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REPRESENTATION OF CAMPYLOBACTER SPP. IN MEAT AND MEAT PRODUCTS IMPORTED INTO R. MACEDONIA

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Abstract

Campylobacteriosis is the most frequently reported zoonosis in the EU and the epidemiology of sporadic campylobacteriosis, especially the routes of transmission, is to a great extent unclear. Poultry easily become colonized with Campylobacter spp., being symptom-less intestinal carriers. Earlier it was estimated that internationally between 50% and 80% of the cases could be attributed to chicken as a reservoir. Campylobacter spp. is the leading bacterial cause of diarrhea in human population in all parts of the world. In most cases the infection with Campylobacter spp. in humans comes from contaminated chicken meat and products from chicken meat. This paper aims to design and to assess the prevalence of Campylobacter spp. in meat and meat products imported into the Republic of Macedonia. Among the analyzed samples, the highest prevalence of Campylobacter spp. was determined in chicken - sample 1 with 84% positive samples, followed by chicken - sample 2 with 81.8%, pork by 10%, and in beef and smoked beef the presence of Campylobacter spp. was not established. The overall prevalence of Campylobacter spp. in all tested meat samples was 55.36%.

From the results obtained it can be concluded that due to the high prevalence in the examined samples and the severity of the disease in humans, the industry for the production and processing of poultry meat and poultry meat products, as well as food operators, are required to consider Campylobacter spp. in assessing risks in the course of making the HACCP plan.

Key words: Campylobacter spp., campylobacteriosis, chicken meat, pork, beef, smoked beef, contamination.

Introduction

Campylobacter spp. is the second most important cause of acute gastroenteritis in humans in Europe. Systems for rapid reporting and warning RASAFF confirm the existence of 55.745 human campylobacterial cases with an incidence of 67.5 cases an 100. 000 inhibitions in Germany. In the Netherlands it is estimated that 80,000 cases of human campylobacteriosis occur annually.

The number of cases is increasing steadily, and *Campylobacter jejuni*, together with *Campylobacter coli* are considered triggers of more than 98% of infections with *Campylobacter spp.*

In the European Union (EU) the incidence of thermotolerant *Campilobacter* infection in humans continues to increase (infection in humans continues to increase, European Food Safety Authority (EFSA) 2005 and European Food Safety Authority (EFSA) 2005. The number of case has now overtaken that for *salmonella species,* European Food Safety Authority (EFSA) 2005. *Campylobacter jejuni* is the most common cause of campylobacteriosis encountered in the European Union, followed by *Campylobacter coli,* Nadeau et al. 2002, Nielsen et al. (1999). In the United States in 2004, the incidence of infection in 100 000 people was 12.9, Centers for Disease Control and Prevention (CDC) 2005, while in the EU in the same year the rate was 41.3 cases per 100 000 people, European Food Safety Authority (EFSA) 2005.

Studies to identify risk factors have confirmed that contact with animals and the consumption of contaminated food (poultry meat, raw milk and contaminated water) are the principal sources of human infection are the consumption of poultry meat. The risk increases when products are consumed away from home, Allerberger et al. (2003), Friedman et al. (2004), Pearson et al. (2000).

Levels of Campylobacter contamination reported in the literature show a wide range of values which, although generally low, are not comparable as they are affected by different sampling methods and by the different poultry samples analyzed, Lake et al. (2003). A 2001 survey in the United Kingdom found that 63.7% of poultry samples may have a contamination level of 1-10 colony-forming units (cfu)/ cm^2 corresponding to about 2-20x10^4 cfu per carcass, Lake et al. 2003.

In the U.S. *Campylobacter spp.* Causes about 2.5 million cases per year (or 12.4% of all defined diseases from pathogens originating from food) and is responsible for 124 deaths. In developed countries, the infection is hyperendemic in young children under 5 years of age.

In recent decades, consumers have been looking for fresh and minimally processed foods. Minimal processing is the application of methods of preservation with minimal thermal processing and without adding preservatives. That is why this pathogen spread in human population through consumption of undercooked poultry, pork, beef, unpasteurized milk, contaminated drinking

water and faeces from infected animals. Food of animal origin, raw milk, pork and lamb meat, seafood, especially poultry meat and its products pass these zoonotic pathogens M/O.

The infectious dose of *Campylobacter spp.* in humans is low and it amounts to approximately **500 bacterial** cells. Thermo tolerant types *Campylobacter spp.* as *Campylobacter jejuni, Campylobacter coli* and *Campylobacter lari*, do not grow and multiply in food or in the vicinity of temperatures lower than 25°C. *Campylobacter spp.* is sensitive to heat and is inactivated by pasteurization.

Campylobacter jejuni grows between 30 and 45°C, with ph 5,5 - 8,0 in the presence of more than 1,75 % NaCl, the most optimal temperature is 40°C, which is close to the body temperature of poultry (about 41°C), one of the most important hosts. It is microaerophilic so that growth can be interrupted in the presence of 21% oxygen. It conserves cellular energy through aerobic respiration and produces oxidase and catalase to neutralize toxic oxygen components. If *Campylobacter jejuni* is inoculated in vacuum packed cooked turkey meat, the number of bacteria is reduced but some remain able to grow another 28 days at a temperature of 4°C.

Infection with *Campylobacter spp.* in domestic animals is widespread. It has been found in the gastrointestinal tract and faecal material in many animal species such as poultry, cattle, pigs and sheep. Infection in humans is primarily caused by thermophilic species such as *Campylobacter jejuni and Campylobacter spp.* Several cases of illnesses associated with the consumption of chicken meat that has not been fully and properly cooked have been proven. In other cases it is assumed that a cross-contamination of chicken meat with *Campylobacter spp.* occurred. Cross-contamination of other foods from raw chicken meat is also taken into account in other cases. Manipulation of raw poultry meat and consumption of undercooked poultry meat are identified as risk factors for campylobacteriosis.

The high prevalence of *Campylobacter spp.* in fresh poultry meat and meat products is an important factor for the occurrence of infections with *Campylobacter spp.* Of particular importance is to assess the prevalence and number of *Campylobacter spp.* in poultry meat in order to have an insight in the risk of infection by this pathogen and to prevent food poisoning caused by contaminated food products.

Materials and methods

Material: *The research for detecting* and determining the prevalence of *Campylobacter spp.* was conducted on 56 samples:

11 samples fresh poultry meat, 25 samples of poultry meat freed from bones, 10 samples fresh pork, 5 samples fresh beef, and 5 samples of products made from smoked beef.

All samples were imported into the Republic of Macedonia from different countries and they were included in the survey randomly regardless of the country of origin.

Methods: Standard reference methods ISO 6778-2 and ISO 10272 were used for the isolation and identification of *Campylobacter spp.* in this research.

The samples obtained for testing were kept in a refrigerator at +4°C by the beginning of the analysis. A 25 g sample was taken in an aseptic manner and transferred to a sterile Stomacher bag. The sample with selective liquid medium for enrichment (Preston broth) was processed in Stomacher for 60 seconds at medium speed in order to achieve greater homogeneity. The resulting suspension was sealed in a Stomacher bag with a plastic zipper seal, with previous extrusion of almost all the air from the bag. Incubation was performed at 42°C microaerophilic atmosphere for 18 hours.

After incubation expiration, the enrichment medium was inoculated with an inoculation loop with a volume of 10 microliters on the surface of the first selective medium for isolation, Karmali agar. The second chosen selective medium for isolation was treated in the same way.

In this research we also used a third selective solid medium for cultivation Charcoal cephoperazon desoxycholate agar (CCDA). The plates were incubated at a temperature of 42°C in microaerophilic atmosphere in sealed plastic containers and a generator for microaerophilic atmosphere was added (approximately 5% O_2, 10% CO_2, 85% N_2) CampyGen (Oxoid). After 24 and 48 hours incubation, the plates were examined for the presence of characteristic colonies of thermo tolerant *Campylobacter spp.*

Pure colonies that were supposed to be *Campylobacter spp.* positive were Gram stained and observed under a phase microscope to see the characteristic spiral movement. Then 26 biochemical testing of the production of catalase and oxidase, hydrolysis of hippurate, sensitivity to nalidexic acid and cephalothins and seeding of TSI agar were performed. As a control strain was used *Campylobacter spp.* from R. Macedonia.

Results

From 55.3% in all samples of examined meat and meat products the individually perceived prevalence was 83.3% (30 of 36) in fresh poultry meat samples and meat freed from bones, and 10% 91 if 10) in pork samples, 0% (0 of 5) in frech samples and 0% (0 of 5) in beef products.

Species	Chicken meat	Meat freed from bones	Pork	Beef	Smoked beef
Campylobacter jejuni	6 (66,6%)	15 (71,4%)	1 (10%)	0 (0%)	0 (0%)
Campylobacter coli	3 (33,3%)	6 (28,5%)	0 (0%)	0 (0%)	0 (0%)
Total					
Campylobacter spp.	9 (81,8%)	21 (84%)	1 (10%)	0 (0%)	0 (0%)
Tested samples	11	25	10	5	5

Table 1. Representation of *Campylobacter spp.* in certain kinds of meat

In fresh poultry meat *Campylobacter jejuni* was frequently isolated 66.6% (6 of 9) compared with *Campylobacter spp.* 33,3%. Identical is the case with the prevalence in meat freed from bones where *Campylobacter jejuni* was isolated 71.4% compared with *Campylobacter coli* 28.5% (6 of 21). *Campylobacter jejuni* was isolated in only one sample of pork. In beef and smoked beef *Campylobacter spp.* was not detected.

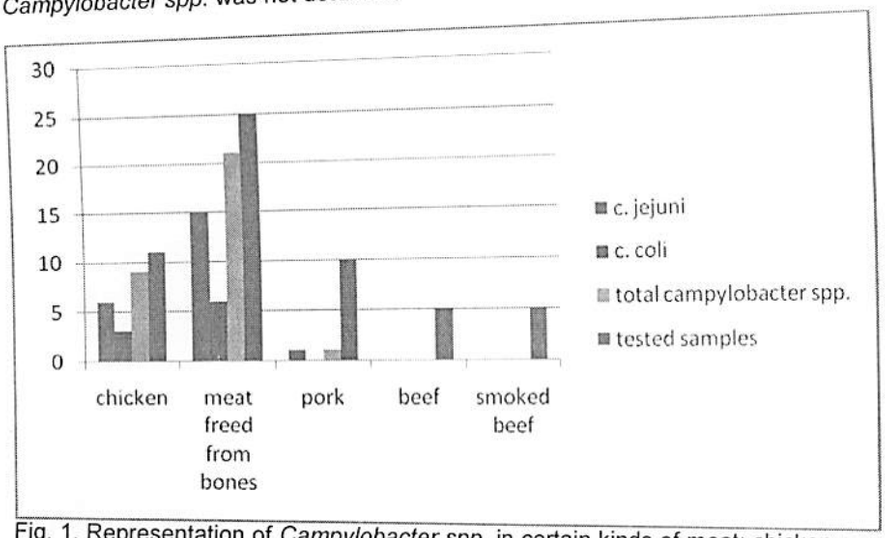

Fig. 1. Representation of *Campylobacter spp.* in certain kinds of meat: chicken meat, meat freed from bones, pork, beef, smoked beef.

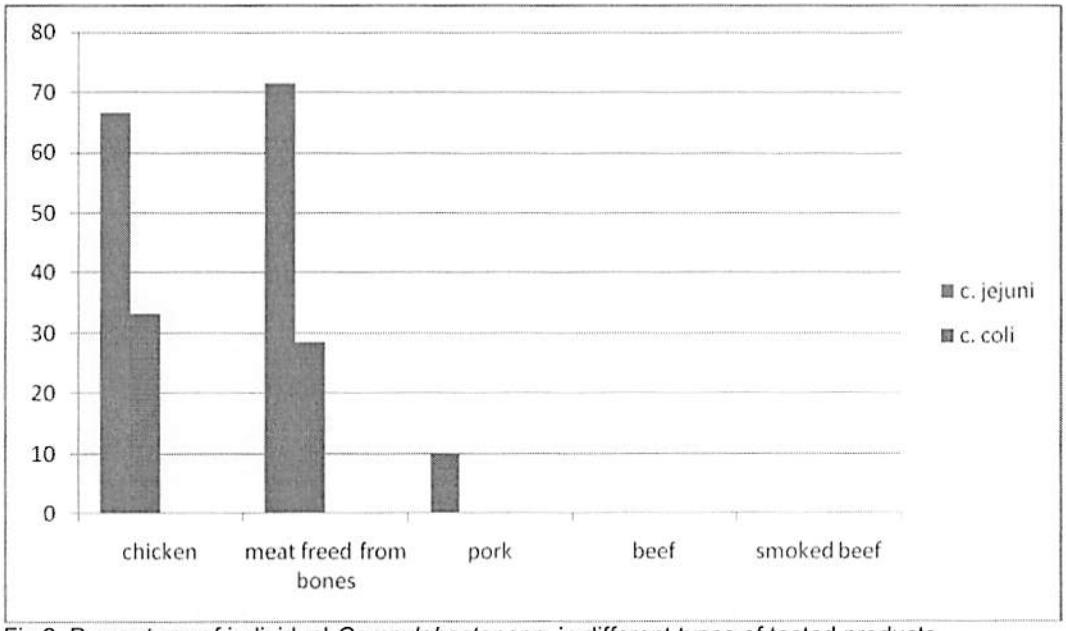

Fig 2. Percentage of individual *Campylobacter spp.* in different types of tested products

Discussion

According to the results obtained it can be concluded that the main source of introduction and spread of contamination by *Campylobacter spp.* In the food chain is poultry meat and raw materials from fresh poultry meat. The proof of it is the prevalence of *Campylobacter spp.* in samples of fresh poultry meat and meat freed from bones, which is 81.8% and 84%.

In beef and beef products from smoked meat which were selected for testing by random method the presence of *Campylobacter spp.* has not been established, pointing to the fact that these foods do not present a significant source of infection or contamination.

Accordingly, undercooked poultry meat treated is most at risk for cross-contamination of food and infection of humans. The percentage of meat freed from bones where *Campylobacter spp.* was detected is particularly high.

As the risk of consuming fresh foods of animal origin in correlation with the emergence of human campylobacteriosis is evident, we need to know the ways to reduce and eliminate the risk. The risk of occurrence of disease can be avoided by consuming thoroughly heat processed red meat, chicken meat and seafood, water from reliable sources, and consumption of pasteurized milk. Proper practical handling of food is important both at home and in facilities where food is prepared.

Conclusion

The overall prevalence of *Campylobacter spp.* in examined samples is 55.36%. From the testing it can be concluded that due to low prevalence in the examined samples and the severity of the disease in humans, industry production and processing of poultry meat and food operators have to consider *Campylobacter spp.* in their assessment of the risks during the preparation of the HACCP plan.

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