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Short communication

Yersinia enterocolitica strains isolated from beavers (*Castor fiber*)

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Abstract

Pseudocloacal swabs and palatine tonsils from beavers have been examined for the *Yersinia enterocolitica* presence. Thirty-six samples from 24 beavers were collected and subjected to bacteriological examinations including sero- and biotypisation. Amplicons confirmed by PCR as *Y. enterocolitica* were sequenced. Positive samples originated from 4 out of the 24 beavers (16.7 %) and all the strains belonged to biotype 1A. The study suggested that *Y. enterocolitica* could be isolated from beavers, which may therefore be treated as a reservoir, a significant factor of water contamination and a vector of the *Y. enterocolitica*.

Key words: beavers, sequence, Yersinia enterocolitica, ystA, ystB

Introduction

Yersinia enterocolitica is a food-borne human pathogen isolated from the intestinal tract of many animal species and humans. Based on differences of the antigen O structure, *Y. enterocolitica* was divided into over 70 serotypes belonging to 6 biotypes, 1A, 1B, 2-5 (Bottone 1997). Most of *Y. enterocolitica* strains isolated so far from free-living animals or from the environment belong to biotype 1A, concerned as an nonpathogenic (Bancerz-Kisiel et al. 2012). Currently, strains of that biotype are increasingly often isolated from clinical cases of yersiniosis but the pathogenicity of these strains is difficult to define (Ramamurthy et. al. 1997).

Pigs are considered as the main reservoir of *Y. enterocolitica* (Bottone 1997). Mechanisms of the environmental *Y. enterocolitica* circulation are complicated and not fully known. The participation of other free-living animal species in the dissemination of *Y. enterocolitica* in the environment cannot be excluded. Beavers (*Castor fiber* L.) are among more numerous animal species inhabiting aquatic-terrestrial ecosystems in Europe. There are also few papers, mainly from North America, which attest to the possibility of isolating *Y. enterocolitica* from beavers (Hacking and Sileo 1974, Kaneko and Hashimoto 1981). Moreover, no thorough molecular analysis of *Y. enterocolitica* strains isolated from beavers has been performed.
The aim of the study was to examine pseudocloacal swabs and palatine tonsils from beavers for the *Y. enterocolitica* presence and characteristics of the isolated strains.

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Table 1. Characteristics of *Y. enterocolitica* strains isolated from beavers.

Y. enterocolitica/ NCBI No	Material		Enrichment		Gene			Serotype	Biotype
	Palatine tonsil	Pseudocloacal swab	ITC/CIN	PSB/CIN	*ail*	*ystA*	*ystB*		
1 KJ592623	–	+	–	+	–	–	+	NI	1A
2 KJ592624	+	–	+	–	–	–	+	0:5	1A
3 KJ592625	+	–	–	+	–	–	+	NI	1A
4 KJ592626	+	–	+	–	–	+	NI	1A	
5 KJ592627		+	–	+	–	–	+	NI	1A

NI – Not identified. There was no agglutination with O:3, O:5, O:8 and O:27 diagnostic sera.

Materials and Methods

The material for examination was received from 24 beavers. Pseudocloacal swabs were taken from all beavers and, additionally, palatine tonsils from 12 animals were collected. In total, 36 samples were examined. The animals originated from emergency hunts, ordered by the Regional Director of Environmental Protection, decision no RDOŚ-28-OOP-6631-0007--638/09/10/pj. The experiment complied with the Resolution of the Local Ethics Committee for Animal Experiments No. 11/2010.

Each specimen was taken in duplicate and placed simultaneously in irgasan-ticarcillin-potassium chlorate medium (Biocorp Ltd, Poland), incubated at 25°C for 48 h (ITC) and in bile salts medium, incubated at 4°C for 3 weeks (PSB). The details of the bacteriological procedure and serotype and biotype determination methods were previously described by Bancerz-Kisiel et al. (2012).

Genomic DNA isolation was performed with a Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's instruction and the products were stored at -20°C for further analyses.

Triplex PCR included the amplification of three genes: *ail, ystA*, and *ystB*. The sequences of the primers and triplex PCR conditions were published previously (Bancerz-Kisiel et al. 2012). One modification was applied, temperature of primer annealing was 45°C.

Positive samples were confirmed in PCR with new *ystB* primers: [YSTBF – 5'GGA CAC CGC ACA GCT TAT ATT TT 3', YSTBR – 5' GCA CAG GCA GGA TTG CAA CA 3']. The primers were designed with the gene sequence from the GeneBank (D88145.1), with the primer – BLAST program, available at the NCBI website. The reactions were performed using a HotStarTaq Plus PCR kit (Qiagen, Germany). The 20 µl of the PCR mixture consisted of 4.8 µl of RNase-free water, 10 µl of HotStarTaq*Plus* Master Mix, 2 µl of CoralLoad, 0.1 µl of ystBF (YSTBF) primer (final concentration 0.5 µM), 0.1 µl of ystBR (YSTBR) (final concentration of 0.5 µM) and 3 µl of DNA. The amplicons were purified using a Clean-up purification kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations. Purified amplicons were independently sequenced (Genomed S.A., Poland) in both directions. Phylogenetic analysis was carried out using the freeware Computational Evolutionary Biology MEGA v. 5.2.1. (Tamura et al. 2011).The nucleotide sequences analysed in this study are available in the GenBank [KJ592623-KJ592627].

Results and Discussion

The bacteriological assays revealed the *Y. enterocolitica* presence in 4 out of 24 beavers (16.7%). In total, 5 isolates were obtained. All the strains indicated affinity to biotype 1A (Table 1).

The multiplex PCR revealed the presence of amplicons consistent with the *ystB* gene (180 bp) and associated with pathogenic properties of *Y. enterocolitica* in 5 out of 36 samples (13.9%). The DNA sequencing of the amplicons confirmed affinity of the strains to the *Y. enterocolitica* species.

The phylogenetic analysis is presented in Fig. 1. Nucleotide sequences of *ystB* – GenBank:. KJ592623 and KJ592625 in comparison with *Y. enterocolitica* DNA for Yersinia Heat-stable Enterotoxin Type B, complete cds [Acc. No. D88145] showed 100% sequence identity. Nucleotide sequences of *ystB* GenBank: [KJ592624, KJ592626 and KJ592627] represent a new sequence variants of *ystB* partial cds detected in beavers in Poland.

Beavers have now become a widespread species of rodents in Europe (Kukuła et al. 2008). Our study indicated that isolation of *Y. enterocolitica* from this animal species is possible. Large areas covered by migrating beavers, their amphibious lifestyle and growing populations enable us to presume that, in the context of animal carriers and bacterial excretion, beavers can be a significant factor of water contamination and *Y. enterocolitica* transmission over long distances.

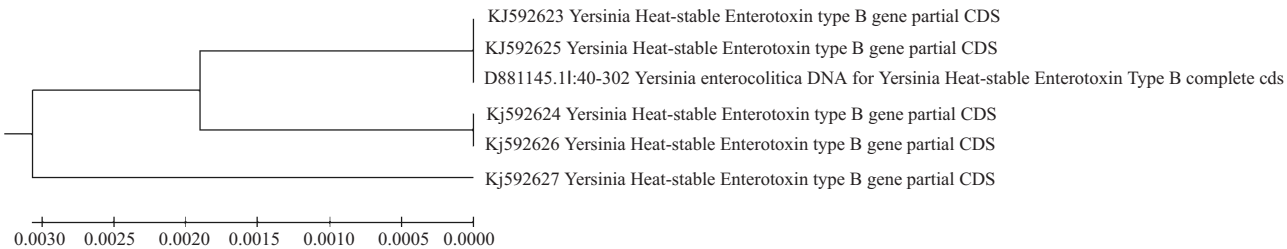

Fig. 1. Evolutionary relationships of taxa.
The evolutionary history was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.00804681 is shown. The analysis involved 6 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 263 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011)

The results of bacteriological examinations showed the presence of *Y. enterocolitica* strains belonging to 1A biotype in the samples. Territoriality and long distance migrations of beavers infected with *Y. enterocolitica* can significantly contribute to the dissemination of the microorganism in the environment, both terrestrial and aquatic. Isolation of *Y. enterocolitica* and the presence of *ystB* gene in the isolated strains suggest their potential pathogenicity and indicate that the beaver can be treated as reservoir and a vector of *Y. enterocolitica* transmission constituting potential source of *Y. enterocolitica* infections as well as a significant factor of water contamination.

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