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CAMPYLOBACTER SPP. IN SOME RAW MATERIALS OF ANIMAL ORIGIN

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ABSTRACT

The aim of this work was to assess contamination level of meat, available at the retail market in Szczecin, with *Campylobacter* spp. In total, 172 samples, including 65 poultry, 57 pork and 50 beef half-carcasses were tested. Campylobacters were isolated from 73.8; 66.7 and 66.0% of smear samples tested, respectively. Numbers of campylobacters on poultry were by one order of magnitude higher than on pork and beef half-carcasses and exceeded 10^3 CFU per $1cm^2$ of skin.
Campylobacter co*li, Campylobacter lari/Campylobacter cryoaerophila* and *Campylobacter jejuni* dominated in poultry samples while pork and beef carcasses were contaminated mostly with *Campylobacter upsaliensis/ Campylobacter coli* and *Campylobacter coli*, respectively.

Key words: campylobacters, beef, pork, poultry, retail market

INTRODUCTION

Campylobacters are presumed to be one of the main causes of acute gastroenteritis in humans lately, with *Campylobacter jejuni*/*Campylobacter coli* being implicated in most of the documented cases (Atabay and Corry, 1998; Franco, 1988; Griffiths et. al., 1990; Scotter et.al., 1993). Pathogenicity of other *Campylobacter* species is less pronounced, though some of them were isolated from particular gastroenteritis cases. Among less frequently identified and isolated species were such enteric pathogens as e.g.: *Campylobacter lari, Campylobacter upsaliensis, Campylobacter fetus* ssp. *fetus, Campylobacter concisus, Campylobacter cryaerophila*, *Campylobacter hyointestinalis* or *Campylobacter sputorum* (Atabay and Corry, 1998; Borczyk et.al., 1987; Griffiths and Park, 1990).

Although poultry was found to be the main source of campylobacteriosis in humans, campylobacters were isolated from milk, water, shellfish, faeces of wild and domestic animals, etc. (Abeyta et al., 1993; Atabay and Corry, 1998; Borczyk et.al., 1987; Gluender and Peterman, 1989; Kotula and Pandya, 1995; Steele et al., 1997; Wallace et al., 1998).

Numerous publications confrmed presence, mainly, of *Campylobacter jejuni*/ *Campylobacter coli* in different types of raw meats and products of animal origin. (Fernandez and Pison, 1996; Madden et al., 1998; Manzano et al., 1995; Willis and Murray, 1997; Vanderlinde et.al., 1998)

The aim of this work was to determine whether and to what extent poultry, bovine and porcine meat, available at retail market in Szczecin of the Western Pomerania district origin, are carriers of *Campylobacter* spp. and which species dominate in the particular environment.

MATERIALS AND METHODS

The subject of surveys were fresh poultry and half-carcasses of bovine and porcine meat available at the retail market in Szczecin. Samples were collected directly from shops on the day of delivery. In total 172 samples were collected, including 65 of poultry, 57 of porcine and 50 of bovine ones.

The samples were collected by swabbing a defined area of meat carcasses/parts with a sterile gauze and transfering immediately into a screw caped bottles containing Preston broth with Campylobacter Growth Supplement (SR 84 E Oxoid) in. The swabbed areas covered 10 cm^2 for poultry or 25 cm^2 for pork and beef half-carcasses. The method accuracy was 1CFU/10 cm^2 for poultry and 1CFU/25 cm^2 for pork and beef samples. The analysis took place within 2 hours after sampling. The contamination level was estimated both, by direct plating and enrichment techniques.

Direct plating was carried out on modified CCDA medium (CM 739 Oxoid) supplemented with selective agent SR 155E (Oxoid). Initial and serial decimal dilutions were plated on selective medium and incubated at 37^{o}C, under microaerophilic atmosphere for 48h. Suspected colonies were counted. Three of each colony type were selected at random, and transfered, paralelly, on Campylobacter Agar Base - CAB (CM 689 Oxoid) with deffibrinated horse blood (SR 48 Oxoid) and selective agent (SR 117E Oxoid), on CCDA medium and Brain Heart Infusion Agar - BHIA (CM 375 Oxoid) and incubated under the above mentioned conditions. Parallel transfers onto BHIA plates were incubated with access of O_2. Strains growing on the media under microaerophilic conditions and not growing in O_2 atmosphere were subjected to primary identification including cell morphology, Gram-staining, oxidase and catalase test (Scotter i wsp., 1993). Gram negative, oxidase positive rods growing on the

above mentiioned media under limmited O_2 tension were presumed to be *Campylobacter* and identified for the species level by API Campy tests (bioMerieux).

For enrichment purposes a selective agent SR 155E (Oxoid) was added to preincubated, initial suspension in the Preston medium. After 24-48 h of selective enrichment at 37°C a multiplied material was spreaded with the loop, over the CCDA medium as to obtain single colonies.

For samples giving negative results in the direct plating method, plates were examined for the presence of suspected colonies. In the case of samples giving positive results in direct plating method, plates were checked only for the types of colonies not present before. In both cases identification procedure was conducted according to the above mentioned scheme.

RESULTS AND DISCUSSION

On the basis of the obtained results, the presence of campylobacters was confirmed in 73.8% of poultry samples and on 66.7% of pork and 66.0% of beef half-carcasses, from the retail market in Szczecin (Table 1).

Table 1. Contamination level of poultry, pork and beef half-carcasses with Campylobacter ssp.

Sample type	No. of samples tested	Positive samples [%]	Contamination level (CFU of *Campylobacter spp.*/cm^2) Number of samples with defined contamination level						Isolated species*
			<1/10 cm^2	1/10cm^2-<1/cm^2	1-10	11-100	101-10^3	>10^3	
Hen	3	3 (100)	-	1	-	-	-	2	C.coli
Chicken	42	30 (71.4)	12	2	6	13	9	-	C. lari/C. cryoaerophila C. hyointestinalis, C. fetus ssp. fetus
Turkey	20	15 (75.0)	5	3	3	4	4	1	C. jejuni, C. coli
Poultry	65	48 (73.8)	17	6	9	17	13	3	C.coli, C. lari/C. cryoaerophila, C. jejuni, C. hyointestinalis, C. fetus ssp. fetus
Sample type	No. of samples tested	Positive samples [%]	Contamination level (CFU of *Campylobacter spp.*/cm^2) Number of samples with defined contamination level						Isolated species*
			<1/25 cm^2	1/25cm^2-<1/ cm^2	1-10	11-100	101-10^3	>10^3	
Pork	57	38 (66.7)	19	12	8	13	5	-	C. upsaliensis/C. coli C. fetus ssp.fetus, C. jejuni
Beef	50	33 (66.0)	17	2	12	16	3	-	C. coli, C. upsaliensis, C. jejuni

* in order of dominating species

Isolation frequency of campylobacters, similar to that noted elsewhere for the poultry meat, was much higher for the pork and beef carcasses, when compared with the data presented e.g. for Ireland (Madden et al., 1998), Australia (Vanderlinde et al., 1998) or Belgium (Korsak et al., 1998).

According to Madden et al. (1998), lamb and beef carcasses from the abbatoirs in Northern Ireland were free of campylobacters. However, retail packs of chicken parts, collected from the lical market for over a one year period, were contaminated with campylobacters in 38%.

Quality assessment of beef carcasses produced in Australia both for domestic market and export, confirmed campylobacters to be present respectively in 0.81 and 0.16% of the tested samples (Vanderlinde et al., 1998).

Contamination level of pork and beef carcass meat, collected from nine Belgian slaughterhouses estimated by Korsak et al. (1998) was not high, either Campylobacters were isolated from 2.0 and 10.0 % of porcine and bovine meat samples, respectively.

Tissue samples as the subject of studies, in most cited cases, were, obviously, less contaminated than the skin ones. Besides the analysis directed to *Campylobacter jejuni/coli* itself could have lowered greatly the numbers of campylobacter positive samples.

Pork and beef carcasses from retail market in Szczecin were contaminated mostly with *Campylobacter coli* and *Campylobacter upsaliensis* while *Campylobacter jejuni* was isolated only from 11% of porcine and 16.5% of bovine campylobacter positive samples.

Data presented by Uradzinski et al., (1987) pointed out to *Campylobacter coli* as predominating species in pigs, with *Campylobacter jejuni* being a dominanting species in beef cattle.

Faecal samples from dairy cows and calves, examined by Atabay and Corry (1998), were campylobacter positive in 37.5 to 79%. Incidence freqency was herd dependant, with most of the animals being carriers of just one species. The dominating species were identified as *Campylobacter sputorum* and *Campylobacter hyointestinalis.* Presence of *Campylobacter jejuni* subsp. *jejuni* was less pronounced, and confirmed only in 7% of the tested animals.

Presented results are an indirect evidence for possible qualitative differences in campylobacter species dominating on porcine/bovine and fresh poultry meats.

In contrast to the beef cattle, poultry was considered to be the main reservoir of *Campylobacter jejuni*. Apart from confirmed predominance of *Campylobacter jejuni/coli* on fresh poultry meat, isolation frequency of campylobacters ranged, due to the country of origin, time of the year, subject of analysis and isolation method chosen, from 0 to 100% (Anonim, 1995; Flynn et al., 1994; Kotula and Pandya, 1995; Manzano et al., 1995; Uyttendaele et al., 1996; Wallace et al., 1998; Willis and Murray, 1997; Varga, 1997).

Manzano et al., (1995) emphasised the relation ship between the isolation frequency and type of the sample. Pericloacal skin samples were contaminated in 100% while back skin samples in 40%, only. Nevertheless *Campylobacter jejuni* predominated in both cases.

Turkeys population examined by Wallace et al., **(**1998) was campylobacter positive in 100% and numbers of *Campylobacter jejuni* in fresh faecal samples of healthy individuals exceeded 10^7 CFU per 1g.

Surveys conducted by Kotula and Pandya (1995) confirmed high contamination of poultry with *Campylobacter* jejuni/coli. The contamination level noted for broiler chicken carcasses and parts followed a similar pattern for *Salmonella* spp. and *Campylobacter jejuni/coli* and

ranged, for the latter, from 6.1 to 7.2 lg_{10} per g for 61.5% of the breast skin samples and 72.5% of chicken feet samples tested.

Numbers of campylobacters on poultry meats from the retail market in Szczecin though on average, by one order of magnitude higher than on pork and beef half-carcasses of the same origin, not exceeded 10^4 CFU per 1 cm^2 of skin. In 62.5% of positive poultry samples contamination ranged from 10^1 to 10^3 CFU per cm^2 (Table 1) and was much lower than that noted by others. In 55 and 85% of positive porcine and bovine samples respectively, the contamination level ranged from 10^0 to 10^2 CFU per 1 cm^2.

Qualitatitve structure of contamination also differed. In our experiment campylobacter species contaminating poultry depended visibly on type of the sample. *Campylobacter jejuni* predominated on the turkey parts, chicken carcasses were contaminated mostly with *Campylobacter lari/Campylobacter cryoaerophila* while *Campylobacter coli* was the only campylobacters representative on hen carcasses (Table 1)

Apart from undoubtfull dominance of *Campylobacter jejuni* on poultry samples. Uyttendale et al. (1998) also confirmed presence of other campylobacters such as *Campylobacter coli*. *Campylobacter lari* and unidentified species. respectively. in 3.75; 3.12 and 1.25% of poultry samples tested.

Both. unfavorable conditions for survival of *Campylobacter jejuni* on the skin surface and possible secondary contamination could affect structure of campylobacter species present in tested samples. Besides. variability in species predominating in various raw meat materials could reflect diversity of microorganisms (campylobacters included) typical for the place of breeding. etc.

CONCLUSIONS

1. Poultry. pork and beef samples. available at retail market in Szczecin.s were contaminated with campylobacters in 73.8; 66.7 and 66.0%. respectively.
2. Numbers of campylobacters. highest on poultry/hens and turkeys. exceeded 10^3 CFU per 1 cm^2 of skin and were by one order of magnitude higher than on pork and beef half-carcasses.
3. Campylobacter species dominating on poultry were *Campylobacter coli. Campylobacter lari/ Campylobacter cryoaerophila* and *Campylobacter jejuni*
4. Pork and beef carcasses were contaminated mostly with *Campylobacter upsaliensis/ Campylobacter coli* and *Campylobacter coli*. respectively.

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