

# **Epidemiology, molecular characterization and toxin regulation of enterotoxigenic *Escherichia coli* (ETEC) isolated from children with diarrhoea.**

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av

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Avhandlingen baseras på följande arbeten:

- I. **Gonzales L.**, Joffre E., Rivera R., Sjöling Å., Svennerholm AM. and Iñiguez V.  
Prevalence, seasonality and severity of disease caused by pathogenic *Escherichia coli*  
in children with diarrhoea in Bolivia.  
*Submitted for publication*
- II. **Gonzales L.**, Sanchez S., Zambrana S., Iniguez V., Wiklund G., Svennerholm AM. and  
Sjöling Å.  
Molecular Characterization of Enterotoxigenic *Escherichia coli* Isolates Recovered  
from Children with Diarrhea during a 4-Year Period (2007 to 2010) in Bolivia  
*J Clin Microbiol.* 51(4):1219-1225 2013
- III. **Gonzales L.**, Nicklasson M. and Sjöling Å.  
Influence of environmental factors on the production and secretion of the heat stable  
(ST) and heat labile (LT) toxins of enterotoxigenic *Escherichia coli* (ETEC).  
*Manuscript*
- IV. **Gonzales L.**, Bagher Ali Z., Nygren E., Wang Z., Karlsson S., Zhu B., Quiding-Järbrink,  
M. and Sjöling Å.  
Alkaline pH is a signal for optimal production and secretion of LT in enterotoxigenic  
*Escherichia coli* (ETEC).  
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# **Epidemiology, molecular characterization and toxin regulation of enterotoxigenic *Escherichia coli* (ETEC) isolated from children with diarrhoea.**

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Acute diarrhoeal diseases are among the major causes of morbidity and mortality in children under 5 years of age in developing countries. Knowledge of the epidemiology of such diseases and the causative agents is important for development of vaccines and other interventions. Interactions with the host expose diarrhoeal pathogens to different environmental conditions such as different pH, osmolarity and nutrients at the site of infection and may be important for the virulence of microbial pathogens. In this thesis we have studied the epidemiology of diarrhoea associated with infections by diarrhoeagenic *Escherichia coli* (DEC) with emphasis on enterotoxigenic *Escherichia coli* (ETEC), as well as the role of host environmental factors in the regulation of the ETEC enterotoxins.

We studied the prevalence, seasonality, antibiotic resistance and severity of disease of diarrhoeas caused by DEC in children aged less than five years in two areas in Bolivia over a period of four years (2007-2010). We showed that enteroaggregative *E. coli* EAEC (11.2%), ETEC (6.6%) and enteropathogenic *E. coli* EPEC (5.8%) were the most prevalent DEC pathogens isolated from children, with a peak in children <2 years, and that these categories were significantly associated with disease. No difference in the severity of the disease was found between EAEC, ETEC and EPEC and antibiotic resistance was found in high frequency among the DEC strains isolated.

Subsequently, we performed a molecular characterization of the enterotoxin profile, colonization factors (CFs), putative virulence genes as well as the severity of disease of all ETEC strains isolated from diarrhoeal cases. Strains expressing heat-labile toxin (LT) or heat-stable toxin (STh) alone were isolated in 40% of the children, respectively; the remaining ETEC isolates produced both toxins. The most common CFs were CFA/I and CS14, which were mainly associated with STh strains whereas LT-only strains were significantly more often CF negative. Severity of disease was not related to the toxin or CF profile of the strains. Presence of the suggested ETEC virulence genes (*clyA*, *EatA*, *tia*, *tibC*, *leoA* and *East-1*) was not associated with disease.

To study host factors that may influence expression and secretion of the two toxins LT and STh, clinical ETEC isolates were cultured under various conditions *in vitro*. LT and STh were shown to be differentially regulated by certain environmental factors, *i.e.* different carbon sources (glycerol, glucose, and amino acids), and osmolarity. Secretion of ST was down-regulated by glucose as carbon source under certain conditions but up-regulated by casamino acids and the osmoprotectant sucrose; LT was only secreted in complex media and up-regulated in the presence of glucose.

We also investigated the impact of external pH, which is known to fluctuate in the gastrointestinal tract, and the activity of the cyclic AMP receptor protein (CRP), which is regulated in response to glucose, on the regulation of the production and secretion of LT. The study was performed by constructing a *crp* mutant in an ETEC strain and subsequent analysis of the wild-type and mutant strains after growth in media buffered to pH 5, 7 and 9. We demonstrated that CRP is a repressor of LT transcription and production but a positive regulator of LT secretion. LT production and secretion increased at neutral to alkaline pH compared to acidic pH which was inhibiting secretion. An important finding was that at pH 9 the transcriptional negative regulation of the *eltAB* promoter was abolished and secretion was favored, resulting in maximal production and secretion of LT. We propose that ETEC is exposed to an environment characterized by low glucose levels and alkaline pH close to the epithelium in the small intestine and that this may be a signal for toxin release.

**Keywords:** DEC, ETEC, LT, ST, environmental factors, CRP