Journal of the Marine Biological Association of the United Kingdom, 2009, 89(6), 1177–1179.
doi:10.1017/S0025315409000447 Printed in the United Kingdom

The effect of infection by *Pomphorhynchus laevis* (Acanthocephala) on the composition of the intestinal bacterial flora in flounder, *Platichthys flesus*

MARTA ZIÓŁKOWSKA-KLINKOSZ[1], JERZY ROKICKI[2] AND ANNA KĘDZIA[1]

[1]Department of Oral Microbiology, Medical University of Gdańsk, ul. Do Studzienki 38 Gdańsk, Poland, [2]Departament of Invertebrate Zoology, University of Gdańsk, Al. Piłsudskiego 46, 81–378 Gdynia, Poland

A total of 112 flounder, Platichthys flesus*, was surveyed for the presence of* Pomphorhynchus laevis *(Acanthocephala). No acanthocephalans were found in 35 fish, 40 fish were infected with 1–5 thorny-headed worms, and in 37 fish the intensity of infection exceeded 6. The fish surveyed were also studied for the composition of the resident microflora of the terminal portion of the intestine—the characteristic site for* P. laevis*. It turned out the number of worms was positively correlated with the bacterial count. The bacteria cultured from the material sampled from intestines of infected- and non-infected fish exhibited similar specific composition and the differences were only in the intensity of individual species. The most frequently isolated bacteria, represented the genera:* Aeromonas, Pseudomonas *and* Shewanella*. The least frequent were species of* Chromobacterium, Escherichia, Providencia *and* Serratia*. The presently reported study results, suggest that bacteria species, constituting physiological bacterial flora, may become, under certain conditions, potentially pathogenic, through substantial increase in their abundance. The factor responsible for disturbing the natural balance in the intestine may be the thorny-headed worm,* Pomphorhynchus laevis.

Keywords: infection, *Pomphorhynchus laevis*, composition, intestinal bacterial flora, flounder, *Platichthys flesus*

Submitted 18 May 2007; accepted 7 January 2009; first published online 15 May 2009

INTRODUCTION

A number of freshwater and marine fish can be definitive hosts for the thorny-headed worm, *Pomphorhynchus laevis*. Within the Polish Exclusive Economic Zone of the Baltic Sea this acanthocephalan has been recorded predominantly from flounder, *Platichthys flesus* (cf. Sulgostowska *et al*., 1987; Rokicki & Morozińska, 1994; Ziółkowska *et al*., 2000). This parasite settles in the lower part of the intestine, perforating its wall with its proboscis. In the course of such action the anterior part of the worm, consisting of the proboscis and bulbous anterior part of the body, protrudes into the peritoneal cavity, leaving the rest of the body within the intestinal lumen. Mass infection of flounder with this acanthocephalan causes not only occlusion of the intestine, but can also be the reason for acute inflammatory changes (Janiszewska, 1938). Physically damaged walls of the lower part of the alimentary tract are convenient sites for establishment and development of various micro-organisms. The bacteria living in flounder have been most frequently collected from skin lesions (Kroon, 1994; Wiklund, 1994). Only sporadically such material has been isolated from the intestine. There has been no conclusive evidence, as to which bacteria, found within the intestine, are pathogenic. Ullrich (1994) believed that bacteria of the genera *Vibrio* and *Aeromonas* constitute natural components of the bacterial flora of this fish. It is commonly known that bacteria of the genus *Vibrio* cause vibriosis and one of the sites, from where they can be isolated, is the intestine. On the other hand, bacteria of the genus *Aeromonas* cause not only pathogenic changes on the external surface of fish, but also in their internal organs. Quite often, the organisms inhabiting the alimentary tract, and believed to be commensals, exhibit their harmful effects only under certain conditions. The above is evident not only for bacteria, but also for fungi, protozoans and worms. The presence of acanthocephalans in the intestine may disturb bacterial equilibrium.

The aim of the present paper was to study the possible effect of the *Pomphorhynchus laevis* infection and the composition of the host fish's intestinal bacterial flora.

Corresponding author:
J. Rokicki
Email: rokicki@univ.gda.pl

MATERIALS AND METHODS

A total of 112 flounder, *Platichthys flesus*, was surveyed between May and December 2005. The fish, measuring 25–37 cm, were caught at the Łeba fishing grounds (ICES subdivision 25, statistical rectangles O, N-7). Each batch of the fish was refrigerated and promptly delivered to the laboratory. The fish were examined for possible presence of thorny-headed worms and for inflammatory changes. The worms were identified and counted.

The contents of the lower intestine of the fish were sampled and placed in brain–heart infusion (BHI) broth. Then, they were passed through dilution series. The suspended material, prepared in such way, was inoculated on nutrient agar and nutrient agar with blood. After 72 hours of incubation at room temperature, the bacterial colonies were counted and Gram-stained preparations were made. The isolated bacteria were inoculated on 5% of blood agar, MacConkey agar, and Hektoen agar. Individual species of bacteria were identified with the aid of API 20E biochemical diagnostic tests (BioMérieux), following the manufacturer's instructions.

RESULTS

The infection levels of flounder with *Pomphorhynchus laevis* were variable and a number of fish did not harbour that parasite (Table 1).

The highest number of *P. laevis*, recorded in the lower portion of the intestine was 24.

The fish infected with the acanthocephalan often had a hyperaemic lower portion of the intestine and proctitis.

There was a relation between the total number of *P. laevis* and the overall bacterial count in the intestine (within the proposed infection-level groups) (Table 2).

We determined that the number of bacteria, occurring in the intestine, increases with the number of parasites living there. Moreover, the genera and species of those bacteria, grown from the samples taken from intestines of infected- and non-infected fish, were similar and they differed in the prevalence of infection within three earlier defined infection-level groups (Table 3).

The most frequently isolated bacteria, represented the genera: *Aeromonas*, *Pseudomonas* and *Shewanella*. The least frequent were species of *Chromobacterium*, *Escherichia*, *Providencia* and *Serratia*.

The fish infected with parasites showed a two-fold increase in the intensity of *Aeromonas hydrophila*/*sobria*/*caviae* gr. 2, *Burkholderia cepacia*, *Chryseobacterium indologenes*, *Ch. meningosepticum* and *Stenotrophomonas maltophilia*, compared with the parasite-free fish. On the other hand, the fish without acanthocephalans harboured more frequently *Pantoea* spp. and *Providencia stuartii*.

Table 1. The infection levels of flounder (N = 112) with *Pomphorhynchus laevis*.

Flounder	Number of acanthocephalans found		
	0	1–5	≥6
Number of fish	35	40	37

Table 2. The overall bacteria count of the lower part of the flounder intestine, in relation to the number of *Pomphorhynchus laevis* acanthocephalans.

Micro-organisms	Number of acanthocephalans		
	0	1–5	≥6
Mean number of bacteria in the lower part of flounder intestine	1×10^6	1×10^8	1×10^9

Table 3. Per cent of fish infected with the studied bacteria species, within three groups of flounder, infected with *Pomphorhynchus laevis.*

Micro-organism	Number of acanthocephalans in fish intestine (N)		
	0	1–5	≥6
	Per cent of fish infected (in groups)		
Aeromonas hydrophila gr 1	46	55	68
Aeromonas hydrophila/*sobria*/*caviae* gr 2	26	33	54
Alcaligenes spp.	26	28	30
Burkholderia cepacia	3	5	14
Chromobacterium violaceum	–	3	–
Chryseobacterium indologenes	20	33	43
Chryseobacterium meningosepticum	17	23	35
Escherichia vulneris	–	3	–
Flavimonas oryzihabitans	31	25	24
Hafnia alvei	6	5	–
Ochrobactrum anthropi	14	15	11
Pantoea spp.	14	8	3
Pasteurella pneumotropica/haemolytica	54	45	43
Proteus vulgaris	6	5	5
Providencia stuartii	6	–	–
Pseudomonas aeruginosa	11	8	5
Pseudomonas fluorescens/putida	11	10	14
Pseudomonas spp.	40	55	59
Serratia liquefaciens	–	3	–
Serratia plymuthica	3	3	–
Shewanella putrefaciens	80	85	100
Stenotrophomonas maltophilia	14	45	51
Vibrio alginolyticus	6	5	5
Vibrio fluvialis	6	5	3

DISCUSSION

The present study demonstrated that the number of the acanthocephalans, occurring in the lower part of the intestine of flounder, had an effect on the quantities of the bacteria present there. An increase of the acanthocephalan number resulted in an increase of the bacteria numbers within the intestine. The reason behind that fact may be the damage to the intestinal wall, inflicted by the proboscis of the acanthocephalan. When, the proboscis of the acanthocephalan penetrates the intestinal wall further, it results in formation of inflammatory changes, visible as hyperaemia of the tissues (Wanstall *et al.*, 1988). The above-mentioned authors observed that the inflamed tissues contained granulocytes and phagocytes of the hosts and at the same time the integument of *P. laevis* was intact. The inflammation inflicted by acanthocephalans, and the related decrease of the fish defence mechanisms, stimulate additional bacteria build-up. High numbers of the acanthocephalans, anchored in the intestinal wall, cause an occlusion of its lumen and compromise its functions. This way the acanthocephalan introduces bacteria to the blood vessels and the peritoneal cavity.

Aeromonas hydrophila was present not only in the infected fish, but also in those without the presence of *P. laevis*. The percentage of fish, however, where these bacteria are present, increased with the rising number of the acanthocephalans. A similar tendency could also be observed in relation to bacteria representing the genera: *Pseudomonas*, *Alcaligenes*, *Chryseobacterium*, as well as *Shewanella putrefaciens* and *Stenotrophomonas maltophilia*.

https://doi.org/10.1017/S0025315409000447 Published online by Cambridge University Press

Aeromonas hydrophila is a Gram-negative rod-like bacterium, widely distributed in the aquatic environment. It is an opportunistic micro-organism capable of causing pathologic changes in both freshwater (Shao *et al.*, 2004) and marine fish (Lilley *et al.*, 1997). It has been found in pathological changes of the skin, ulcers, infected liver and kidneys (Johnson, 1993). The pathological changes are more likely to occur, when the fish shows decreased immunity levels, e.g. during spawning or under influence of external stress factors.

Aeromonas hydrophila has been considered a prime infection factor (Shome & Shome, 1999) or a bacterium responsible for continuing pathological processes (Thampuran *et al.*, 1995).

Shewanella putrefaciens was observed in the majority of the flounder surveyed. It occurred in 80% of non-infected fish and in 100% of the flounder infected with 6 or more specimens of *P. laevis*. Species of the genus *Shewanella* are common in the marine environment. They are often isolated from sick people, who had contact with seawater (Heller *et al.*, 1990; Leong *et al.*, 2000). It is possible that those bacteria constitute a component of physiological bacterial flora of the intestine and are opportunistic micro-organisms that reproduce when the immunity of the organism decreases.

Stenotrophomonas maltophilia is a pathogen capable of infecting people (Robin & Janda, 1996). *Stenotrophomonas maltophilia* has been isolated in cases of bacteraemia, endocarditis, inflammation of central nervous system, dermatitis, ocular inflammation and inflammation of urinary tract (Denton & Kerr, 1998). It naturally occurs in water, soil, on plants, animals and in hospitals (Denton & Kerr, 1998).

Bacteria of the genera *Pseudomonas* and *Chryseobacterium* are very common in nature (water, soil and air). They may also cause infections. Also the percentage of fish with those bacteria, increased with the number of the acanthocephalans.

In both fish groups, those non-infected as well as infected with the acanthocephalans, the bacteria detected were, in most cases, the same (Table 3). Some micro-organism species, however, were more frequent in the fish infected with *P. laevis*. It is possible that the attachment process of those acanthocephalans in the flounder intestine induces some stress. Opportunistic bacteria, which were not pathogenic as yet, increase in their numbers and cause inflammatory changes. At the same time, some species of bacteria, becoming predominant, take over available ecological niches and displace those micro-organisms, which are not so well adapted to the defined conditions.

The specific composition of the intestinal microflora of flounder can also be affected by the external environment, which is related to the pollution level of the Baltic Sea. Some evidence confirming this assumption is the fact that the bacteria species listed in Table 3, except *Chryseobacterium* spp., *Flavimonas oryzihabitans*, *Ochrobactrum anthropi* and *Stenotrophomonas maltophilia* have also been found in municipal sewage or in the air above a sewage treatment plant (Cyprowski *et al.*, 2005, 2006).

REFERENCES

Cyprowski M., Szarafińska-Kraszewska J., Dudkiewicz B., Krajewski J. and Szadkowska-Stańczyk I. (2005) Ocena narażenia pracowników oczyszczalni ścieków na czynniki szkodliwe występujące w miejscu pracy. *Medycyna Pracy* 56, 213–222.

Cyprowski M., Buczyńska A. and Szadkowska-Stańczyk I. (2006) Ocena narażenia na bioaerozole pracowników kanalizacji, *Medycyna Pracy* 57, 525–530.

Denton M. and Kerr K.G. (1998) Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. *Clinical Microbiology Reviews* 11, 57–80.

Heller H.M., Tortora G. and Burger H. (1990) *Pseudomonas putrefaciens* bacteremia associated with shellfish contact. *American Journal of Medicine* 88, 85–86.

Janiszewska J. (1938) Studien über die Entwicklung und die Lebensweise der parasitischen Würmer in der Flunder (*Pleuronectes flesus* L). *Mémoires de L'Académie Polonaise des Sciences et des Lettres* 14, 1–68.

Johnson M. (1993) The veterinary approach to channel catfish. In Brown L. (ed.) *Aquaculture for veterinarians: fish husbandry and medicine.* Oxford: Pergamon Press, pp. 249–270.

Kroon K. (1994) Characterization of the microflora isolated from ulcerated flounder (*Platichthys flesus*) in Estonian coastal waters. Diseases and parasites of flounder in the Baltic Sea. *BMB Publication* 15, 27–30.

Leong J., Mirkazemi M. and Kimble F. (2000) *Shewanella putrefaciens* hand infection. *Australian and New Zealand Journal of Surgery* 70, 816–817.

Lilley J.H., Hart D., Richards R.H., Roberts R.J., Cerenius L. and Soderhall K. (1997) Pan-Asian spread of single fungal clone results in large-scale fish kills. *Veterinary Record* 140, 653–654.

Robin T. and Janda J.M. (1996) *Pseudo-*, *Xantho-*, *Stenotrophomonas maltophilia*: an emerging pathogen in search of a genus. *Clinical Microbiology Newsletter* 18, 9–16.

Rokicki J. and Morozińska J. (1994) Diseases and parasites of flounder *Platichthys flesus* (L.) from the mouth of Vistula River. Diseases and parasites of flounder in the Baltic Sea. *BMB Publication* 15, 57–60.

Shao J., Liu J. and Xiang L. (2004) *Aeromonas hydrophila* induces apoptosis in *Carassius auratus* lymphocytes *in vitro*. *Aquaculture* 229, 11–23.

Shome R. and Shome B.R. (1999) Atypical chronic form of *Aeromonas hydrophila* infection in Indian major carp, *Catla catla* from Andaman. *Current Science* 76, 1188–1190.

Sulgostowska T., Banaczyk G. and Grabda-Kazubska B. (1987) Helminth fauna of flatfish (*Pleuronectiformes*) from Gdańsk Bay and adjacent areas (south-east Baltic). *Acta Parasitologica Polonica* 31, 231–240.

Thampuran N., Surendran P.K., Mukundan M.K. and Gopakumar K. (1995) Bacteriological studies on fish affected by epizootic ulcerative syndrome (EUS) in Kerala, India. *Asian Fisheries Science* 8, 103–111.

Ullrich S. (1994) Epidemiology and aetiology of bacterial flounder diseases in the lower Elbe and Eider (Germany): a comparison of two differently polluted rivers. Diseases and parasites of flounder in the Baltic Sea. *BMB Publication* 15, 31–34.

Wanstall S.T., Thomas J.S. and Robotham W.J. (1988) The pathology caused by *Pomphorhynchus laevis* Müller in the alimentary tract of the stone loach, *Noemacheilus barbatulus* (L.). *Journal of Fish Diseases* 11, 511–523.

Wiklund T. (1994) Skin ulcer disease of flounder (*Platichthys flesus*): a review. Diseases and parasites of flounder in the Baltic Sea. *BMB Publication* 15, 17–26.

and

Ziółkowska M., Chibani M., Kijewska A. and Rokicki J. (2000) The occurrence of *Pomphorhynchus laevis* (Müller, 1776) (Acanthocephala) and the age of the flounder (*Platichthys flesus* L.). *Helminthologia* 37, 159–161.

Correspondence should be addressed to:
J. Rokicki
Departament of Invertebrate Zoology, University of Gdańsk
Al. Piłsudskiego 46, 81–378 Gdynia, Poland
email: rokicki@univ.gda.pl