tehnologija mesa
Osnivač i izdavač: Institut za higijenu i tehnologiju mesa, Beograd
UDK: 637.5.05/.07 : 579.67 : 616.993
BIBLID: 0494-9846

INTEGRATED MONITORING OF ZOONOTIC FOODBORNE PATHOGENS IN THE MEAT CHAIN*

Nastasijević I.

A b s t r a c t: Zoonoses are diseases or infections, which are transmissible from animals to humans. These diseases can be acquired directly from animals but are most often acquired through ingestion of contaminated foods. The severity of these diseases in humans can vary from mild symptoms to life-threatening conditions. Although various foods can serve as sources of foodborne illness, meat and meat products are important sources of human infections with zoonotic foodborne pathogens: Salmonella spp., Campylobacter jejuni/coli, Yersinia enterocolitica, VTEC E. coli (including E. coli O157:H7) and, to some extent, Listeria monocytogenes. The most frequent chain of events leading to meat borne illness involves food animals as healthy carriers of the pathogens; these organisms are faecally excreted and subsequently transferred to humans through production, handling and consumption of meat and meat products. In order to prevent zoonoses from occurring, it is important to identify which animals and foodstuffs are the main sources of infections. Zoonoses Directive (2003/99/EC) covers the collection, evaluation and reporting data on: zoonoses, zoonotic agents, antimicrobial resistance, food-borne outbreaks and epidemiological investigation in the Member States of the EU. Zoonotic pathogens in foods, including meats, have to be controlled through a complete, continuous farm-to-fork system (i.e. Longitudinal and Integrated Safety Assurance – LISA) and should take into account not only the risk assessment, but also technical possibilities, consumers' attitude/behaviors, and cost–benefit analysis. This means that integrated concept for monitoring in all major phases along the meat chain should be implemented through modular approach: 1. Pre-harvest (on the farm), 2. Harvest (in abattoir), and 3. Post-harvest (meat processing-distribution-retail-consumer). This approach includes sampling, testing and reporting on pathogens` occurrences in those three main production modules. It is of utmost importance to control direct and indirect faecal contamination of carcasses, in abattoir, through efficient GHP/GMP and HACCP based process hygiene management systems.

***Key words**: zoonoses, contaminated foods, zoonotic foodborne pathogens, antimicrobial resistance, modular approach*

Integrisani monitoring zoonotskih alimentarnih patogena u lancu mesa*

S a d r ž a j: Zoonoze su oboljenja ili infekcije koje su prenosive sa životinja na ljude. Ove bolesti mogu da nastanu direktno preko životinja, ali su najčešće stečene ingestijom kontaminirane hrane. Težina ovih oboljenja kod ljudi može da varira od blagih simptoma do stanja koja ugrožavaju život. Iako različita hrana može da bude izvor alimentarnih oboljenja, meso i proizvodi od mesa predstavljaju važne izvore infekcija ljudi, sa zoonotskim alimentarnim patogenima: Salmonella spp., Campylobacter jejuni/coli, Yersinia enterocolitica, VTEC E. coli (uključujući E. coli O157:H7) i, do određenog stepena, Listeria monocytogenes. Najčešći sled događaja koji dovodi do alimentarnih oboljenja preko mesa, uključuje zdrave životinje koje se koriste za proizvodnju hrane, kao nosioce patogena; ovi mikroorganizmi se fekalno izlučuju i posledično dospevaju do ljudi u toku proizvodnje, rukovanja i konzumiranja mesa i proizvoda od mesa. Radi sprečavanja nastajanja zoonotskih oboljenja, važno je da se identifikuju životinje i hrana koji predstavljaju glavne izvore infekcije. Direktiva o zoonozama (2003/99/EC) pokriva prikupljanje, ocenjivanje i izveštavanje o: zoonozama, zoonotskim agensima, antimikrobnoj rezistenciji, alimentarnim oboljenjima i epidemiološkim istragama u zemljama članicama EU. Zoonotski patogeni u hrani, uključujući meso, treba da budu kontrolisani preko kompletnog, kontinuiranog sistema od farme do trpeze (tj. Longitudinalno i integrisano osiguranje bezbednosti – LISA), pri čemu treba da se uzme u obzir ne samo ocena rizika, već takođe tehničke mogućnosti, stav/ponašanje potrošača i ekonomska opravdanost. To znači da koncept integrisanog monitoringa u svim glavnim fazama duž lanca mesa treba da bude primenjen kroz modularni pristup: 1. farma (pre−harvest), 2.klanica (harvest), i 3. prerada mesa−distribucija−maloprodaja−potrošač (post−harvest). Ovakav pristup uključuje uzorkovanje, testiranje i izveštavanje o učestalosti patogena u ova, tri glavna proizvodna modula. Od najvećeg je značaja kontrolisanje direktne i indirektne fekalne kontaminacije trupova, u klanici, kroz efikasnu primenu GHP/GMP i HACCP – baziranih menadžment sistema za procesnu higijenu.

***Ključne reči:** zoonoze, kontaminirana hrana, zoonotski alimentarni patogeni, antimikrobna rezistencija, modularni pristup.*

Introduction

Zoonoses are diseases or infections, which are transmissible from animals to humans. These diseases can be acquired directly from animals but are most often acquired through ingestion of contaminated foods. The severity of these diseases in humans can vary from mild symptoms to life-threatening conditions. Zoonotic agents reportedly affected over 368, 000 persons in the EU in 2007 (Figure 1).

**Plenary paper on International 55th Meat Industry Conference held from June 15-17th 2009 on Tara mauntain*
**Plenarno predavanje na Međunarodnom 55. savetovanju industrije mesa, održanom 15-17. juna 2009. na Tari*

AUTHOR: Ivan Nastasijevic, ivann@inmesbgd.com, Institut of Meat Hygiene and Technology, Kacanskog 13, Belgrade Serbia.
AUTOR: Ivan Nastasijević, ivann@inmesbgd.com, Institut za higijenu i tehnologiju mesa, Kaćanskog 13, Beograd, Srbija.

Although various foods can serve as sources of foodborne illness, meat and meat products are important sources of human infections with *Salmonella spp., Campylobacter jejuni/coli*, *Yersinia enterocolitica*, VTEC *E. coli* (including *E. coli* O157: H7) and, to some extent, *Listeria monocytogenes*. All these foodborne pathogens can be harbored in the gastrointestinal tract of food-producing animals. The most frequent chain of events leading to meat borne illness involves food animals as healthy carriers of the pathogens; these organisms are faecally excreted and subsequently transferred to humans through production, handling and consumption of meat and meat products. Occurrences of *Salmonella spp*., *C. jejuni/coli*, *Y. enterocolitica*, VTEC *E. coli* and *L. monocytogenes* in fresh red meat are variable, although most often are between 1% and 10%, depending on a range of factors including the organism, geographical factors, farming and/or meat production practices (*Norrung and Buncic*, 2008).

Zoonotic pathogens in foods, including meats, have to be controlled through a complete, continuous farm-to-fork system (i.e. Longitudinal and Integrated Safety Assurance – LISA) and should take into account not only the risk assessment, but also technical possibilities, consumers' attitude/behaviors, and cost–benefit analysis.

However, some aspects of the control system are pathogen-specific. Thus some pathogens in meats (e.g. *Salmonella* spp., *Campylobacter* spp., *Y. enterocolitica* and VTEC *E. coli* are most efficiently controlled by the main interventions applied in the primary production, combined with optimization of the slaughter hygiene. For some others, such as more environmentally ubiquitous *L.* monocytogenes, the main control measures are focused on later stages of the meat chain (*Norrung and Buncic,* 2008).

In order to prevent zoonoses from occurring, it is important to identify which animals and foodstuffs are the main sources of infections. For this purpose and to follow the developments on food safety in the European Union, information aimed at protecting human health is collected and analysed from all European Union Member States. Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents (Zoonoses Directive) covers the epidemiological investigation and reporting of food-borne outbreaks in the Member States (MSs) of the European Union (EU). Each MS has the obligation to collect relevant and, where applicable, comparable data of zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks. Thorough investigation of foodborne outbreaks aims to identify: 1. the pathogen, 2. the food vehicle involved, and 3. the factors in the food preparation and handling, contributing to the outbreak.

The data collection may allow the identification of emerging trends in the causative agents, and vehicles. Data regarding food-borne outbreaks provides important information on the number of humans affected annually and complements the picture of the burden of food-borne disease given by the total number of cases of disease in the Community. The added value concerns especially the information on the causative agent-food vehicle combinations responsible for the food-borne outbreaks. This information is necessary when targeting actions to improve food safety (EFSA, 2009a).

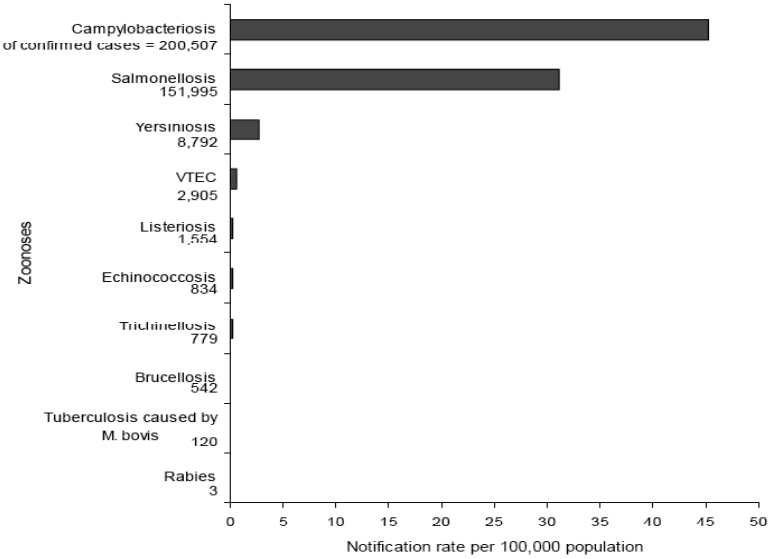

Figure 1. The reported notification zoonoses rates in confirmed human cases in the EU, 2007 (Adapted from EFSA, 2009b)
Slika 1. Incidenca prijavljenih zoonoza kod potvrđenih slučajeva u ljudi, na nivou EU, 2007 (preuzeto iz EFSA, 2009b)

2. Materials and methods

The present paper is not an detailed review of microbial zoonotic foodborne pathogens along the meat chain, but rather gives an overview of the main microbial meatborne risks, aspects of their control, and system of integrated monitoring (*Campylobacter* spp., *Salmonella* spp., *Yersinia enterocolitica*, VTEC *E. coli* and *Listeria monocytogenes*). Therefore, for the purposes of better understanding and explanation of the monitoring/surveillance and reporting system of microbial zoonotic foodborne pathogens, as well as, their control, reporting system and subsequent epidemiological investigation, the related documents issued by EFSA (European Food Safety Authority) and DG SANCO (EU Commission, Directorate General Health and Consumer Protection) have been used (Manual for Reporting of Food-borne Outbreaks in the framework of Directive 2003/99/EC; EFSA, 2009a, The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007; EFSA, 2009b); as well as, the other relevant documents.

3. Main microbial meatborne infections in Europe

In 2007, campylobacteriosis was again the most frequently reported zoonotic disease in humans in the European Union, with 200,507 reported confirmed cases; most Member States (MSs) reporting an increased number of cases. Salmonellosis was still the second most commonly recorded zoonosis accounting for 151,995 confirmed human cases. However, the incidence of salmonellosis continues to decrease in the European Union with a statistically significant trend over the last four years.

3.1. Campylobacter spp.

Humans. In total, 200,507 confirmed cases of campylobacteriosis were reported by 24 MSs, which was a 14.2% increase compared to 2006. Children under the age of five had the highest notification rate (120 cases per population of 100,000). Other age groups varied between circa 32 to 53 cases per population of 100,000 (Figure 2, Figure 3).

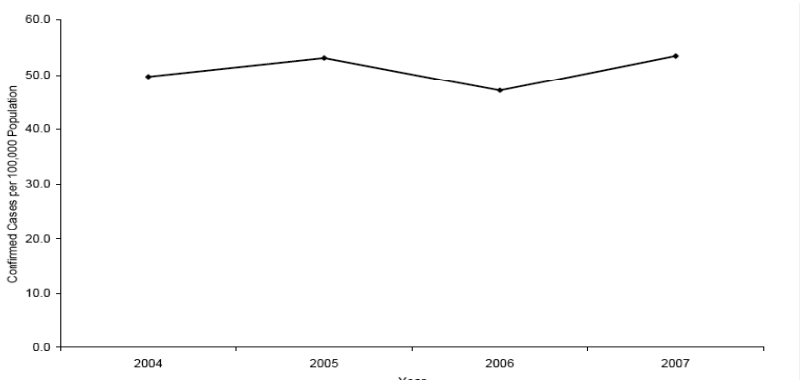

Figure 2. Notification rates of reported confirmed cases of human campylobacteriosis in the EU, 2004-2007 (Adapted from EFSA, 2009b)
Slika 2. Nivoi prijavljenih i potvrđenih slučajeva humanih kampilobakterioza u EU, 2004-2007 (preuzeto iz EFSA, 2009b)

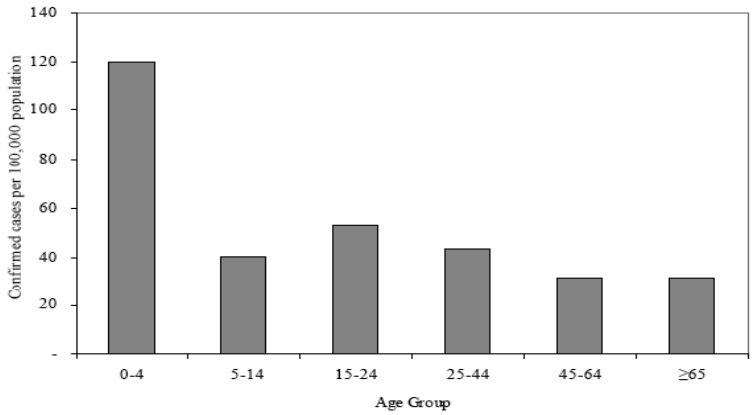

Figure 3. Age-specific distribution of reported confirmed cases of human campylobacteriosis (TESSy, 2007)
Slika 3. Distribucija prijavljenih i potvrđenih slučajeva humanih kampilobakterioza prema starosnoj kategoriji (TESSy, 2007)

Foodstuffs. Broiler meat was the most frequently sampled food category in 2007 and the reported occurrence of *Campylobacter* was generally at the same high level as in previous years. On average, 26.0% of fresh broiler meat samples tested *Campylobacter* positive at EU level and findings ranged from 0% to 86.5%. In samples of pig meat and bovine meat, *Campylobacter* was detected less frequently: 0.9% and 1.2% of the samples, respectively. Poultry meat appears still to be the most important food-borne source of *Campylobacter* as the occurrence of the bacteria remained at high levels throughout the food chain, from live animals to meat retail level (Figure 4).

Animals. In 2007, as in previous years, the majority of data on *Campylobacter* in animals was from investigations of broilers, but data from pigs and cattle was also reported. The recorded prevalence of *Campylobacter* positive broiler flocks was generally high: 25.2% at EU level ranging from 0% to 82.8% in MSs. High prevalence was also observed from the monitoring of pigs, 56.1% at EU level (ranging from 0.9% to 78.5%). In cattle, reported occurrences were somewhat lower, 5.9% on average in the EU, but prevalence up to 70.5% was reported by some MSs. However, *Campylobacter* contamination rates in pig and bovine meat typically decrease sharply following slaughter and remain low at retail (Figure 5).

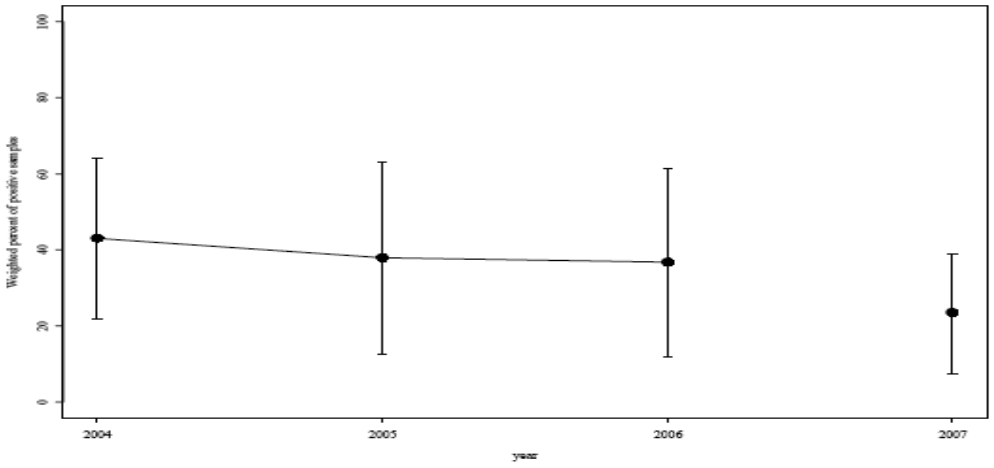

Figure 4. *Campylobacter* in fresh broiler meat* (Adapted from EFSA, 2009b)
*****Combined data (samples taken at slaughter, at processing/cutting plant or at retail)
Slika 4. ***Campylobacter*** u svežem živinskom mesu (preuzeto iz EFSA, 2009b)
*****Kombinovani podaci (uzorci uzeti na klanju, u pogonu za rasecanje ili u maloprodaji)

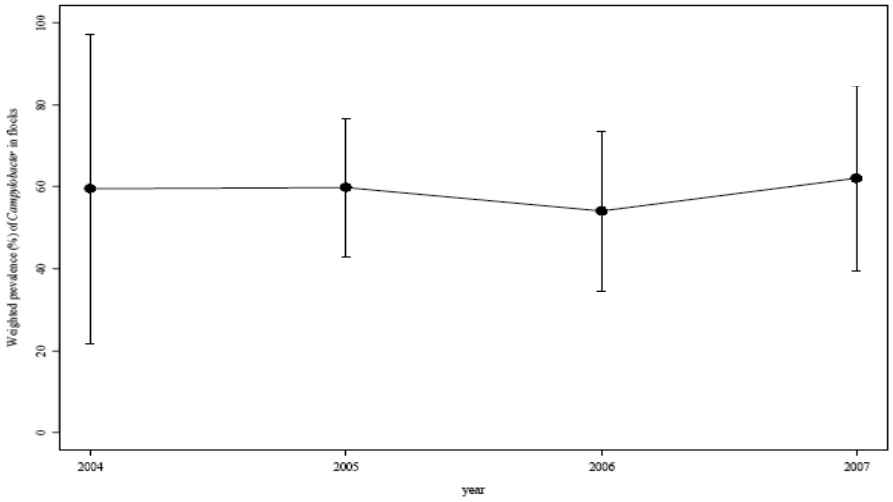

Figure 5. *Campylobacter* in broiler flocks (Adapted from EFSA, 2009b)
Slika 5. *Campylobacter* u jatima živine (preuzeto iz EFSA, 2009b)

3.2. Salmonella spp.

Humans. In 2007, a total of 151,995 confirmed cases of human salmonellosis (TESSy) were reported in the EU. The EU incidence rate was 31.1 cases per population of 100,000, ranging from 2.9 to 171.6 confirmed cases. In 2007, there was a 7.3% decrease comparing with 2006 and this was part of a significant, decreasing trend over the past four years. As in previous years, *S. Enteritidis* and *S. Typhimurium* were the most frequently reported serovars (81% of all known serovars in human cases) (Figure 6). The highest notification rate for human cases was for age groups 0 to 4 years and 5 to 14 years. A seasonal peak in the number of cases during the late summer and autumn was generally observed in all MSs and *S. Enteritidis* demonstrates a much more prominent peak than the other serovars.

Foodstuffs. Reported *Salmonella* findings were most frequently from investigations of poultry meat, followed by those of pig meat. The highest proportions of positive samples were also observed in investigations of these food categories. The overall proportion of positive samples in fresh broiler meat was 5.5%, at EU level, varying between 0% and 55.6%. 1.1% of fresh pig meat samples were on average found *Salmonella* positive in the EU, ranging from 0% to 19.4% (this data is strongly influenced by the high numbers of samples reported by the Nordic MSs that have low prevalence). In bovine meat, most MSs reported very low (<1.0%) proportions of positive samples (Figure 7, Figure 8). Overall, 0.8% (range 0% to 5.8%) of tested egg units were found positive, which is the same level as in 2006 (0.8%). However, in general, the level of samples in noncompliance with the *Salmonella* criteria in 2007 was comparable to the findings in 2006.

Animals. 2007 was the first year when the new *Salmonella* control programmes in breeding flocks of *Gallus gallus* were implemented on a mandatory basis (Regulation (EC) No 2160/2003). The aim of the programmes is to meet the *Salmonella* reduction target set down by the Regulation (EC) No 1003/2005. The target states that the occurrence of *S. Enteritidis*, *S. Hadar, S. Infantis, S. Typhimurium* and *S. Virchow* should be reduced to 1% or less in adult breeding flocks comprising at least 250 birds by 31 December 2009. 15 MSs reported in 2007 a prevalence of

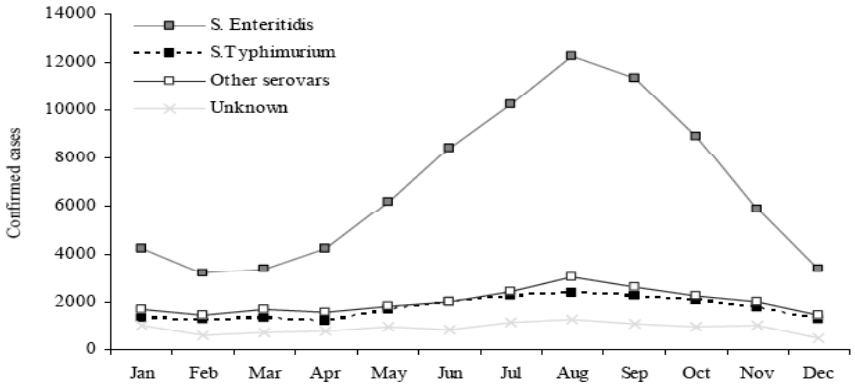

Figure 6. Number of reported confirmed salmonellosis cases in humans by month and serovar (Adapted from EFSA 2009b; TESSy, 2007)
Slika 6. Broj prijavljenih i potvrđenih slučajeva salmoneloza kod ljudi, prema mesecu i serovaru (preuzeto iz EFSA 2009b; TESSy, 2007)

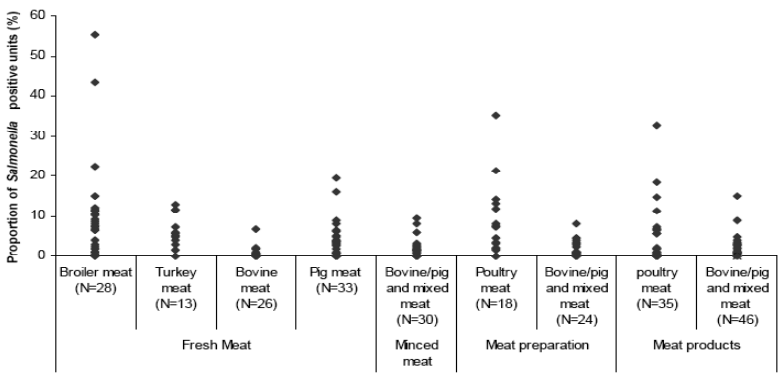

Figure 7. Proportions of *Salmonella* positive units, by meat category (Adapted from EFSA, 2009b)
Slika 7. Proporcija *Salmonella* pozitivnih proizvodnih jedinica, prema kategoriji mesa (preuzeto iz EFSA, 2009b)

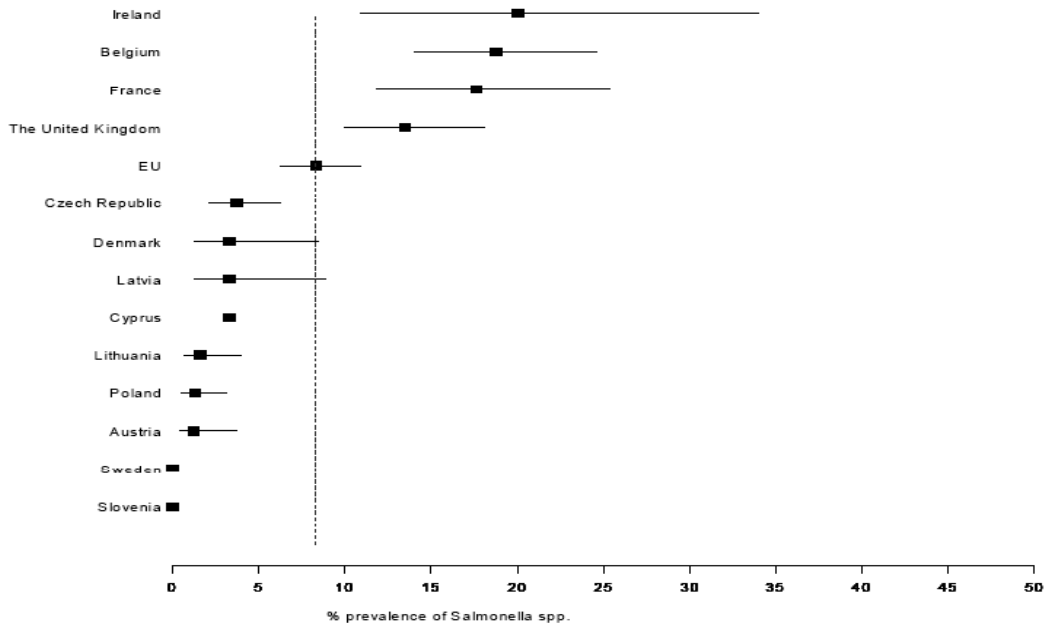

Figure 8. Observed prevalence of carcasses contaminated with Salmonella spp., baseline survey 2006-2007 (Adapted from EFSA, 2009b)

Slika 8. Prevalenca kontaminiranih trupova sa *Salmonella spp*., osnovno istraživanje 2006-2007 (preuzeto iz EFSA, 2009b)

these five target serovars that was lower than the target, whereas eight MSs reported prevalence of the five serovars ranging from 1.1% to 15.4%. A total of 4.3% (ranging between 0% and 27.1%) of the tested laying hen flocks were found infected during 2007, an overall occurrence slightly higher than in the two previous years. An EU-wide *Salmonella* baseline survey was carried out in slaughter pigs in 2006 to 2007 (*S. Typhimurium*). In total, 19,071 ileo-caecal lymph node samples were collected from slaughtered pigs and the EU weighted mean prevalence in pigs was 10.3% ranging between 0% and 29.0% in MSs. Few MSs have active monitoring of *Salmonella* in cattle, but two MSs both reported slaughter prevalence of 0.1% in cattle (*S. Typhimurium*, *Salmonella Dublin*) (Figure 9).

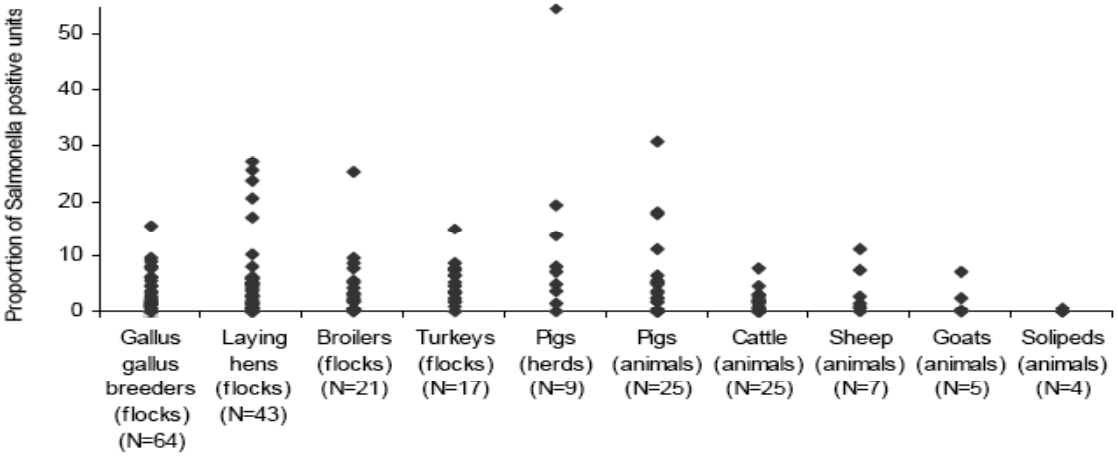

Figure 9. Reported Salmonella prevalence by animal species within the EU, in 2007* (Adapted from EFSA, 2009b)

*****Data are only presented from sample size ≥25. Results from HACCP and baseline surveys are excluded, as well as data based on suspicion or trace-back sampling.

Slika 9. Prevalenca *Salmonella*, prema vrstama životinja na nivou EU, u 2007* (preuzeto iz ESFA, 2009b)

*Podaci su predstavljeni samo za veličinu uzorka ≥25. Rezultati iz HACCP-a i osnovnih studija nisu prikazani, kao i podaci o suspektnom ili retroaktivnom uzorkovanju.

3.3. Yersinia enterocolitica

Humans. In 2007, 8,792 confirmed human cases of yersiniosis were reported in the EU. *Foodstuffs*. Findings of *Y. enterocolitica* were reported on average in 2.0% of pig meat samples. *Animals*. Findings of *Y. enterocolitica* were reported in 0% to 52% of pigs.

3.4. VTEC E. coli

Humans. In 2007, a total of 2,905 confirmed human VTEC cases were reported from 23 MSs. This is a slight decrease compared to 2006. The EU incidence rate was 0.6 per population of 100,000. The most commonly identified VTEC serogroup was O157 (54%), although other serogroups were detected (i.e. O26, O103, O91, O145, O111, O128, O113, O146) (Figure 10). The notification rate was highest in 0 to 4 year old children and this group also accounted for almost 60% of the 103 HUS cases reported, mainly associated with VTEC O157 infections (Table 1).

Foodstuffs. The reported occurrence of VTEC bacteria in food was generally low, and has been relatively constant during the 2005 to 2007 period. In fresh bovine meat the proportion of samples positive for VTEC was 0.3% at EU level and 0.1% for the serogroup VTEC O157. Some MSs also reported, from bovine meat, the O26, O103, O111, and O113

Table 1. VTEC serogroups by country (TESSy, 2007)
Tabela 1. VTEC serogrupe po zemljama (TESSy, 2007)

	Serogroup										
Country	**O157**	**NT**	**O26**	**O103**	**O91**	**O145**	**O111**	**O128**	**O113**	**O146**	**Other**
Austria	17	41	1	3	2	7	2			2	7
Belgium	25	3	5	2	1	2	2		1	2	4
Denmark	25	1	28	16	9	5	4	8	5	8	47
Estonia	2							1			
Finland	9	3									
France	14	29	10		1		1	1			1
Germany	66	577	61	46	26	13	12	9	8	1	51
Hungary	1										
Ireland	94	5	13			1	1	1			
Italy	5	20	1				1				
Luxembourg	1										
Malta	4			3							
Netherlands	80	1	3	1	1						
Poland	2			6							
Slovakia	3	3									
Slovenia											4
Spain	18										
Sweden	85	138	13		3	1		1	2	1	12
United Kingdom	1,120	21	1			2					4
Total (19 MSs)	**1,571**	**842**	**136**	**77**	**43**	**31**	**23**	**21**	**16**	**14**	**130**
Iceland	13										
Norway	5	7	3	1		4		2		1	3

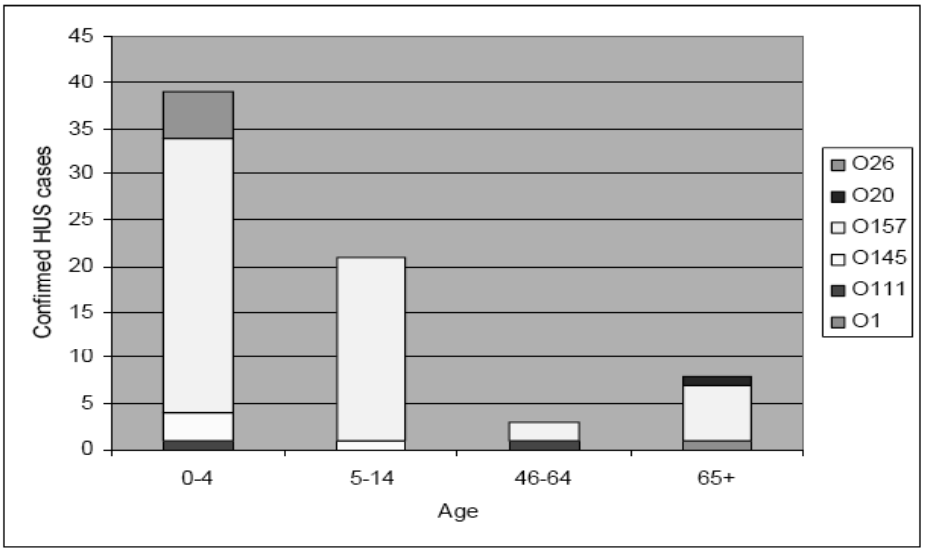

Figure 10. Haemolytic Uremic Syndrome (HUS) by age and serogroup (Adapted from EFSA, 2009b)
Slika 10. Hemolitički Uremički Sindrom (HUS) po godinama i serogrupama (preuzeto iz EFSA, 2009b)

serogroups that are all frequently isolated from human VTEC cases. (Figure 11).

Animals. In bovine animals the average VTEC prevalence was 3.6% and the proportion of VTEC O157 positive animals was 2.9%. The reported occurrence of VTEC ranged from 0% to 22.1%.

3.5. Listeria monocytogenes

Humans. A total of 1,554 confirmed cases of listeriosis were reported in 2007. The EU incidence rate was 0.3 per population of 100,000. The highest notification rates were observed in Scandinavian countries. The number of confirmed cases of listeriosis almost reached the same level as in 2006. Listeriosis mainly occurred among elderly people, with 53.1% of cases (notification rate was 1.0 per population of 100.000) occurring in individuals over the age of 65. The notification rate among children under the age of five was 0.5 cases per population of 100.000. The case fatality rate for human listeriosis was 20% (mainly in elderly people) (Figure 12).

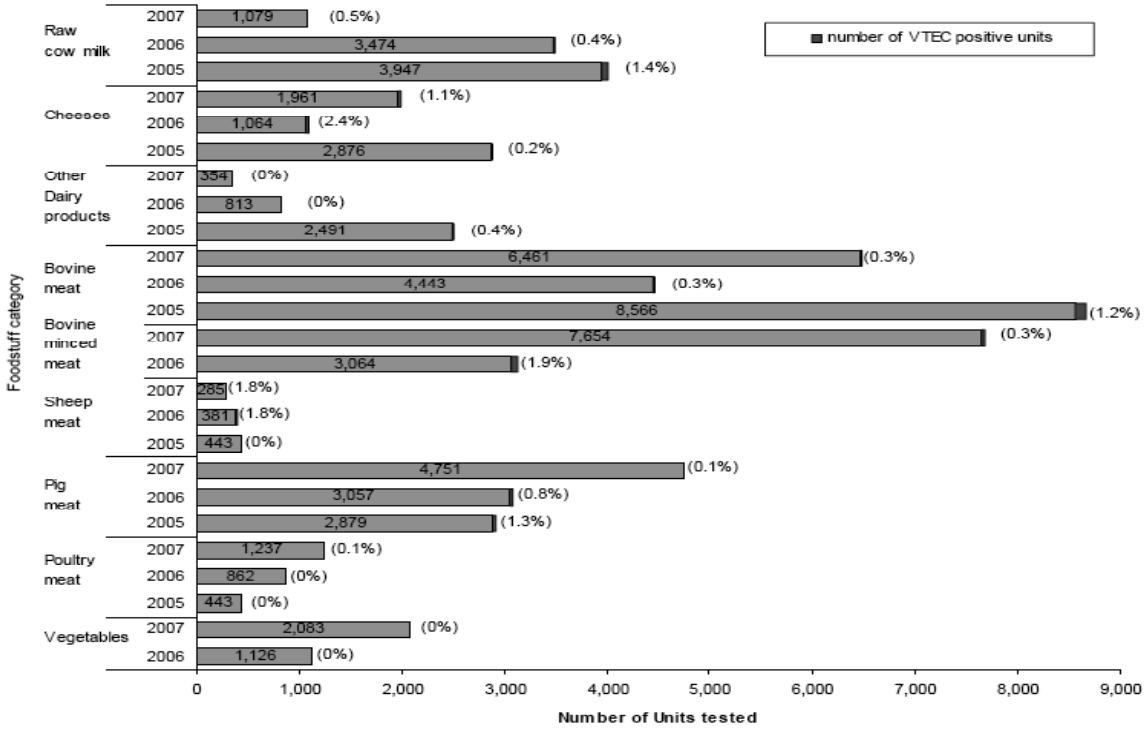

Figure 11. Number of food samples tested for VTEC by food category and number of VTEC positive units, 2005-2007 (Adapted from EFSA, 2009b)

Slika 11. Broj uzoraka hrane testiranih na VTEC prema kategoriji hrane i broju VTEC pozitivnih proizvodnih jedinica (preuzeto iz EFSA, 2009b)

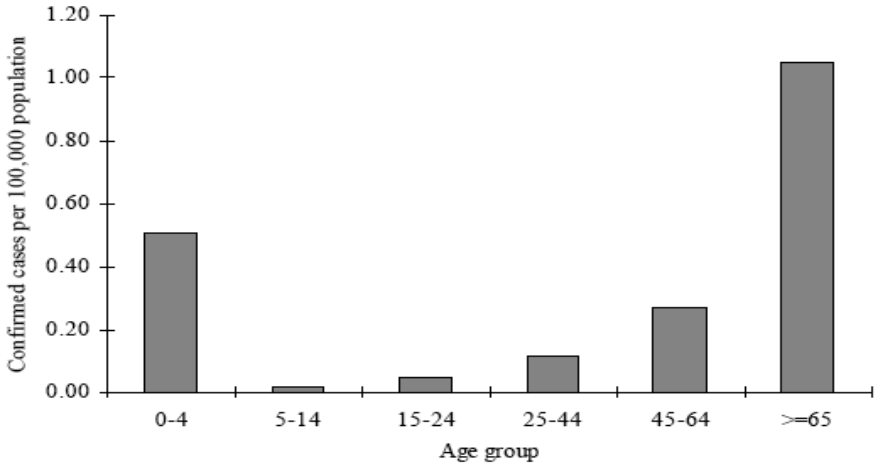

Figure 12. Age-specific distribution of reported confirmed cases of human listeriosis (TESSy, 2007)

Slika 12. Distribucija prijavljenih i potvrđenih slučajeva listerioza kod ljudi, prema starosnoj kategoriji (TESSy, 2007)

Foodstuffs. In 2007, a large number of investigations concerning ready-to-eat (RTE) foodstuffs were reported by MSs. The food categories most often covered were RTE meat products, dairy products, cheeses and fishery products (Figure 13). In general, *L. monocytogenes* was rarely detected in quantities exceeding the legal safety limit of 100 cfu/g (Regulation 2073/2005/EC). The proportion of the samples in non-compliance with the criterion was most often observed at retail in fishery products (1.7% and 2.2% for single products and batches, respectively), particularly in smoked fish, followed by meat products (0.3% and 0.7%).

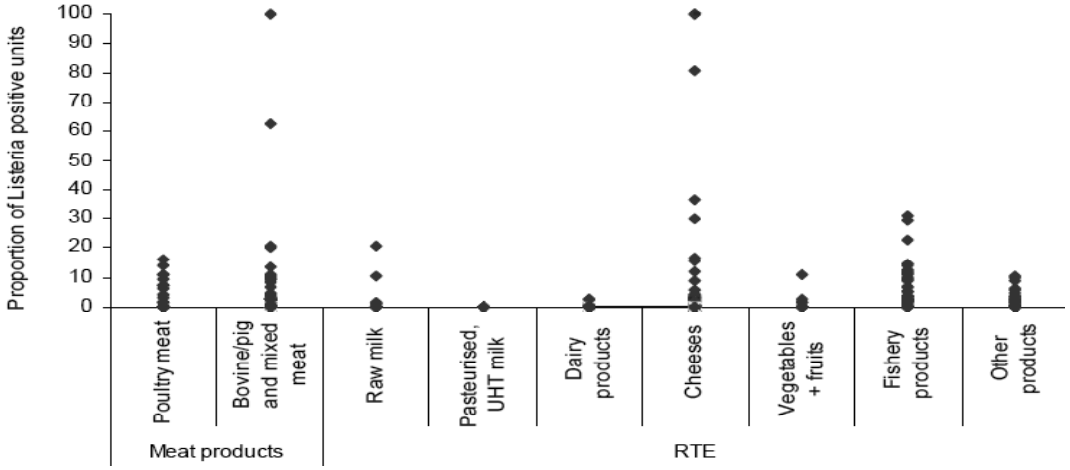

Figure 13. Proportions of *Listeria* positive samples by ready-to-eat food category (Adapted from EFSA, 2009b)
Slika 13. Proporcija *Listeria* pozitivnih uzoraka kod proizvoda spremnih za konzumiranje (preuzeto iz EFSA, 2009b)

Animals. In 2007, data on *L. monocytogenes* in animals and the bacterium was reported from various animal species. In some MSs the detected proportion of positive samples reached a moderate level in cattle and in small ruminants.

4. Structure of the web-based integrated monitoring system

The European Community (EC) system for monitoring and collection of information on zoonoses is established by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents. This Directive requires Member States (MS) to collect, evaluate and report data on: zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks to the European Commission each year. Monitoring on zoonoses, antimicrobial resistance and foodborne outbreaks is web-based and accessible on the EFSA zoonoses reporting homepage: (www.efsa.europa.eu/zoonoses).

For each reporting year, a national report is created in the web-based reporting system. For each zoonoses or other subject, text forms and reporting tables are provided. The text forms are used to enter the narrative part of the report, *e.g.* description of the monitoring system and the analyses of the results. The reporting tables are used to enter the results, *e.g.* number of samples and number of positive results.

The national report on zoonoses, antimicrobial resistance and foodborne outbreaks is divided into three sections:

1. Description of the national reporting system and national evaluation of the reported food-borne outbreaks;
2. Total number of food-borne outbreaks;
3. Data to be reported for verified food-borne outbreaks.

4.1. Relevant outbreaks and causative agents to be reported

The annual reporting system covers the results of the investigations of all food-borne outbreaks carried out in MSs. "Food-borne outbreak" is defined in the Zoonoses Directive "*as an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source"* (Directive 2003/99/EC).

For the purpose of the reporting system, this is understood to include food-borne outbreaks caused by any virus, bacterium, alga, fungus, parasite, and its products, such as toxins and biological amines (e.g. histamine). Reporting should not be limited to foodborne outbreaks caused by zoonotic agents only, but should include food-borne outbreaks caused by any of the agents above. Outbreaks caused by ingestion of drinking water are also considered food-borne (Regulation 178/2002), while food-borne outbreaks caused by chemical agents are not covered at this stage.

4.2. Mandatory reporting

In accordance with the Zoonoses Directive 2003/99/EC, all MS have to report on the following zoonoses, zoonotic agents (list A) and other subjects:

- Brucellosis and agents thereof;
- Campylobacteriosis and agents thereof;
- Echinococcosis and agents thereof;
- Listeriosis and agents thereof;
- Salmonellosis and agents thereof;
- Trichinellosis and agents thereof;
- Tuberculosis due to Mycobacterium bovis;
- Verotoxigenic Escherichia coli;
- Antimicrobial resistance in Salmonella and Campylobacter isolates from poultry, pigs and cattle and foodstuffs derived from these species;
- Food-borne outbreaks;
- Susceptible animal populations.

4.3. Reporting based on epidemiological situation

Other zoonoses are to be included in the monitoring and reporting according to the epidemiological situation in each MS. This means that if a certain zoonosis is of public health importance in a MS, this MS should report on that zoonosis, but the other MSs do not have the same obligation to report on it.

The zoonoses to be reported based on the epidemiological situation are listed in Directive 2003/99/EC (list B):

Viral zoonoses:

- Calicivirus;
- Hepatitis A virus;
- Influenza virus;
- Rabies;
- Viruses transmitted by arthropods.

Bacterial zoonoses:

- Borreliosis and agents thereof;
- Botulism and agents thereof;
- Leptospirosis and agents thereof;
- Psittacosis and agents thereof;
- Tuberculosis other than in point A;
- Vibriosis and agents thereof;
- Yersiniosis and agents thereof.

Parasitic zoonoses:

- Anisakiasis and agents thereof;
- Cryptosporidiosis and agents thereof;
- Cysticercosis and agents thereof;
- Toxoplasmosis and agents thereof.

Other zoonoses and zoonotic agents

Other non-zoonotic pathogenic microbiological and toxicological agents in foodstuffs (e.g. *Enterobacter sakazakii*, staphylococcal enterotoxins and histamine).

4.4. Monitoring system for zoonotic foodborne pathogens in the meat chain

Sampling strategy. The framework of the sampling is an important part of the strategy, and it should be stated if the sampling is part of a permanent or temporary monitoring programme, linked to surveillance or control programmes or if it is a question of a single survey, e.g. the sampling strategy chosen and the purpose of the sampling: whole country covered or only part of it; target population (entire or subset of animal population, categories of foodstuffs and feedingstuffs); geographical regions; size of the holdings; sampling protocol (objective, selective, suspected, convenient or census sampling); who is performing the sampling (competent authority – official sampling, by owners of animals, food or feed businesses in the context of HACCP / owncheks); where the samples are taken (at farm, at slaughterhouse, at hatchery, at food processing plant or at retail); stage of sampling (animal rearing period, production period, before of after a chilling of carcase in the slaughterhouses, before or after expiration of the shelf-life of foodstuffs).

Frequency of the sampling. This part is intended to explain how often samples are taken. The standard terms (*e.g.* every week, once a month, *x* times a year)

Type of specimen taken. The specimen taken from the units sampled is described (Table 2):
Animal species – cattle, pigs, broilers (specimens: faeces, blood, organs or milk)
Foodstuffs – beef, pork, poultry meat
Stage in the meat chain – preharvest (on the farm), harvest (slaughter), postharvest (processing/distribution/retail)

Methods of sampling. This should include information on the site of sampling (*e.g.* part of a car-

Table 2. Description of the sampling strategy for monitoring of *Salmonella* spp. in the meat chain (EFSA, 2009a; adapted by *Nastasijevic I.*)
Tabela 2. Opis strategije uzorkovanja za monitoring *Salmonella spp*. u lancu mesa (EFSA, 2009a; preuzeo i modifikovao *Nastasijević I.*)

Salmonella spp.
The sampling strategy
The control, surveillance and monitoring programmes
Who performs the sampling: competent authority (official sampling) or industry (own checks)
The type of sampling i.e. objective, selective or suspect
The place or stage at which the sample was taken (e.g. farm, slaughterhouse, processing plants, retail, border inspection posts)
Type of specimen taken
Meat and meat products
Animal species: broiler, bovine and pig meat, duck meat
Pre-harvest phase: faeces, environmental surfaces, animal waste, animal` hides, etc.
Harvest phase: carcase, fresh meat, trimming
Post-harvest phase: minced meat, meat preparations, meat products, retail
Status of meat: fresh/frozen/cooked *Intended to be consumed: raw or cooked*

case, part of the facilities for environmental sample), size of sample taken (*e.g.* in g, cm^2, ml), use of swabs or other instruments in the sampling, when relevant, the number of (sub)samples / sample units taken, pooling of samples when conducted (always refer the number of samples combined by pooling), the possible storage of samples, and the length of this storage.

*Case definition / definition of a positive finding***.** This covers the description of when the sample is considered to be positive for the zoonotic agent or when the animal, herd or flock is considered to be infected with the zoonotic agent. Regarding food and feed, it should describe when the foodstuff, feedingstuff or the batch sampled is considered to be positive or contaminated with the zoonotic agent.

*Diagnostic / analytical methods used***.** Under this title, the diagnostic or analytical methods used in the laboratory to test the specimens are described. Whenever possible, a reference to standard methods used is made (such as national, ISO or EN standard methods), or to the methods prescribed by the legislation.

*Vaccination policy***.** This policy can cover different kinds of situations: vaccination of animal populations against the zoonotic agent may be prohibited or it may be mandatory or voluntary. There can be recommendations in place to vaccinate certain animal populations or to use a certain type of vaccination scheme. It may also be that there is no official policy regarding vaccination. If a vaccination policy exists, it should be described and if no policy exists, the established way of using the vaccines in the MS can be explained. The description should include, at least, a description of the vaccine, characteristics of the animals to be vaccinated (age, sex), area where vaccination is to be implemented, special measures for marking the vaccinated animals, etc.

*Other preventive measures than vaccination in place***.** Other preventive measures may include actions taken at different levels of the food chain. Regarding animals, it may cover bio-security measures at the farms. For the foodstuffs, it may include recommendations on meat consumption for susceptible consumer groups.

4.5. Reporting on antimicrobial resistance

Trends on antimicrobial resistance. The information to be reported each year or at regular intervals (*e.g.* every 2. or 3. year).

4.5.1. Mandatory

Antimicrobial resistance on Salmonella spp.

Relevant animal species / food categories to be reported: Laying hens and broilers (*Gallus gallus*), turkeys, pigs and cattle, broiler meat, pig meat, bovine meat.

Relevant agent species / serovars to be reported:

In the qualitative antimicrobial susceptibility tables: *S. Enteritidis* and *S. Typhimurium* and the

next 5 most prevalent serovars in the country and the other serovars group together. In the quantitative antimicrobial susceptibility tables: *S. Enteritidis* and *S. Typhimurium for poultry species* and meat thereof*; S. Typhimurium and S. Derby* for pigs and pig meat, *S. Typhimurium* and *S. Dublin* for cattle and bovine meat, and other *Salmonella* serovars grouped together for all species.

Recommended antimicrobials to be reported: Ampicillin; Cefotaxime; Chloramphenicol; Ciprofloxacin; Gentamicin; Nalidixic acid; Streptomycin; Sulphonamides; Tetracycline; Trimethoprim.

Antimicrobial resistance on Campylobacter spp.

Relevant animal species / food categories to be reported: Broilers (*Gallus gallus*), turkeys, pigs, cattle, broiler meat, other poultry meat

Relevant agent species / serovars to be reported: *C. jejuni and C. coli* separately. Reporting of susceptibility data for *Campylobacter spp*. overall is discouraged because resistance patterns vary for different species.

Recommended antimicrobials to be reported: Erythromycin; Ciprofloxacin; Tetracycline; Streptomycin; Gentamicin.

4.5.2. Optionally

Antimicrobial resistance on E. coli (non-pathogenic).

Relevant animal species / food categories to be reported: Laying hen, broilers (*Gallus gallus*), turkeys, pigs, cattle, broiler, pig and bovine meat.

Recommended antimicrobials to be reported: Ampicillin; Cefotaxime; Chloramphenicol; Ciprofloxacin; Gentamicin; Nalidixic acid; Streptomycin; Sulphonamides; Tetracycline; Trimethoprim.

Antimicrobial resistance on Enterococcus spp.

Relevant animal species / food categories to be reported: Broilers (*Gallus gallus*), pigs, cattle, broiler meat, pig meat, bovine meat

Relevant agent species to be reported: *E. faecium* and *E. faecalis,* separately

Recommended antimicrobials to be reported: Aminoglycosides: streptomycin, gentamicin; Amphenicols: chloramphenicol; Beta-lactams or ß-lactam inhibitors: ampicillin or amoxicillin; Glycopeptides: vancomycin; Macrolides: erythromycin; Streptogramins: preferably quinopristin/dalfopristin; Tetracyclines: tetracycline.

Diagnostic/analytical methods typically used. Three types of methods are used in antimicrobial resistance testing for *Salmonella* and indicator bacteria: disk diffusion, agar dilution and broth dilution. For *Campylobacter*, only dilution methods are considered reproducible.

4.6. Control programmes/mechanisms

*The control programmes / strategies in place***.** Under this title, the control programmes in place are described. The control programmes may be national or regional, and they may be approved nationally or by the Commission and co-financed by the Community (Council Decision 90/424/EEC). The nature of the control programmes, *e.g.* voluntary, mandatory, national, regional, Community or national approval and co financing should be indicated.

*Measures in case of the positive findings or single cases***.** Actions required by the legislation or control programmes as a consequence of findings of positive animals, foodstuffs or feedingstuffs are explained (e.g. withdrawal of the products from the market, destruction of animals and others).

Notification system in place. The notification system is described, including its legal basis and since when the disease or infection has been notifiable.

Recent actions taken to control the zoonoses. Specific measures undertaken during the recent ye ars to control zoonoses, are described (Table 3).

4.7. Results of the investigation

*National evaluation of the recent situation, the trends and sources of infection***.** The results are interpreted in relation to their importance to public health. It is essential to evaluate the trend when compared to the previous year, *e.g.* is there a decreasing or increasing trend or is the situation stabilized. The important sources of infections are also discussed.

*Relevance of the findings in feedingstuffs / animals / foodstuffs and to human cases (as a source of infection)***.** The importance of the feedingstuffs / animals / foodstuffs as sources of the human infections is evaluated. The role of feedingstuffs as a source of infection for animals, and similarly the role of animals as a source of contamination for foodstuffs are considered, as well.

History of the disease and / or infection. The history of the zoonoses cases in humans and animals in the past is reflected. For example, issues such as the number of cases in the past and the impact of control and eradication programmes can be addressed.

Additional information…

Table 3. Example of integrated monitoring and control programmes for VTEC ***E. coli*** in the meat chain (EFSA, 2009a; adapted by *Nastasijevic I.*)
Tabela 3. Primer integrisanog monitoringa i programa za kontrolu VTEC *E. Coli* u lancu mesa (EFSA, 2009a; preuzeo i modifikovao *Nastasijević I.*)

Verotoxigenic *Escherichia coli* (VTEC) in foodstuffs
The sampling strategy
The control, surveillance and monitoring programmes in place
Who performs the sampling: competent authority (official sampling) or industry (own checks)
The type of sampling i.e. objective, selective or suspect
The place or stage at which the sample was taken *(e.g. farm, slaughterhouse, processing plants, retail, border inspection posts)*
Type of specimen taken
Meat and meat products
*Animal species: broiler**, bovine**, sheep**, goat, game (ruminants)***
Harvest phase: carcase, fresh meat, trimming
Post-harvest phase: minced meat, meat preparations, ready-to-eat fermented meat products, retail
Status of meat: fresh/frozen/cooked *Intended to be consumed: raw or cooked*
Relevant agent species / serovars / phage types to be reported:
Strains of E. coli that are capable of producing vero- (shiga-) cytotoxin (i.e. VT+) and/or possess the genes coding for VT production. *Information on the serotype* *or the serogroup (O antigen) should be reported.* *Serotypes of particular interest: O157 and non-O157, (e.g. O111, O103, O26, O145, O91).*
Case definition / definition of a positive sample
***VTEC positive sample / batch** – a sample / batch from which verotoxigenic E. coli has been isolated using a method specified below.*
***VTEC O157 or other serotype positive sample / batch** - a sample / batch from which verotoxigenic E. coli O157 or other serotype has been isolated using a method specified below.*
Diagnostic/analytical methods typically used
The recommended method: EN/ISO ISO 16654 *- molecular subtyping (PCR)*
Currently, there is no internationally recognised standard method for detection of VTEC non-O157
Details should be provided on the diagnostic method used, including how verification of VTEC is carried out and the serotypes for which screening is carried out.
Other methods *(the performance characteristics of the methods* *should be given in comparison to the EN/ISO or ISO standard reference methods or other reference methods- evidence of validation: ISO 16140:2003)*
Reporting the results in the tables
For reporting of data, use tables named: • *"VT E. coli in food"; Specific guidelines for reporting data in the prevalence table:* • ***Sampling unit – "**Single" or "Batch" should be used as the terms to be reported;* • ***Total units positive for VTEC** - the total number of units positive for Verotoxigenic E. coli (VTEC);* • ***VTEC O157 and other serotypes** – the number of units positive for the specific VTEC serotype;* • ***VTEC, unspecified -** the number of units positive for VTEC where the serotype is unknown.*
Preventive and control measures in place
National microbiological criteria or guidelines for foodstuffs
Provisions or recommendations concerning use of certain foodstuffs containing potentially hazardous agents
Special recommendations for susceptible populations of consumers

5. Conclusions

There are many routes by which the zoonotic pathogens can reach consumers via meats including consumption of contaminated, uncooked or improperly cooked ready-to-eat (RTE) product and cross--contamination from raw to RTE foods. Better knowledge on the relative importance of these different routes is needed. For that, both epidemiological and microbiological approaches as well as risk assessments of specific pathogens in specific foods need to be applied. Such knowledge is important to tailor and optimise the risk management strategies and activities.

Therefore, zoonotic pathogens in meat have to be monitored and controlled through a complete, continuous farm-to-fork system. This means that integrated concept for monitoring in all major phases along the meat chain should be implemented through "modular approach". Risk mitigation options were identified according to three lines of defence formulated by the World Health Organization (WHO): the first line focuses on the control of foodborne pathogens in the food producing animal (Pre-harvest control / on the farm), the second line deals with improvement of hygiene during slaughter and further processing of meat (Harvest control / in abattoir) and the third line concentrates on measures during the final preparation of the food and the education of the industry and the consumer concerning the application of effective hygienic measures (Post-harvest control / meat processing-distribution-retail-consumer) (WHO, 1980; EFSA, 2006). This approach includes sampling, testing and reporting on pathogens` occurrences in those three main production modules. In addition, it is of utmost importance to control direct and indirect faecal contamination of carcasses, in abattoir, through efficient GHP/GMP and HACCP based process hygiene management systems.

In Serbia, the integrated system for monitoring of zoonotic foodborne pathogens in the food (meat) chain should be developed and implemented in the foreseeable future, in the scope of necessary harmonization with Zoonoses Directive 2003/99/EC. The implementation of harmonized survey methods is needed. This will include targeted research to obtain top quality baseline data on occurrences of different pathogens along the meat chain and the characteristics on their antimicrobial resistance. Further on, proper science-based risk assessment of consumer exposure to related pathogens can be only achieved by effective intersectoral cooperation between veterinary and health authorities. This includes: 1. assessment of the monitoring systems for foodborne hazards in the food (meat) chain (veterinary authorities); 2. assessment of surveillance systems for foodborne diseases (health authorities); and, 3. assessment the interface, between the monitoring systems for foodborne hazards in the food (meat) chain and the surveillance systems for foodborne diseases (Figure 14). Finally, the ultimate objective regarding adequate level of public health protection, can be only achieved by effective professional integration of veterinary and health authorities, setting up all related activities to the integrated health concept.

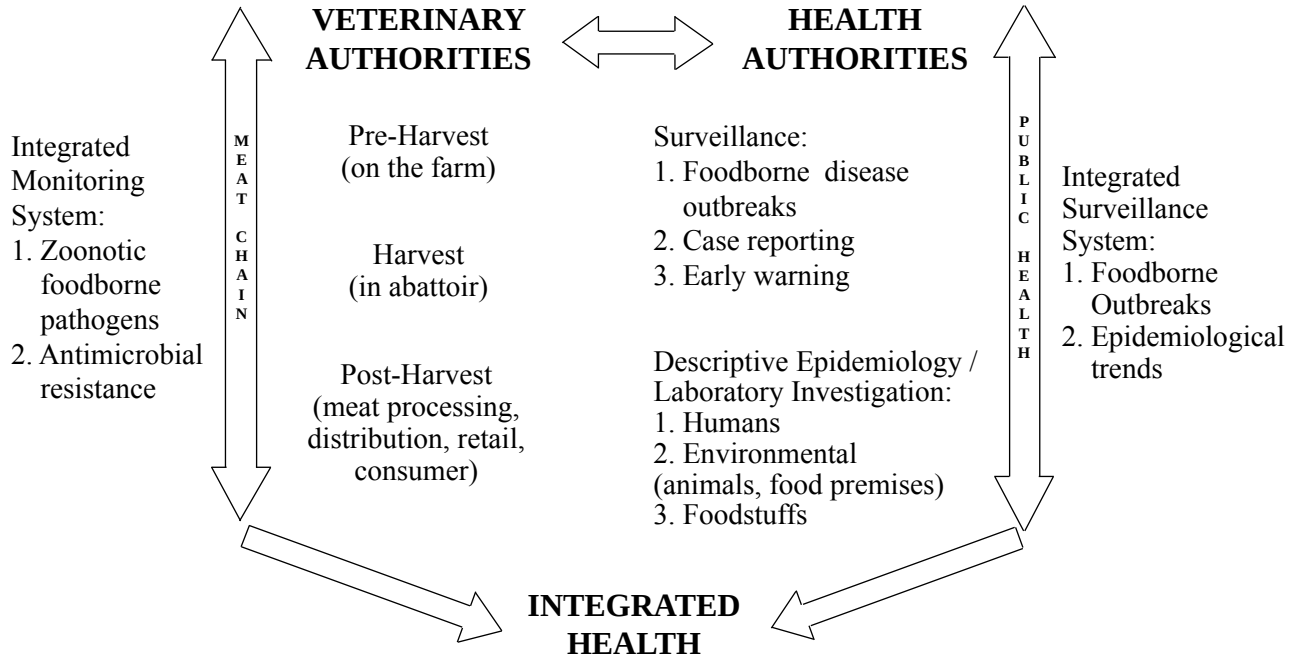

Figure 14. Integrated Health Concept (schematic overview, by Nastasijevic, I.)
Slika 14. Koncept integrisanog zdravlja (šematski prikaz, prema Nastasijević, I.)

References

Outbreaks in the Framework of Directive 2003/99/EC. The EFSA Journal, 257, 1−46;

Anonymous, 2009 b. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal, 223, 1−217;

Anonymous, 2006. Risk Assessment and Risk Mitigation Options of *Salmonella* in pig production. The EFSA Journal, 341, 1−131;

Commission Regulation (EC) No 1003/2005 of 30 June 2005 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in breeding flocks of *Gallus gallus* and amending Regulation (EC) No 2160/2003;

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs;

Commission Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003, on the control of salmonella and other specified food-borne zoonotic agents;

Commission Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety;

Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field;

Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC;

Norrung, B., Buncic, S., 2008. Microbial Safety of meat in the European Union. Meat Science, 78, 14−24;

TESSy (The European Surveillance System). 2007. European Centre for Disease Prevention and Control (ECDC). http://ecdc.europa.eu/documents/pdf/TESSy_workgroup.pdf (access on 5th May, 2009.);

WHO 1980. Report of the WHO/WAVFH Round Table Conference on the Present Status of the Salmonella Problem (Prevention and Control), Bilthoven, the Netherlands, WHO/VPH/81.27;

Paper recieved 11.05.2009.