HIV-infection in sub-Saharan Africa

From quantity to quality of care

Huibers, M.

Citation for published version (APA):

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Chapter 5

Multiplex real-time PCR detection of intestinal protozoa in HIV-infected children in Malawi, Enterocytozoon bieneusi is common and associated with gastrointestinal complaints and may delay BMI (nutritional status) recovery


*Paediatric Infection Disease Journal. 2018 Sep; 37(9):910-915.*
Abstract

**Background:** Intestinal protozoa are common opportunistic infections in HIV patients. Longitudinal studies on either the clinical relevance, or the effects of immune reconstitution by anti-retroviral therapy on intestinal protozoan infections are children is lacking however. This study investigates prevalence and clinical relevance of intestinal protozoa in HIV-infected Malawian children prior to and during their first year of ART.

**Methods:** Stool samples collected at enrolment and during follow-up were tested for non-opportunistic (Giardia lamblia, Dientamoeba fragilis, Entamoeba histolytica) and opportunistic protozoa (Entroctytoon bieneusi, Encephalitozoon spp., Cryptosporidium spp and Cystoisospora belli) using multiplex real-time Polymerase Chain Reaction (PCR). Associations between infections and clinical symptoms were evaluated using univariate methods.

**Results:** Non-opportunistic and opportunistic protozoa were detected in 40% (14/35) and 46% (16/35) of children at baseline respectively. E. bieneusi was the most prevalent protozoa (37%, 13/35) and associated with gastrointestinal complaints (43% in positive (10/13) versus 18% (4/22) in E. bieneusi-negative children, p=0.001). Body Mass Index (BMI) recovery during 12 months of ART was more commonly delayed in E. bieneusi-positive (+0.29 +SD 0.83) than E. bieneusi-negative children (+1.03 +SD 1.25; p=0.05). E. bieneusi was not detected after 12 months of ART.

**Conclusion:** E. bieneusi was the most prevalent opportunistic intestinal protozoa, present in over a third of study participants prior to initiation of ART. Although all children cleared E. bieneusi after 12 months of ART, E. bieneusi was associated with gastro-intestinal complaints and may delay BMI recovery. Trials to assess effect of treatment of E. bieneusi on nutritional status should be considered in HIV-infected African children.
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Background

Intestinal protozoal infections, caused either by opportunistic or non-opportunistic species, can complicate HIV infection in adults and children. Protozoal infections can cause chronic diarrhoea and thereby malabsorption leading to malnutrition and dehydration, contributing to the high rates of morbidity and mortality observed in resource limited settings (1-3). Prevalence data on protozoal infections in patients with chronic diarrhoea range widely between 1-75%, which can be explained by differences in demographics, seasonal variance and diagnostic methods (4, 5). Prevalence rates are higher in those studies employing modern, more sensitive, Polymerase Chain Reaction (PCR) techniques (6, 7). However PCR techniques are not commonly available in resource limited settings and therefore reliable data from these areas are scarce.

Treatment of HIV infection has greatly improved over the last decade due to the massive scale up of ART availability. In general, ART reduces HIV viral load, permits immune reconstitution and reduces the risk of new opportunistic infections (8). Reduction of the prevalence of intestinal protozoa in HIV-infected adults after introduction of ART has been documented both in industrialized nations and resource limited settings (9-11). However, prospective data on children receiving ART treatment is lacking. Longitudinal data is needed in order to clarify which opportunistic infection may resolve through immune reconstitution due to ART alone and which infections might require more targeted therapy.

Therefore this prospective cohort study was conducted to document the prevalence of opportunistic and non-opportunistic intestinal protozoa in HIV-infected children in Malawi, during the first year of first line ART, using multiplex real-time PCR techniques. Additionally, the study investigated the dynamics and clinical aspects of the most common infections in order to evaluate their contribution to clinical symptoms and morbidity in Malawian children.
Methods

Methods| Study population
The study cohort included ART-naïve HIV-infected children initiating ART at the Queen Elizabeth Central Hospital, Blantyre, Malawi. Children aged 18 months to 18 years were enrolled prior to initiating ART, if they met ART initiation criteria outlined in the Malawi National ART program guidelines (2010-2012)(12, 13). Local guidelines, applicable at the time of the study have been described in detail elsewhere (14). In short, ART was initiated in children based on WHO staging as well as immune status. Clinical criteria included: ≥ 18 months of age with WHO clinical stage 3 and severe immune suppression was based on the WHO guideline at the time of the study: for children aged < 59 months by CD4% < 25% and/or CD4 < 750 cells/mm and children aged > 59 months CD4 < 350 cells/mm (12).

Methods| Data collection
Following recruitment and informed consent, a standardised history was collected, including socio-economic data and gastro-intestinal symptoms. A physical examination was performed including collection of anthropometry. Children were monitored via data collection evaluating symptoms and anthropometry throughout the first 12 months of ART. Blood and stool samples were collected at 0, 6 and 12 months. This intestinal protozoa study was nested within a larger ART follow-up cohort study. Stool samples were stored and determined at a later point in time. All physicians were unaware of the PCR results.

Methods| Laboratory
HIV infection was confirmed using two HIV antibody tests (Abbott Determine HIV-1/2 Test and Uni-Gold HIV test). Full blood count (Beckman Coulter HMX, Beckman coulter, CA USA) and CD4 count (flow cytometry, Becton Dickinson, CA USA) were analysed at the MLW Clinical Research Programme laboratory. Aliquots were stored for batch analysis of HIV-1 viral load (Roche Amplicor; Roche, Basel, Switzerland). Stool samples were stored at -20 degrees Celsius within 24 hours after sampling and subsequently shipped to LUMC, Leiden, The Netherlands. DNA isolation, amplification and detection were performed at LUMC as described elsewhere with some modifications, in particular the combinations of targets (15-17). One
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Multiplex real-time PCR was used for the detection of DNA of the non-opportunistic protozoa *G. lamblia, D. fragilis* and *E. histolytica* (15) and another multiplex real-time PCR targeted opportunistic protozoa including microsporidia *E. bieneusi* and *Encephalitozoon spp.* (i.e. *E. intestinalis*) (18) with *Cryptosporidium spp* (16) and *C. belli* (17). Appropriate positive and negative controls were included in each assay. The PCR output consisted of a cycle threshold (Ct-) value representing the amplification cycle in which the level of fluorescent signal exceeded the background fluorescence. Intensity of infection was categorized for each target into low, moderate and high DNA levels based on Ct-value, respectively: higher than 35, between 35 and 30 and lower than 30, while negative PCR results were recoded as Ct=50 (19).

**Methods** | **Definitions**

Gastrointestinal symptoms were actively asked for at each planned clinic visit and included abdominal pain, diarrhoea or vomiting. Anaemia was classified using age and gender specific haemoglobin (Hb) cut-offs: children aged 18 months-59 months Hb< 11.0 g/dl, children 5.0-11.9 years < 11.5 g/dl, boys 12-15 year Hb< 12.0 g/dl, girls > 12 years Hb 12.0 g/dl; boys > 15 years: Hb < 13.0 g/dl (20). Severe immune suppression was defined using the WHO definitions according to age (20): 18-59 months CD4% < 10% or CD4 count < 200 cells/mm3; > 59 months: CD4 count < 100 cells/mm3(21). Anthropometric data, based on weight, length and age related Z-score were calculated based on WHO Multicentre Growth Reference Study Group (22, 23). Primary outcome was BMI for age z-score (BMIZ) as this parameter was applicable to children of all ages whilst weight for age (WAZ) only applies to children <10 years and height for age (HAZ) is a late marker for growth recovery (>6-12 months). Malnutrition was defined as a body mass index z-scores (BMIZ) < -2 SD (24).

**Methods** | **Statistical analysis**

Data were double entered, cleaned and analysed using SPSS version 19.0. The study was primarily powered to detect prevalence of protozoa at baseline and their change over time. Secondly univariate analysis assessed risk factors for protozoal infection using chi square test or Fisher exact test for categorical variables and independent sample t-test for continuous variables. All p-values presented are two-tailed and a significance level of < 0.05 was used.
Chapter 5

Methods | Ethical considerations
The purpose of the study was explained to the guardians of each patient in Chichewa and written informed consent was obtained before inclusion into the study from the parents or guardians and assent from the children, if applicable. The study protocol was approved by the Research Ethics Committee of the College of Medicine, University of Malawi.

Results

A total of 35 children were included with a mean age of 7.9 (SD 4.2) years. Baseline characteristics are shown in table 1. At baseline, severe immune suppression was prevalent among 30% (10/33). Prevalence of severe immune suppression did not differ significantly between the different age groups: 26% (5/19) in children <10 years, versus 36% (5/14) if > 10 years; p=0.56. Fifteen percent (5/34) of children were malnourished (BMIZ< -2 SD) at baseline, which dropped to 3% (1/33) after 12 months on ART. Gastrointestinal complaints occurred in 40% (14/35) of children during the first 6 months of follow-up, but these complaints were not associated to immunosuppression however (p=0.411). Stool samples were available for 35 children at baseline, 27 (77%) children at 6 months and 26 (74%) children at 12 months of follow-up.

Results | Non-opportunistic protozoa at baseline
Non-opportunistic protozoa were detected in 40% (14/35) of the children at baseline (table 2). *G. lamblia* was the most common protozoa and detected in 26% (9/35) of the samples. *D. fragilis* was prevalent in 17% (6/35) of children whilst *E. histolytica* was not detected. Three of nine children (33%) with *G. lamblia* infection at baseline had gastrointestinal complaints. Gastrointestinal complaints among children with *G. lamblia* were not more common than among children without *G. lamblia* (42% (11/26), p=0.22). At baseline, BMI for age z-scores (BMIZ) were -0.60 (SD 1.0) for *G. lamblia* positive children versus a mean of -0.55 (SD 1.4) for negative children, p=0.92. Also *D. fragilis* infections at baseline were not associated with gastrointestinal complaints or a significant difference in BMIZ scores. Respectively 50% (3/6) of the *D. fragilis* positive children showed gastrointestinal complaints.
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Results | Opportunistic protozoa at baseline

Opportunistic protozoa were detected in 46% (16/35) of children at baseline. *E. bieneusi* was the most common opportunistic protozoa, present in 37% of samples (13/35). Gastrointestinal complaints were more common among children with *E. bieneusi* at baseline than those without infection (43% (10/13) versus 18% (4/22), p=0.001 (table 3). Children with and without *E. bieneusi* had a similar BMIZ at
baseline; respectively BMIZ -0.6 (SD 1.0) versus BMIZ -0.6 (SD 1.4), p=0.92. The clinical relevance of and *E. bieneusi* infection is described in detail below. *Cryptosporidium spp* was seen in 11% (4/35) of children, whilst *Encephalitozoon spp* (i.e. *E. intestinalis*) and *Cystoisospora belli* were not detected at baseline. *Cryptosporidium spp* infections at baseline were not associated with gastrointestinal complaints (42% (13/31) versus 25% (1/4) for *Cryptosporidium spp* positive and negative children respectively, p=0.42). No difference in mean BMIZ at baseline was seen: -0.16 (SD 0.4) versus -0.57 (SD 1.3), p=0.56 for *Cryptosporidium spp* positive and negative children respectively.

**Results | Effect of 12 months ART on intestinal protozoa**

The prevalence of non-opportunistic protozoa and opportunistic protozoa infections over time are described in table 2. Non-opportunistic protozoa were present in 43% (15/35) of the children at baseline and 42% (11/26) after 12 months of ART. The prevalence of *G. lamblia* dropped from 26% (9/35) to 11% (3/26), however new infections also occurred, table 2. Prevalence of *D. fragilis* increased from 17% (6/35) towards 31% (8/26) following ART initiation. All opportunistic protozoa, including *E. bieneusi*, were cleared after 12 months of ART.

**Results | *E. bieneusi***

To identify children at risk for *E. bieneusi* and to assess the clinical importance of the infection, potential risk factors and symptoms were compared between children with and without *E. bieneusi*, table 3. *E. bieneusi* was more common in children older than 10 years of age when compared to younger children (57% (8/14) vs 24% (5/21) respectively, p=0.05). Children with *E bieneusi*, when compared to children without, did not suffer from more severe immune suppression at baseline (38.5% (5/13) versus 25.0% (5/15), p=0.46). However children with and without *E. bieneusi* had a similar BMIZ at baseline, significant differences were seen after 12 months of ART. Children with an *E. bieneusi* infection showed a trend towards lower BMIZ when compared to those without; BMIZ -0.36 (SD 0.97) versus BMIZ +0.56 (SD 1.39), p=0.052 (figure 1a). BMIZ recovery after 12 months was +0.29 (SD 0.83) versus +1.03 (SD 1.25) (p=0.050) for *E. bieneusi* infection versus non-infected children. Children with high levels of *E. bieneusi* DNA in their stool showed a non-significant trend towards a more delayed BMI recovery, figure 1b. BMIZ at 12 months of follow-up was -0.47
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...versus +0.56 (SD 1.39) for high, low to moderate and negative baseline *E. bieneusi* DNA levels, respectively (p=0.25).

**Discussion**

This study presents important new PCR prevalence data evaluating non-opportunistic and opportunistic intestinal protozoa infections in HIV-infected African children receiving ART. *E. bieneusi*, an opportunistic infection, was the most prevalent intestinal protozoa. Despite being a rather unknown opportunistic infection amongst paediatric clinicians, *E bieneusi* was not only common but might be clinically relevant, as it was associated with both gastro-intestinal complaints and possible reduced BMI recovery. Although all children cleared *E. bieneusi* by 12 months of ART and gastro-intestinal complaints diminished, the BMI recovered poorly in those with an *E. bieneusi* infection.

Thirty seven percent of the ART naïve HIV-infected children were infected with *E. bieneusi* in this cohort. The reported prevalence of *E. bieneusi* in the literature shows wide ranges (0.8-79%) of *E. bieneusi* prevalence. This may be explained by differences in diagnostic methods, demographics and study populations, including ages, presence of clinical symptoms, other underlying conditions and severity of the immune suppression (25-27). Two other studies which used PCR-based assays in HIV-infected African children reported prevalence rates ranging from 20% - in a general population of HIV-infected children in South Africa to 79% - in children with HIV and diarrhoea in Uganda of *E. bieneusi* infection(5, 27). In the current study, all children cleared *E. bieneusi* and other opportunistic protozoa following immune recovery after 12 months of ART. This is the first paediatric cohort study evaluating non-opportunistic and opportunistic intestinal protozoa infections including *E. bieneusi* during ART treatment. However the data collected in children is corroborating with data in adults showing clearance of the *E. bieneusi* infection after receiving several months of ART (9, 10). The finding that children clear opportunistic protozoa are especially reassuring as children are considered to be more vulnerable to *E. bieneusi* as it is more common in children than adults (25).

Gastrointestinal complaints are commonly reported in microsporidian infections such as *E. bieneusi* (28, 29). In the current study 43% of the children
with a PCR-detected *E. bieneusi* infection described gastrointestinal complaints. The prevalence of gastrointestinal symptoms corroborates data in HIV-infected adults where *E. bieneusi* infection has been associated with diarrhoea and vomiting (28, 29). More importantly this cohort demonstrated that *E. bieneusi* is associated with a delayed BMI recovery, which persisted despite protozoal clearance and reduction of gastrointestinal complaints. Other, cross-sectional studies have also reported an association between malnutrition and lower rates of weight gain *E. bieneusi* infected HIV-patients (28, 30, 31). Malnutrition and lower rates of weight gain are usually attributed to ongoing diarrhoea and impaired absorption of micronutrients due to mucosal damage as well as malabsorption caused by direct replication of protozoa in the epithelium of small intestine (29). In the presenting study, the gastrointestinal complaints resolved within 6 months and immune status improved during 12 months of ART treatment, while a poor nutritional state, signified by delayed BMI recovery, persisted. Secondly there was no association between potential confounders such as immune suppression at baseline, (table 3), neither tuberculosis infection at enrolment, nor ART failure at 12 months and BMI recovery in this population (data not shown). Further studies are needed to assess if the effect on BMI is secondary to a direct effect of *E. bieneusi* on the mucosa or if more factors are involved in the delayed BMI recovery.

Like other studies, a large part of patients with an *E. bieneusi* infection detected by PCR are asymptomatic (5, 25). However other studies have not assessed if the lower protozoal load may explain why some do not have symptoms (5, 25). Low detectable DNA levels may, for instance, reflect spore shedding rather than actual infection (6, 31-33). *E. bieneusi* spores are detected in stool specimens by microscopy for 9-33 days, while stool specimens evaluated by PCR are positive for 3-40 days longer (25). PCR results should be therefore be interpreted with some care, especially if low DNA levels are detected. Despite this we did identify a trend towards a poorer BMI recovery among children with high *E. bieneusi* DNA levels in this relatively small study.

None of the children received treatment following their PCR results in this study. Given our finding of a significant association between *E. bieneusi* and a poor BMI-recovery and clinical symptoms, the differences found were small and both may have multiple causes. Therefore the effect of therapy for *E. bieneusi* on these outcomes should be tested in a placebo-controlled trial. Effective treatment for
E. bieneusi is complex, however. Albendazol has shown conflicting results in the treatment of different E. bieneusi subtype infections (34, 35). Fumagillin, a newer agent was shown to be effective in adults and was approved in 2005, but severe adverse effects limit its use and availability (35, 36). If these restrictions also apply to children is unclear as paediatric data are very limited despite showing good effectiveness and no side effects (36). TPN 470, the fumagillin analogue, may be a potentially safer alternative though it again lacks paediatric testing (2, 34, 35). Given the serious reduced BMI recovery in these HIV-infected children, phase 2 and 3 trials should be considered.

Besides E. bieneusi, G. lamblia was a common non-opportunistic pathogen. Prevalence of G. lamblia worldwide can differ enormously due to diagnostic test, population, demographics, season and ages (37, 38). Our reported PCR detected prevalence of 26% is similar to a previously reported prevalence of 30% among severely malnourished children in Blantyre, Malawi (39). Immune status recovery has a questionable effect on infection while the prevalence of G. lamblia dropped from 26% towards 11% during 12 months of ART, new infections also occurred. G. lamblia infection can present with gastro-intestinal complaints, but carriers may also be asymptomatic (37, 40). In this study, gastrointestinal complaints and malnutrition could not be linked to G. lamblia infection. As we detected mainly moderate and low DNA loads (78%), we may have identified a large proportion of asymptomatic carriers. As G. lamblia is not an opportunistic infection, it is unlikely that prevalence will be reduced by restored immunity due to ART.

Cryptosporidium spp occurred in this study but infections resolved after immune recovery during ART treatment and DNA levels were only high in two children. Earlier PCR-based studies in Malawi (Blantyre) among pre-school children with diarrhoea and an unknown HIV status showed a comparable prevalence of Cryptosporidium spp of 5-9% (41). Cryptosporidiosis is commonly associated with symptoms of diarrhoea, however in this study gastrointestinal complaints were not associated with infection (42). This effect may be explained by the relatively low to moderate infection prevalence of all Cryptosporidium spp infection at baseline as compared to other pathogens. Considering the low prevalence and the lack of an effective treatment in children, routine testing will likely not benefit children in an outpatient setting (43). Combined infections were not common among the presenting cohort. Clinical symptoms are therefore not related and influenced by co-infections.
This study had several limitations. Firstly, this study used local guidelines to start ART in Malawi in 2010, which meant that the studied cohort was severely immune compromised in comparison to current cohorts of children starting ART. Current WHO guidelines suggest start ART early in the course of HIV disease (21). Current HIV-infected populations may therefore have lower protozoal prevalence rates. Given the high prevalence in our study and the fact that HIV diagnosis still is often delayed in African settings we believe our findings are still important. Secondly, the number of children recruited is small and follow-up was stopped at 12 months; therefore associations may have been missed or suggested that required a larger sample size or prolonged follow-up. Still this study is the first paediatric cohort from Africa using PCR techniques with 12 months of follow-up during ART. Thirdly, the use of PCR may have overestimated the prevalence of pathogens over time as PCR also detects spores, which are not active pathogens (6, 31-33). However, we assessed the effect of DNA load and were able to show a trend towards a more delayed BMI recovery in children with a higher load. To confirm this effect more research is needed to investigate the duration of diminished growth after 24 months and the eventual effect of (repeated presumptive) treatment on growth over time. Lastly, we have not performed of E. bieneusi subtype analysis, this information could be of use as clinical presentation is influenced by genotype and therefore may vary in different geographic regions (44, 45).

Conclusion

This prospective cohort reports on the prevalence of intestinal protozoa in HIV-infected children in Malawi, during the first year of first line ART using multiplex real-time PCR techniques. E. bieneusi is a very common pathogen at start of ART and clinically important as it was associated with gastrointestinal complains and may be associated with prolonged reduced growth, a predictor of poor prognosis. Future studies should focus on trials to assess treatment options for E. bieneusi to improve symptoms and poor nutrition status.
Acknowledgments

We thank all children, the parents and guardians of the children and the staff of the Queens Elisabeth Central Hospital for participation and cooperation. The study was supported by a grant of the NWO- NACCAP, Emma foundation Amsterdam Medical Centre and the Wellcome Trust. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.
# Table 1. Patient characteristics at baseline and during first 12 months on ART.

Abbreviations: SD = standard deviation. *" Gastrointestinal-symptoms include: abdominal pain or diarrhoea or vomiting. "Complaints during 0-6 months follow-up */" complaints during 6-12 months follow-up. */ Severe immune suppression: age < 59 months: CD4% < 10% or a CD4 count < 200 cells/mm³ (20); age > 59 months: CD4 count < 100 cells/mm³ (21). */ Anaemia: 18-59 months: haemoglobin level (Hb) < 11.0 g/dl, 5.0-11.9 years Hb < 11.5 g/dl, boys 12-15 years: Hb < 12.0 g/dl, girls > 12 years: Hb < 12.0 g/dl; boys > 15 years: Hb < 13.0 g/dl.
## Table 2. Multiplex real-time PCR results of protozoal infections in HIV-infected children during the first year of ART.

*E. histolytica* and *Encephalitozoon spp* (i.e. *E. intestinalis*) were not detected. Combined *E. bieneusi-Cryptosporidium spp* infection at baseline was seen in 3%; 1/35 and *E. bieneusi* combined with *G. lamblia* infection at baseline was seen among 11%; 4/35. Detected DNA level based on Ct-value: Total positive: Ct<50, low DNA level: Ct 35-50, moderate DNA level: Ct 30-35 and high DNA level: Ct<30. *1* no new infections *2* 2 new infections, *3* 6 new infection *4* 7 new infections, *5* 4 new infections; *6* 3 new infections, *7* all new infections.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=35)</th>
<th>6 months (n=27)</th>
<th>12 months (n=26)</th>
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<tbody>
<tr>
<td><strong>Non-opportunistic protozoa</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>G. lamblia</em></td>
<td></td>
<td></td>
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<tr>
<td>Total positive</td>
<td>9 (26%)</td>
<td>4 (15%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>High DNA level</td>
<td>2/9 (22%)</td>
<td>2/4 (50%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Moderate DNA level</td>
<td>5/9 (56%)</td>
<td>0/4 (0%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Low DNA level</td>
<td>2/9 (22%)</td>
<td>2/4 (50%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td><em>D. fragilis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total positive</td>
<td>6 (17%)</td>
<td>6 (22%)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>High DNA level</td>
<td>4/6 (67%)</td>
<td>2/6 (33%)</td>
<td>1/8 (13%)</td>
</tr>
<tr>
<td>Moderate DNA level</td>
<td>2/6 (33%)</td>
<td>2/6 (33%)</td>
<td>5/8 (63%)</td>
</tr>
<tr>
<td>Low DNA level</td>
<td>0/6 (0%)</td>
<td>2/6 (33%)</td>
<td>3/8 (34%)</td>
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<tr>
<td><strong>Opportunistic protozoa</strong></td>
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<tr>
<td><em>E. bieneusi</em></td>
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<td></td>
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<tr>
<td>Total positive</td>
<td>13 (37%)</td>
<td>6 (22%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>High DNA level</td>
<td>7/13 (54%)</td>
<td>3/6 (50%)</td>
<td></td>
</tr>
<tr>
<td>Moderate DNA level</td>
<td>3/13 (23%)</td>
<td>2/6 (33%)</td>
<td></td>
</tr>
<tr>
<td>Low DNA level</td>
<td>3/13 (23%)</td>
<td>1/6 (17%)</td>
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<tr>
<td><em>Cryptosporidium spp</em></td>
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<td></td>
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</tr>
<tr>
<td>Total positive</td>
<td>4 (11%)</td>
<td>3 (11%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>High DNA level</td>
<td>0/4 (0%)</td>
<td>1/3 (33%)</td>
<td></td>
</tr>
<tr>
<td>Moderate DNA level</td>
<td>0/4 (0%)</td>
<td>1/3 (33%)</td>
<td></td>
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<tr>
<td>Low DNA level</td>
<td>4/4 (100%)</td>
<td>1/3 (33%)</td>
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<tr>
<td><em>C. belli</em></td>
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<tr>
<td>Total positive</td>
<td>0 (0%)</td>
<td>2 (7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>High DNA level</td>
<td>0/2 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate DNA level</td>
<td>1/2 (50%)</td>
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<tr>
<td>Low DNA level</td>
<td>1/2 (50%)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>E. bieneusi Negative DNA level</td>
<td>E. bieneusi Positive DNA level</td>
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<tr>
<td>----------------</td>
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<td>-------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Boys</td>
<td>9/22 (41%)</td>
<td>8/13 (62%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Years (mean ±SD)</td>
<td>6.85 ±3.6</td>
<td>9.8 ±4.5</td>
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</tbody>
</table>

**Symptoms**

<table>
<thead>
<tr>
<th></th>
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<th>E. bieneusi Negative DNA level</th>
<th>E. bieneusi Positive DNA level</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrointestinal symptoms</strong></td>
<td>0-6 months</td>
<td>4/22 (18%)</td>
<td>10/13 (43%)</td>
<td>0.001</td>
<td>15.0</td>
<td>2.8-80.9</td>
</tr>
<tr>
<td><strong>Diarrhoea</strong></td>
<td>0-6 months</td>
<td>1/22 (5%)</td>
<td>6/13 (46%)</td>
<td>0.003</td>
<td>18.0</td>
<td>1.8-176.6</td>
</tr>
<tr>
<td><strong>Vomiting</strong></td>
<td>0-6 months</td>
<td>1/22 (5%)</td>
<td>7/13 (54%)</td>
<td>0.001</td>
<td>24.5</td>
<td>2.5-240.3</td>
</tr>
</tbody>
</table>

**Immune status**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>E. bieneusi Negative DNA level</th>
<th>E. bieneusi Positive DNA level</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WHO</strong></td>
<td>I</td>
<td>10/22 (46%)</td>
<td>6/13 (46%)</td>
<td>0.85</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5/22 (23%)</td>
<td>2/13 (15%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>7/22 (32%)</td>
<td>5/13 (39%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>0</td>
<td>0/39 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Severe immune suppression</strong></td>
<td>Baseline</td>
<td>5/20 (25%)</td>
<td>5/13 (39%)</td>
<td>0.41</td>
<td>1.9</td>
<td>0.4-8.5</td>
</tr>
</tbody>
</table>

Table 3. Factors associated at baseline and over 12 months follow-up with E. bieneusi infection at baseline.

Abbreviation: SD= standard deviation. *1 Gastrointestinal symptoms include; abdominal pain or diarrhoea or vomiting. *2 Complaints during 0-6 months follow-up *3 Severe immune suppression: age < 59 months: CD4% < 10% or a CD4 count < 200 cells/mm3 (20); age > 59 months: CD4 count < 100 cells/mm3 (21). *4 p-significant < 0.05. Toilet use and water availability did not differ significant between children with and without E. bieneusi infection (data not shown).
References


Comorbidities in HIV: Intestinal protozoa


Chapter 5


