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Pregledni rad

STEC in the beef chain – One Health Approach

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A b s t r a c t: Since the early 1980s, E. coli O157 has emerged as one of the most significant pathogens of public health relevance not because of the incidence of the illness, which is much lower than that of other food borne pathogens such as Campylobacter or Salmonella, but because of the severity of the symptoms, the low infectious dose and potential sequelae. Shiga toxin – producing (STEC) Escherichia coli is a human pathogen that can cause hemorrhagic colitis (bloody diarrhea) and sometimes hemolytic uremic syndrome (HUS), a life-threatening disease that causes kidney damage. Cattle carry mixtures of O157 and non-O157 STEC in their intestines that are not necessarily pathogenic to humans. The O157 STEC serogroup was until recently responsible for the majority of disease outbreaks reported for STECs in North America, however now the non-O157 STECs account for almost 50% of the reported human disease outbreaks in North America and Europe. The STECs are shed at significant levels by healthy/asymptomatic cattle, e.g. cattle with jejunal hemorrhage syndrome. The shedding leads to contamination of the farm environment. This may lead to direct or indirect contamination of cattle hides, which, in turn, can serve as the main source of carcass contamination during slaughter and dressing of cattle at abattoirs or contamination of fresh beef and beef products. A science-based risk assessment is needed to assess the public health impact, the consumer exposure to the pathogen and for design of the most effective risk mitigation strategies regarding prevention and reduction of beef-borne O157 and non-O157 STECs.

Key words: *STEC, risk assessment, public health, risk mitigation.*

1. Introduction

Escherichia coli O157 is a potential food borne pathogen and a toxin-producing serogroup that-after ingestion-can cause severe damage to the intestinal mucosa and, in some cases, other internal organs of the human host. Since the early 1980s, *E. coli* O157 emerged as one of the most significant pathogens of public health relevance not because the incidence of the illness, which is much lower than that of other food borne pathogens e.g. *Campylobacter* or *Salmonella*, but because of the severity of the symptoms, the low infectious dose and potential sequelae. Shiga toxin-producing *Escherichia coli* (STEC) is a human pathogenic *E. coli* bacterium that is able to cause hemorrhagic colitis (HC; bloody diarrhea), which sometimes develops into hemolytic uremic syndrome (HUS). HUS is a life-threatening disease that causes kidney damage and is a severe complication of STEC infection (*Fairbrother and Nadeau*, 2006) (Figure 1). In most of cases, HUS is developed in children and immuno-suppressed individuals, while HC (Haemorrhagic Colitis) is usually associated with elder people. Faecally excreted by healthy, asymptomatic cattle, STEC can be spread to environment, water and/or foods directly or indirectly contaminated by the fecal material.

Cattle can carry different types of STEC in their intestines that are not necessarily pathogenic for humans. *E. coli* O157 is STEC serogroup that is responsible for the majority of outbreaks reported for shiga (verotoxin)-producing strains, although other non-O157 serotypes are also involved in human outbreaks (e.g. serogroups O26, O103, O111 and O145). Most available information relates to serotype O157:H7, since it is easily differentiated biochemically from other *E. coli* strains.

Cattle carry mixtures of STEC O157 and non-O157 in their intestines which are not necessarily pathogenic to humans and those healthy cattle may intermittently excrete VTEC seropathotypes, by fecal shedding, (*Nastasijevic,* 2011). The infectious dose of *E. coli* O157 is not known. In some cases of food borne human disease, only a few cells, perhaps lower than 100 CFU, may have been ingested (*Tilden et al.,* 1996). Therefore, the prevention of foodborne *E. coli* O157 infections requires not only growth suppression in foods, but also elimination of the pathogen from foods (*Buncic et al.,* 2004).

Advancement of the knowledge and development of efficient risk mitigation strategies for *E. coli*

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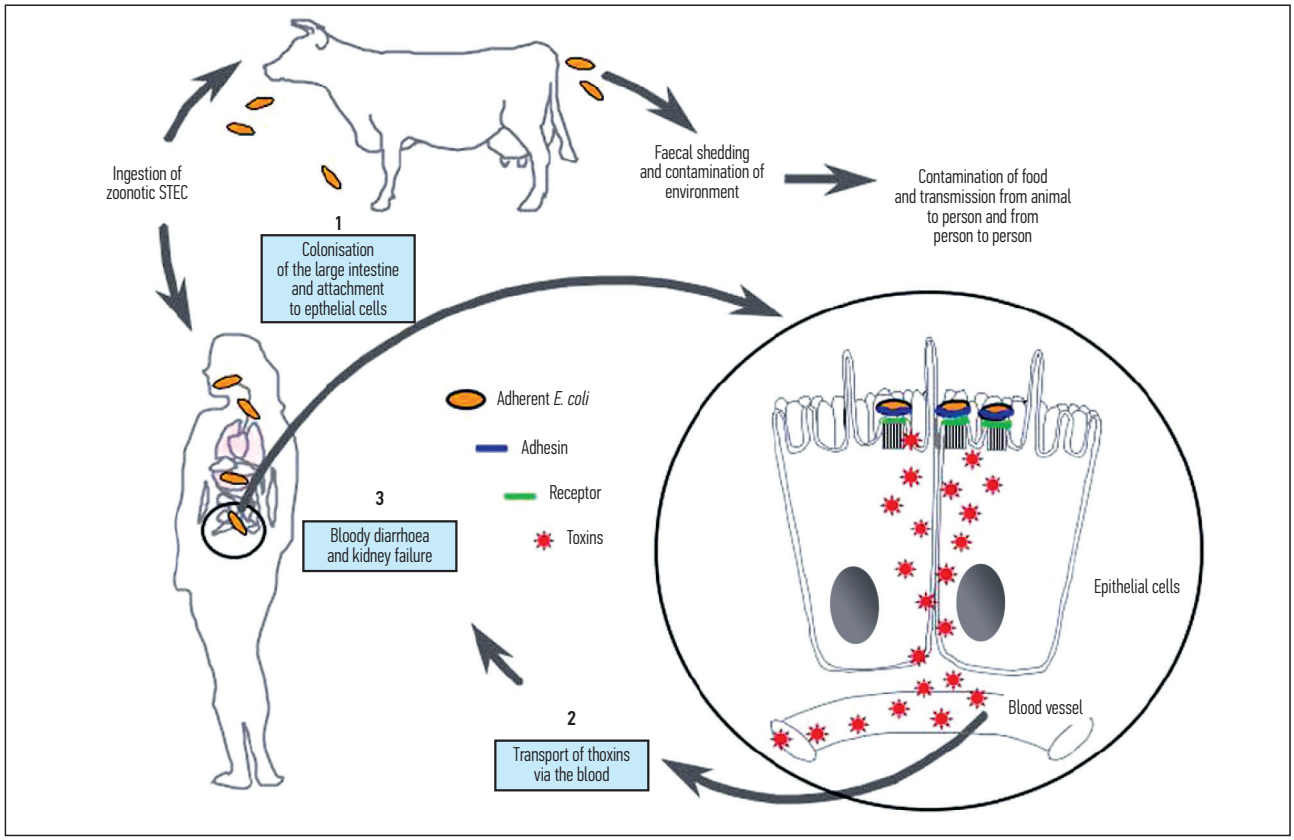

Figure 1. Bloody diarrhea and hemolytic uremic syndrome in humans caused by STEC O157 (*Fairbrother and Nadeau*, 2006)

Slika 1. Krvava dijareja i hemolitički uremički sindrom kod ljudi uzrokovan sa STEC O157 (*Fairbrother i Nadeau*, 2006)

O157 in the context of science-based risk assessment, longitudinal and integrated approach to meat safety assurance, are and will remain for the foreseeable future, one of the priorities for both – researchers and regulators in the area of meat safety (*Buncic,* 2006). Availability and quality of relevant data at different points of the meat chain is one of the key pre-requisites.

2. Risk assessment and risk management

Risk assessment. This is the science-based component of risk analysis (risk assessment, risk management and risk communication) consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization. In this process, the hazards are identified and the risk posed by that particular hazard (i.e. pathogen) is calculated (*Codex*, 1999). Therefore, the risk assessment has the aim to estimate prevalence/occurrence/numbers of *E. coli* O157 at different points along the beef chain. In this paper, only first three components of risk assessment will be addressed. This is due to the reason that risk characterization should be completed only after in-depth interpretation of national baseline data, originated from targeted research.

Risk management. This is the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (*Codex,* 2001). It aims to define the main and most effective control options of *E. coli* O157 along the beef chain, which are also technically and financially sustainable.

2.1. Hazard identification

E. coli pathogenic for humans can be faecally shed by humans and healthy animals; they can be divided into different groups including:

- Enteropathogenic *E. coli* (EPEC): associated with infantile diarrhoea;

- Enteroinvasive *E. coli* (EIEC): cause dysentery-like disease;
- Enterotoxigenic *E. coli* (ETEC): produce enterotoxins and diarrhoea;
- Enteroaggregative *E. coli* (EAEC): express aggregative adherence;
- Diffusely adherent *E. coli* (DAEC): adhere to the surface of epithelial cells;
- Enterohaemorrhagic *E. coli* (EHEC), including serotype O157, is the subset of STEC: produce Shiga-like toxin (Stx) or Verocytotoxin (Vtx) and can provoke hemorrhagic colitis (HC) in humans, with some cases developing to Hemolytic Uremic Syndrome (HUS).

The primary reservoir of EHEC are farm ruminant shedders i.e. cattle, sheep, goats. These farmed ruminants are healthy carriers of EHEC. The bacteria reside in the gut and can be intermittently fecally excreted. Therefore, STEC (EHEC) is a zoonotic pathogen that can be transmitted from animals to humans via direct contact with fecally contaminated animals, or more commonly through the food chain or through water. Person-to-person faecal-oral route of transmission can also occur.

2.1.1. Definitions associated with STEC/VTEC

VTEC. In Europe, most commonly, the cytotoxin produced by *E. coli* serotypes O157 or non-O157 has been called verotoxin (verocytotoxin) due to its lethal *in vitro* effects on Vero cells.

STEC. In US, *E. coli* serotypes who has the ability to produce cytotoxin are usually called shigatoxin-producing *E. coli*.

STEC O157. It usually denotes shiga(vero)toxin producing *E. coli* of O (somatic) serogroup O157, but with either "unspecified" or "undetermined" H (flagellar) serovar.

STEC O157:H7. It denotes shigatoxin producing *E. coli* of O157 serogroup and of H (flagellar) 7 serovar. It does not indicate whether the strains produce other virulence factors (apart from shigatoxin) necessary for causing food borne illness.

STEC non-O157. It denotes the number of serogroups, other than O157, which are associated with ability to produce shigatoxin, as well as other virulence factors and thus have significant impact on public health (e.g. O26, O45, O91, O103, O111, O145).

EHEC. Those STEC that cause enterohemorrhagic colitis (i.e. a subset of STEC) have been called enterohemorrhagic *E. coli* (EHEC; including O157).

HP-VTEC. Use of term Human pathogenic verotoxigenic *E. coli* (including *E. coli* O157) has been proposed in an attempt to cover both key aspects; ability to cause illness of "any" clinical manifestation in humans and the ability to produce shiga(vero)toxin (SCVMPH, 2003).

2.1.2. Overview of E. coli O157 infections

Reporting according to the new rules in Zoonoses Directive 2003/99/EC, instated as of 12 June 2004, started with data collected during 2005.

In 2012, the total number of confirmed VTEC cases in the EU was 5,671 based on data submitted by 22 member states (MSs). This represents a decrease of 40% compared with 2011 (9,487 reported cases), when a large outbreak of STEC O104:H4 occurred in Germany. The outbreak was associated with the consumption of contaminated raw sprouted seeds affecting more than 3,800 persons alone in Germany and linked cases in an additional 15 countries; the EU-incidence was 1.15 cases per population of 100,000 (*EFSA*, 2014). Overall, the highest notification rates were reported in Ireland, the Netherlands and Sweden (8.99, 6.27 and 4.98 cases per 100,000 population, respectively), while the lowest rates were reported in Bulgaria, Cyprus, the Czech Republic, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Romania and Spain (<0.1 cases per 100,000). The different sensitivities of the reporting systems of the MSs may have also influenced these figures. Consequently, comparison between countries should be done with caution. Comparison between years within a country is, in general, more valid (Table 1).

Data presented in Table 1. could lead to conclusion that number of reported and confirmed STEC cases in humans generally increased over time, within the three years period of time (2008-2011), e.g. 3,162 to 9,487, respectively. However, this increase may be attributed not only to ineffectiveness of current risk management strategies in place, but also to improvement of surveillance and reporting systems in respective EU Member States (e.g. Austria, Denmark, Ireland, Italy, Netherlands, Spain, Sweden), as well as the massive STEC O104:H4 outbreak in Germany, 2011.

In addition, more than half (53.0%) of EU reported confirmed human STEC infections in 2008 were associated with the O157 serogroup, while the rest belonged to the most frequent non-O157 serogroups, i.e. O26, O103, O145, O91, O111, O128, O146, O117, respectively (Table 2).

Table 1. Reported STEC cases in humans, 2008-2011 and notification rates for confirmed cases, 2012[1] (adapted from *EFSA*, 2014)

Tabela 1. Prijavljeni slučajevi STEC kod ljudi, 2008–2011. i stepen prijavljivanja za potvrđene slučajeve, 2012[1] (adaptirano iz *EFSA*, 2014)

Country	2012				2011	2010	2009	2008
	Report type[2]	Cases	Confirmed cases	Confirmed cases/ 100,000	Confirmed cases			
Austria	C	131	130	1.54	120	88	91	69
Belgium	C	105	105	0.95	100	84	96	103
Bulgaria	U	0	0	0	1	0	0	0
Cyprus	U	0	0	0	0	0	0	0
Czech Republic	C	9	9	0.09	7	-	-	-
Denmark	C	193	193	3.46	215	178	160	161
Estonia	C	3	3	0.22	4	5	4	3
Finland	C	30	30	0.56	27	21	29	8
France	C	208	208	0.32	221	103	93	85
Germany	C	1587	1573	1.93	5558	955	887	876
Greece	U	0	0	0	1	1	0	0
Hungary	C	3	3	0.03	11	7	1	0
Ireland	C	554	412	8.99	275	197	237	213
Italy	C	68	50	0.08	51	33	51	26
Latvia	U	0	0	0	0	0	0	0
Lithuania	C	2	2	0.07	0	1	0	0
Luxembourg	C	21	21	4.00	14	7	5	4
Malta	C	1	1	0.24	2	1	8	8
Netherlands	C	1049	1049	6.27	845	478	314	92
Poland	C	3	1	< 0.01	5	3	0	3
Portugal[3]	-	-	-	-	-	-	-	-
Romania	C	1	1	< 0.01	2	2	0	4
Slovakia	C	9	9	0.17	5	10	14	8
Slovenia	C	29	29	1.41	25	20	12	7
Spain	C	31	31	0.07	20	18	14	24
Sweden	C	472	472	4.98	477	334	228	304
United Kingdom	C	1339	1339	2.17	1501	1110	1339	1164
EU Total		**5848**	**5671**	**1.15**	**9487**	**3656**	**3583**	**3162**
Iceland	C	1	1	0.31	2	2	8	4
Liechtenstein	-	-		-				0
Norway	C	75	75	1.50	47	52	108	22
Switzerland	C	63	63	0.79	71	31	40	72

Legend:

1. C: case-based data reported; –: no report; U: unspecified.
2. Mandatory notification of VTEC in 2008 and reported to ECDC from 2011.
3. No surveillance system.
4. Switzerland provided data directly to EFSA.

Table 2. Reported confirmed STEC cases in humans by serogroup (top 10), 2007-2008. (Adapted from *EFSA,* 2010)

Tabela 2. Prijavljeni potvrđeni slučajevi STEC kod ljudi prema serogrupama (prvih 10), 2007–2008. (adaptirano iz *EFSA*, 2010)

2008				2007			
Serogroup	**No. of cases**	**% Total**	**% Known**	**Serogroup**	**No. of cases**	**% Total**	**% Known**
O157	1,673	53.0	53.0	O157	1,571	54.1	54.1
NT	819	25.9	25.9	NT	842	29.0	29.0
O26	166	5.3	5.3	O26	136	4.7	4.7
O103	88	2.8	2.8	O103	77	2.7	2.7
O145	49	1.6	1.6	O91	43	1.5	1.5
O91	50	1.6	1.6	O145	31	1.1	1.1
O111	43	1.4	1.4	O111	23	0.8	0.8
O128	28	0.9	0.9	O128	21	0.7	0.7
O146	25	0.8	0.8	O113	16	0.6	0.6
O117	20	0.6	0.6	O146	14	0.5	0.5
Other	198	6.3	6.3	Other	130	4.5	4.5
Total: 3,159				**Total: 2,904**			

Legend: Source: Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, Malta, the Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden and the United Kingdom
NT = non typed/untypeable

The large majority of the STEC infections had diarrhea as a clinical manifestation (i.e. non-HUS infections), whilst STEC infections with HUS manifestations were markedly less frequent. The largest proportion (34.2%) of reported STEC infections occurred in infants aged 0 to 4 years and the most frequent detected serogroup was O157 (Figure 2).

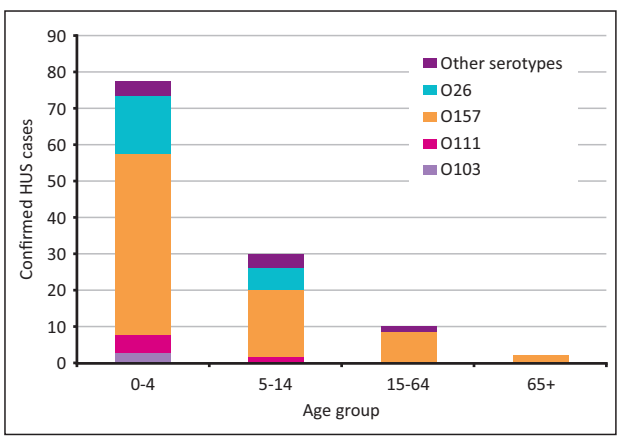

Figure 2. Hemolytic Uremic Syndrome (HUS) by age and serogroup in reporting MSs, 2008 (Adapted from *EFSA*, 2010)

Slika 2. Hemolitički uremički sindrom (HUS) po starosnoj kategoriji i serogrupama u zemljama članicama EU, 2008 (adaptirano iz *EFSA,* 2010)

However, in the massive outbreak recorded in Germany (May-July 2011), provoked by STEC O104:H4, it was reported that majority of HUS cases were adults (>87%), with a clear predominance of women (68%). Cases in children of school age are also reported. It was the unusual clinical manifestations having in mind that in the majority of previous STEC outbreaks HUS was developed mainly in children <5 years old. The number of people affected and the severity of disease (e.g. development of HUS) confirmed that it was the biggest ever recorded STEC outbreak in EU. By the end of the outbreak, the number of 3774 (750 HUS cases and 3024 non-HUS cases) of infected people was reported. The leading hypothesis was that seeds used for sprouting (distributed to local producers or retail outlets) contained a level of *E. coli* O104:H4 contamination, ultimately leading to contaminated sprouts destined for human consumption. The implicated food source was also attributed to consumption of faecally contaminated fresh produce/vegetables (cucumbers, tomatoes, etc.). However, the presence of the STEC O104:H4 in implicated foods was not precisely confirmed; the exact point of contamination in the food chain was not established (ECDC, 2011). The molecular characterization (e.g. PCR) of isolated STEC strain from the stools of infected people confirmed the unusual combination of virulence factors,

belonging both – to Enteroaggregative (EAEC) and Shigatoxin-producing (STEC) *E. coli*, as follows: Stx1-negative, Stx2-poisitive, Intimin (eae)-negative, enterohemolysin (hyl)-negative, EAggEC virulence plasmid-positive (aatA, aggR, aap) (*RKI*, 2011). This led to hypothesis that the acquisition of plasmid encoded gene for Stx2, possibly happened via direct horizontal transfer between STEC (animal/ruminant-host) and EAEC (human host). The combination of these factors – Stx2 and enteroaggregative characteristics of the isolated pathotype O104:H4, was the probable reason for the unusually high level of virulence of this epidemic strain. This biological indication that probably humans – and not animals – are the reservoir for this strain, backed up the finding of the epidemiology that the outbreak was not linked to meat or dairy products. Before this outbreak, there were some sporadic cases of O104 infection, reported in EU since 2008, e.g. Belgium (two cases in 2008), Denmark (one case in 2008), Norway (three cases in 2009), Austria (one case in 2010) and Sweden (one case in 2010).

In United States, the Center for Disease Control and Prevention (CDC) has estimated that 112,752 food borne illnesses annually are due to non-O157 STEC, which is nearly twice the number of illnesses attributed to *E. coli* O157:H7. According to United States Department for Agriculture (USDA), an estimated 36,700 illnesses annually due to non-O157 STEC could be attributed to beef products (*USDA FSIS,* 2011a). Therefore, USDA has declared six additional serogroups of Shiga toxin-producing *E. coli* (STEC) – O26, O103, O45, O111, O121 and O145 – adulterants in non-intact raw beef. The regular testing of these pathotypes, together with *E. coli* O157:H7, had begun in 2012 (*USDA FSIS,* 2011b). Such scheme had been introduced within the regular verification protocols related to cattle slaughter hygiene. To understand the prevalence of non-O157 STEC in beef, USDA Food Safety Inspection Service (FSIS) initiated a nationwide microbiological baseline survey on beef carcasses which ended in 2011. In the meantime, the FSIS advised establishments that manufacture raw, non-intact beef products or intact raw beef components of those products to evaluate whether non-O157 STEC are hazards reasonably likely to occur in their products (*USDA FSIS,* 2011a).

STEC – EU versus USA. It should be noted that certain differences exists between EU and USA, regarding the proposed methodology for monitoring of STEC in the beef chain. Namely, the detection and enumeration of STEC in EU is required to be carried out along the meat chain – at different, selected points (e.g. animals, food) in order to provide high quality data (*Nastasijevic,* 2014). Internationally recognized testing methodology should be also applied for obtaining the valid results. The EU regulations require that Member States should carry out regular monitoring/testing in three-year interval basis. The testing should primarily include VTEC O157, but also VTEC non-O157 (e.g. serogroups O26, O103, O111 and O145). On the other hand, the US Department of Agriculture, Food Safety and Inspection Service (*USDA FSIS*, 2011b) introduced verification procedures, including sampling and testing for raw ground/trimmed beef products, to ensure control of both *Escherichia coli* O157:H7 and six other serogroups of STECs (O26, O45, O103, O111, O121, and O145). These STECs are declared as adulterants of non-intact raw beef products within the meaning of the Federal Meat Inspection Act (FMIA). Therefore, it is evident that there is a certain difference regarding the scientific approach in prevention and control of STEC in the meat (beef) chain, between the EU and USA. For example, the recommended testing of STEC non-O157 in the EU doesn`t include serogroups O45 and O121, as it is the case in USA. It can be assumed that, in the light of ongoing negotiation between EU and USA within the framework of Transatlantic dialogue (Free Trade Agreement/FTA) and related food safety/public health issues, e.g. GMOs, pesticides, hormone-treated beef, growth promoters (*Hilary*, 2014) – the further harmonization regarding monitoring and testing protocols for STEC in the meat chain, might be also initiated.

2.1.3 Routes of infection

Person-to-person transmission. The faecal-oral route of infection of *E. coli* O157 appears to be commonly occurring in patients' homes, pre-schools, geriatric homes and hospitals (*CDCP,* 1993; *Bell et al.*, 1994; *CDCP, 1995*; *Chapman et al.,* 1997; *Paunio et al.,* 1999).

Contact with animals. *E. coli* O157 was found in a range of animal species including farm animals (primarily cattle, but also sheep and pigs), companion animals (horses, rabbits) and wild animals (gulls, rats, flies). Contact with faecally contaminated animals or animal-related environments can lead to the faecal/oral route of infection. For example, three children developed HC or HUS after animal contact on an open farm during a school visit in England (*Milne et al.,* 1999). Animal-to-person transmission was also confirmed by PFGE typing in Canada (*Louie et al.,* 1999). Hand-mouth contact and nail biting were significantly associated with disease (*CDCP*, 2001).

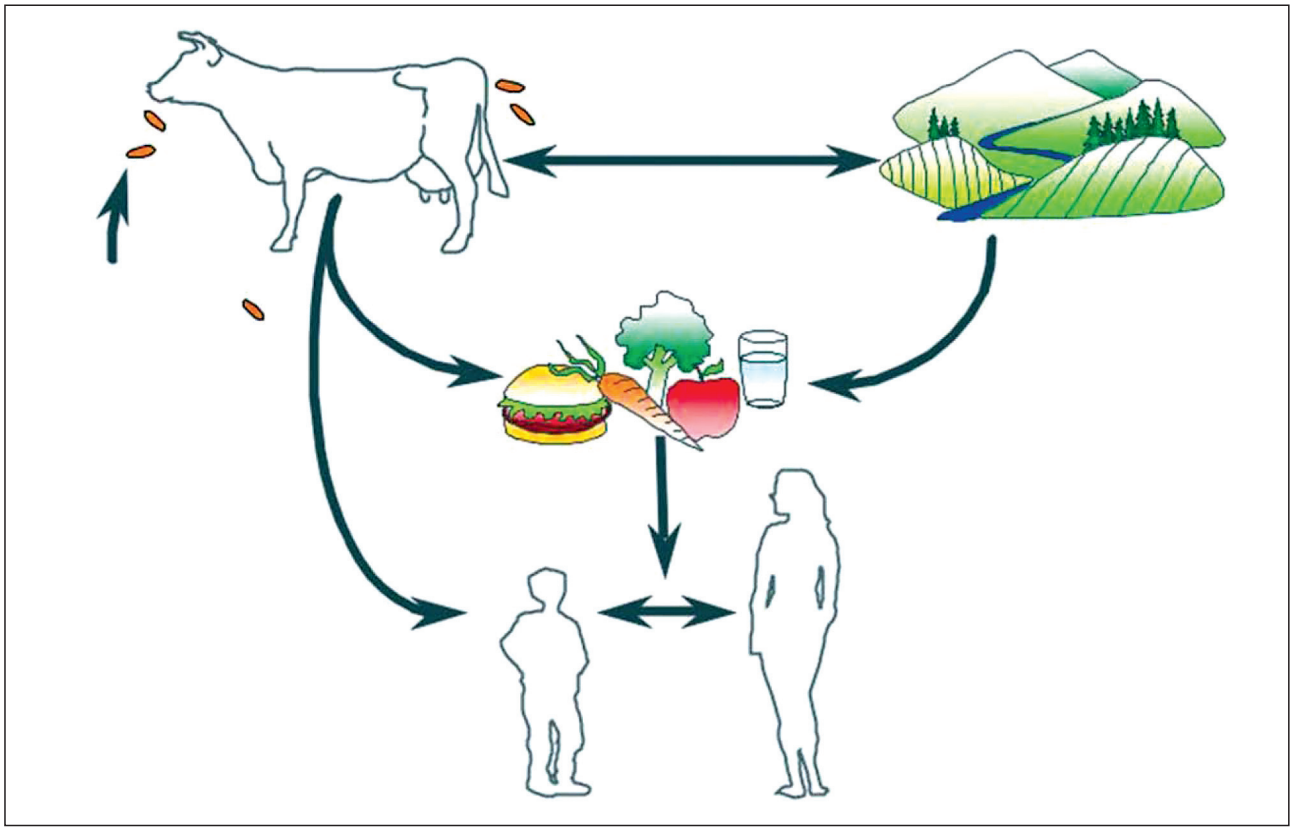

Figure 3. Exposure of humans to zoonotic Shiga toxin-producing *Escherichia coli* (STEC) (*Fairbrother and Nadeau*, 2006)

Slika 3. Izloženost ljudi ka zoonotskoj Šiga toksin-produkujućoj *Escherichia coli* (STEC) (*Fairbrother i Nadeau*, 2006)

Food borne. This is considered, overall, to be the main infection route, at least in the outbreaks (*Fairbrother and Nadeau*, 2006) (Figure 3). The implicated sources of food borne infections include:

- meats (e.g. meat patties, fermented sausages, deer jerky);
- milk/dairy (e.g. un-pasteurized milk, heat--treated milk, cheese from raw milk);
- produce (e.g. potato, alfalfa/radish sprouts);
- drinks (e.g. apple cider); and
- water (well water, reservoir water, mains' water supply).

Systemically presented information on the relative relevance of the food borne route *vs* other routes for *E. coli* O157 infections, is scarce. In the UK during 1995−2004 period, within O157 infections with identified infection routes, the food borne route was the most common and was responsible for 25% to 40% of the cases (*Smith,* 2004). In the US, over 20-year period, 52% of outbreaks were food borne, amongst which ground beef was implicated as a food vehicle in 41% of outbreaks (*Rangel et al.,* 2005). However, the situation with the routes of the food borne diseases can – and do – markedly vary between countries, and also both spatially and temporally within a given country.

2.2 Hazard characterization

2.2.1. Basic mechanism of E. coli O157 infection

Infection process in humans. After ingestion and incubation of around 4 (3 to 9) days, *E. coli* O157 is thought to be non-invasive, presumably colonizing the GI tract (large intestine, i.e. colon) by adhering to the external surface of gut epithelial cells (mediated by the Locus of Enterocyte Effacement-LEE). It seems that the exact location associated with A/E (attaching/effacing) lesions in the guts is not fully understood, possibly because human colon biopsy specimens are collected relatively late in the disease process, and lesions would only be visible during the early stages of infection (*Nataro and Kaper*, 1998). However, *Phillips et al.* (2000) found one *E. coli* O157 isolate did form microcolonies and attaching/effacing (A/E) lesions on human Payer's patches

in vitro, but not on proximal or distal small intestine nor on colon tissue. Once established in the GI tract, *E. coli* O157 cells do not move from the gut lumen, but produce one or more shiga(vero)toxins in the large intestine (*Griffin et al.,* 1990).

2.2.2. Manifestations of human infection

Hemorrhagic colitis (HC). HC or bloody diarrhea is usually accompanied by abdominal cramps causing severe pain. It may start with non-bloody diarrhea that progresses to bloody diarrhea within 2 to 3 days, and may be accompanied by vomiting and sometimes relatively mild fever (*Nataro and Kaper,* 1998).

Hemorrhagic uremic syndrome (HUS). HUS is characterized by acute kidney failure (Figure 1); it is the leading cause of kidney failures, overall, in children (*Park et al.,* 1999).

Thrombotic thrombocytopaenic purpura (TTP). In adults, *E. coli* O157 infection may result in thrombotic thrombocytopenic pupura (TTP). This disease is similar in course to HUS, but the central nervous system is involved in addition to the kidneys. Neurological complications occur in about 25% of HUS patients (*Mead and Griffin,* 1998).

Morbidity and mortality. Approximately 30% to 45% of the *E. coli* O157 infection cases are hospitalized. Long term complications are possible in patients that recovered from the infection, particularly in case of HUS (Hemolytic Uremic Syndrome). Fatalities are usually associated with HUS and the mortality rate is usually between 2 and 7% (*Mahon et al.,* 1997; *Bantavala et al.,* 2001; *Roberts and Upton,* 2001).

Asymptomatic carriers. Humans can be asymptomatic carriers of *E. coli* O157 (*Curnow*, 1999). Cattle carry *E. coli* O157 in their GIT (gastrointestinal tract) and they remain healthy. The organism may be a constituent of their naturally-occurring microflora, and longitudinal studies show most cattle occasionally carry *E. coli* O157 in their feces (*Hancock et al.,* 1997; *Lahti et al.,* 2003).

*DALY*s. In order to evaluate the public health impact regarding STEC-associated disease, a disability-adjusted life years model (DALY) is regularly used by public health agencies and international organizations (e.g. the World Health Organization) to assess a metric that combines the burden of mortality and morbidity (non-fatal health problems) into a single number. The DALY measure combines the years of life lost due to premature death (YLL) and the years lived with disability (YLD) for varying degrees of severity, making itself a valuable public health indicator. One DALY is a health measure, equating to 1 year of healthy life lost. (*WHO*, 2006). For instance, the mean disease burden of VTEC O157 in the Netherlands was 116 DALY per year (*Havellar et al,* 2003).

2.2.3. Beef meats associated with E. coli O157 infections

Around 52% of these cases were caused from ground beef (minces-burgers-patties), around 33% from other meats, around 12% from various fermented sausages, and around 0.5% from dried venison. These data outlined the dominant role of raw meats intended for cooking – particularly ground beef – followed by ready-to-eat sausages, i.e. fermented salamis (*Wells et al.,* 1983; *CDCP,* 2000; *CDCP,* 2003; *Pennington*, 2010).

2.2.4 Dose-response relationship

Assumption that exposure to a relatively low number of *E. coli* O157 cells can lead to the development of the illness is generally accepted (<100 CFU). If the infectious dose is very low, the consequence would be that infection may occur without pathogen's growth occurring in contaminated food (*Anon*. 1999).

2.2.5. Virulence factors affecting dose-response

Shigatoxins (Stx1, Stx2, Stx2c). Among the most important virulence characteristics of *E. coli* O157 is the ability to produce one or two Shiga toxins (verocytotoxins) (*Mead and Griffin*, 1998).

Enterohemolysin (hyl). Nearly all strains of *E. coli* O157 produce a hemolysin (termed enterohemolysin) that is encoded on the 60-MDa plasmid. Patients with HUS develop antibodies to enterohemolysin (*Schmidt et al.,* 1995), but it is still unclear whether/how it is involved in pathogenesis of disease.

Intimin (eae). Encoded by the *eae* gene, is an adherence factor that plays a role in intestinal colonization of *E. coli* O157 *in vivo* and in animal model (*Nataro and Kaper,* 1998).

pO157 plasmid. Encodes a catalase-peroxidase with unknown function. The plasmid is widely distributed among human EHEC isolates, but its role in the pathogenesis of disease is not yet determined and the results of *in vivo* and *in vitro* studies have been conflicting (*Nataro and Kaper,* 1998).

Iron transport. *E. coli* O157:H7 strains contain an iron transport system (a 69-kDA protein encoded by the *chu*A gene) allowing the use haeme/hemoglobin as an iron source (*Torres and Payne*, 1997), which possibly aids infection as it stimulates the growth of the pathogen.

EAST1. Many strains of *E. coli* O157:H7 possess the *ast*A gene encoding EAST1 (*Savarino et al*., 1996), the role of which in pathogenesis of disease is not known although it may be involved in non-bloody diarrhea.

Other intestinal adherence factors. Some adherence factors other than intimin have been reported for *E. coli* O157:H7 but they have not been well characterized or specifically demonstrated *in vivo*. For example, a 94-kDa OMP (distinct from intimin) mediated adherence adherence to Hep-2 epithelial cells (*Sherman et al.,* 1991) but it was not further characterized.

2.3. Exposure assessment

2.3.1. Introduction

Although this review reflects the STEC distribution along the beef chain, the majority of data are based on the published research on *E. coli* O157:H7. Therefore, it is hypothesized that different serotypes belonging to non-O157 STEC will most likely behave in a broadly similar fashion as *E. coli* O157:H7, in different ecological compartments – the gastrointestinal tract (GIT) and farm environment; this should be considered as an educated and informed assumption. It should be also considered that ecological distribution related to *E. coli* O157:H7 may not apply always to all STECs.

Presently, there is no single point along the meat (food) chain at which *E. coli* O157 can be reliably eliminated so to entirely prevent exposure of consumers to the pathogen, apart from sufficient heat treatment and reliable post-heating control of contamination. A longitudinally integrated approach to the meat (food) chain (i.e. LISA/Longitudinally Integrated Safety Assurance) including reduction of the pathogen at multiple points is necessary to reduce the risk of *E. coli* O157 infections occurring via meats (foods).

2.3.2. STEC in the beef chain

On-farm. Healthy cattle appear to shed STEC O157 and non-O157 serotypes sporadically, with high numbers being excreted in intermittent "bursts" in their faeces. The factors that contribute to a burst of shedding from a particular animal or herd are not fully defined yet (e.g. mixing of individual animals and/or herds, diet and the watering system). Published data have indicated that transmission via feedstuffs (*Davis et al.,* 2003), drinking/irrigation water (*Barham et al.,* 2002) and wilde-life (rats, flies, birds) may be involved. Recently, the study done by *Baines et al.* (2011) aimed to prove the link between mouldy feeds, mycotoxins, STEC colonization and development of Jejunal Hemorrhage Syndrome (JHS) in beef cattle. According to this study, until recently there have been no reports of STEC O157 disease in mature cattle (*Baines et al.,* 2008), but STECs do affect calf health from birth to weaning (*Hall et al.,* 1985; *Schoonderwoerd et al.,* 1988; *Sandhu and Gyles,* 2002). In some other studies (*Cray and Moon*, 1995; *Brown et al.,* 1997) it was confirmed that STEC infections cause high mortality in neonatal calves resulting from acute enteritis. Older calves may have transient watery diarrhea but are not seriously affected by STEC O157 infections. In addition, the similar A/E lesions, presented in hemorrhaged tissues in humans, were also found in the jejunum, ileum, cecum, colon, and rectum in neonatal calves, but not in older calves (*Cray et Moon,* 1995; *Brown et al.,* 1997; *Dean-Nystrom et al.,* 1997; *Dean-Nystrom et al.,* 1998). However, if STECs do cause disease in mature cattle, the most likely candidates are diseases with unclear etiologies such as JHS (*Puntenney et al.,* 2003). Current treatments for JHS include an aggressive medical and surgical therapy that can be effective, but the prognosis for long term survival relies upon early detection (*Peek et al.,* 2009).

While a number of factors have been identified and suggested as playing a role in the on-farm population dynamics of this pathogen, only season has been repeatedly and consistently shown to have an effect on shedding. Fecal shedding is typically low in the winter, increasing in the spring to peak levels during the summer months, then tapering off in the late autumn to very low winter levels (*Chapman et al.,* 1997; *Hancock et al.,* 1997; *Van Donkersgoed et al.,* 1999). The frequency of human outbreaks of *E. coli* O157:H7 occurring predominantly in the summer months, are complement with seasonal shedding patterns in cattle (*Besser et al.,* 1999; *Rangel et al.,* 2005).

Taking into consideration the intermittent/sporadic shedding of *E. coli* O157, the detected prevalence of *E. coli* O157 in the bovine faeces at farm was very low – 0.5% (*Buncic and Avery,* 1997), medium – 2.6% (*Nastasijevic et al.,* 2009a), 4.4% (*Conedera et al.,* 2001) and up to very high levels – 22.7% (*Smith et al.,* 2001). The detected level on the hides was 18% (*Barham et al.,* 2002), while higher levels, e.g. 24.6% were detected on farm surfaces (*Lahti et al.,* 2003). Commercial stock feeds sampled on-farm contained *E. coli*, but not *E. coli* O157 (*Lynn et al.,* 1998). It seems that proper silage fermentation reduces and, depending on initial levels, even can eliminate *E. coli* O157. This pathogen was

also found in slurry collected from cattle feedlots (*Cízek et al.,* 1999) and a dairy farm (*Porter et al.,* 1997). It is proved that *E. coli* O157 can survive up to 99 days in soil (*Bolton et al*., 1999) or even can proliferate in various soil types such as silt loam, sandy loam and clay loam (*Gagliardi and Karns*, 2000). Lastly, potential for "mechanical" spread of *E. coli* via vectors (e.g. rodents, birds, flies, vehicles, workers-visitors, feeds) exist on farms, in the same (well-known) way as with other food borne pathogens such as *Salmonella*.

Transport-market-lairage. Information on the fate of *E. coli* O157 during the transport and in livestock markets is limited. A recent study showed that the prevalence of marker organisms inoculated onto the hides of cattle entering the market process increased 2– to 5-fold on those animals post-market (*Collis et al.,* 2004). In some other studies, the prevalence of *E. coli* O157 dropped on cattle hides after transport and lairage (*Barham et al.,* 2002). In addition, *E. coli* O157 was also isolated from 7.3% of clean transport surfaces before cattle were loaded, indicating environmental spread of the pathogen could occur (*Barham et al.*, 2002). The detected prevalence of *E. coli* O157 in the bovine faeces post-transport, pre-lairage was 1.7% and 13% (*Minihan et al.,* 2003).

Lairage-to-dressing. A number of data accumulated in the past few years showed that lairage-to-dressing environment could play an important role in the spread of *E. coli* O157 in cattle at slaughterhouses through animal-animal and/or animal-environment-animal contacts (e.g. surfaces in lairage pens or stunning boxes). In addition to that, the occurrence of this pathogen in lairage varied from 7.8% (*Small et al.*, 2002) and even very high level – 50% (*Tutenel et al.,* 2003). It was confirmed that *E. coli* O157 survives very well on environmental surfaces; decimal reduction times (D values) on hide, concrete, metal or straw ranged between 3 and 15 days (*Small et al.,* 2003). An additional meat safety concern is that naturally-occurring *E. coli* O157 can persist on surfaces even after routine washing; not only in lairage areas (*Small et al.,* 2002), but also on surfaces on farm (*Lahti et al.,* 2003) and in transporters (*Barham et al.,* 2002). It should be taken into account that cattle lairage washing rarely includes treatments with detergents/sanitizers (*Small et al.,* 2003). Therefore, carry-over of *E. coli* O157 contamination on lairage surfaces from one day to subsequent days seems likely. One of the most important sites is the stunning box, the surfaces of which all animals contact in succession (*Small et al.,* 2002; *Avery et al.,* 2002). It means that through animal-environment-animal contacts the transfer and spread of persisting *E. coli* O157, amongst livestock slaughtered on different days, may occur and such cross-contamination could negate any successful control of the pathogen achieved on individual farms.

Slaughter-to-dressing. Visible cleanliness of hides and levels of microorganisms on carcasses may (*McEvoy et al*., 2000) or may not (*Kain et al.,* 2001) be correlated with quantitative total microbial viable counts. However, reported on-hide *E. coli* O157 prevalences on cattle at slaughter ranged from 4.5% (*Barham et al.,* 2002), 28.2% (*Nastasijevic et al.,* 2008a) to 56% (*Tutenel et al.,* 2003). This indicates the hides may be the very important sources of pathogen. This is a major concern, as in modern industrial slaughterhouses, carcass contamination is mainly due to hide-to-meat microbial cross-contamination, directly or via equipment, tools (*Tutenel et al.,* 2003) or airborne (*Rahkio and Korkeala,* 1997), rather than due to direct spills of digesta/faeces during evisceration. The skinning may be considered as a high-risk operation and *Elder et al.* (2000) found that most of the carcasses were contaminated with *E. coli* O157 in the pre-evisceration phase. The detected levels of carcass contamination were low – 2.8% (*Nastasijevic et al.,* 2009a), medium – 11.1% (*McEvoy et al.,* 2003) and high – 43.4% (*Elder et al.,* 2000).

Chilling-processing-retail. In a large 3-year survey in Belgium, 1.02% of the carcasses during chilling at the slaughterhouse were contaminated with *E. coli* O157 (*Tutenel et al.,* 2003), whilst other studies found higher prevalences – 5.5% (*McEvoy et al.,* 2003). *Carney et al.* (2006) found prevalence of pathogen in 2.4% samples of beef trimmings. Occurrences of *E. coli* O157 in minced beef (without ingredients) and completed sausage batter (i.e. intended for production of fermented sausages) were 6.2% and 2.1%, respectively (*Nastasijevic et al.,* 2009a). Other surveys of beef at retail level indicated a wide range of *E. coli* O157 occurrence, ranged from 0% (*Uhtil et al.,* 2001) and up to 36% (*Radu et al.,* 1998) with a median prevalence of around 6% in studies were pathogen was detected. Even if the contamination level on beef was low, that would still represent a serious public health risk because beef is often eaten undercooked (e.g. beef burgers) or even raw (e.g. beef tartar).

Catering-consumer level. Food safety problems associated with *E. coli* O157 in meats at catering and consumer levels relate to final preparation of food for consumption. The catering-level issues have been recently summarized in the form of brief guidelines (*Bolton and Maunsell*, 2004). At consumer level, epidemiological data from Europe (*Tirado and Schmidt*, 2000), North America, Australia, and

Table 3. Examples of confirmed *E. coli* O157 cases in meat borne catering outbreaks
Tabela 3. Primeri potvrđenih *E. coli* O157 slučajeva u alimentarnim epidemijama u vezi sa keteringom, nastalim nakon konzumacije mesa

Implicated meats (country)	Cases (deaths)	Reference
Beef tacos (USA)	13	*Conway* (1995)
Beef ("seeme rolle") (USA)	11	*Werber et al.* (2002)
Beef (roast) (USA)	65	*CDCP* (1990)
Cooked meat (Scotland)	496 (20)	*Pennington* (1998)
Cooked meat (UK)	30	*Rajpura et al*. (2003)
Genoa salami (Canada)	39	*Williams et al.* (2000)
Sausages (mortadella and teewurst) (Germany)	28 (3)	*Ammon et al.* (1999)

New Zealand indicate that substantial proportions of foodborne disease can be attributed to food preparation practices used in the domestic environment. The main risk factors include:

a) cross-contamination from raw to cooked foods via refrigerators, contaminated hands, cutting boards and kitchen towels;
b) inadequate refrigeration;
c) improper cooking; and
d) inadequate post-cooking handling including slow cooling and/or re-contamination.

In North and South America eating undercooked ground beef continues to pose a risk to the population (Table 3), but this has not been implicated as a food vehicle in the UK according to a study by *Parry et al*. (1998).

According to *Eblen* (2007) the prevalence of STEC O157 and some other non-O157 STEC strains in retail foods and retail meats can be very variable ranging from 0.1-12% in raw ground beef and up to 4.8% and 3.3% in fresh and dry sausages, respectively (Table 4).

Table 4. The prevalence of STEC O157 and non-O157 STEC strains in retail foods and retail meats
Tabela 4. Prevalenca STEC O157 i non-O157 STEC sojeva u hrani iz maloprodaje i mesa iz maloprodaje

Country	Organism	Reported prevalence	**Reference**
Argentina	*E. coli* O157:H7	4.8% of fresh sausages; 3.8% of raw ground beef; 3.3% of dry sausages	*Chinen et al.,* 2001
Belgium	All STEC	4.6% of raw meat samples (beef, mutton and venison)	*Pierard et al*., 1999
Botswana	*E. coli* O157:H7	5.2% of meat cubes; 3.8% of raw ground beef; 2.3% of fresh sausages	*Magwira et al*., 2005
England	*E. coli* O157:H7	2.9% of lamb products, 1.1% of beef products	*Chapman et al*., 2000
France	All STEC	11% of beef	*Pradel et al*., 2000
France	*E. coli* O157:H7	0.1% of raw ground beef	*Vernozy-Rozand et al*., 2002
Italy	*E. coli* O157:H7	0.4% of raw ground beef	*Conedera et al*., 2004
New Zealand	All STEC	12% of beef, 17% of lamb; 4% of pork	*Brooks et al*., 2005
Sweden	*E. coli* O157:H7 All STEC	0.06-0.5% of raw ground beef 4% of raw ground beef	*Lindqvist et al*., 1998

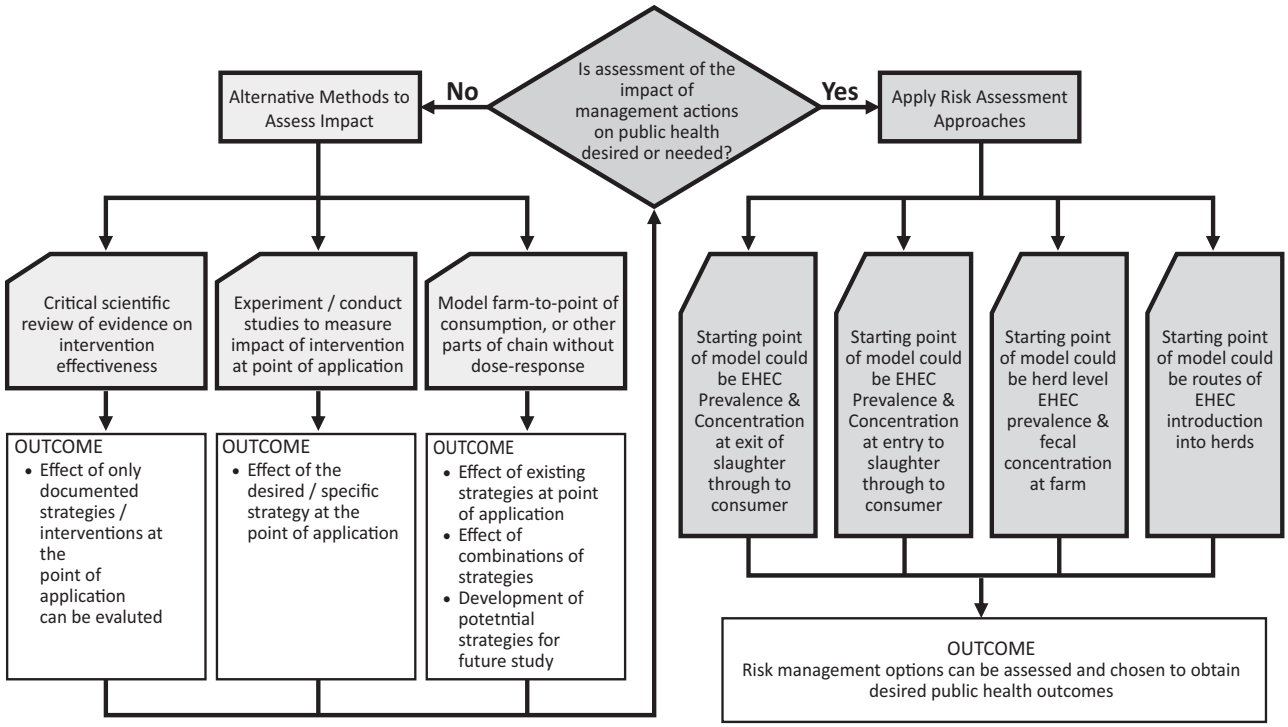

Figure 4. Roadmap for the application of risk assessment approaches in managing the public health impact of EHEC (*WHO*, 2011)

Slika 4. Mapa puta za primenu pristupa u oceni rizika za upravljanje uticaja EHEC na javno zdravlje (*WHO*, 2011)

Lastly, for successful science-based risk assessment, risk assessors need to have good communications with risk managers to provide guidance on what risk assessment can and cannot answer, i.e. to condition the expectations of the risk manager (e.g. depending on the risk question it may not be necessary to consider the full chain (e.g. if only interested in interventions at the consumer level, then no model, or a less detailed model, would be needed at the pre-harvest and harvest levels). Examples of the types of risk assessments that could be undertaken to assist risk managers in controlling STEC/EHEC in beef and beef products and their potential areas of application are as follows (*WHO,* 2011) (Figure 4):

- Prevalence and concentration of STEC/EHEC in beef products exiting processing plant
- Prevalence of contaminated cattle and contamination level of STEC/EHEC on animals entering the slaughter and processing plant
- Herd prevalence and fecal contamination levels at the farm
- Routes of introduction and spread of STEC/EHEC into and within herds

3. Risk management

In considering the risk management issues associated with STEC it has to be noted that to date most of these have been addressed in the absence of quantitative microbial risk assessments. In some countries a risk assessment has been carried out, irrespective of whether or not the risk assessment contributed to the risk management decision. For the most effective risk management decisions, it is suggested that it will be useful to look at one country where no risk assessment has been undertaken, as such an example may also reflect the situation in many other countries, especially with respect to the adoption of HACCP and GHP in slaughterhouses (WHO, 2011). In the process of risk management, the main and the most effective control options of *E. coli* O157 along the beef chain, which are also technically and financially sustainable, have to be selected. Risk management strategies should be implemented by the Competent Authorities responsible for the enforcement of official controls in the food (beef) chain and should be based on holistic and integrated approach to the food safety – "farm to fork". These strategies may include: managing farms to reduce fecal shedding in cattle and spread of pathogen

in environment; transport-market-lairaging procedures to control cross-contamination; GHPs and HACCP procedures in slaughter operations and beef processing; proper handling during transport and by retail outlets; proper handling and cooking by consumers. Over-reliance on any one sector may result in a false sense of security of the final product.

3.1. Pre-harvest control options

Currently, there are no on-farm measures that can be relied upon to guarantee complete absence of *E. coli* O157 carriage in individual cattle or herds. This is very difficult even if herds are subjected to an on-farm testing regime, because the intermittent nature of the shedding. Due to multi-factorial nature of the *E. coli* problem on-farm, the efficacies of individual control measures or their combinations at pre-harvest level are difficult to quantify and carry many uncertainties. Therefore, when cattle destined to enter the human meat chain are transported from farms for slaughter, their *E. coli* O157-carriage status is largely unknown. Presently, on-farm control measures could only reduce *E. coli* O157 incidences/prevalences, and only to uncertain extents. The main considerations related to on-farm controls include:

3.1.1 Prevention of the pathogen's recycling

Slurry used as a fertilizer. E. coli O157 was found in slurry collected from cattle feedlots (*Cízek* et al., 1999) and a dairy farm (*Porter et al.,* 1997). Additionally, *E. coli* O157 occurring naturally in manure originated from bovine shedders can survive for very extensive times – ranging from a few weeks to 21 months (*Kudva et al*., 1998; *Bolton et al.,* 1999).

Soil. *E. coli* O157 survived up to 99 days in soil (*Bolton et al.,* 1999) or proliferated in various soil types including silt loam, sandy loam and clay loam (*Gagliardi and Karns*, 2000). Therefore, farmers have a responsibility to manage land and organic agricultural materials, i.e. slurry and manure, in ways that prevent contamination of ready-to-eat food crops, water supplies, feed and grassland and also to apply other necessary biosecurity measures such as deratization, disinfection of farm and surrounding environment, control of wildlife, movement of workers and mechanical vectors. Farmers are also obliged to present animals for slaughter with the minimum amount of soil and faecal contamination on their hides, so that cross-contamination of carcasses/meat during slaughter and dressing will be minimized.

3.1.2. Prevention of ingestion of the pathogen

Feed. STEC *E. coli* was detected in 6.3% of fresh grass samples, indicating that pastures have the potential to act as sources of transmission of the pathogen for grazing livestock (*Hutchison et al.,* 2006). On the other side, feeding hay, grass, or silage high in propionic or acetic acids may reduce the likelihood of STEC shedding by cattle (*Lynn et al.,* 1998). Due to changes in farm husbandry practices, cattle nowadays are fed more grain and concentrates. It has been suggested that these practices may promote the growth of *E. coli* populations. However, further studies are required before definitive advice can be formulated in relation to the effect of feeds on the incidence of STEC (*FSAI,* 2010).

Water. Studies have shown that *E. coli* O157:H7 can survive in water for up to 109 days (*Scott et al.,* 2006). Water supplies contaminated with livestock effluent have been implicated in a number of outbreaks (*Locking et al.,* 2006). STEC survives in water trough sediments for at least four months and appears to multiply there, especially in warm weather. Farmers should clean water troughs frequently to prevent the accumulation of sediments. Water troughs should be positioned away from feed troughs/feed passageways, as contamination of water with feed can providing a nutrient-rich substrate for bacterial growth and survival at the bottom of the trough (*Lejeune et al.,* 2001). Specific water treatments (i.e. disinfection) may be also needed.

Animal interactions (suckling, licking, etc). The design of the farm holding should allow logistic organization of animal feeding and breeding so to avoid unnecessary contacts between them. This is because by suckling and licking of fecally contaminated hides, pathogen can be easily transmitted, by fecal-oral route (*Pearce et al.* 2004).

3.1.3. Suppression of the ingested pathogen

Dietary manipulation. It has been shown that zoonotic STEC O157 and non-O157 survive in acid conditions and persist in rumen contents (*Boukhors et al.* 2002), which supports the proposal that a grain-rich diet may induce acid resistance of STEC in the rumen and permit the bacteria to survive in the abomasum, leading to increased fecal shedding. However, numerous field studies have demonstrated the opposite effect: hay-fed sheep (*Kudva et al.,* 1997) and cattle (*Hovde et al.,* 1999) shed STEC O157 for shorter periods than grain-fed animals of the same species. In another study, (*Grauke et al.,* 2003) no difference in fecal shedding of STEC O157

was observed between hay-fed and grain-fed cattle. Further investigation is needed in order to give proper risk management recommendations regarding dietary regimes/manipulations which could minimize shedding of STEC O157.

Probiotics, prebiotics, competitive exclusion. Treatment with different probiotic strains has had variable effects on fecal shedding of STEC in cattle. Encouragingly, daily treatment of finisher beef cattle with direct-fed microbials, such as certain strains of *Lactobacillus acidophilus* (*Younts-Dahl et al.,* 2005), reduced fecal shedding of STEC O157 by over 50%. Treatment with a competitive exclusion probiotic containing *E. coli* strains reduced fecal shedding of both O157 and O111, but not O26 zoonotic STEC in weaned calves (*Tkalcic et al.,* 2003). Hence, these results suggest that a judicious choice of probiotic bacterial strains for the treatment of cattle could eventually permit a reduction in fecal shedding of not only STEC O157 but also a variety of zoonotic STEC non-O157 serotypes. Lastly, the study performed by *Baines et al.* (2011) confirmed that mycotoxins and STEC are part of the disease complex for JHS (Jejunal Hemorhagic Syndrome) in beef cattle. A prebiotic treatment alleviated the development of disease in symptomatic beef calves. Future studies should examine the role of STECs and mycotoxins in the infection process that leads to JHS and the mode of action of prebiotics.

Phage therapy. Antibacterial viruses, known as bacteriophages, that specifically target STEC O157 appear to be able to control the growth of these bacteria under laboratory conditions and have shown promising results in sheep; however, further work is necessary before the viruses can be considered a feasible approach for the control of STEC in cattle (*Callaway et al.,* 2004; *Niu et al.,* 2012).

3.1.4. Modification of the animal's response

Vaccination. Vaccination performed with type III secreted proteins, resulted in significant decrease of the number of animals shedding faecally the organism and the number of challenge organisms shed per animal. These studies were done in experimentally infected cattle and in clinical trials in feedlot cattle, demonstrating the potential benefits of such an approach (*Potter et al.,* 2004; *Allen et al.,* 2011). Nevertheless, this approach still requires some optimization as faecal shedding was not reduced after administration of the same vaccine to feedlot cattle in commercial operations (*Van Donkersgoed et al.,* 2005). Furthermore, in the recent study carried out by *Cernicchiaro et al.* (2014) it was revealed that the *E. coli* O157:H7 vaccine, which reduced STEC O157 fecal shedding, didn't significantly affected fecal shedding of non-O157 STEC serogroups, despite the fact that the most prevalent non-O157 STEC serogroups tended to occur concurrently with O157 STEC strains within fecal samples; O157, O26 and O103 were the most prevalent STEC O serogroups that have been fecally shed by feedlot cattle.

3.2. Transport-market-lairage

3.2.1. Transport

Significant spread of the pathogen contaminating animal coats during transport can occur. This occurs through the same mechanisms of animal-to-animal and/or animal-surfaces-animal cross-contamination taking place during lairaging (*Childs et al.,* 2006). Therefore, transportation of cattle intended to slaughter should be always performed in properly sanitized vehicles and with minimal duration because stress that may happen can increase shedding of STEC *E. coli* O157 and even increase subsequent cross-contamination between animals and/or animal-surface-animal.

3.2.2. Livestock market

A recent study showed that the prevalence of marker organisms (including generic *E. coli*) inoculated onto the hides of cattle entering the market process increased 2– to 5-fold on those animals post-market (*Collis et al.,* 2004). It is recommended that livestock markets should be avoided, if possible. That is because the mixing of animals from different farms (e.g. pathogen-free farms and others) can increase the cross-contamination of non contaminated cattle either through direct contact and/or through contamination of environment.

3.3. Lairage-to-dressing

3.3.1. Lairaging

The lairage-to-dressing environment plays an important role in the spread of *E. coli* O157 in cattle at slaughterhouses through animal-animal and/or animal-environment-animal contacts (*Avery and Buncic*, 2005). Cattle should be kept in sanitized pens and with minimal duration, because carry-over of pathogens on surfaces from one day to another may increase the probability for cross-contamination of subsequent animal batches coming from different farms (e.g. animals lying on contaminated floor).

3.3.2. Sanitation of stun boxes

During the process of stunning, the first slaughter operation, animals can contaminate the surfaces of stun box via contact of their fecally contaminated hides and surfaces-mediated cross-contamination of consecutively stunned animals may occur (*Small et al.,* 2006). Therefore, the proper sanitation of stun box between different slaughter batches is highly recommended.

3.4. Slaughter-to-dressing (Harvest)

3.4.1. Efficient cleaning-sanitation of the slaughter-hall environment

Effective cleaning-sanitation of the slaughter-hall environment is necessary and beneficial for microbial safety of the meat (*Nørrung and Buncic*, 2008), because the potential for transfer of pathogen via cross-contamination between carcasses and slaughter equipment, floor and walls, is minimized.

3.4.2. Minimizing microbial contamination through application of GHP/GMP and HACCP principles

A range of standard operational procedures are used at slaughter line to prevent/minimize microbial cross-contamination during slaughter. According to the best GHPs, it is advisable to slaughter only visually clean animals and to reject dirty ones; to perform mechanical skinning, bagging of anus and tying ("rodding") of esophagus before evisceration; and to apply procedures, such as regular hot water/steam "sterilization" of all tools and equipment coming in direct contact with meat, so to avoid and/or minimize the possibility of cross-contamination (*Nastasijevic et al.,* 2008a; *Nastasijevic et al*., 2008b). The HACCP principles should ensure science-based hazard analysis and risk categorization of all steps along the beef slaughter line. The potential Critical Control Points (CCPs) may be visual assessment of cattle hides` contamination, skinning, evisceration and rapid chilling (<4ºC/24h). Lastly, the use of indicator organisms may be helpful to assess the probability of the presence of *E. coli* O157 on bovine carcasses (e.g. APC, Generic *E. coli*, *Enterobacteriaceae*), through continuous monitoring of microbial process hygiene (*USDA FSIS*, 2002).

3.4.3. Decontamination treatments

The higher level of safety assurance against STEC, regarding prevention and minimization of hide-mediated cross-contamination of carcasses, can be achieved by "pro-active" decontamination of hides before skinning and "reactive" decontamination of carcasses (Table 5). Decontamination treatments of hides (*Castillo et al.*, 1998a; 1998b; *McEvoy et al.,* 2001; *Bosilevac et al.,* 2005) and dressed carcasses (e.g. steam vacuuming, steam pasteurization, hot water washes, organic acid washes, etc.) (*Dickson et al.,* 1994; *Carneiro et al.,* 1998; *Uyttendaele et al.*, 2001) and their combinations can be used. Carcass decontamination is a mandatory CCP (Critical Control Point in HACCP-based food safety management system) at abattoirs in USA, whilst it is still not widely used in the EU. Namely, *EC Regulation 853/2004* (article 3 and 12) allows decontamination treatments to be considered as a supplement to good hygiene practices. In the EU, risk

Table 5. Example of the effects of decontamination treatments on *E. coli* O157 on hide or meat

Tabela 5. Primer efekata dekontaminacionih tretmana u odnosu na *E. coli* O157 na koži ili mesu

Treatment	Anti-E. coli O157 effects achieved (approx.)	Reference
Hide decontamination		
Sodium sulphide-hydrogen peroxide combination (chemical dehairing)	5 log reduction	*Castillo et al*. 1998a
Steam (condensing at 80ºC; sub-atmospheric pressures)	4 to 6 log reduction	*McEvoy et al*. 2001
Sodium hydroxide wash plus chlorinated (1 ppm) water rinse	Prevalence reduced from 44% to 17%	*Bosilevac et al*. 2005
Meat decontamination		
Hot water (74-80ºC)	3.7 log reduction	*Castillo et al.* 1998b
Steam pasteurization (above atmospheric pressure)	3.7 to 4.4 reduction	*Phebus et al.* 1997

assessors and risk managers need to ensure any such substance is first shown to be safe and effective at significantly reducing microbial contamination before it can be approved. In addition, EU authorities (e.g. EFSA, EU Commission) are also seriously considering a possibility of microorganisms developing resistance to substances used for decontamination of carcasses/meat – as a result of their use. Currently, only lactic acid is approved to reduce microbiological surface contamination on bovine carcasses, and no other substances are authorized for this purpose within the EU (*EC Regulation 101/2013*).

3.4.4. Novel approaches

Microbial immobilization treatments. Recently, the novel approaches regarding microbial immobilization treatment of hides are considered. It is proved that bacterial on-hide immobilization, rather than decontamination of hide, could be very effective in reducing transmission of bacteria (including *E. coli* O157) from cattle hide onto the meat. This novel approach is more proactive and preventative because it aims to prevent pathogen transfer from their main source – surface of hide, to the carcass. This is achieved by immobilization (fixation) of pathogens on the hair. With this approach the killing of the entire target hide microbiota may not be necessary. Rather, the hide could be treated with some compound(s) "glueing" the microorganisms to the hair so as to prevent their detachment from the hair and transmission onto the carcass during the skinning operation at slaughter line. This approach can be even more effective from decontamination treatments, by reducing swab-recoveries of TVC (Total Viable Counts) by 6.6 logs, fecal indicators (GEC-Generic *E. coli* and EC-Enterobacteriaceae) by 2.9 and 4.8 logs, respectively and *E. coli* O157 by 2.1 logs (*Antic et al.*, 2010).

Revision of post-mortem protocol in the cattle slaughter line. Current abattoir protocols do not include examination of the jejunum for lesions suggestive of hemorrhagic disease (JHS) and as such, it provides a novel approach to identifying suspect animals and removing them from the food chain (*Baines at al.,* 2011).

3.5. Chilling-processing-retail (Post-harvest)

The cold chain during all stages after slaughter should be maintained. Effective cleaning and sanitation in related premises should be performed in order to prevent cross-contamination during cutting, de-boning and further processing. Bactericidal step (e.g. heating/cooking) should be included in the process, e.g. >71°C/1min, throughout the product (*USDA,* 2003); recontamination of the heated products during further handling (e.g. slicing, packaging) should be prevented; For non-heated products, e.g. fermented sausages, the "hurdle" concept should be applied, e.g. validated 5D inactivation treatment (*Reed,* 1995); cross-contamination of ready-to-eat products from raw meats (and other raw ingredients) during food preparation should be prevented.

3.6. Catering-consumer level

To avoid/prevent the cross-contamination of foods with *E. coli* O157, as well as other food borne pathogens, general hygiene principles of WHO Five Keys for Safer Food (WHO, 2006) should be applied. The core messages of the document are: (1) keep clean; (2) separate raw and cooked; (3) cook thoroughly; (4) keep food at safe temperatures; and (5) use safe water and raw materials. It is noteworthy to emphasize that adequate cooking is currently the only bactericidal step in the meat chain by which any level of *E. coli* O157 can be reliably and completely eliminated (*Duffy et al.,* 1999).

4. One health approach to diagnosis, treatment and prevention of STEC

Earlier and timely diagnosis and effective responses to infection provoked by VTEC may be achieved if current recommendations for EHEC diagnosis in humans, issued by clinical laboratories, are followed (*Gould et al.*, 2009). The efficient protocol for detection/diagnosis of VTEC/EHEC O157 and non-O157 pathotypes should encompass culturing on selective and differential agar (e.g. CTSMAC, ChromAgar) and simultaneous molecular methods that can detect shiga-toxins or genes that encode them (e.g. multiplex PCR). No specific treatments are available for HUS in humans. Supportive therapy includes intravenous fluids and volume expansion *(Ake et al,* 2005). Antibiotic treatments are contraindicated in suspected or confirmed cases of O157:H7 infection or infection provoked by other non-O157 pathotypes, due to the possibility of increased risk of HUS by lysis of pathogen` cells and induction of Stx-encoding bacteriophages, which may subsequently lead to increased release of shiga-toxin into blood stream (*Ahn et al.,* 2009; *Zhang et al.*, 2000). Therefore, the recommended intervention strategies in humans consist of vaccines (Gb3 receptor analogues), and monoclonal antibodies against Stx (*Bitzan*, 2009; *Orth et al.*, 2008). Prevention of STEC/EHEC O157 and non-O157 infection is the

best approach to avoid HUS. Hand washing is the most important step for reducing the risk of EHEC O157 and non-O157 transmission (*NASPHV*, 2009).

The overall success of risk mitigation strategies regarding STEC occurrence in the beef chain and protection of public health, is inevitably associated with the implementation of targeted and synergistic control measures within the "One Health" concept which encompass environment/wildlife-animal-human interface. Such integrated approach should enable active monitoring and control of pathogen entering routes along the beef chain (*EC Regulation* 99/2003; *Nastasijevic,* 2009b; *Nastasijevic,* 2014; *Buncic et al.*, 2014), e.g. environment (soil, water, on-farm surfaces, wildlife/pests, birds, workers, vehicles movement), animal (hygienic conditions on-farm, vaccination, dietary manipulations – prebiotics/probiotics, monitoring of fecal shedders, introduction of new animals, etc.) and human (public health education, prevention strategies, early diagnosis, prevention and effective disease management). The "One Health" concept is widely accepted approach in mitigating the public health risks of zoonotic origin and also advocates active and structured inter-sectoral cooperation between the key stakeholders involved in the public health – environmental, veterinary, food and health authorities (Figure 5).

5. Conclusion

Many beef-borne STEC O157 and non-O157 serotypes (O26, O45, O103, O91, O111, O121, O145) are intermittently fecally shed by healthy/asymptomatic cattle and may represent a significant threat to human consumers and public health. STECs colonization and growth in small and large intestine (jejunum, ileum, caecum, colon and rectum) may play important role in that. Calves may develop life-threatening STEC infections in the first months of their life (JHS) and this may also contribute to long term carriage or shedding. The seasonal variations of shedding are reported, with increase in the spring – to peak levels during the summer months, then tapering off in the late autumn to very low winter levels. The shedding leads to contamination of farm environment (slurry, water, soil) which may lead to direct or indirect contamination of hides, which, in turn, serves as the main source of carcass

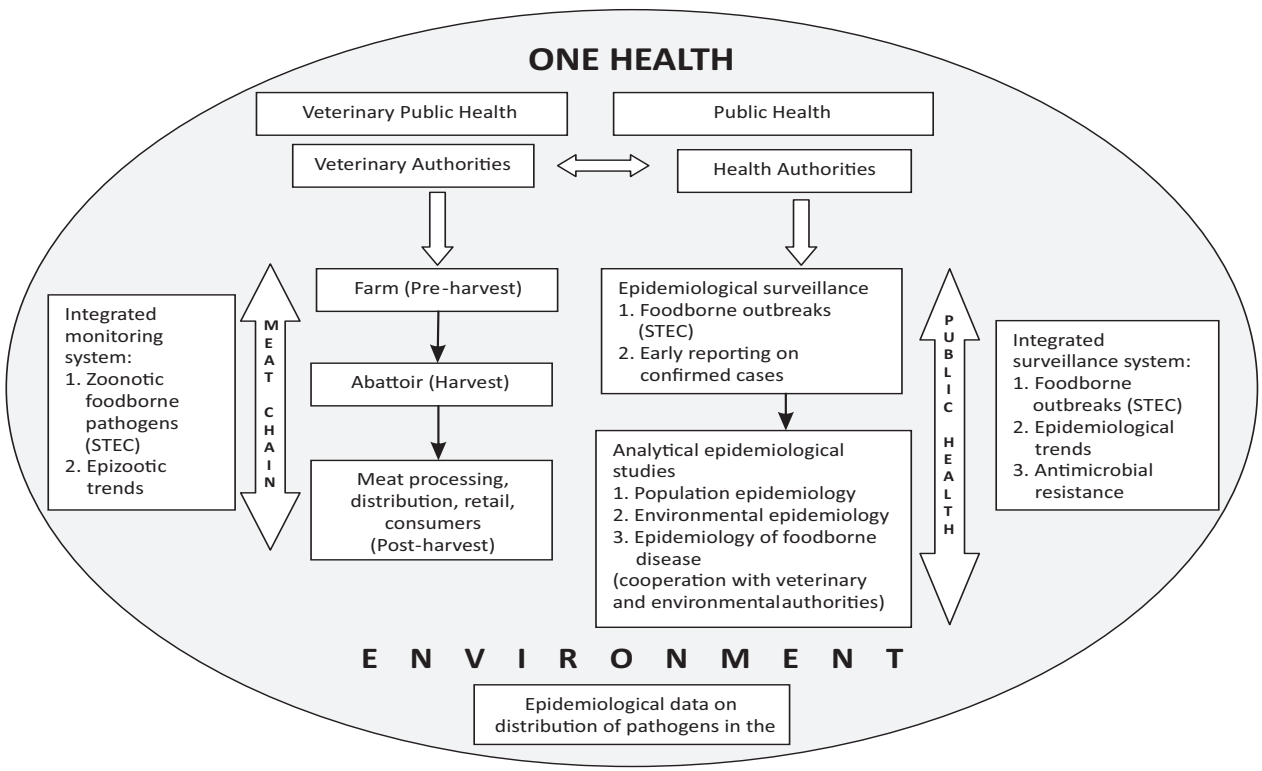

Figure 5. Inter-sectoral collaboration between environmental, veterinary and health authorities in mitigating the public health risks originated from foodborne hazards (adapted from *Nastasijevic*, 2009b)

Figure 5. Inter-sektoralna saradnja između agencija nadležnih za životnu sredinu, veterinarstvo i zdravstvo u suzbijanju rizika po javno zdravlje poreklom od hazarda koji se prenose hranom (adaptirano iz *Nastasijević*, 2009b)

contamination during slaughter and dressing of cattle at abattoirs, and further spread to fresh beef and products thereof.

Therefore, the main control options to reduce the beef-borne risk of *E. coli* O157 include: 1) on-farm interventions to reduce its shedding in cattle and prevent contamination of environment and hides – risk management strategies should correspond with peak shedding times during the year (pre-harvest control strategies); and, 2) at-abattoir interventions to reduce hide-to-carcass cross-contamination (harvest control strategies).

It is known that pre-harvest controls in cattle hold great potential to reduce STEC dissemination on farms, in the environment, and entering the food chain. However, none of the on farm management-based controls can completely eliminate STEC from cattle and will certainly not eliminate the need for proper procedures in the processing plant. Therefore, risk-based and well designed meat safety management system (GHP/HACCP), which should include decontamination treatments of hides and carcasses and/or microbial immobilization treatment of hides, regular monitoring of microbial process hygiene (APC – general hygiene indicator, EC/Generic *E. coli* – indicators of fecal contamination) and pathogen occurrence (STEC) on carcasses/meat, as well as targeted post-mortem examination of jejunum, will present the novel approach (harvest controls). The integrated meat safety approach should be also continuously applied in further processing (meat boning, meat processing, distribution, retail). These aforementioned in-plant interventions should maximize the reduction in pathogen entry to the food supply.

At the catering-consumer level, the basic hygienic principles should be applied in meat handling and preparation (e.g. WHO guideline "Five Keys for Safer Food"; *WHO*, 2006).

Lastly, it should be considered that relatively limited *E. coli* O157 reductions are achieved by applying only one control measure (e.g. decontamination treatments of hides and carcasses or adequate cooking). Therefore, a longitudinally integrated approach to the beef chain, within farm-to-fork continuum, with coordinated and targeted control measures at multiple points is necessary to manage the risk of beef-borne STEC infections. Such approach should be based upon: on-farm controls; transport-livestock market-lairage controls; slaughter-dressing controls; chilling-processing-retail controls; and controls at the catering-consumer level. The competent authorities (veterinary, health, food, environmental) should be encouraged to strengthen the inter-sectoral cooperation within `One Health` concept and to support further, deeper research regarding occurrence and distribution of STEC in the meat chain, so that valid and top quality data needed for the science-based risk assessment and design of effective control measures are generated.

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STEC u lancu goveđeg mesa – Koncept „Jedno zdravlje“

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R e z i m e: Još od ranih 80-ih godina prošlog veka E. coli O157 se pojavila kao jedan od najznačajnijih patogena relevantnih za javno zdravlje, ne zbog incidence oboljenja, koja je mnogo manja u odnosu na druge patogene koji se prenose hranom poput Campylobacter ili Salmonella, već zbog težine simptoma, niske infektivne doze i potencijalnih hroničnih posledica. Šiga toksin-produkujuća Escherichia coli (STEC) je pathogena za ljude i može da izazove hemoragični colitis (krvava dijareja) i ponekad hemolitički uremički sindrom (HUS), bolest koja je opasna po život jer dovodi do oštećenja bubrežne funkcije. U svom intestinalnom traktu, goveda nose mešavinu O157 i non-O157 sojeva koji nisu uvek patogeni za ljude. Donedavno se smatralo se da je O157 STEC serogrupa odgovorna za većinu alimentarnih epidemija u vezi sa STEC u Severnoj Americi, ali je nedavno potvrđeno da su non-O157 STEC serogrupe odgovorne za skoro 50% alimentarnih epidemija, odnosno oboljenja ljudi, u Severnoj Americi i Evropi. STEC sojevi se najčešće fekalno izlučuju u značajnim nivoima od strane zdravih/asimptomatskih goveda, npr. goveda sa jejunalnim hemoragičnim sindromom. Takvo izlučivanje dovodi do kontaminacije farmskog okruženja. To može da uzrokuje direktnu ili indirektnu kontaminaciju koža goveda, koja, sa druge strane, može da posluži kao glavni izvor kontaminacije trupa u toku klanja i obrade goveda u klanicama ili kontaminacije sirovog goveđeg mesa, odnosno proizvoda od mesa. Naučno bazirana ocena rizika je potrebna da bi se utvrdio uticaj na javno zdravlje, izloženost potrošača patogenu i za dizajn najefektivnijih strategija za redukciju rizika, odnosno za prevenciju i redukciju alimentarnih O157 i non-O157 STEC sojeva poreklom od goveda.

Ključne reči: *STEC, ocena rizika, javno zdravlje, redukcija rizika.*

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