

OCCURRENCE OF *CAMPYLOBACTER*, *SALMONELLA*, *YERSINIA ENTEROCOLITICA* AND *LISTERIA MONOCYTOGENES* IN SOME RETAIL FOOD PRODUCTS IN NOVI SAD

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SUMMARY

The official reporting system in the Province of Vojvodina (PV) indicates that cases of human salmonellosis were partly covered by complete epidemiological investigation including laboratory analysis of the suspected food. Intestinal campylobacteriosis and yersiniosis and four cases of septicemias caused by *Listeria monocytogenes* were not fully epidemiologically investigated. Actual country legislation on food safety does not include provisions for a routine control of the above mentioned pathogens except for *Salmonella*. In the PV, there are no other sources of data that contribute to risk assessment of the above food-borne diseases.

A pilot investigation, performed in Novi Sad, indicated that 8.17% out of the total number of 257 retail food samples (90 of fresh meat and 167 of ready-to-eat food) had been contaminated with one of the tested bacteria *Campylobacter* or *Salmonella* or *Listeria monocytogenes*. *Yersinia enterocolitica* was not detected in any of the tested samples. Fresh poultry meat and other fresh meats were the dominant sources of the detected pathogens compared to samples of ready-to-eat food ($p < 0.05$). *Campylobacter* was detected in 18.8% and 10.0% samples of fresh poultry and other fresh meat respectively, which was not statistically significant ($p > 0.05$). *Salmonella* was detected in 3.3% samples of fresh poultry meat. *Listeria monocytogenes* was detected in 5.0% samples of fresh poultry and in 3.3% samples of other fresh meat, the difference was not statistically significant ($p > 0.05$). One sample (0.6%) of ready to eat food was contaminated with *Campylobacter* and one (0.6%) with *Salmonella*.

Key words: food, *Campylobacter*, *Salmonella*, *Yersinia*, *Listeria monocytogenes*, Novi Sad

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INTRODUCTION

Data obtained from countries with good reporting system brought evidence that *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica* are frequent causative agents of food borne diseases (1, 2, 3). Infections in vulnerable population groups caused by *Listeria monocytogenes* are also a growing problem (3). Different degrees in severity of food-borne diseases were observed, from mild gastrointestinal disorders to more serious ones that required long-term hospitalization. Systematic manifestations and, even, lethal outcome, usually occur in vulnerable population groups and immunocompromised patients. There are increasing number of studies indicating that alimentary infections and intoxications may result in renal insufficiency, chronic reactive arthritis, damage of central nervous system etc. (2, 4, 5). A special problem in treatment of these patients is the increasing resistance to antibiotic therapy of this group of microorganisms (6, 7).

The Province of Vojvodina (PV) covers one third of the territory of the Republic of Serbia and has a population of about

two million inhabitants. According to data obtained through the official reporting system, the incidence rate of food-borne diseases in the PV has a rising tendency (8, 9, 10). Around 30% of the total number of reported causes of food-borne diseases has a full laboratory confirmation of the causative agent. The most frequently identified causative agent was *Salmonella* spp., followed by *Campylobacter* spp. *Yersinia enterocolitica* was also identified, but proportionally in much lesser extent compared to *Campylobacter* spp. and *Salmonella* spp. (10). *Listeria monocytogenes* has not been identified as a causative agent of food-borne diseases, but was involved as causative agent in several septicemias in patients with other primary illness. Only in part of recorded cases of intestinal infections caused by *Salmonella* spp. full epidemiological investigation including laboratory testing of the suspected food was performed. The reported cases of intestinal campylobacteriosis and intestinal yersiniosis and cases of septicemia due to *Listeria monocytogenes* were not fully epidemiologically investigated and there were no data on food suspected as a route of transmission available (10).

As to the existing country legislation, a routine foodstuff control for *Salmonella* spp. is obligatory (11, 12), but this does not go for *Campylobacter* spp., *Listeria monocytogenes* and *Yersinia enterocolitica*. Presence of these three bacteria in food products can be investigated provided this is from the epidemiological point of view indicated (13).

There were no other available data for food microbiological hazard assessment. The purpose of this paper was to determine the frequency of *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* in certain, common in use, food products retailed in Novi Sad.

MATERIAL AND METHODS

In the period July–October 2004 and July–October 2005, a pilot project of laboratory examination of the presence of *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* in 257 samples of foodstuffs retailed in Novi Sad was performed. The project was conducted during a routine microbiological safety control of food retailed in Novi Sad by the Sanitary Inspectorate of the Secretariat for Health and Social Welfare of the PV. All samples were chosen randomly between 9 a.m. and 2 p.m. Samplers were trained to avoid any contamination during sampling. They did not have any instructions to chose, or to avoid, specific shops. The investigation included 90 samples of fresh meat (60 samples of fresh poultry meat, 30 samples of other kinds of fresh meat ready-made for grill) and 167 ready-to-eat foodstuffs (30 samples of sausage products, 37 samples of heat-treated meat of “fast food” meal, 39 samples of “fast food toppings”, 31 samples of milk and dairy products and 30 samples of ice-cream), as shown in Fig. 1.

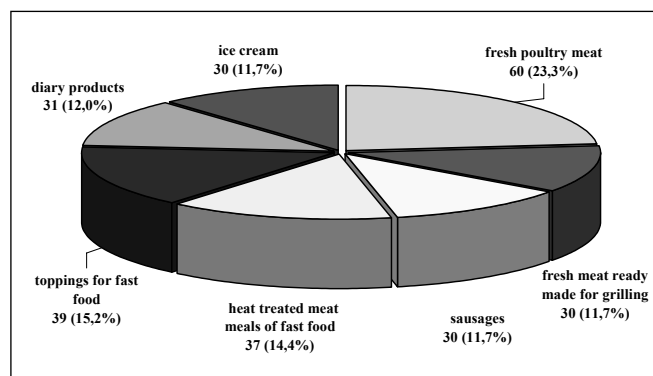


Fig. 1. Food samples. Total number of the tested samples: 257.

The laboratory methods recommended by FAO were applied for *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* identification. (14).

Detection of *Campylobacter* spp.: Food samples were aseptically weighed (25 g), inoculated into 100 ml of *Campylobacter* enrichment broth, blended and incubated for 24 hours at 42 °C under microaerobic conditions. Small drops of the broth were transferred onto Columbia agar plate and incubated at 42 °C for 24 hours under microaerobic conditions. Typical colonies were microscopically examined and tested for catalase reaction.

Detection of *Listeria monocytogenes*: Food samples (25 g) were aseptically inoculated into 225 ml *Listeria* enrichment broth, blended and incubated for 48 hours at 30 °C. Small drops of broth were streaked onto Lithium chloride-phenylethanol-moxalactam agar and Blood agar and then incubated for 24–48 hours at 30 °C. Colonies were streaked for isolation on trypticase soy agar with 0.6% yeast extract and incubated at 30 °C for 24 hours. Typical colonies were examined for biochemical and fermentation reactions.

Detection of *Yersinia enterocolitica*: Food samples were aseptically weighed (25 g), inoculated into 225 ml Peptone sorbitol bile broth, homogenized and incubated at 10 °C for 10 days. On the tenth day, 0.1 ml of enrichment was transferred to 1 ml of 0.5% potassium hydroxide and into 0.5% saline, and mixed for 5–10s. One loopful was streaked onto MacConkey agar plate and one loopful to Cefsulodin-irgasan-novobiocin agar plate. The selected colony was inoculated into lysine arginine iron agar slant, triple sugar iron agar slant by streaking slant and stabbing butt, Christensen's urea agar slant, bile esculin agar plate and incubated for 48 hours at room temperature. Also two MR-VP media (Methyl Red and Voges-Proskauer broth) were inoculated, one at room temperature for 3 days and the other at 35–37 °C for 24 hours. For confirmation of cultures presumptively identified as *Yersinia*, *Yersinia* serological test was performed. API 20E system was used for biochemical identification.

Detection of *Salmonella* spp.: Food samples were aseptically weighed (25 g), inoculated into 225 ml of Selenite F broth, homogenized and incubated at 35 °C for 18–24 hours. One loopful was transferred to *Salmonella-Shigella* selective agar plate, one to Wilson–Blair bismuth sulfite agar plate, and then they were incubated for 24–48 hours at 35 °C. Typical colonies were transferred onto triple sugar iron agar slant by streaking slant and stabbing butt, and incubated at 35 °C for 24–48 hours. For confirmation of cultures presumptively identified as *Salmonella*, biochemical and serological tests were performed.

Serotyping of the tested pathogens was not performed.

A statistically significant difference between percentages of contaminated samples of various food groups with examined pathogen microorganisms using Student's t-test was calculated.

RESULTS

The laboratory investigation showed that 21 samples (8.17%), out of the total of 267 tested samples, were contaminated by *Campylobacter* spp., *Salmonella* spp., or *Listeria monocytogenes* except for one sample that was contaminated with two species of the tested pathogens (*Campylobacter* spp. and *Salmonella* spp.). *Yersinia enterocolitica* was not identified in any of the tested samples.

The frequency of contaminated fresh meat samples with one of the three identified pathogens was 22.2%, and frequency of contaminated ready-to-eat food samples was 1.19%. The established difference was statistically significant ($p < 0.05$), (Table 1).

Campylobacter spp.

Campylobacter spp. was identified in 11 samples (18.3%) of poultry meat and in 3 samples (10.0%) of other fresh meat. A difference was statistically not significantly recorded ($p > 0.05$) (Table 2).

Table 1. Number of contaminated samples with one of the tested pathogens

Food	Total number of samples	Number of contaminated samples	
		n	%
Fresh meat	90	20	22.2
Ready to eat food	167	2	2.0

t = 4.5; df = 255; p<0.05

Table 2. *Campylobacter* occurrence in fresh meat samples

Food	Number of tested samples	Samples contaminated with <i>Campylobacter</i> spp.	
		n	%
Fresh poultry meat	60	11	18.3
Fresh meat ready made for grilling	30	3	10.0
Total	90	14	15.6

t = 1.1; df = 88; p>0.05

This bacteria was identified in one sample (0.6%) of ready-to-eat food (cheese salad, a topping for fast food).

Salmonella spp.

Salmonella spp. was determined in 2 samples (3.3%) of fresh poultry meat (Table 3).

Table 3. *Salmonella* occurrence in fresh meat samples

Food	Number of tested samples	Samples contaminated with <i>Salmonella</i> spp.	
		n	%
Fresh poultry meat	60	2	3.3
Fresh meat ready made for grilling	30	0	0.0
Total	90	2	2.2

t = 1.4; df = 88; p>0.05

This bacteria was identified in one sample (0.6%) of ready-to-eat food (white soft cheese),

Listeria monocytogenes

Listeria monocytogenes was determined in 3 samples (5.0%) of fresh poultry meat and in one sample (3.3%) of other types of fresh meat. Difference was not statistically significant (Table 4).

This pathogen was not identified in any ready-to-eat food sample.

Yersinia enterocolitica

Yersinia enterocolitica was not identified in any of the controlled sample.

Table 4. Occurrence of *Listeria monocytogenes* in fresh meat samples

Food	Number of tested samples	Samples contaminated with <i>Listeria monocytogenes</i>	
		n	%
Fresh poultry meat	60	3	5.0
Fresh meat ready made for grilling	30	1	3.3
Total	90	4	4.4

t = 0.4; df = 88; p>0.05

DISCUSSION

Epidemiological data have implied that exposure to food of animal origin is a major risk factor for campylobacteriosis, salmonellosis, listeriosis and yersiniosis. The majority of experimental investigations indicated an exponential like dose-dependent association between the number of live cells of pathogenic microorganisms in consumed foodstuffs and risk for occurrence of intestinal infectious diseases (15–18). These results emphasized the importance of clear identification and control of factors that can contribute to survival and growth of microorganisms. Implementation of risk analysis method appeared to be the most appropriate tool that links presence of pathogen microorganisms in food with public health outcome (3, 15–18).

According to data published in the last two decades, *Campylobacter* spp. was a very frequent causative agent of foodborne diseases (19–23). It was identified in various kinds of animal foodstuffs, and as a great problem for meat industry, particularly for chicken meat industry (23–28). Our results are in line with these investigations. Obtained results indicated that *Campylobacter* spp. was the most frequently isolated pathogen of all tested samples. Samples of fresh meat were most frequently contaminated with this pathogen. There was no recorded statistically significant difference between number of samples of fresh poultry meat and other fresh meat as to their contamination with *Campylobacter* spp. This pathogen was also isolated from one sample of ready-to-eat-food (cheese salad topping for fast food). It is difficult to speculate about the real reason of contamination of this particular food sample. *Campylobacter* could originate from raw unpasteurized milk and cheese (24, 25, 29), or secondary contamination due to poor handling practice (30, 31).

In this investigation, *Listeria monocytogenes* was isolated from samples of fresh poultry meat and fresh ready-made grilling meat. These results are in line with the results of other investigations (32, 33). *Listeria monocytogenes* was not identified in tested samples of “chilled” foodstuffs such as various kinds of soft cheese, dairy products and fast food toppings as it was expected (34, 35, 36). The possible reasons for the absence of *Listeria monocytogenes* in 25 g of the tested samples were consequence of either good thermal treatment (pasteurization or refrigeration) of ready to eat food, or absence of food samples with long self life (vacuum packaged food stuffs) (34, 36, 37).

Salmonella spp. was the most commonly identified causative agent of food borne diseases in many countries including Serbia and PV (10). This pilot investigation indicated that *Salmonella*

spp. was not the most frequently isolated bacteria compared to *Campylobacter spp.* and *Listeria monocytogenes*. The main source of *Salmonella spp.* was fresh poultry meat, as it was expected (16, 22, 38). This pathogen was also identified in one sample of fresh white cheese. This kind of ready-to-eat food can be placed on the market as a white cottage cheese produced from raw, unpasteurised milk. *Salmonella spp.* isolated from one sample of fresh white cheese can originate from primary contamination of raw milk, or can be a consequence of secondary cross-contamination due poor hygienic practice during manufacturing process or/and bad handling at a retail place (39, 40).

Although *Yersinia enterocolitica* is a ubiquitous microorganism, the pig is the major reservoir of the human foodborne pathogen strains (41). According to published data, *Yersinia enterocolitica* was isolated from chicken carcasses (42), pasteurized milk and ice-cream (43, 44), which were also foodstuffs controlled in our pilot investigation. In this pilot investigation, *Yersinia enterocolitica* was not detected in any of the tested samples. One can speculate that in this pilot investigation relatively small number of fresh pork meat samples was examined. The other reason could be that the investigation was performed during warm seasons, summer and autumn. *Yersinia enterocolitica*, as a psychotropic microorganism, grows better under lower environmental temperature, although some papers reported that it was detected during summer and autumn period (43, 45). An additional reason could be that a culture method was applied for *Yersinia enterocolitica* isolation (14). Low rate of isolation of *Yersinia enterocoliticae* in clinical material, foodstuffs and environment has been attributed to limited sensitivity of classical culture methods, compared to the PCR ones (46, 47).

Data obtained throughout the official reporting system on infectious diseases in PV indicate that food is an important route of transmission of *Salmonella spp.*, *Campylobacter spp.*, *Yersinia enterocolitica* and *Listeria monocytogenes* (8, 9, 10). A systematic investigation, that could support exposure assessment of the population with examined pathogens through food, has not been performed in AP. This pilot study was an initial attempt to gather data relevant for further investigations with the more specific goals.

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