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Molecular characterization of non-O157 verotoxigenic Escherichia coli isolated from slaughtered cattle in Poland*)

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Summary

Aim of the study: The study was conducted to assess the molecular relationship of verotoxigenic E. coli (VTEC) isolated from cattle slaughtered in abattoirs in the eastern part of Poland.

Materials and Methods: A total of 50 non-O157 VTEC strains isolated from bovine hides and the corresponding carcasses were tested with the pulsed field gel electrophoresis (PFGE) method.

Results and discussion: The XbaI restriction analysis enabled the clustering of the isolates into 19 PFGE profiles. Seven of them contained more than two strains with two groups of 8 isolates each. Furthermore, three profiles grouped two isolates and the remaining nine PFGE types were represented by only one strain. The PFGE results were analyzed in relation to identified VTEC serotypes (O186:H16, O185:H7, O181:H49, O177:H25, O175:H21, O174:H2, O153:H25, O153:H2, O148:H8, O139:H19, O117:H4, O91:HNT, O84:H28, O36:H19, O21:H25, O2:H6, O2:H32, ONT:H34) and presence of their virulence genes. It was found that strains of the same PFGE type were usually of the same serotype and possessed the same pathogenic markers. The most numerous profile was represented by eight isolates: all of these strains were identified as O2:H32 serotype and had the same virulence genes – vtx2 and vtx2e. Furthermore, the majority of the PFGE profiles grouped the strains isolated during the same day. PFGE analysis revealed that among the isolates obtained from hides and the corresponding carcasses five pairs of strains had an identical molecular profile. The present study provided valuable information concerning the molecular characterization of VTEC isolated from cattle at the slaughter level. The results reflected a low genetic diversity among VTEC isolates tested and may suggest a common source of contamination within the abattoir.

Keywords: VTEC, PFGE, molecular relatedness, cattle

Verotoxigenic *Escherichia coli* (VTEC) is an important group of pathogenic *E. coli* which can contaminate food and cause serious illnesses, such as hemorrhagic colitis (HC) that may result in hemolytic uremic syndrome (HUS). The pathogenicity of VTEC is associated with various virulence factors, of which the most important is the ability to produce the verocytotoxins VT1 and/or VT2 (12, 14, 16). The majority of clinical cases of VTEC infections are associated with O157:H7 serotype, but in recent years a growing number of non-O157 serogroups have been isolated from animals. Many of these VTEC have been associated with severe illnesses in humans (8). Cattle and other ruminants are well recognized as the primary reservoir of VTEC of public health significance (10).

The objective of the present study was to identify the genetic diversity of VTEC isolated from slaughtered cattle in Poland through determination of their PFGE profiles and to compare them to serotypes and presence of virulence genes.

Material and methods

Fifty VTEC strains were investigated with the pulsed field gel electrophoresis (PFGE) method for their genetic relatedness and analyzed in relation to their serotypes and the presence of virulence marker genes. The isolates were recovered from bovine hides (n = 26) and the corresponding carcasses (n = 24) collected at the slaughterhouse level in

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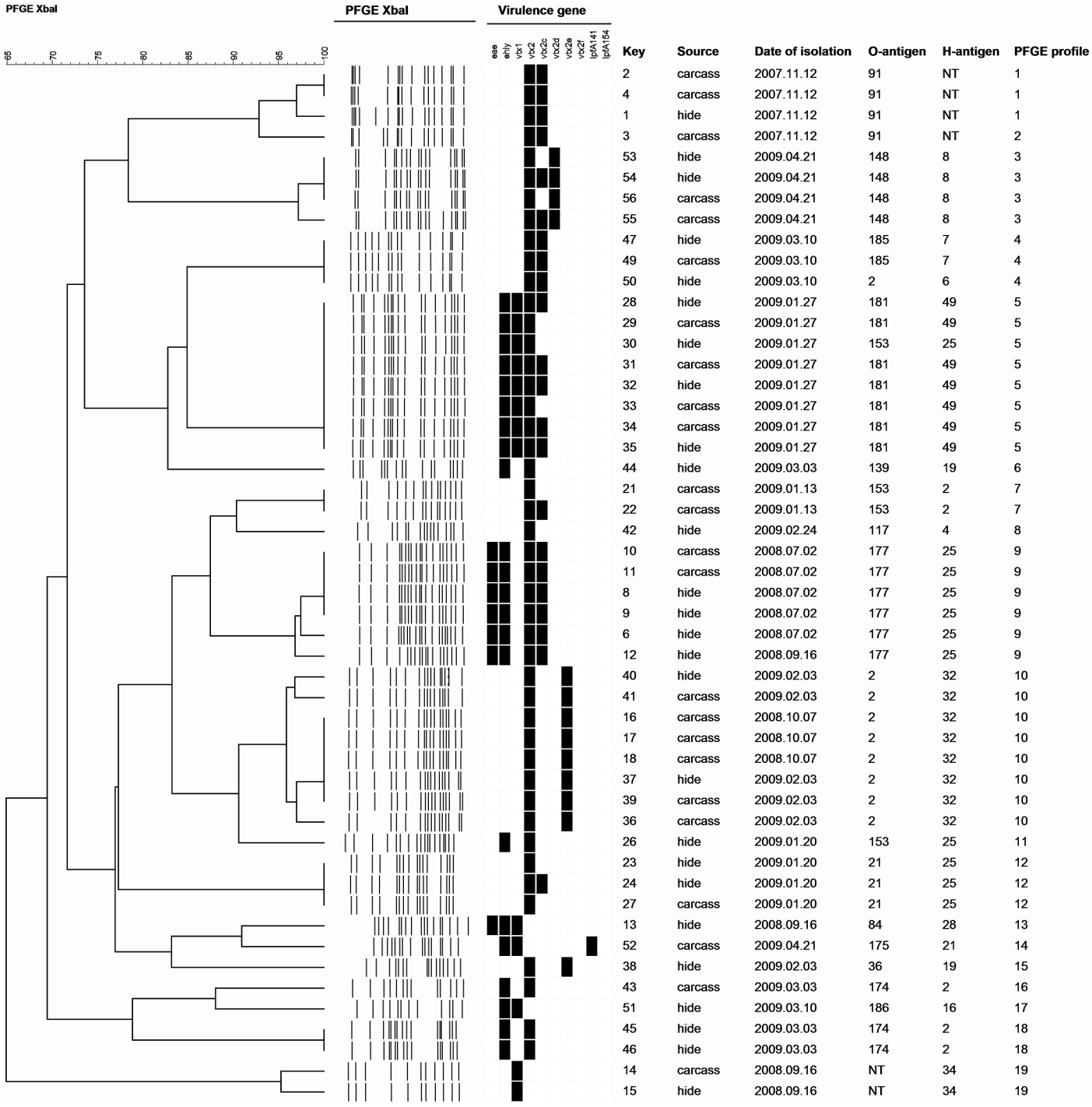
the eastern part of Poland between 2007 and 2009 as previously described (19). VTEC were subjected to serotyping with anti-O and anti-H specific sera and PCR as described (2, 18). Moreover, the presence of virulence genes (*vtx1*, *vtx2*, *vtx2c*, *vtx2d*, *vtx2e*, *vtx2f*, *eae*, *ehly*, $lpfA_{141}$, $lpfA_{154}$) were also investigated using PCR (15). PFGE was carried out following the PulseNet procedure with the CHEF DR II System (Bio-Rad, USA). The DNA banding pattern was captured with the Gel Doc 2000 (Bio-Rad) and analyzed using Bionumerics® software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were generated based on the Dice correlation co-efficient for similarity, while the unweighted-pair group method with arithmetic means (UPGMA) was employed for cluster analysis.

Results and discussion

PFGE analysis with the *Xba*I restriction enzyme grouped isolated VTEC into 19 different macrorestriction profiles (with 95% similarity). Seven PFGE types (numbers 1, 3, 4, 5, 9, 10, 12) covered more than two isolates, with two clusters of eight isolates each (numbers 5 and 10). Furthermore, three profiles grouped two isolates and the remaining nine PFGE types were represented by one strain only (Fig. 1). PFGE clusters were analyzed in relation to the identified serotypes (O186:H16, O185:H7, O181:H49, O177:H25, O175:H21, O174:H2, O153:H25, O153:H2, O148:H8, O139:H19, O117:H4, O91:HNT, O84:H28, O36:H19,



Key	Source	Date of isolation	O-antigen	H-antigen	PFGE profile
2	carcass	2007.11.12	91	NT	1
4	carcass	2007.11.12	91	NT	1
1	hide	2007.11.12	91	NT	1
3	carcass	2007.11.12	91	NT	2
53	hide	2009.04.21	148	8	3
54	hide	2009.04.21	148	8	3
56	carcass	2009.04.21	148	8	3
55	carcass	2009.04.21	148	8	3
47	hide	2009.03.10	185	7	4
49	carcass	2009.03.10	185	7	4
50	hide	2009.03.10	2	6	4
28	hide	2009.01.27	181	49	5
29	carcass	2009.01.27	181	49	5
30	hide	2009.01.27	153	25	5
31	carcass	2009.01.27	181	49	5
32	hide	2009.01.27	181	49	5
33	carcass	2009.01.27	181	49	5
34	carcass	2009.01.27	181	49	5
35	hide	2009.01.27	181	49	5
44	hide	2009.03.03	139	19	6
21	carcass	2009.01.13	153	2	7
22	carcass	2009.01.13	153	2	7
42	hide	2009.02.24	117	4	8
10	carcass	2008.07.02	177	25	9
11	carcass	2008.07.02	177	25	9
8	hide	2008.07.02	177	25	9
9	hide	2008.07.02	177	25	9
6	hide	2008.07.02	177	25	9
12	hide	2008.09.16	177	25	9
40	hide	2009.02.03	2	32	10
41	carcass	2009.02.03	2	32	10
16	carcass	2008.10.07	2	32	10
17	carcass	2008.10.07	2	32	10
18	carcass	2008.10.07	2	32	10
37	hide	2009.02.03	2	32	10
39	carcass	2009.02.03	2	32	10
36	carcass	2009.02.03	2	32	10
26	hide	2009.01.20	153	25	11
23	hide	2009.01.20	21	25	12
24	hide	2009.01.20	21	25	12
27	carcass	2009.01.20	21	25	12
13	hide	2008.09.16	84	28	13
52	carcass	2009.04.21	175	21	14
38	hide	2009.02.03	36	19	15
43	carcass	2009.03.03	174	2	16
51	hide	2009.03.10	186	16	17
45	hide	2009.03.03	174	2	18
46	hide	2009.03.03	174	2	18
14	carcass	2008.09.16	NT	34	19
15	hide	2008.09.16	NT	34	19

Fig. 1. Molecular characteristic of VTEC tested in the study

O21:H25, O2:H6, O2:H32, ONT:H34) and the presence of virulence genes. The results were also compared with the date of sampling. It was found that most of the identified PFGE types grouped the strains of the same serotype with an identical or similar virulence pattern and that were sampled during the same day (Fig. 1). The most numerous profile number 10 was represented by eight isolates; six of them were recovered from bovine carcasses and two from hides. All these strains were identified as O2:H32 serotype and had the same virulence markers – *vtx2* and *vtx2e*. The profiles 1, 9, 18, and 19 were also represented by the strains of the same serotype (O91:HNT, O177:H25, O174:H2 and ONT:H34, respectively) and with the identical virulence pattern. The VTEC isolates of profiles 3, 7, and 12 were of the same serotype (O148:H8, O153:H5 and O21:H25, respectively) but had a slightly different virulence gene pattern. On the other hand, among the isolates from two other PFGE pulsotypes (numbers 4 and 5), different VTEC serotypes were identified. The majority of the PFGE profiles grouped the strains isolated at the same day; however, the isolates with the same restriction profile that were recovered at different days were also found. PFGE analysis showed that among the isolates obtained from hides and the corresponding carcasses five pairs of strains had identical molecular profiles (Fig. 1).

There are several reports regarding the molecular characterization of VTEC strains based on the PFGE analysis. Albihn et al. (1) investigated 37 O157:H7 VTEC isolates from bovine feces and found 23 different PFGE profiles. A similar study in Ireland revealed 15 restriction patterns among 44 VTEC O157 isolated in beef and sheep abattoirs (13). On the other hand, an investigation of 117 O157 strains of bovine hide origin revealed a high heterogeneity, since among 109 PFGE profiles identified 101 were unique (7). In the same study 32 isolates from beef trimming were clustered into 28 PFGE profiles of which 26 were represented by only one strain. Similar observations were described by other authors. Dos Santos et al. (6) detected 28 restriction types among 35 VTEC O113:H21 of different animal and human origins. In the study performed by Karama et al. (11), among 91 VTEC O103:H2 strains recovered from cattle and humans 66 PFGE patterns were found.

In the present study, 19 distinct PFGE profiles were identified among 50 VTEC isolates. Ten profiles included 41 isolates whereas the remaining nine VTEC strains were of unique profiles. Furthermore, characterization of distinguished PFGE types in relation with serotypes and virulence genes revealed that the strains of the same profile were generally of the same serotype and possessed identical or only slightly different pathogenic markers. Similar results were obtained in the study of Blanco et al. (3) on 24 isolates of pig origin that were clustered into 21 distinct PFGE patterns and VTEC of the same serotype were classified together. On the other hand, Eklund et al. (9) revealed a poor correlation between PFGE profiles and serotypes, since 56 human VTEC isolates were clustered into 41 PFGE types, which covered the same but also different serotypes, and within the same serotype several different PFGE profiles were identified. Furthermore, in the present study the majority of the PFGE types covered the strains isolated during the same day, which may suggest bacterial transmission between the slaughtered animals within the abattoir or may be due to the common origin of the cattle. Similar findings were obtained in the studies performed in Ireland (7, 16). However, the opposite results were also described (4, 5, 13, 17). Furthermore, Cobbaut et al. (4) classified 324 VTEC isolates into 83 distinguishable PFGE types and found that the majority of the macrorestriction clusters (n = 76) were farm specific. In the present study, PFGE analysis revealed that five isolates recovered from carcasses had the identical molecular profile as those identified on bovine hides and cross-contamination during the slaughter process could have occurred. Moreover, the isolates with the same PFGE profile that were sampled at different days were also found, which suggests the persistence of the strains within the abattoir. However, to support these results further molecular investigations are needed.

In conclusion, PFGE used in the present study in correlation with serotypes and virulence gene profiles provided valuable information concerning the molecular characterization of VTEC isolated from cattle at the slaughter level. The obtained results reflected a low genetic diversity among the examined VTEC which may suggest a common source of contamination within the abattoirs.

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