

IMPORTANT STEPS FOR QUALITATIVE RISK ASSESSMENT OF CAMPYLOBACTER PRESENCE IN POULTRY MEAT IN SERBIA

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Review paper

Abstract: The aim of this paper was to answer some questions important for a qualitative risk assessment in poultry production related to *Campylobacter*. These steps are described: hazard identification, hazard characterisation, exposure assessment and risk characterization. Hazard identification: *Campylobacter* is important cause of food borne diseases. Hazard characterization: human campylobacteriosis is assumed to be dose-independent based on some studies on healthy human volunteers. Exposure assessment: *Campylobacter* colonisation and spread of *Campylobacter* on poultry carcasses were described. Our results indicate high prevalence of *Campylobacter spp.* in intestines of poultry, the clinical symptoms of campylobacteriosis usually depends of the age and additional immunosuppressive factors, such as *Salmonella*. According to our experimental results artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after 5 days, with slight decrease in next 20 days and return to starting level in next 20 days. According to our experimental results, slaughtering of *Campylobacter* positive flock appears where 100% birds (with 3.02 log cfu/g feces) is contaminated leads to 50% contaminated carcasses. High prevalence of *Campylobacter spp.* was also found on poultry carcasses in our slaughterhouses. Risk characterization: on the basis of the performed examinations it can be concluded that even there is high exposure of human population to *Campylobacter* from poultry meat; the incidence of human campylobacteriosis is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis.

Key words: risk assessment, *Campylobacter*, poultry

Introduction

Although various foods can serve as a source of food borne illness, meat and meat products are important sources of *Campylobacter*. The *Campylobacter* is most frequently reported cause of zoonotic diseases in EU in 2007, incidence was 45.2 cases per 100 000 population *EFSA* (2009, 2010). This bacteria is widespread within the poultry production in Europe. *C. jejuni* is frequent commensal in poultry and cattle, and *C. coli* in swine and poultry. The most common way people become infected with zoonotic enteric pathogens is through the ingestion of food contaminated with animal feces (contamination usually occurs during processing).

An understanding of thermophilic *Campylobacter* and specifically *C. jejuni* in broiler chickens is important from public health. The food safety risk analysis usually is used as a tool for the control of biological, chemical and physical hazards associated with foods. Risk analysis comprises of three functions: risk assessment, risk management and risk communication. Risk assessment can be quantitative and qualitative.

The aim of this paper was to answer some questions important for a qualitative risk assessment in poultry production related to *Campylobacter*. The qualitative risk assessment attempts to understand how the incidence of human campylobacteriosis is influenced by various factors during poultry breeding and broiler meat production “from farm to table”. In the estimation of risks, the following steps are usually involved: hazard identification, hazard characterization, exposure assessment and risk characterization. During the research *Campylobacter* colonisation and spread of *Campylobacter* on poultry carcasses were examined.

Materials and methods

Experimental design: The control group (group A) consisted of 30 chickens which were not artificially infected with *Campylobacter*. The group B consisted of 32 chickens which were artificially infected with 6.77 log cfu *C. jejuni* ATCC 29428 per chicken on day 21 of life. Total count of *Campylobacter* was examined in chicken feces five times in the following twenty days. The chickens were slaughtered in the poultry abattoir on day 42 of life (10 chickens from the control group and 10 from the infected group). The prevalence of *Campylobacter* on carcasses and livers were determined immediately after processing.

The identification and total number of *Campylobacter* was determined according to procedures to the standard ISO 10272 and ISO 4833 procedures.

Results and discussion

Hazard identification

The genus *Campylobacter* now comprises 17 member species most of which are microaerophils, i.e. grow preferentially in low oxygen concentrations. The majority of cases of human campylobacteriosis are caused by two species: *C. jejuni* and the closely-related *C. coli*. These two species are often referred to as the “thermophilic” *Campylobacter* as they grow preferentially at 42°C *EFSA* (2009, 2010). Thermophilic *Campylobacter spp* are a leading cause of zoonotic enteric disease in most developed countries. They are usually indirectly transmitted to humans through the consumption of contaminated food. The principal reservoir of these organisms is the digestive tract of food producing animals. In the vast majority of cases, the organisms are constantly shed in feces by asymptomatic animals *Stojanov et al.* (2011). Handling raw poultry and eating poultry products are important risk factors for sporadic campylobacteriosis *EFSA* (2010). In our study hazard was identified as risk of human campylobacteriosis associated with thermophilic *Campylobacter* in poultry meat.

Hazard characterization

Hazard characterization provides a description of the public health outcomes following infection, including sequelae, pathogen characteristics influencing the ability of organism to elicit infection and illness, host characteristics that influence the acquisition of infection, and food-related factors that may affect the survival of *Campylobacter* in the human gastrointestinal tract.

Food poisoning data usually are obtained after an outbreak but in the case of campylobacteriosis there is no necessary data from outbreak, because this disease occurs in sporadic cases.

Until the mid of XX century there was a strong belief that human infections caused by campylobacteria are rare, and the organism was considered an opportune human pathogen. The researches dating from last 30 years established the importance of *Campylobacter* species in the pathogenesis of human diseases *Stojanov et al.*(2008a). Human campylobacteriosis occurs sporadically, as small-scale family infections or epidemics in particular communities. Campylobacteriosis is characterized by poor general health status of the patient, recurrent high body temperature, tremor, headache, abdominal pain, vomiting and diarrhea. Occurrence and severity of these symptoms is determined by the virulence of the organism and by the immune status of the patient. Results of some recent research revealed that campylobacter infections mostly affect the newborns and children, as well as patients with an immune deficiency syndrome or malignant diseases of the immune system *Zonios et al.* (2005) and *EFSA* (2005).

Human campylobacteriosis is assumed to be dose-independent based on some studies on healthy human volunteers *WHO* (2002).

Exposure assessment

Risk models which estimate the exposure to *Campylobacter* from poultry meat include two important steps at beginning: flock prevalence and concentration of campylobacter on poultry carcasses before cooling.

Exposure assessment at farm level

The occurrence of zoonotic pathogens at farm varies depending on the range of factors including the organism, geographical factors, production practice. Some animal production conditions facilitate the spread of bacteria, like poor infection control *Stojanov et al.* (2008a). *Campylobacter* colonisation in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalence from 18 to 90%. Higher prevalence was obtained in southern countries *Barrios et al.* (2006).

Initial introduction of *Campylobacter* into poultry flock still remains poorly understood and the phenomenon may be multi-factorial. *Campylobacter* usually colonise chickens in the third week of life. The level of colonisation and the spread of *Campylobacter* between animals depends on different factors: breeding conditions, flock size, hygiene measures, carry over from previous flock, air and water contamination, immune response of animals, other infected livestock on the farm, mechanical transmission via insects and birds *Barrios et al.* (2006); *Stojanov et al.* 2011).

According to our experimental results *Stojanov et al.* (2011) infection of the one day old chickens induces diarrhea, while infection of the three days old chickens with 10^9 cfu does not induce diarrhea. If diarrhea occurs, it is usually 6 hours after infection and lasts for 10 days. The distal part of intestines usually are affected (jejunum and cecum), pathological changes are not characteristic and are described as enlargement of the intestine, as a consequence of accumulation of the water and jelly content inside the intestine. Hemorrhages are not always present.

According to our experimental results *Stojanov et al.* (2011) artificial infection of chickens with 4 log cfu *C. jejuni* and 4 log cfu *Salmonella enteritidis* per chicken on day 14 of life leads to watery diarrhea and traces of blood in first two weeks after the infection. Campylobacteriosis can be expected if an additional factor is present, which affects the immune system and its ability to cope with the infection.

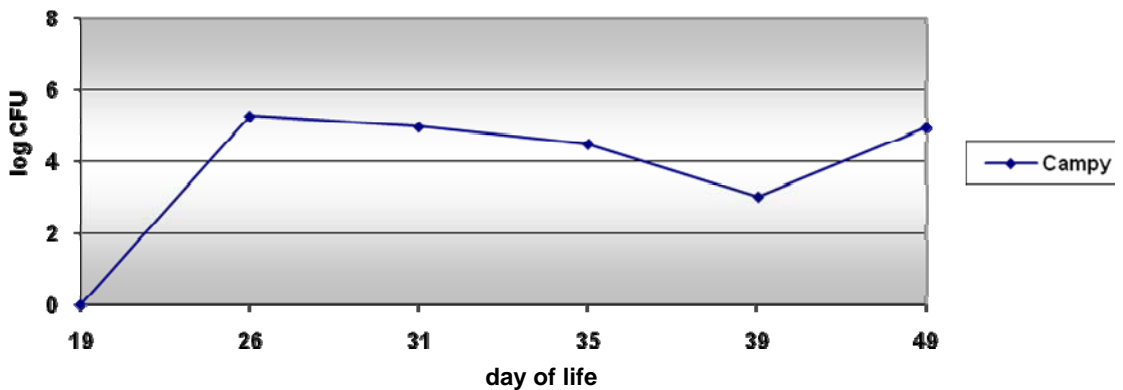
The clinical symptoms usually depend of the strain, number of bacteria, stress and immune suppression *Kazwala et al.* (1992). In spite of widespread nature of infections caused by *Campylobacter*, the clinical symptoms and pathology changes caused by these bacteria are rather rare. Table 1 presents findings of *Campylobacter spp.* in intestines from poultry, pigs and calves in Serbia.

According to our quantitative examinations of *Campylobacter* contamination and spread in poultry flock, artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after

only 5 days, with a tendency to decrease 4.97 log cfu/g (31st day of life), 4.49 log cfu/g (35th day of life) and 3.02 log cfu/g (39th day of life). After this day the increase in *C. jejuni* count was noticed 4.95 log cfu/g (49th day of life). Tendency of decrease in *Campylobacter* count was found also in the work of *Van Boven et al. (2003)*. The results are shown in Graph 1.

Table 1. The findings of *Campylobacter spp.* in the samples originating from poultry, pigs and cattle *Stojanov et al. (2008b)*

Material	No of samples	<i>C. jejuni/coli</i> positive	%
Poultry	60	44	73.33
Pigs	12	7	58.33
Calves	6	4	66.66



Graph 1. Average number of *C. jejuni* log cfu/g feces by days of life

In Slovenia a nation-wide genotyping study was performed by PFGE to determine the genetic profiles of 500 isolates of *C. jejuni* obtained from humans (n=156), animals (n=133) and food (n=214). The isolates exhibited marked genetic diversity, however, identical PFGE profiles were identified for animal, human and food isolates, indicating the animals as a direct infection source for humans. The isolates from animals of the same owner, bred in different time periods, exhibited different PFGE profiles suggesting the *C. jejuni* strains do not persist on the farm *Ocepek et al. (2011)*.

Exposure assessment at slaughterhouse

According to our experimental results the prevalence of *Campylobacter* - contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g feces) to 50% of chicken carcasses - Table 2.

The *Campylobacter* can be transmitted to the production facility and can contaminate the processing environment and the final product. According to our results Petrović et al. (2007a, b, c) the occurrence of *Campylobacter* is very frequent in poultry carcasses in slaughterhouses in Vojvodina region (Table 3). The influence of production management is great, since in poultry abattoirs the prevalence varies from 11.43 to 90.00% carcasses Petrović et al. (2008a, b, c). The prevalence of *Campylobacter* positive carcasses increased during evisceration and decreased upon the method of chilling.

Table 2. Prevalence of *Campylobacter* on broiler carcasses

Sample	No samples	Group A control <i>C. jejuni</i> posit (%)	Group B infected <i>C. jejuni</i> posit (%)
Liver	10	0 (0.00%)	5 (50.00%)
Carcase	10	0 (0.00%)	5 (50.00%)
Total	20	0 (0.00%)	10 (50.00%)

Table 3. Occurrence of *Campylobacter* in poultry samples Petrović et al. (2007a,b,c)

Sample		Abattoir mark/ Prevalence of <i>Campylobacter</i> (%)						
		A	B	C	D	E	F	G
	liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71
	carcasse	90.00	14.28	51.43	20.01	68.57	11.43	31.43

Risk characterization

This step links the probability and magnitude of exposure to *Campylobacter* associated with consumption of meat to adverse outcomes that might occur. *Campylobacter* is frequently found in feces of live animals in poultry farms. Overall *Campylobacter* contamination decreased through processing with temporary increases occurring during poultry transport and evisceration. According to the Report of the Institute of public health of Serbia »dr Milan Jovanović Batut«, in Serbia in 2010 there were 357 cases of *Enteritis Campylobacterialis* diagnosed

with the incidence of 4.88. Incidence in Vojvodina was between 5,22 in 2007 and 11,25 in 2008. *Hrnjak Cyjetković et al. (2011)*. While in the EU in 2005 the incidence rate of 38.2-51.6 cases per 100 000 population was noticed. But despite high exposure of population to *Campylobacter* in Serbia, the incidence of human campylobacteriosis from raw meat is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis. *Enteritis Campylobacterialis* is a sporadic disease; symptoms usually do not require hospitalisation and many sick people do not go to the doctor and only small number of ill people has laboratory confirmation of campylobacteriosis. Also significant factor of exposure to the decrease are cooking habits in Serbia: meat is usually well cooked.

Conclusions

Hazard identification: thermophilic *Campylobacter spp* are important cause of zoonotic enteric illness. Hazard characterization: it is assumed to be dose-independent based on some studies on healthy human volunteers. Exposure assessment: Initial introduction of *Campylobacter* into poultry flock still remains poorly understood and the phenomenon may be multi-factorial. The clinical symptoms usually depend of the age of chickens, stress and immune suppression. Campylobacteriosis can be expected if an additional factor is present, such as *Salmonella* which affects the immune system and its ability to cope with the infection. The high prevalence of *Campylobacter spp.* in intestines from poultry, pigs and calves was found. The artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after 5 days, with slight decrease in next 20 days and return to starting level in next 20 days. Also the prevalence of *Campylobacter* -contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g feces) to 50% of chicken carcasses. Identical PFGE profiles were identified for animal, human and food isolates, indicating the animals as a direct infection source of *Campylobacter* for humans. Risk characterization: even there is high exposure of human population to *Campylobacter* in Serbia; the incidence of human campylobacteriosis from raw meat is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis.

Acknowledgement

Research was financed by the Ministry of Education and Science, Republic of Serbia project TR31071.

Neophodni koraci za kvalitativnu procenu rizika od prisustva kampilobakterija u mesu živine u Srbiji

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Rezime

Cilj ovog rada je da odgovori na neka od pitanja koja su neophodna da bi se izvršila kvalitativna procena rizika vezana za kampilobakterije u proizvodnji brojlera u Srbiji. Opisani su sledeći koraci: identifikacija hazarda, karakterizacija hazarda, procena izloženosti i karakterizacija rizika. Identifikacija hazarda: kampilobakterije su važan uzročnik alimentarnih oboljenja. Karakterizacija hazarda: na osnovu studija na zdravim dobrovoljcima pretpostavlja se da je humana kampilobakterioza nezavisna od koncentracije kampilobakterija u hrani. Procena izloženosti: opisana je kroz ispitivanja kolonizacije kampilobakterijama kod živine širenje na klanično obrađene trupove. Naši rezultati ukazuju na visoku prevalencu kampilobakterija u crevima živine, klinički simptomi kampilobakterioze obično zavise od starosti i dodatnih imunosupresivnih faktora, kao što je prisustvo *Salmonella*. Prema našim eksperimentalnim rezultatima veštačka infekcija pilića sa 6.77 log cfu *C. jejuni* 21. dana života nakon pet dana dovodi do kolonizacije od 5.26 log cfu/g fecesa, sa laganom tendencijom opadanja u narednih 20 dana i povratkom na početni nivo u sledećih 20 dana. Prema našim eksperimentalnim rezultatima, klanje jata u kom je prevalenca kampilobakterija 100% (sa 3.02 log cfu/g feces) dovodi do pojave 50% kontaminiranih trupova. Ustanovljena je visoka prevalenca termofilnih *Campylobacter spp.* na klanično obrađenim pilićima u našim klanicama. Karakterizacija rizika: na osnovu izvršenih ispitivanja u našoj zemlji može se zaključiti da iako je visoka izloženost humane populacije kampilobakterijama iz živinskog mesa, incidenca humane kampilobakterioze je mala, ovakvi podaci prvenstveno ukazuju na nedovoljnu evidenciju i potvrdu humane kampilobakteroze.

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