

PRESENCE AND VIABILITY OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) IN AQUATIC ENVIRONMENTS

AKADEMISK AVHANDLING

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av

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Umeå Universitet

Avhandlingen baseras på följande arbeten:

- I **Å. Lothigius**, A. Janzon, Y. Begum, Å. Sjöling, F. Qadri, A.-M. Svennerholm, I. Bölin
Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area
by real-time PCR.
J Appl Microbiol. 2008;104(4):1128-36
- II **Å. Lothigius**, Å. Sjöling, A.-M. Svennerholm and I. Bölin
Survival and Gene Expression of Enterotoxigenic *Escherichia coli* during Long Term
Incubation in Sea Water and Freshwater.
J Appl Microbiol. 2009; in press
- III B. Hernroth , **Å. Lothigius** and I. Bölin
Factors Influencing Survival of Enterotoxigenic *Escherichia coli*, *Salmonella enterica*
(serovar Typhimurium) and *Vibrio parahaemolyticus* in Marine Environments.
Accepted for publication in FEMS Microbiology Ecology
- IV **Å. Lothigius**, S. Attridge, A.-M. Svennerholm and I. Bölin
Resuscitation of Viable but Non-Culturable Forms of Enterotoxigenic *Escherichia coli*
in an Infant Mouse Model.
Submitted

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ABSTRACT

Presence and viability of enterotoxigenic *Escherichia coli* (ETEC) in aquatic environments

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Enterotoxigenic *Escherichia coli* (ETEC) is one of the major causes of diarrhoea among children in developing countries and in travelers to these regions. The bacteria are spread via contaminated water and food, and surface and drinking water in developing countries has been found to harbor these organisms. The standard methods for detection of ETEC include culturing and subsequent detection of ETEC enterotoxins by ELISA or the corresponding genes by PCR or DNA-DNA hybridisation. Identification of ETEC based on culturing of specimens may be unsuitable when analysing water samples for ETEC since it has been shown that enteric bacteria that enter the stressful environment of water can convert to a dormant, so called “viable but non-culturable” (VBNC) state.

In this thesis we have developed a real-time PCR assay with primers against the enterotoxin genes of ETEC for detection and quantification of ETEC bacteria in different types of water samples. The assay was proven to be highly specific for ETEC and allow detection of as few as three bacteria. The sensitivity was found to be considerably higher compared to phenotypic methods when analysing water samples from an endemic area in Bangladesh, where 67 % of the samples were positive for ETEC with the real-time PCR assay compared to 15 % based on culturing followed by toxin detection with ELISA.

The survival of ETEC bacteria was evaluated in sea- and freshwater microcosm experiments. Clinically isolated ETEC strains were incubated in the different water types for 12 weeks and the morphology, culturability and expression of virulence factors and housekeeping genes were studied over time. We could show that the ETEC bacteria remained intact and expressed virulence and housekeeping genes, despite the fact that all strains in seawater and one of the strains in freshwater were non-culturable *in vitro* using standard culturing media. It was also shown that ETEC was ingested by and survived in mussels at different temperatures indicating that consumption of contaminated seafood might be a route of infection for ETEC.

To show that the VBNC ETEC from the water microcosms were potentially viable the different strains were used to infect infant mice. Results showed that all strains from the seawater microcosms and one of six strains from freshwater were able to regain their culturability in mice suggesting that VBNC forms of ETEC present in water may be infectious. These results may be of importance for public health since previously used diagnostics based on culturing methods cannot detect VBNC forms of the bacteria and hence the risk of getting infected with ETEC might have been underestimated.

Keywords: Enterotoxigenic *Escherichia coli*, ETEC, real-time PCR, aquatic environments, VBNC, resuscitation, infant mouse model

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