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Fish microbiology

CAMPYLOBACTER SPP. IN FRESHWATER FISHES

BAKTERIE Z RODZAJU *CAMPYLOBACTER* W RYBACH SŁODKOWODNYCH

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The aim of this work was to assess to what extend freshwater fishes are carriers of *Campylobacter* spp. and what species dominate in this environment.

Analysis of 106 alimentary canals representing 13 freshwater fish species originated from 5 different water bodies confirmed *Campylobacter* spp. presence in 8.5% of the samples tested. Numbers of campylobacters did not exceeded 10 CFU/g. The dominating species being *C. coli*.

INTRODUCTION

Campylobacters are presumed, lately, to be one of the most often identified causes of gastroenteritis in humans, in developed countries. Presence of *C. jejuni* and *C. coli* was confirmed in various environments. Though with various frequency, they were isolated from saline waters, deposits and surface waters of freshwater basins and sewages (Bolton et al. 1982; Jones et al., 1990a, b, c; Stelzer and Jacob 1991; Krogulska and Maleszewska 1994; Arvantidou et al. 1995a, b; Fransen et al. 1996; Hernandez et al. 1996; Andersson et al. 1997).

Few publications, confirming presence of *Campylobacter* spp. in crustaciens and molluscs (Reinhard et al. 1995; Wilson and Moore 1996; Abeyta et al. 1997; Van Doorn et al. 1998), pointed out to their linkage with particular cases of campylobacteriosis in humans (Abeyta et al. 1993). So far, however, there was no evidence for the presence of *Campylobacter* spp. in fish.

The aim of this work was to state whether and to what extent fishes are carriers of *Campylobacter* spp. and what species are representative for this environment.

MATERIAL AND METHODS

The subject of surveys were alimentary canals of fresh fishes, from five different freshwater bodies, including fish cultured in the warmwater channel of the "Dolna Odra" power station.

In total 106 alimentary canals of thirteen freshwater fish species were tested both, by a direct plating method and enrichment technique (Tab. 1).

Quantitative analysis was conducted by direct plating of diluted (1:5) and homogenised, with buffered peptone water, alimentary canals onto modified CCDA medium (CM739 Oxoid) supplemented with selective agent (SR 155E Oxoid). followed by incubation of the plates at 37°C for 48 h under microaerophilic atmosphere. Different morphological colonies were counted separately. Three of each colony type were selected, at random, and transferred, paralelly, on Campylobacter Agar Base—CAB (CM 689 Oxoid) with defibrinated 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 conditions mentioned above. Parallel transfers on BHIA plates were incubated under O_2 atmosphere. Strains growing on the media under microaerophilic conditions and not growing under O_2 atmosphere were subjected to primary identification including cell morphology, Gram-staining, oxidase and catalase test (Scotter et al. 1993).Gram negative, oxidase positive rods growing on the above mentioned media under limited O_2 tension were presumed to be *Campylobacter* and identified to the species level by API Campy tests (bioMerieux).

Simultaneously a qualitative analysis was carried out. For enrichment purposes 5 ml of initial dilution of each sample was transferred to 5 ml of double strength Preston broth supplemented with selective agents (SR 84; SR 155E Oxoid). After 24–48 h of selective enrichment at 37°C a multiplied material was spreaded out on CCDA medium as to obtain single colonies. For samples negative in direct plating method, types of colonies growing were characterised and identified according to the above mentioned scheme.

RESULTS AND DISCUSSION

Conducted surveys confirmed *Campylobacter* spp. presence in alimentary canals of nine freshwater fishes, representing four of the thirteen tested freshwater fish species, namely in perch (*Perca fluviatilis*), bream (*Abramis brama*), roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophtalmus*) (Tab. 1). Fishes carrying campylobacters originated from two of five different water basins, that is the Szczecin Lagoon and Dąbie lake. Noted differences could reflect differences in contamination level of the water bodies, the fishes originated from.

Table 1

Campylobacter spp. presence in freshwater fishes

Fish species	Water body	Time of capture	Presence of *Campylobacter* spp.		Isolated strains
			Quantitative analysis *	Qualitative analysis *	
White bream (*Blicca bjöerkna*)	Warmwater canal power station "Dolna Odra"	Aug. 96	0 / 3	0 / 3	—
Carp (*Cyprinus carpio*)		Aug. 96	0 / 3	0 / 3	—
Carp (*Cyprinus carpio*)		Sept. 98	0 / 10	0 / 10	—
Tench (*Tinca tinca*)		Aug.. 96	0 / 3	0 / 3	—
Perch (*Perca fluviatilis*)	Szczecin Lagoon	Nov. 96	0 / 20	4 / 20	*C. coli*
Pike-perch (*Stizostedion lucioperca*)		Dec. 96	0 / 10	0 / 10	—
Pike-perch (*Stizostedion lucioperca*)		Apr. 97	0 / 10	0 / 10	—
Bream (*Abramis brama*)		Apr. 97	0 / 10	2 / 10	*C. coli, C. upsaliensis*
Roach (*Rutilus rutilus*)	Dąbie lake	Apr. 97	0 / 2	1 / 2	*C. coli*
Asp (*Aspius aspius*)		Apr. 97	0 / 3	0 / 3	—
Rudd (*Scardinius erythrophtalmus*)		Apr. 97	0 / 1	1 / 1	*C. coli*
Bream (*Abramis brama*)		Apr. 97	0 / 1	1 / 1	*C. coli*
Rainbow trout (*Oncorhynchus mykiss*)		Apr. 97	0 / 2	0 / 2	—
Ide (*Leuciscus idus*)		Apr. 97	0 / 1	0 / 1	—
Crucian carp (*Carassius carassius*)		Apr. 97	0 / 1	0 / 1	—
Rainbow trout (*Oncorhynchus mykiss*)	Łożnia river	Dec. 97	0 / 5	0 / 5	—
Pike (*Esox lucius*)	Vistula river (Bydgoszcz)	Mar. 98	0 / 21	0 / 21	—

* number of positive samples / number of samples tested

Relation between contamination level of water basin and campylobacters presence in the environment was suggested by Brennhovd et al.(1992); Krogulska and Maleszewska (1994).

According to Brennhovd et al.(1992) campylobacters were recovered from 22.2 to 71.4% of surface water samples collected from three water sources in Norway, with higher numbers of positive samples noted for water basins with higher pollution and eutrophication.

Isolation frequency of campylobacters from Vistula River surface waters ranged from 27 to 55%, with higher number of positive results noted for waters of the 3rd purity class and beyond the class (Krogulska and Maleszewska 1994).

Apart from the isolation frequency contamination level of water environment with campylobacters also differed. Due to the type of water basin, water purity or time of the year, numbers of campylobacters in water samples ranged from <1.1/100 ml (Stelzer and Jacob 1992) to > 240/100 ml (Stelzer et al. 1989).

Campylobacter presence in water, in opinion of most of the cited authors comes, primarily, from defined sources. The main sources of such contamination being wild birds, untreated or only partly treated sewages, farm run-offs and other types of impurities washed out by the heavy rainfalls (Bolton et al. 1982; Jones et al. 1990a, b, c; Abeyta et al. 1993; Arvanitidou et al. 1996a, b; Fransen et al.1996; Hernandez et al. 1996; Popowski et al. 1997).

Presence in water environment means, usually, contamination of living resources within the environment.

Surveys conducted on estuarine filter-feeders, from industrial cultures, confirmed campylobacters presence in shellfish. According to Endtz et al. cited by Van Doorn et al. (1998), *Campylobacter* spp. were isolated from 80% batches of mussels and 26.8% batches of oysters grown in The Netherlands.

Analysis of 331 marine bivalve molluscs, such as: cockles (*Cardium edule*), mussels (*Mytilus edulis*) and scallops (*Pecten maximus*), collected from authorised harvesting beds, let to identify *Campylobacter* spp. in 47.1% of the samples tested (Wilson and Moore 1996). Depurated and ready to eat oysters (*Crassostrea gigas*) from the same source were contaminated with campylobacters in 6.1%.

Fresh blue crab meat samples collected from twelve different blue crab processing facilities analysed for campylobacters by Reihard et al. (1995) were contaminated with *Campylobacter jejuni* and *Campylobacter coli* in 15.0 and 5.8%, respectively.

A rather low number of positive samples in total number tested (8.5%), in own experiment, can signify, that contamination levels of the fishes' natural water environments were relatively low, if any. Equally low were the campylobacter numbers found in alimen-

tary canals of the tested fishes. In all positive samples numbers of *Campylobacter* spp. did not exceeded 10 CFU/g.

Still lower were campylobacter numbers of water-borne raw materials noted by Teunis et al. (1997) and Fernandes et al. (1997).

According to Teunis et al. (1997), though the numbers of *Campylobacter* spp. in molluscs meat samples were lower than 1 CFU/g, were, nevertheless, presumed higher than for the oysters.

None of two hundred and forty 25 g samples of fresh fillets of aquaculture channel catfish (*Ictalurus punctatus*), collected from three catfish processors in the south-eastern United States and tested by Fernandes et al. (1997) was positive for *C. jejuni / coli*.

With generally low contamination level of water environment and its living resources with campylobacters, a threat to consumer's health from this source, although less pronounced when compared with other sources of inland origin, such as poultry, milk or beef cattle, is still possible.

A laboratory experiments carried out by Minet et al. (1995) proved concentrations of *Campylobacter jejuni* and *C. coli* in digestive tracts of mussels to be up to 100 to 500 folds the concentrations of contaminated filtered water. It is worth mentioning, that, after a 6 days' experiment, numbers of viable *C. jejuni* and *C. coli* cells had not changed, though there was a drastic decrease in numbers of culturable *C. jejuni* cells. At the same time *C. coli* cells surviving a 6 days experiment in seawater and mussels maintained intact virulence factor (Minet et al. 1995).

Campylobacter coli happened to be the dominating species in the alimentary canals of the freshwater fishes tested, in own experiment (Tab. 1). Bream from the Szczecin Lagoon, was the only fish species, where, apart from *C. coli*, *C. upsaliensis* was isolated from the alimentary canals samples.

None of the tested fish species harboured *C. jejuni* at the level detected by the method applied.

Surveys addressed to various water basins and water-borne organisms, conducted by others, confirmed domination of *C. jejuni* and *C. coli* in most of the tested environments, mainly because most of the works done in the field was directed initially on thermotolerant *C. jejuni* and *C. coli* only. Few included other *Campylobacter* species than just *C. jejuni* and *C. coli* in such environments.

According to Endtz et al. cited by Van Doorn et al. (1998), *Campylobacter lari* was the species dominating among campylobacters contaminating batches of molluscs and oysters grown in The Netherlands.

Wilson and Moore (1996) isolated different strains of campylobacters from marine bivalve molluscs, collected from authorised harvesting beds in Northern Ireland. Urease-

positive thermophilic campylobacters (UPTC) dominated among the isolates (57%), with *C. jejuni* isolated much less frequently (2.0%).

It is quite possible for atypical species of campylobacters to be typical for the water environments and its inhabitants. *C. jejuni*, being a consequence of an inland origin contamination, dies off quickly and is less frequently represented in water environments.

CONCLUSIONS

1. Presence of campylobacters, at the level detected by the applied method, was confirmed in alimentary canals of 8.5% freshwater fish, representing four of thirteen fish species tested.
2. Numbers of campylobacters were lesser than 10 CFU per 1g of alimentary canals of the fish.
3. *C. coli* was the dominating campylobacter species in the alimentary canals of fishes tested.
4. None of the fish was contaminated with *C. jejuni*, at the level detected by the applied method.

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BAKTERIE Z RODZAJU *CAMPYLOBACTER* W RYBACH SŁODKOWODNYCH

STRESZCZENIE

Celem pracy było określenie, w jakim stopniu ryby słodkowodne mogą być źródłem infekcji oraz które gatunki *Campylobacter* dominują w tym środowisku. Analizie poddano łącznie 106 przewodów pokarmowych 13 gatunków ryb słodkowodnych z pięciu różnych zbiorników wodnych. Obecność bakterii z rodzaju *Campylobacter*, na wykrywalnym zastosowaną metodą poziomie, potwierdzono w 8,5% przebadanych ryb, należących do 4 gatunków, tj. krasnopióry, leszcza, okonia i płoci. Liczebność Campylobakterii w 1 g przewodu pokarmowego ryby nie przekraczała poziomu 10 JTK. Gatunkiem *Campylobacter* dominującym w zanieczyszczeniu był *C. coli*. W żadnej z prób nie stwierdzono obecności *C. jejuni*.

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