		
stx2	GK3	CCCGGATCCAGTAAGAAGATGTTTATGGCG	260	56
	GK4	CCCGAATTCTCAGTCATTATTAAACTGCAC		
eae	SK1	CCCGAATTCGGCACAAGCATAAGC	863	56
	SK2	GCCGGATCCGTCTCGCCAGTATTCG		
eggR	aggRks1	GTATACACAAAAGAAGGAAGC	254	52
	aggRks2	ACAGAATCGTCAGCATCAGC		

CI = 46.73–77.02), whereas the *stx1* gene was detected in 10 isolates (23.26%, 95% CI = 11.76–38.63). Six isolates (13.95%, 95% CI = 5.3–27.93) harboured both *stx1/stx2* genes: 2 STEC and 4 AE-STEC (Table 3).

The prevalence of *stx1* subtypes in 16 *stx1* isolates (7 STEC and 9 AE-STEC) was as follows: 5 *stx1a* (1 AE-STEC) and 11 *stx1*NS (non-subtype) (8 AE-STEC). The prevalence of *stx2* subtypes in 33 *stx2* isolates (12 STEC and 21 AE-STEC) was as follows: 3 *stx2a* (1 AE-STEC), 2 *stx2b* (1 AE-STEC), 4 *stx2e* (4 AE-STEC), 14 *stx2g* (9 AE-STEC), 8 *stx2NS* (3 AE-STEC) and 2 isolates with a combination of *stx2* subtypes: 1 *stx2e/stx2g* (virulence profile: *stx2e/stx2g/eae*) and 1 *stx2a/stx2d* (virulence profile: *stx1NS/stx2a/stx2d/eae*).

Six out of the 152 tested samples (43 STEC/AE-STEC isolates and 47 EPEC isolates) belonged to serogroup O157 (3.95%, 95% CI = 1.46–8.39), 5 isolates belonged to O157:H7 (1 AE-STEC isolate – virulence profile *stx2g/eae*; the remaining EPEC isolates) and one isolate belonged to O157:H21 (EPEC isolate), respectively (Table 3). Fourteen isolates belonged to serogroup O103 (9.21%, 95% CI = 5.13–14.97) (4 AE-STEC isolates – virulence profile: *stx1NS/stx2e/eae*, *stx1NS/eae*, *stx1NS/eae*, *stx2NS/eae*), 14 isolates belonged to serogroup O146 (9.21%, 95% CI = 5.13–14.97) (4 AE-STEC isolates - virulence profile: *stx2a/eae*, *stx2e/eae*, *stx1a/stx2g/eae*, *stx2g/eae*), 9 isolates belonged to serogroup O145 (5.92%, 95% CI = 2.74–10.94) (3 AE-STEC isolates - virulence profile: *stx2e/eae*, *stx2g/eae*, *stx1NS/stx2b/eae*), 6 isolates belonged to serogroup O45 (3.95%, 95% CI = 1.46–8.39) (1 AE-STEC isolate - virulence profile: *stx2g/eae*), 3 isolates belonged to serogroup O91 (1.97%, 95% CI = 0.41–5.66), 2 isolates belonged to O113 (1.32%, 95% CI = 0.16–4.67) (1 AE-STEC isolate - virulence profile: *stx2NS/eae*), and 2 isolates belonged to serogroups O26, O113 and O128 (1.32%, 95% CI = 0.16–4.67). One isolate was serotyped as O104 (0.66%, 95% CI = 2e-04 - 3.61). None of the analysed isolates belonged to serogroups O55 or O111 (Table 3).

4. Discussion

The growing significance of wild animals as reservoirs of zoonotic

Table 2
Primers and annealing temperature used in this study for molecular serotyping.

Target gene	Primer	Oligonucleotide sequences (5′ – 3′)	Amplicon size (bp)	Annealing temp. (°C)	Literature
wzx O26	*5′ O26*	ACTCTTGCTTCGCCTGTT	268	60	(Monday et al., 2007)
	3′O26	CAGCGATACTTTGAACCTTAT			
wzx O45	O45 wzx2 F	TATGACAGGCACATGGATCTGTGG	255	60	(DebRoy et al., 2005)
	O45 wzx2 R	TTGAGACGAGCCTGGCTTTGATAC			
vbgN (O55)	O55F	TGTAATTCGATGCACCAATTCAG	70	60	(Perelle et al., 2004)
	O55R	CGCTTCGACGTTCGATACATAA			
wzy (O91)	O91F	CGATTTTCTGGAATGCTTGATG	105	60	(Perelle et al., 2004)
	O91R	CAATACATAGTTTGATTTGTGTTTAAAGTTTAAT			
wzx (O103)	5′O103	TATCCTTCATAGCCTGTTGTT	320	60	(Monday et al., 2007)
	3′O103	AATAGTAATAAGCCAGACACCTG			
rbf (O104)	O104rfbO-f	TGAACTGATTTTTAGGATGG	351	60	(Bielaszewska et al., 2011)
	O104rfbO-r	AGAACCTCACTCAAATTATG			
wzx (O111)	5′O111.3	GTTGCGAGGAATAATTCTTCA	829	60	(Monday et al., 2007)
	3′O111.2	CCATAGATATTGCATAAAGGC			
wzy (O113)	O113F	AGCGTTTCTGACATATGGAGTG	593	60	(Paton and Paton, 1999)
	O113R	GTGTTAGTATCAAAAGAGGCTCC			
wzx (O121)	5′O121	GTAGCGAAAGGTTAGACTGG	651	60	(Monday et al., 2007)
	3′O121	ATGGGAAAGCTGATACTGC			
wzy (O128)	O128 13F	ATGATTTCTTACGGAGTGC	782	50	(Li et al., 2006)
	O128 13R	CTCTAACCTAATCCCTCCC			
wzx (O145)	5′O145.6	TTGAGCACTTATCACAAGAGATT	418	60	(Monday et al., 2007)
	3′O145·B	GATTGAATAGCTGAAGTCATACTAAC			
wzy (O146)	O146 F	ATTCGGGTAACGACCCTGTGTTGA	378	50	(Liu et al., 2007)
	O146 R	AGACTGCTAATGCAAGGAACATGG			
wzx (O157)	5′O157	GCTGCTTATGCAGATGCTC	133	56	(Monday et al., 2007)
	3′O157	CGACTTCACTACCGAACACTA			
fliC-H7	H7-F	GCGCTGTCGAGTTCTATCGAGC	625	55	(Gannon et al., 1997)
	H7-R	CAACGGTGACTTTATCGCCATTCC			
fliC-H8	H8-F	TAACAGCGCAAAAGACGATG	393	58	(Mora et al., 2012)
	H8-R	CCGAGAAGTTTTCGCATCAAT			
fliC-H11	fliCRH11–1	ACTGTTAACGTAGATAGC	248	54	(Durso et al., 2005)
	fliCRH11–2	TCAATTTCTGCAGAATATAC			
fliC-H21	H21-F	GGCGATTGCTAACCGTTTTA	549–556	58	(Mora et al., 2012)
	H21-R	CGTAAGTGAACCATCCGCAG			
fliC-H28	H28-F	ACGAAATCAAATCCCGTCTG	856	66	(Mora et al., 2012)
	H28-R	GCCGATTGAAGAGACTCAGC			

Table 3
Pathotypes, *stx* subtype and O serogroups of *E. coli* isolated from wild boars (*Sus scrofa*).

Pathotypes	Virulence profile	No. of positive samples	No. of isolates	Subtype *stx1* (No. isolates)	Subtype *stx2* (No. isolates)	Serogroups (No. isolates)
STEC	*stx1* +	7	5	*stx1a* (3)		O146:H21(1), O103:H7(1), ONT:H21(1)
				stx1NS[a] (2)		ONT:HNM(1), O103:H7(1)
	stx2 +	12	10		*stx2a* (1)	O45:H21(1)
					stx2g (4)	O91:H7(1), O91:H21(1), O103:H7(1), O145:H21(1)
					stxNS[a] (5)	ONT:HNM(2), ONT:H21(1), O104:H21(1), O113:H21(1)
	stx1 + *stx2* +	4	2	*stx1a* (1)	*stx2a* (1)	O146:H7(1)
				stx1NS[a] (1)	*stx2g* (1)	O45:H7(1)
No. isolates (%)	17/152 (11.18%)					
AE-STEC	*stx1* + *eae* +	8	5	*stx1NS*[a] (5)		ONT:HNM(3), O103:H7(2)
	stx2 + *eae* +	25	17		*stx2a* (1)	O146:H7(1)
					stx2b (1)	ONT:HNM(1)
					stx2e (3)	O26:H7(1), O145:H21(1), O146:H7(1)
					stx2e/stx2g(1)	O128:H7(1)
					stx2g (8)	ONT:NM(4), O45:H7(1), O145:H7(1), O146:H7(1), **O157:H7(1)**
					stxNS[a] (3)	ONT:H21(1), O103:H21(1), O113:H7(1)
	stx1 + *stx2* + *eae* +	8	4	*stx1a* (1)	*stx2g* (1)	O146:H7(1)
				stx1NS[a] (3)	*stx2a/stx2d*(1)	ONT:H7(1)
					stx2b(1)	O145:H7(1)
					stx2e (1)	O103:H7(1)
No. isolates (%)	26/152 (17.11%)					
EPEC	*eae* +	60	47			O26:H7(1), O45:H7(1), O45:H21(2), O91:H28(1), O103:H7(8), O104:H7(1), O121:H7 (2), O121:H8(1), O128:H11(1), O145:H7(3), O145:H8(1), O145:H21(1), O146:H7(6), O146:HNM(2), **O157:H7(4)**, **O157:H21(1)**, ONT:HNM(11)
No. isolates (%)	47/152 (30.92%)					

[a] NS, not-subtype with used primers for the subtypes of Shiga toxin encoding genes (*stx*).
ONT, O antigen Non-Typeable.
HNM, H antigen Non-Motile.

diseases is an important public health concern (Kruse et al., 2004; Langholz and Jay-Russell, 2013). Wild boar (*Sus scrofa*) food game species is one of the most popular, after red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), hare (*Lepus europaeus*) and pheasant (*Phasianus colchicus*) (Schulp et al., 2014). In recent decades, the wild boar population has increased rapidly in Poland and in other European countries. The above has given rise to two main problems. First, in Poland hunters and foresters have more frequent contact with wild boars, because they are one of the main reservoirs of the ASF virus. Second, contaminated wild boar meat is consumed by hunters and their families, and carcasses are not always processed in full observance of sanitary requirements (Regulation EU No. 853/2004 and No. 854/2004) (Regulation (EC) No. 853/2004, 2004; Regulation (EC) No. 854/2004, 2004). Practices such as skinning and evisceration in the field or in private barns or garages may increase the risk of faecal contamination of meat cuts. Offal is often abandoned in the field, which can contribute to environmental contamination and pathogen transmission to other animal species, including scavengers. Contamination of the environment and meat could lead to a small local outbreak of O157:H7 infections (Cody et al., 1999; Jay et al., 2007). O157:H7 is the most important STEC serotype in relation to public health, but little is known about its prevalence in wild boars and virulence factors in Europe. Studies of wild boar populations have been performed in Spain and Sweden. In different regions of Spain, the prevalence of *E. coli* O157:H7 was estimated at 3.41% (Navarro-Gonzalez et al., 2015), 3.30% (Sanchez et al., 2010) and 0.38% (Mora et al., 2012). In Sweden, the above serotype was not detected in two studies, and its prevalence was estimated at 1.50% in one study (Sannö et al., 2014; Wahlstrom et al., 2003). In north-eastern Poland, the prevalence of *E. coli* O157 was determined at 3.95%, but only one isolate belonged to AE-STEC with virulence profile *stx2g/eae*, which does not appear to be dangerous for humans, because subtype *stx2g* is commonly isolated from cattle and is not implicated in the pathogenesis of human diseases (Leung et al., 2003). The study on this strain should be continued because this is the first note that *E. coli* O157:H7 carry *stx2g*, the nucleotide sequences of *stx2g* gene was deposit in GenBank of National Centre for Biotechnology Information (Acc. No. MH320768).

According to the most recent FAO/WHO report (2018), the health risk associated with STEC infections in humans cannot be reliably assessed based on serotype data alone. STEC strains pathogenic for humans usually harbour *stx2a/stx2d* and other virulence traits such as *eae* or *aggR* genes (Haddad et al., 2018). In a study by Mora et al. (2012), the prevalence of O157 STEC strains in wild boars was low (0.38%), but the prevalence of non-O157 STEC strains was significantly higher (8.4%). In our study, the prevalence of STEC/AE-STEC strains in wild boars was 28.29% (43 isolates). Twenty-six of the 152 tested samples were contaminated by AE-STEC strains (17.11%). The most dangerous virulence profile associated with HUS was detected in 2 isolates: *stx1NS/stx2a/d/eae* serotype ONT:H7 and *stx2a/eae* serotype O146:H7 (1.32%). In Belgium, the results of a screening study conducted for 27 years (1987–2014) in a university hospital revealed the presence of STEC strain subtypes *stx1c*, *stx2b*, *stx2d*, *stx2e*, *stx1a* + *stx2b*, *stx1a* + *stx2c* and *stx1c* + *stx2b* in BD patients and subtypes *stx1a*, *stx2a*, *stx2c*, *stx2a* + *stx2c* and *stx1a* + *stx2c* in patients diagnosed with HUS and BD (De Rauw et al., 2018). In Norway, a survey on STEC infections between 1992 and 2012 demonstrated that young age (≤ 5 years) and strains with virulence profile *stx2a/eae* were the risk factors for HUS development (Brandal et al., 2015). A 16-year study (1997–2013) conducted by the German National Reference Centre for *Salmonella* and other Enteric Bacterial Pathogens also revealed that STEC strains with virulence profile *stx2a/eae* posed the greatest risk of HUS. However, the researchers concluded that DNA-based analyses involving a wide range of virulence and phylogenetic markers and serotyping are required to reliably assess the pathogen's virulence profile and pathogenicity (Fruth et al., 2015). In our study, *stx2g* (14 isolates) and *stx2e/stx2g* (1 isolate) were the most prevalent *stx2* subtypes in wild boars, and 9 of them were identified in AC-STEC strains. In Spain, subtypes *stx1c* and *stx2b* were most frequently identified in the wild boar population (Mora et al., 2012). In Norway, subtype *stx2g* was isolated from 2 hospitalised patients who were not diagnosed with HUS (Brandal et al., 2015). Subtype *stx2e* strains are rarely isolated from humans, and they often cause uncomplicated diarrhoea (Friedrich

et al., 2002). However, these strains may also cause HUS with a fatal outcome, as reported in a 65-year-old male in Switzerland (Fasel et al., 2014). Subtype *stx2e* is commonly associated with oedema disease in pigs, and it is a source of contamination in pork and wild boar meat (Martin and Beutin, 2011). In this study, subtype *stx2e* was detected in 4 AE-STEC isolates, including one isolate with virulence profile *stx1NS/stx2e/eae* and serotype O103:H7, and 3 isolates with virulence profile *stx2e/eae* and serotypes O146:H7, O145:H21 and O26:H7. One strain had mixed *stx2e* and *stx2g* subtype, with virulence profile *vtx2e/vtx2g/eae* and serotype O128:H7.

In summary, the prevalence of *E. coli* O157 in wild boar population in north-eastern Poland was determined at 3.95%, one strain carry *stx2g/eae* and it is the first note that *E. coli* O157:H7 has such a virulence profile. STEC and AE-STEC strains were detected in 28.29%. The predominant *stx* gene subtype was *stx2*. The most dangerous AE-STEC virulence profile associated with HUS was found in 2 isolates: *stx1NS/stx2a/d/eae* serotype ONT:H7 and *stx2a/eae* serotype O146:H7. These results are not overwhelming, nevertheless, highly pathogenic strains have been detected in the examined samples. The presence of AE-STEC in the populations of wild boar has implications for the spread of zoonotic diseases among venison consumers and people who come into contact with animals and their faeces. Meat and faces of wild boars infected with different STEC strains pose a health risk not only for hunters, veterinary practitioners and foresters, but also for other individuals.

5. Conclusions

The results of our study indicate that wild boars in north-eastern Poland can carry STEC/AE-STEC strains that are potentially pathogenic for humans and contribute to environmental contamination with STEC. Further studies are needed, including other regions, to elucidate the role of wild boars as reservoirs of STEC/AE-STEC strains and their role in public health. This is the first report documenting subtypes of the *stx* gene detected in Shiga-toxin producing *E. coli* shedded by wild boars in Poland.

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Competing interests

The authors declare that they have no competing interests.

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