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Age-dependent decline and association with stunting of *Giardia duodenalis* infection among schoolchildren in rural Huye district, Rwanda

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ABSTRACT

Giardia duodenalis infection is highly prevalent and a cause of underweight in pre-school children in rural Rwanda. The present study aimed at assessing the age-pattern of *Giardia* infection and its manifestation in older children, i.e., during school age.

Stool samples were collected from 622 schoolchildren at two schools in the Huye district of southern Rwanda (rural, 301; urban, 321) and subjected to *G. duodenalis* specific PCR assays. Clinical and anthropometric data, socio-economic status and factors potentially associated with *G. duodenalis* infection were assessed.

Of the 622 children (mean age, 10.4 years), 35.7% were infected with *G. duodenalis* (rural, 43.9%; urban, 28.0%; $P < 0.0001$). Only few indicators of low socio-economic status were found to be associated with infection. In rural but not urban schoolchildren, infection prevalence declined significantly with age. *G. duodenalis* infection more than doubled the odds of stunting in both rural (adjusted OR, 2.35 (95%CI, 1.25–4.41)) and urban children (adjusted OR, 2.27 (95%CI, 1.01–5.09)).

In the study area of rural southern Rwanda, *G. duodenalis* prevalence among children declined throughout school-age. The data suggest that while lacking overt clinical manifestation at high endemicity, *G. duodenalis* infection is a common cause of stunting in schoolchildren.

1. Introduction

Giardia duodenalis is one of the most frequent intestinal protozoa worldwide. Acute symptomatic giardiasis including diarrhoea, abdominal pain, bloating and stomach cramps is seen particularly in low-endemicity, high-income countries, e.g., presenting as traveller's diarrhoea (Gautret et al., 2012). In contrast, infections in highly endemic areas commonly lack overt clinical signs (Veenemans et al., 2011; Ignatius et al., 2012) and may even protect children against acute diarrhoea (Veenemans et al., 2011; Muhsen and Levine, 2012). On the other hand, chronic or recurrent *G. duodenalis* infection in childhood may cause malabsorption and has been associated with malnutrition, wasting (acute malnutrition, low weight-for-height) and stunting (chronic malnutrition, low height-for-age), and with reduced cognitive functions at later age (Loewenson et al., 1986; Fraser et al., 2000; Berkman et al., 2002; Nematian et al., 2008; Al-Mekhlafi et al., 2005, 2013; Prado et al., 2005; Carvalho-Costa et al., 2007; Ignatius et al., 2012). Because of the double-edged clinical role of *G. duodenalis* in highly endemic areas and because of frequent re-infection, treatment of children with asymptomatic infections in such settings is controversially discussed (Gilman et al., 1988; Sullivan et al., 1988; Prado et al., 2005; Veenemans et al., 2011).

At high endemicity, *G. duodenalis* is thought to be most common in pre-school children and less frequent at higher ages (Téllez et al., 1997; Kang et al., 1998; Cook et al., 2009; Johnston et al., 2010; Ankarklev et al., 2012; Schmidlin et al., 2013). The actual age distribution and peak age in highly endemic areas, however, is not well

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established. In addition, prevalence data based on microscopy may underestimate the actual situation, e.g., up to two thirds of people in rural western Uganda were found by PCR to be (asymptomatically) infected (Johnston et al., 2010).

In southern highland Rwanda, we recently observed *G. duodenalis* infection by PCR in 60% (by microscopy, 20%) of rural children under five years of age, with prevalence rising from 33% in infants to 73% in children aged four years. Infection was not associated with gastrointestinal symptoms but with malnutrition (Ignatius et al., 2012). In the present study, we aimed at assessing the presence of an age-dependence of *G. duodenalis* infection in older schoolchildren, at identifying factors associated with infection, and at examining the clinical manifestation of infection in this age group.

2. Materials and methods

2.1. Study area and participants

Study procedures and the characteristics of the 622 children have previously been reported (Staudacher et al., 2014). Briefly, we conducted a cross-sectional study in May 2012 alongside routine school-based deworming activities in the area of Butare (population, 100,000), Huye district, southern province of Rwanda. Based on similar school size and accessibility, we purposely selected two primary schools in the district (average altitude, 1800 m; mean temperature, 19 °C; yearly rainfall, 1200 mm) as study sites, namely in a rural village (Gatovu; 18 km out of town; 708 schoolchildren) and in an urban quarter of Butare (Ngoma, 547 schoolchildren). Preceding study initiation, meetings were held at both schools to provide information on study purpose and procedures and to answer questions of parents and teachers. At both schools, we invited a pragmatic number of 400 children each to participate. This number was determined by the laboratory capacity of stool microscopy. Parents or guardians provided informed written consent, and additional assent was obtained from students of eight years or older. The Rwanda National Ethics Committee reviewed and approved the study protocol (RNEC 105/RNEC/2012), and further approval was obtained from the Rwandan Ministry of Education (MINEDUC/S&T/0079/2012).

2.2. Clinical and laboratory examinations

Children were instructed to bring a fresh stool sample in a pre-labelled container to school at the scheduled date. Age (cross-checked with birth certificates if available) and sex were documented. Weight, height (electronic scales and statometer Seca 213, Seca, Germany), and axillary temperature were measured. A venous blood sample was collected into EDTA, and the study physician (JMH) clinically examined all children. Weight-for-age Z (WAZ)-scores and height-for-age Z (HAZ)-scores were calculated using WHO Anthro Plus (http://www.who.int/childgrowth/software/en/). Underweight and stunting were defined as WAZ- and HAZ-scores of <−2 standard deviations (SD), respectively. Clinically assessed malnutrition was evaluated by the study physician (JMH) based on clinical signs, growth percentiles and/or mid-upper-arm circumference. Household characteristics of the children's families as well as proxy indicators reflecting socio-economic status (SES) were assessed by trained health workers interviewing household heads at their homes using pre-tested questionnaires. Interview items included information on maternal and paternal education and occupation, ability to pay school fees and health insurance, household assets, ownership, type, and number of livestock, water use, eating habits, waste disposal, toilet facilities and hygiene measures. As part of the overall assessment of the children's health, haemoglobin (Hb) concentrations were measured by a HemoCue photometer (Angelholm, Sweden). Anaemia was defined according to age-related and altitude-adjusted cutoff values (<5 years, Hb < 11.8 g/dL; 5 < 12 years, Hb < 12.3 g/dL; 12 < 15 years or ≥15 years (female), Hb < 12.8 g/dL; ≥15 years (male), Hb < 13.8 g/dL; Sullivan et al., 2008). Immunochromatographic dipstick tests (Malaria Total Quick Test, Cypress Diagnostics, Langdorp, Belgium) were used to detect malaria parasites. Stool samples were microscopically examined using the Kato-Katz technique (WHO, 1994) to detect infection with soil-transmitted helminths. Adding phocine herpesvirus 1 (PhHV-1) as an internal control for the extraction process (Niesters, 2002), DNA was extracted from stool samples (Qiamp DNA Stool Mini Kit, Qiagen, Hilden, Germany). Real-time PCR assays on a Lightcycler 480 (Roche Diagnostics) identified *G. duodenalis* (Verweij et al., 2004) and *Ascaris lumbricoides* (Basuni et al., 2011), assays including respective positive and negative controls. Assays yielding a cycle threshold (Ct) value of >36 were considered to reflect limited reproducibility due to low copy numbers, and were repeated. Evidence of faecal inhibitory factors (Ct-value for the internal control PhHV-1 > 36) was not observed.

2.3. Statistical analysis

Of 777 consenting parents, 712 (92%) children attended school at the recruitment day. Of these, 662 (93%) provided a stool sample, and 622 (87%) samples were available for analysis by PCR. Data was double-entered, cross-checked, and analysed using Statview 5.0 (SAS Institute Inc.). We compared continuous variables between groups by Student's *t*-, Mann–Whitney or Kruskal–Wallis tests, and proportions by χ^2 test or Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were computed considering e.g., *G. duodenalis* infection as outcome and socio-demographic factors as exposures, or, vice versa, *G. duodenalis* as exposure and stunting as outcome. Following univariate analysis, factors associated at a level of $P < 0.10$ with, e.g., *G. duodenalis* infection, were evaluated by multivariate logistic regression analysis. We used logistic regression models with stepwise removal of factors found to be not associated in multivariate analysis ($P > 0.05$) to identify independent predictors of stunting. A P-value <0.05 was considered to reflect statistical significance.

3. Results

3.1. Baseline characteristics

The main characteristics of the 622 children (321 urban, 301 rural) are displayed in Table 1. *G. duodenalis* infection was present in 44% of rural schoolchildren and in 28% of their urban peers ($P < 0.0001$). As compared to their urban counterparts, rural children were slightly older, more frequently had a poor nutritional status, clinically assessed malnutrition, any clinical finding, a positive malaria test, and *A. lumbricoides* infection. Most children (each >99%) had unsuspicious results of abdominal and neurological examinations and of pulmonary and cardiac auscultation. One child was febrile.

G. duodenalis infection was most common in the youngest schoolchildren, and its prevalence declined with age, but significantly only in rural schoolchildren ($P < 0.0001$; Fig. 1).

3.2. Factors associated with G. duodenalis infection

Interview data were available for 531 children. Most indicators of socio-economic status were significantly lower for rural as compared to urban children (Staudacher et al., 2014), and analysis was therefore performed separately. Overall, only few factors were