

Rotavirus and polymicrobial enteric infections and their short-term course in East African children

Akademisk avhandling

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Avhandlingen baseras på följande delarbeten

- I. **Andersson ME**, Kabayiza J-C, Elfving K, Nilsson S, Msellem MI, Mårtensson A, Björkman A, Bergström T, Lindh M. Co-infection with enteric pathogens in East African children with acute gastroenteritis – associations and interpretations.
Manuscript
- II. **Andersson ME**, Elfving K, Shakely D, Nilsson S, Msellem MI, Trollfors B, Mårtensson A, Björkman A, Lindh M. Rapid clearance and frequent reinfection with enteric pathogens among children with acute diarrhea in Zanzibar.
Clinical Infectious Diseases 2017; 15;65(8):1371-1377.
- III. **Andersson M**, Lindh M. Rotavirus genotype shifts among Swedish children and adults – application of a real-time PCR genotyping.
Journal of Clinical Virology 2017; 96:1-6
- IV. **Andersson ME**, Kabayiza J-C, Elfving K, Nilsson S, Bergström T, Lindh M. Rotavirus infections and their genotype distribution in Rwanda before and after the introduction of rotavirus vaccination.
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SAHLGRENKA AKADEMIN



Rotavirus and polymicrobial enteric infections and their short-term course in East African children

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ABSTRACT

Diarrhoeal diseases in children under five years are the second leading cause of deaths in children worldwide, and especially in low-income countries in sub-Saharan Africa and in southern Asia where about 450,000 children die every year as a result of diarrhoea. The main cause of diarrhoeal diseases is acute gastroenteritis that is due to infection with viruses, bacteria or protozoa, most often acquired by ingestion of contaminated water or food, or through contact between persons. Studies of acute gastroenteritis in children in low-income countries have identified rotavirus, norovirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* and *Shigella* as the most frequent aetiologies to diarrhoea. Rotavirus has been the cause of more than half of all deaths caused by diarrhoea in children, but its impact is declining due to increased use of the rotavirus vaccines Rotarix and RotaTeq.

Enteric infections are frequent in small children in low-income countries, both in those with diarrhoea and in healthy controls, and often two or more pathogens are present at the same time. How co-infecting pathogens are associated, and if multiple infections aggravate symptoms, is not well known. We investigated polymicrobial infections among 1318 children in Rwanda and Zanzibar and found negative associations between the agents that alone are capable of causing diarrhoea. Positive associations between agents only in the patient group were unusual and rarely aggravated the symptoms. Positive associations in both patients and controls were found between two pairs of targets, and these results were useful for estimating the proportion of *Escherichia coli* that carried both or only either of some important virulence factors (ST or LT; *eae* or *bfpA*).

Clearance and acquisition of enteric pathogens were studied in 127 children in Zanzibar with diarrhoea. Faeces samples were collected on admission and at a follow-up 14 days later. The majority of the pathogens detected at baseline had been eradicated or decreased in amount on follow-up, but in parallel new infections occurred at a high rate. The clearance rates were independent of the children's nutritional status. The findings suggest that the high rates of enteric infections in children in low-income settings depend on living conditions with high exposure rather than failure to eradicate pathogens because of malnutrition and poor immune responses.

Rotavirus vaccines were introduced in Rwanda in May 2012. Analysis of samples from children with diarrhoea during the pre- and post-vaccine period showed a significantly lower rate of rotavirus in vaccinated children less than one year of age compared with unvaccinated children in the same age group, as presented in Paper IV. In children aged 1–5 years the rate of rotavirus was independent on vaccination status. Severe dehydration was more rare in vaccinated children, independently of age.

To allow simple distinction between rotavirus genotypes in large numbers of samples, we developed a multiple real-time PCR method. This assay was used for genotyping of rotavirus in samples from Sweden ($n = 775$) and Rwanda ($n = 549$). In Sweden, where vaccination has not yet been implemented, the predominant rotavirus genotype in patients with diarrhoea changed significantly over time during 2010–2014, and these shifts differed also between age groups. Likewise, in Rwanda there were significant genotype shifts during 2009–2015, i.e. both before and after the introduction of vaccination. These results indicate that changes in genotype frequencies observed after the start of vaccination most likely were part of natural fluctuations rather than reflecting that the vaccine induced poorer protection against certain genotypes.

In summary, this work provides new knowledge on the importance of enteric co-infections and shows that children in poor settings are heavily exposed to enteric pathogens that they effectively clear. By the introduction of a new and simple rotavirus genotyping method we show how rotavirus genotypes change extensively over time in both Sweden and Rwanda, irrespective of vaccination. Furthermore, the results demonstrate that the introduction of rotavirus vaccination in Rwanda in 2012 has reduced the number of rotavirus infection in children below, but not above, the age of 12 months. Finally, vaccination has reduced the proportion of rotavirus infections that cause severe dehydration, but resulted in a relative increase of other viruses detected in children with diarrhoea.

Keywords: gastroenteritis, diarrhoea, children, co-infections, aetiology, real-time PCR, follow-up, rotavirus, genotypes, vaccine