# Studies on bacterial transmission pathways in a high endemic area, with a focus on *Helicobacter pylori*

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

I. Lothigius Å., Janzon A., Begum Y., Sjöling Å., Qadri F., Svennerholm A.-M. and Bölin I. Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area by real-time PCR.
*Journal of Applied Microbiology* 2008 104 (4):1128-1136.

II. Janzon A., Sjöling Å., Lothigius Å., Ahmed D., Qadri F. and Svennerholm A.-M. Failure To Detect *Helicobacter pylori* DNA in Drinking and Environmental Water in Dhaka, Bangladesh, Using Highly Sensitive Real-Time PCR Assays.
*Applied and Environmental Microbiology* May 2009 75: 3039-3044

III. Janzon A., Bhuiyan T., Lundgren A., Qadri F, Svennerholm A.-M. and Sjöling Å.. Presence of high numbers of transcriptionally active *Helicobacter pylori* in vomitus from Bangladeshi patients suffering from acute gastroenteritis.
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IV. Janzon A., Svennerholm AM and Sjöling Å.
*Helicobacter pylori* virulence gene expression in a mouse model.
*Manuscript*

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# Studies on bacterial transmission pathways in a high endemic area, with a focus on *Helicobacter pylori*

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## Abstract

Even though half of the world's population is infected with *Helicobacter pylori*, which causes gastritis, peptic ulcer and gastric cancer, the transmission routes of these bacteria remain unknown despite extensive epidemiological studies. Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* are two of the most common causes of acute watery diarrhea in developing countries. The main aim of this thesis was to study transmission pathways of these bacteria, with a focus on *H. pylori*, through analyses of clinical and water samples from Dhaka, Bangladesh, an area with high prevalence of gastrointestinal diseases.

To determine the bacterial numbers in clinical and water samples we developed highly sensitive quantitative real-time PCR assays targeting specific and conserved virulence genes of *H. pylori* (*cagA, flaA, glmM, hpaA, ureA* and *vacA*), ETEC (*eltA* and *estB*) and *V. cholerae* (*ctxB* and *tcpA*). The assays were used for quantification of bacterial DNA and reverse-transcribed gene transcripts.

Twenty-six of 39 (67 %) drinking and environmental water samples from a poor area in Dhaka were positive by real-time PCR for ETEC, whereas all 75 drinking and environmental water and 21 drinking water biofilms from the same location were negative for *H. pylori*, suggesting that ETEC may be waterborne while *H. pylori* is not.

*H. pylori* transmission during epidemics of gastroenteritis was then explored by analyzing vomitus and stool samples collected from diarrhea patients admitted to the ICDDR, B hospital in Dhaka. All samples were positive for *V. cholerae*, with higher numbers in stool (median 2.5 x $10^6$ genomes) than vomitus (median 2.7 x $10^4$ genomes) and a strong correlation between DNA real-time PCR and quantitative culture. Analyses for *H. pylori* showed that 23 of 26 (88 %, median genome number = 4.35 x $10^5$ $ml^{-1}$) vomitus and 17 of 23 (74 %, median genome number = 7.33 x $10^2$ $ml^{-1}$) stool samples were positive in real-time PCR, but *H. pylori* could not be isolated by culture. The results indicate that high numbers of *H. pylori* are shed in vomitus during acute gastroenteric disease and indicate that *H. pylori* may be transmitted by this route. To establish possible infectivity of these bacteria, the gene expression of *H. pylori* in vomitus, stool and biopsies from infected individuals and in in vitro cultures was analyzed. Vomitus, biopsies and in vitro cultures showed high expression of *cagA*, *flaA* and *ureA* and lower expression of *hpaA* and *vacA*, whereas no expression was detected in diarrheal stool. Expression analyses of the same genes in a C57Bl/6 *H. pylori* strain SS1 infection mouse model showed a similar relative transcription pattern as in biopsies, in vitro cultures and vomitus and that expression is up-regulated during exponential growth.

In conclusion, our results suggest that *H. pylori* may be disseminated through vomitus during outbreaks of gastrointestinal infections in Bangladesh and that waterborne transmission is less likely whereas waterborne transmission of ETEC may occur. Furthermore, the studies indicate that experimental murine infection and vomitus from *H. pylori* infected subjects may be suitable models of *H. pylori* virulence gene expression in vivo.

**Keywords:** *H. pylori*, enterotoxigenic *E. coli*, *V. cholerae*, real-time PCR, transmission pathways, gastroenteritis, mouse infection models, bacterial gene expression.
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