Detection and identification of *Escherichia coli* (VTEC) in food samples – microbiological safety of vegetables and seeds in Slovenia

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INTRODUCTION

Verocytotoxin-producing *Escherichia coli* (VTEC) can cause illness ranging from mild intestinal disease to severe kidney complications. VTEC include the relatively important serotype *E. coli* O157:H7 and more than 100 other non-O157 strains such as O111 and O26. VTEC are of zoonotic origin and can be transmitted directly or indirectly from animals to humans.

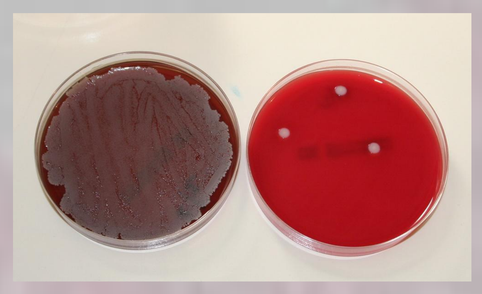
On 22 May Germany informed the European Commission's Early Warning and Response System (EWRS) of a significant increase in the number of patients with haemolytic uremic syndrome (HUS) and bloody diarrhea caused by enterohaemorrhagic *Escherichia coli* (VTEC). Investigations confirmed that bacteria *Escherichia coli* (VTEC) O104:H4 was responsible for this outbreak. At first it has been assumed that the source of infection were raw vegetables (tomatoes, cucumber and leaf salad), but then the analysis of the data from the French and the German outbreaks led to the conclusion that seeds for sprouting, imported from Egypt by a German importer, were the most likely source of infection.

In order to ascertain whether Slovenian vegetables, seed and infant food in Slovenia were microbiologically safe, the Slovenian authorities tested 275 samples for presence of VTEC up until 15 July 2011 .

LABORATORY METHODS

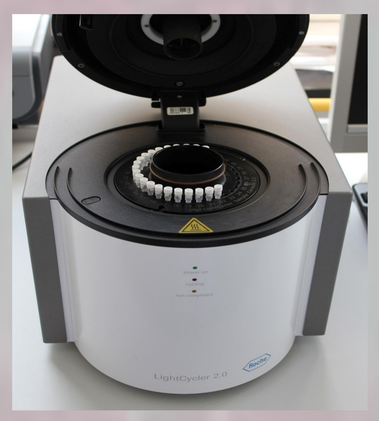
Standard bacteriological method and PCR confirmation test (NIPH)

1. Enrichment cultures are performed by adding a 25 g test portion of food to a stomacher bag with filters containing the appropriate amount of mTSB or BPW. Homogenise in a stomacher (see ISO 7218)
2. Enrichment broth streaked onto TBX, SMAC and EO
3. 10 – 50 colonies with *E. coli* morphology inoculate on NA, Identify isolates with biochemical test for *E. coli*, qualitative detection of Verotoxins 1 and 2 from *E. coli* (GLISA Duopath Verotoxins)
4. PCR confirmation test for *E.coli* isolates: Genes for *vtx1* (verocytotoxin 1), *vtx2* (verocytotoxin 2) and *eae* (intimin) were detected with PCR using primers described by Person et al. (2007).



Molecular method - Polymerase chain reaction - PCR detection of VTEC from enrichment broth (IPH - MB)

1. Enrichment cultures are performed by adding a 25 g test portion of food to a stomacher bag containing 225 ml of TSB broth. Homogenise in a stomacher (see ISO 7218)
2. DNA extraction is performed from 1 ml of enrichment broth
3. Detection of genes encoding verocytotoxin 1 and 2 (*vtx1* and *vtx2*) from extracted DNA is performed with real time PCR assay, carried out with LightCycler® 2.0. (Roche Applied science).



RESULTS

All results from microbiological tests were negative for presence of VTEC.

Table 1: Inspectorate of the Republic of Slovenia for Agriculture, Forestry and Food

Laboratory	No.of samples – vegetables	No.of samples – sprouts	Results – negative for VTEC
NIPH	98	20	118
IPH - MB	62	23	85
	160	43	203

Table 2: Health Inspectorate of the Republic of Slovenia

Laboratory	No.of samples – infant food	No.of samples – vegetables	Results – negative for VTEC
NIPH	20	15	35
IPH - MB	10	15	25
	30	30	60

Table 3: Food businesses operators

Laboratory	No.of samples – vegetables	Results – negative for VTEC
NIPH and IPH - MB	12	12

DISCUSSION

In Slovenia two laboratories (NIPH and IPH – MB) were testing food samples taken in connection with VTEC outbreak in Germany. Both laboratories are capable to perform correct methods and have many years of experience. Two different methods were used, standard bacteriological method and polymerase chain reaction. While the advantage of the standard bacteriological method is the certainty of bacteria isolation, the advantage of the molecular method (PCR) is swiftness of the results.

Based on the results of the investigation, none of the samples was positive; thus, indicating that there was no food contaminated with VTEC O104:H4 - strain isolated from the large outbreak in Germany earlier this year - in Slovenia.

During the investigation, the excellent cooperation between inspectors and microbiologists has proved to be of the greatest significance. The question of vital importance now is how to prepare for a similar event in Slovenia and Europe, where the structures involved in exchanging data and experiences are indispensable. Consequently, it is necessary to work on establishment of direct linkages between inspectorates and independent laboratories.