PHENO- AND GENOTYPIC CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 ISOLATED FROM CATTLE HIDES IN ESTONIA

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INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), which are also quoted as verocytotoxin-producing *E. coli* (VTEC), are a group of *E. coli* that are characterised by their capability to produce toxins closely related with the Shiga-toxin produced by *Shigella dysenteriae* [1]. Nowadays a substantial number of STEC serotypes have been recognised as a causative agent of human disease, but severe infections reported have been mostly associated with the O157 serogroup [2]. Contaminated ruminants' meat is considered to be a main source of foodborne STEC infections in humans, particularly by *E. coli* O157:H7 [3]. Sampling cattle hides at abattoir for STEC monitoring is preferable, since the highest prevalence is presumed at this stage of the meat production and hides represent a major source of carcass contamination by *E. coli* O157 [4], [5]. The monitoring results reflect the probability of further contamination of carcasses and meat, and represent a tool to evaluate the efficacy of good hygiene practices as a control measure of carcass contamination [6].

The aim of the present study was to evaluate the prevalence of STEC O157:H7 in cattle at slaughter in Estonia by investigating the contamination of cattle hides. The isolates were further characterised by identifying the presence of genes of main virulence factors, the subtypes of the Stx-coding genes and determining the antimicrobial susceptibility and genetic relationship among the isolates.

MATERIALS AND METHODS

During the period from January 2011 to December 2013 the abrasive sponge samples from cattle hides were collected from abattoirs in Estonia. Starting with the slaughterhouses of largest throughput, those covering at least 80% of the national throughput were included into the monitoring program. Animals to be sampled were chosen randomly throughout the year and only one animal per slaughterhouse was sampled per selected day. Furthermore, the prerequisite that the animals to be sampled had to originate from different herds was followed. Samples were analysed for the presence of *E. coli* O157 using the procedure described by standard method ISO 16654 [7] followed by the conventional serotyping and detection of *stx1*, *stx2* and *eae* genes in compliance with ISO/TS 13136 [8]. Identifying of *sxt1* and *stx2* genes subtypes was performed as described by Scheutz and colleagues [9]. The genetic relationship among isolates was investigated by PFGE in accordance with the standard operating procedure of EFSA external scientific report [10] and analyses were performed by European Union Reference Laboratory for *E. coli*. The susceptibility to antimicrobials was determined by minimal inhibitory concentration (MIC) microdilution method using VetMIC GN panel. Based on epidemiological cut-off values, the results were categorized as susceptible or resistant according to the guideline of European Committee on Antimicrobial Susceptibility Testing and EU Reference Laboratory for Antimicrobial Resistance [11].

RESULTS

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 was isolated from 30 (4%, CI_{95} 2.8% - 5.7%) of 744 cattle hide swab samples collected at Estonian abattoirs within a three year monitoring program. All isolates turned out to be motile, possessed H7 antigen and showed enterohaemolytic phenotype. Twelve isolates (40%) showed resistance to at least one of the 14 antimicrobials tested and the isolates were predominantly resistant to streptomycin, sulfamethoxazole and ampicillin. The resistance to three or more unrelated antimicrobials was detected in eight (27%) isolates while no extended spectrum beta-lactamase producing isolates were confirmed. Seventeen isolates carried both *stx1* and *stx2* genes and the presence of *stx2* gene were solely detected in case of 13 isolates. Most frequently detected *stx* subtype, was *stx2c* occurring alone (n = 12) or in combination with subtype *stx1a* (n = 13). Subtype *stx2a* alone was detected in one isolate and in combination with *stx1a* in four isolates. All isolates harboured the intimin-encoding *eae* gene. Comparison of PFGE profiles showed that the isolates presented an overall similarity more than 75%. In total, 20 different *Xba*I PFGE patterns were differentiated among the 30 isolates, of which 14 grouped into 4 clusters based on a genetic relatedness criterion of 100%.

CONCLUSIONS

Current study provides a first description of the *E. coli* O157:H7 strains spreading in Estonian cattle population including their virulence gene profiles and *stx* gene subtypes. Isolates with indistinguishable PFGE pattern were found from cattle originating from different localities and in different years, suggesting that some persistent clones are present in Estonian beef production chain. The high proportion of strains showing multiple drug resistance raises the question about appropriate use of antimicrobials in the Estonian cattle herds. Up to now, STEC state monitoring in Estonia has been focused only to *E. coli* O157, but also other STEC serogroups are important in STEC epidemiology and should be included into national monitoring program.

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