Title: Intestinal infection with *Campylobacter* in a rural cohort in Moramanga, Madagascar

Short title: *Campylobacter* infection in Madagascar

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Abstract

Background

Campylobacter infection is the most common cause of bacterial gastroenteritis in developing countries, as demonstrated by a previous study in Madagascar. Campylobacter is endemic to developing countries and Campylobacter infections seem to lead to the development of protective immunity. We tested this hypothesis in a rural setting in Madagascar, by exploring the association between infection and age, the recurrence of infection and the pathogenicity of Campylobacter.

Methods

We carried out a cohort study of children under the age of 24 months in Moramanga, with twice-weekly follow-up until the age of 36 months.

Results

Between January 2010 and May 31st 2012, 508 children were included in the cohort. We detected 319 episodes of Campylobacter infection in total, and 43.3% (n=220) of the children had at least one episode of intestinal Campylobacter infection. The rate of Campylobacter isolation from stool specimens was 9.3%, with the highest isolation rates recorded for children aged 6 to 11 months and 12 to 17 months, at 13.4% and 15.2%, respectively. The annual incidence rate for symptomatic Campylobacter infection was 0.05 episodes/child. The probability of
Campylobacter infection was highest between the ages of six and 23 months. Taking children under six months of age as the reference group, the age-specific odds ratio for the association was 5.0 (95% CI: 2.9-8.6) for children aged six to 11 months, 5.7 (95% CI: 3.3-10.0) for children aged 12 to 17 months and 3.3 (95% CI: 1.8-5.8) for children aged 18 to 23 months. A second episode of infection occurred 63 days after the first episode in children with primary infections, and after 137 days in children with multiple infections (p<0.01). First episodes of Campylobacter infection were associated with diarrhoea (odds ratio=16.1; 95% confidence interval: 1.8-140.8)

Conclusion
Our findings suggest that protective immunity to Campylobacter may be acquired over time, following repeated exposure. Care should be paid to children, in the first year of life, as this age seems to be associated with the highest risk of diarrhoea during Campylobacter infection. Our observation raises questions about the priorities for interventions, such as the development of vaccines for prevalent Campylobacter serotypes.

Key words: Campylobacter infection, cohort study, diarrhoea, rural area, Madagascar

Introduction
Diarrhoea-related mortality is decreasing by about 4% per year, but remains high. In total 3.6 million children each year die before reaching their fifth birthday in Africa, and diarrhoeal disease accounts for 11% of these deaths [1]. Disease morbidity, and the incidence of diarrhoea in particular, is declining more modestly [2]. The importance of pathogens such as rotavirus and Escherichia coli in the aetiology of severe childhood diarrhoea in developing countries is well recognised [3,4]. However the role of Campylobacter is not well understood either in the community setting or in hospitalised subjects and outpatients.

The epidemiological features of Campylobacter infection differ between developed and developing countries. Campylobacter is endemic to developing countries, in which it is, one of
the bacteria most frequently isolated from both healthy children and children with diarrhoea [5]. Current estimates are derived from only a small number of studies, but the proportion of diarrhoea cases attributable to *Campylobacter* infection is believed to be high, at between 5 and 20% [5]. A diarrhoeal case-control study was conducted in 14 districts of Madagascar in 2008, by the Pasteur Institute of Madagascar [6]. Its findings suggested that *Campylobacter* was the second most frequently isolated enteropathogen, after parasites, in children under the age of five years, with a prevalence of 9.7%. However, its frequency did not differ between cases and control subjects.

Many studies have suggested that the development of protective immunity in children from developing countries may account for the high rates of asymptomatic *Campylobacter* infection and for the decrease in the proportion of infected subjects presenting illness with increasing age [7,8]. *Campylobacter* isolation rates are highest during the first two years of life and appear to decline with age [5, 9]. The development of immunity might also affect the recurrence of infection. Immunity following a first episode of campylobacteriosis might decrease the risk of subsequent events, which might also vary over time and with patient characteristics [10].

We investigated the possible effect of immunity following *Campylobacter* infection, by conducting a community-based longitudinal study of a cohort of children aged under the age of two years in Moramanga, Madagascar, an area in which diarrhoeal diseases are endemic. We assessed the association between *Campylobacter* infection and age, and studied the pathogenicity of *Campylobacter* and the recurrence of infection.

**Methods**

**Setting and study population**
This study was conducted in the low-income rural areas of Befotsy and Ampitambe, Moramanga, towards the middle of the eastern region of Madagascar. These two villages were the pilot areas of the Health and Demographic Surveillance Site of Moramanga (HDSS-Moramanga) and were investigated during the case-control study carried out in 2008 [6]. The highest prevalence of Campylobacter infection in children with diarrhoea was found in the district of Moramanga (20.7%). The prevalence of Campylobacter was 20% in Befotsy and 10% in Ampitambe. The local population is 4231 inhabitants, most of whom are engaged in agricultural activities. The 1006 households lack basic sanitary facilities, use water from the river or a traditional well for drinking and often have free-range domestic chickens at home; the risk of faecal contamination of the environment is therefore likely to be high.

**Cohort enrolment**

We used data from HDSS Moramanga, in which longitudinal demographic surveillance was carried out on the population of four communities in the district of Moramanga. The HDSS Moramanga conducted a door-to-door census, and collected demographic data and data for each individual household in the two villages at a given time point (water supply, goods, ownership of animals, etc.). Children under the age of 24 months living in either of the two villages were eligible for enrolment in this study. An open cohort of children enrolled before the age of 24 months was followed up from January 2010 to May 2012. All the children were monitored until the age of 36 months. Children who moved within the study area were followed up at their new homes, whereas those who moved outside the study area were withdrawn from the study. On enrolment, an interview was conducted with one of the child’s parents, to obtain information about the child, including breastfeeding and nutritional status. Each of the children enrolled provided a stool specimen for the isolation of Campylobacter spp. Enrolment continued
throughout the study, for new infants born into a household and for young children moving into the village.

**Surveillance activities**

**Twice-weekly diarrhoeal surveillance**

From the day of enrolment in the study until 36 months of age, a study physician and a locally recruited community health worker (CHW) visited each of the children twice weekly at home. The children’s mothers were asked about any episodes of diarrhoea since the last visit. If a child had diarrhoeal illness, the physician carried out clinical and anthropometric examinations, collected a stool sample for *Campylobacter* culture and provided oral rehydration therapy and/or other treatment if indicated. **Treatment was given in accordance with Ministry of Health guidelines.** Antimicrobial treatment was provided if diarrhoea was associated with fever or blood/mucus in the stools. A pictorial diarrhoea diary was given to the mothers of children with diarrhoea, for recording the number of bowel movements and the consistency of the faeces, from the first day of diarrhoea until its cessation. Movements of children to areas outside the study area, deaths and other losses to follow-up were recorded by the CHW during the twice weekly visits.

**Bimonthly surveillance**

We also carried out cross-sectional surveillance. All children included in the cohort, regardless of their history of diarrhoea, were surveyed once every two months (60 days), with the collection of a stool specimen for *Campylobacter* isolation. **In these surveys, weight and length/height data were also collected.** This cross-sectional provided us with data for asymptomatic *Campylobacter* infection.

**Definitions**
A day with diarrhoea was defined as a 24-hour period in which three or more loose stools taking the form of the container or any number of stools containing blood were obtained [11]. For small, exclusively breastfed children, if the stools were not bloody, diarrhoea was defined as an increase in the frequency or a reduction of the consistency of the stools with respect to what the mother considered normal for her child.

Episodes of diarrhoeal illness were defined using a minimum three-day diarrhoea-free gap to mark the beginning of a new episode.

- Campylobacter infection was defined as symptomatic if Campylobacter was isolated from diarrhoeal stools or within a period of five days before and after an episode of diarrhoea.

- Campylobacter infection was defined as asymptomatic if there were at least five consecutive symptom-free days before and after the isolation of Campylobacter from faeces.

- The days at risk included the three-day period following the occurrence of an episode of diarrhoea.

- Primary Campylobacter infection was defined as the first isolation of Campylobacter from a stool specimen collected during enrolment, or at the twice weekly or bimonthly surveillance visits, from a child enrolled within 28 days of birth.

- Multiple Campylobacter infection was defined as the isolation of Campylobacter from a stool specimen collected during enrolment, or at the twice weekly or bimonthly surveillance visits, from a child enrolled after the age of 28 days.

Microbiological analyses

For the isolation of Campylobacter spp., we cultured fresh faecal specimens from children with and without diarrhoea directly in the field on selective agar plates (Karmali). Plates were incubated at 37°C under microaerophilic conditions (Campygen, Oxoid France) for 48 to 72 hours. The identification of Campylobacter isolates was confirmed with the Campy dry spot kit, a
haemagglutination test from Oxoid (England), as recommended by the manufacturer. We
differentiated between *Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*) and other
species by the polymerase chain reaction (PCR) method. **This differentiation was conducted on**
271 samples of *Campylobacter* isolates selected at random.

**Data analysis**

We analysed data from 19<sup>th</sup> January 2010 until 31<sup>st</sup> May 2012. Person-time at risk was calculated
as the observed number of days at risk between episodes. Incidence rates were calculated by
dividing the number of episodes by the number of child-years of observation. We estimated the
incidence rates for diarrhoea and symptomatic infection. Children were assigned to age groups: 0
to 5 months, 6 to 11 months, 12 to 17 months, 18 to 23 months, 24 to 29 months and 30 to 36
months. **This classification was chosen in accordance with our hypothesis that exposure and
infection risk vary with age.**

**Association between infection and age**

**At the time of enrolment**

The Chi-squared test was used to measure associations between *Campylobacter* infection and age
group at the time of enrolment. If a significant result was obtained in the Chi-squared test, we
assessed the strength of association between *Campylobacter* infection and age group at the time
of enrolment by logistic regression. The unit of analysis was the individual child. **Data collected
at the time of enrolment were used, with age group considered as the explanatory variable and the
infection status of the faecal specimen as the outcome.**

**During follow-up**

During follow-up, the association of *Campylobacter* infection and symptomatic *Campylobacter*
infection with age group was assessed with a logistic mixed regression model. **The outcome
variable was *Campylobacter* infection or symptomatic *Campylobacter* infection status, and age**
group was the main explanatory variable. For the two analyses, we incorporated socioeconomic and household data collected at the time of enrolment, nutritional status data obtained during enrolment and follow-up as potential confounding variables. A list of the potential confounding variables considered in this study is shown in Table 1. We specified potential confounding variables at the outset of the multivariate analyses, on the basis of the results of univariate analyses (we included in the mixed models all potential confounding variables with a $p$-value $<0.2$). We then used backward elimination to identify robust confounding variables for inclusion in the final model. A Wald Chi-squared test was used to assess the significance of each of the variables tested and odds ratios were calculated to quantify their effects. The follow-up visits for a given child were not statistically independent entities. We therefore took into account the correlation between repeated observations for each individual, between multiple children living in the same household and in the same village. Thus, for the two mixed models, we added each observation for each child, household and village as a random effect. The use of a random effect made it possible to adjust for the correlation between data obtained from the same individual, the same household or the same village and to measure the variability between these data.

The unit of analysis was the individual visit, with the collection of a stool sample, for investigations of the relationship between *Campylobacter* infection and age. For analysis of the relationship between symptomatic *Campylobacter* infection and age, we considered each visit at which a diarrhoeal stool sample was obtained (diarrhoeal episode) as the unit of analysis. We took the 0-5 months age group as the reference group.

Data for *Campylobacter* infection status (overall infection, symptomatic infection) were obtained at the time of enrolment, and at the twice weekly and bimonthly surveillance visits.

*Pathogenicity of Campylobacter*
The association between *Campylobacter* infection and diarrhoeal episodes was investigated with logistic regression models. This analysis was performed on newborns enrolled within 28 days of birth. **Children with diarrhoea were considered as the outcome, with *Campylobacter* infection as the explanatory variable.**

**Recurrent episodes**

Survival analysis was used to assess the time to recurrent episodes of *Campylobacter* in children with primary infection and those with multiple infection. We used log-rank tests to compare the two Kaplan-Meier survival curves. Time to event was estimated as the interval between the first and second episodes of infection.

Data were analysed with R software version 2.12.1 (R Development Core Team (2007). For all statistical tests, a *p*-value below 0.05 was considered statistically significant.

**Ethics**

Written informed consent was obtained from the parent or guardian of each child before enrolment. The study was approved by the National Ethics Committee of the Ministry of Health of Madagascar (Number 002-CE/MINSAN – 01/13/2012).

**Results**

We recruited 210 children at the start of the study. Thereafter, 68 new births, 54 immigrants to the study and 152 children included after the age of 28 days were enrolled. The mean age of children at the start of the study was 11.7 months (standard deviation: 7.4; range: 0.2-23 months; median: 11.6 months). **During the observation period, four children died from causes other than diarrhoea during their first year of life and 68 (13.4%) dropped out of the study due to a withdrawal of consent (22/508) or migration out of the study area (46/508).** The study population comprised 508 children, who were followed for a total of 256,366 child-days (702.4 child-years).
The median follow-up time was 505.7 days (interquartile range [IQR]: 373 days). The cohort of 508 children consisted of 259 girls and 249 boys (male/female ratio = 0.9).

Over the two 2-year study period, 3424 stool samples were tested for *Campylobacter*. In total, 2965 (87%) of these samples were collected from children without diarrhoea during the cross-sectional survey, which was conducted at two-month intervals. The rate of *Campylobacter* isolation from all samples was 9.3% (319/3424): 8.9% (41/459) from diarrhoeic samples and 9.4% (278/2965) in non-diarrhoeic samples. Overall, 43.3% (220/508) of children had at least one *Campylobacter* infection; 16.4% (36/220) of the infected children had diarrhoea and 32.3% (71/220) had more than one episode of *Campylobacter* infection. Overall, 13.9% (71/508) of all children had more than one episode of infection. Identification to the species level was carried out for 271 of the 319 *Campylobacter* isolates: 190 (70.1%) were *C. jejuni*, 64 (23.6%) were *C. coli* and 17 (6.3%) belonged to other species. *Campylobacter* isolation rates from diarrhoeic and non-diarrhoeic samples, by age group, are shown in Table 2.

At the time of enrolment, 6.3% (n=32) of the children were already infected with *Campylobacter*: 6.2% (2/32) were symptomatic and the remaining (30/32) children were asymptomatic. The prevalence of *Campylobacter* infection, by age group, at the time of enrolment was 4.6% (17/369) for children under 12 months of age and 10.8% (15/139) for children aged 12 months or older. The frequency of *Campylobacter* infection at the time of enrolment differed significantly between age groups (Chi-squared test, \( p < 0.001 \)). The age-specific odds ratios for *Campylobacter* infection at the time of enrolment were 8.6 (95% confidence interval [CI]: 3.0-24.1), 5.9 (95% CI: 1.9-18.3), 5.7 (1.9-17.2) for the 6-11 month, 12-17 month and 18-23 month age groups, respectively.

The probability of infection during follow-up varied with age. In the logistic mixed model, taking children under six months of age as the reference group, the odds of being infected with
*Campylobacter* were five times higher in children aged between six and 18 months, the odds ratio were 5 (95% CI: 2.9-8.6) for children aged six to 11 months and 5.7 (95% CI: 3.3-10) for those aged 12 to 17 months (Table 3). The association for living in a household that owned livestock (versus living in a household that did not own livestock) was of borderline significance (odds ratio: 1.3; 95%CI: 1.0-1.7). The variables with a *p*-value below 0.2 included in the mixed regression model were the construction of a floor, the ownership of goods, domestic animals, livestock and the availability of toilets.

Antimicrobial treatments were given to children with diarrhoea who had fever and to children with blood or mucus in their faeces. Antimicrobial treatment was given to 18.5% (85/459) of patients with diarrhoeal episodes due to fever and to 17.8% due to the presence of blood or mucus in the faeces (3.1% for blood and 14.8% for mucus in the faeces). Mild dehydration was observed in four episodes of diarrhoea.

We observed 475 diarrhoeal episodes with a median duration of 4.5 days (IQR: 5 days), resulting in an annual incidence rate of 0.7 episodes/child (95% CI: 0.7-0.8 episodes/child). We were able to collect stool samples for 96.6% (459/475) of all diarrhoeal episodes. Overall, 53.7% (*n*=273) of the children presented at least one episode of diarrhoea during the observation period. The highest incidence rates were obtained for children under the age of 12 months. The annual incidence was 0.8 episodes/child (95% CI: 0.7-0.9 episodes/child) in children aged 0 to 11 months and 0.5 episodes/child (95% CI: 0.6 episodes/child) for children over the age of 12 months.

The overall annual incidence of symptomatic *Campylobacter* infections was 0.05 episodes/child. The median duration of symptomatic *Campylobacter* infection was five days, with an IQR of 4.3 days. The mean age for symptomatic *Campylobacter* infection was 13.8 months (standard deviation: 7.1; range: 2.7-33.8 months; median: 11.8 months). Among the
children with diarrhoea, 13.2% (36/273) excreted *Campylobacter* and 16.7% (6/36) received antimicrobial treatments. The frequency of symptomatic *Campylobacter* infections did not differ between age groups ($p=0.3$, Wald $z$-statistic test).

*Campylobacter* was isolated from 201(39.6%) asymptomatic children. The mean age for asymptomatic *Campylobacter* infection was 14.7 months (standard deviation: 7; range: 1.3-35.2 months; median: 13 months).

There was a statistically significant association between *Campylobacter* primary infection and diarrhoea ($p<0.001$, Wald $z$-statistics test). In the birth cohort of 64 children, the odds ratio for shedding *Campylobacter* for the first time among children with diarrhoea was 16.1 (95%CI: 1.8-140.8), taking children without diarrhoea as the reference group.

Infection recurred more rapidly in children with primary infections than in those with multiple infections: 25% of children with primary infections had their second infection episode a median of 63 days after the first episode, whereas the second episode occurred after a median of 317 days after the first episode in children with multiple infections ($p<0.01$). Figure 1 shows the survival curves for recurrent infection over time for the two groups.

**Discussion**

To our knowledge, this is the first cohort study on diarrhoea in children in Madagascar. Our results provide an indication of the role of the immunity on the occurrence of *Campylobacter* infection in children in Moramanga, because we observed an age-related decrease in infection rates and the time to recurrence was shorter in children with primary infections than in those with multiple infections.

We found an association between age group and infection, as reported in other developing countries [12,13], with infection rates increasing up to the age of six months and then decreasing after 30 months. In our cohort of children, immunity seemed to decrease the probability over time.
of individuals developing infections in response to further exposure [14, 15]. The shorter time to recurrence of infection in children with primary infection may reflect the immature and naive nature of the immune system in this group. The immune system is known to be immature at birth, with microbial colonization of the intestine playing a role in the maturation of the immune cell response [16, 17]. Repeated exposures may be required to achieve immunity. Prior immunity has also been demonstrated to be important for protection against Campylobacter in other studies [18]. Primary infection in our study occurred at a median age of eight months, suggesting a contribution of antibody transfer from the mother during the first eight months of life. Susceptibility to Campylobacter infection in early infancy may thus be reduced by the acquisition of passive immunity from placentally transferred antibodies from immune mothers [15]. A previous study conducted in the Central African Republic also suggested a protective role for maternal antibodies. Children who had Campylobacter infections during the first six months of life had significantly fewer anti-flagellum antibodies at birth than those who did not have Campylobacter infections during this period [19, 20].

We conclude that the first Campylobacter infection was pathogenic. An analysis performed in a subset of the cohort, in newborns enrolled during the first 28 days of life, corroborated this finding. Reports of the pathogenicity of Campylobacter in different studies from around the developing world have not been consistent [7, 8, 13]. The development of diarrhoea during Campylobacter infection may depend on bacterial virulence and host susceptibility factors [21], and strains of Campylobacter may differ in pathogenicity. There may also be animal host-adapted genotypes that never or rarely cause diarrhoea in humans [22]. Despite the many studies carried out on Campylobacter, we still need to elucidate the mechanism by which Campylobacter causes diarrhoea in humans and its interaction with the human immune system.
The rate of *Campylobacter* isolation in our study area was 9.3% and the annual incidence of symptomatic infection was 0.05 episodes/child. The rate of isolation of *Campylobacter* was lower than that reported for Peruvian children [13] but higher than that estimated for an Egyptian cohort [8]. However, the annual incidence of symptomatic infection in our study was only one tenth that reported in Peru [13], Mexico [7] and Egypt [8]. These differences may reflect differences in environment, context and study design between settings. In addition, technical difficulties in the isolation of *Campylobacter* species in developing countries, due to their fastidious growth requirements and/or the relative insensitivity of culture techniques, may influence detection rates [23].

One of the limitations of our study was the lack of availability of data about breastfeeding during the follow-up period. Breastfeeding may also affect the development of immunity during early infancy. In this study, we followed up a small sample of children included before the age of 28 days. An epidemiological study on this birth cohort might provide a better assessment of the role of immunity in determining the occurrence of infection.

**Conclusion**

In this study, we provide evidence of the high burden of *Campylobacter* infection in the rural areas of Befotsy and Ampitambe. Our findings suggest that protective immunity to this infection may be acquired over time. The association between infection and diarrhoea during the first year of life highlights the need for greater awareness of diarrhoeal disease prevention measures in infants and the importance of controlling *Campylobacter* infection. Repeated exposure is required for the generation of acquired immunity, but exposure to different strains of *Campylobacter* and overwhelming challenge may overcome immunity [18]. In developing countries, repeated exposure may subvert or suppress immune responses rather than leading to protection [24].
Prioritising interventions, such as the development of vaccines for prevalent *Campylobacter* serotypes, might have an impact on diarrhoeal illnesses in the developing world. The Moramanga HDSS constitutes a potential research platform for *Campylobacter* infection studies and vaccine trials.

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**Competing interest**

The authors have no competing interests to declare.

**Authors’ contributions**

RVR conceived and co-ordinated the study, performed the statistical analysis and drafted the manuscript; FR participated in the design of the study and carried out the biological analysis of stool samples; PS participated in the design of the study and was involved in revising the manuscript critically for important intellectual content; HCR performed the PCR analysis; AR performed the field study and participated in the coordination of the study; IMR performed the PCR analysis; RR participated in the design of the study and the collection of data; VR participated in the design of the study and was involved in revising the manuscript critically for important intellectual content. All authors have read and approved the final manuscript.

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the Institut Pasteur of Madagascar. We would also like to thank the local community health workers and the people of the Fokontany of Befotsy and Ampitambe for their participation.

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V. Case-control study of the etiology of infant diarrheal disease in 14 districts in Madagascar. PLoS ONE 2012, 7(9):e44533


### Table 1. Potential confounding variables collected during a 28-month longitudinal community study of *Campylobacter* infection, Moramanga, 2010-2012

<table>
<thead>
<tr>
<th>Individual characteristics</th>
<th>Sex</th>
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<tr>
<td></td>
<td>Weight</td>
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<td>Length/height</td>
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<tr>
<td>Household data</td>
<td>Number of inhabitants/room, Construction of a floor</td>
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<tr>
<td></td>
<td>Ownership of goods[^e]</td>
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<td>Ownership of domestic animals</td>
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<td></td>
<td>Ownership of livestock[^h]</td>
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<td>Ownership of fowl[^l]</td>
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<td></td>
<td>Availability of toilet</td>
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<td>Availability of a shower</td>
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<td>Availability of a cooking area</td>
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<td>Source of drinking water</td>
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<td>Protection of drinking water</td>
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<td></td>
<td>Availability of soap</td>
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<td></td>
<td>Type of fuel for cooking</td>
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<td></td>
<td>Type of fuel for lighting</td>
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<table>
<thead>
<tr>
<th>Characteristics of the child’s mother</th>
<th>Age</th>
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<td></td>
<td>Education level</td>
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[^e]: Ownership of one of the following: radio, television, bicycle, sewing machine, mobile phone, rice fields, house
[^h]: Ownership of one of the following: cow, ox, sheep, pig, rabbit
[^l]: Ownership of one of the following: chicken, duck, goose
Table 2. Rate of isolation of enteric *Campylobacter* from diarrhoeic and non-diarrhoeic samples, Moramanga, 2010-2012.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Child-days at risk</th>
<th>Number of episodes</th>
<th>Number positive (%)</th>
<th>Number of tests</th>
<th>Number positive (%)</th>
<th>Number of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 months</td>
<td>27,616</td>
<td>64</td>
<td>4 (6.2)</td>
<td>564</td>
<td>14 (2.5)</td>
<td>18</td>
</tr>
<tr>
<td>6-11 months</td>
<td>77,208</td>
<td>171</td>
<td>16 (9.3)</td>
<td>641</td>
<td>93 (14.5)</td>
<td>109</td>
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<tr>
<td>12-17 months</td>
<td>68,184</td>
<td>102</td>
<td>10 (9.8)</td>
<td>557</td>
<td>90 (16.1)</td>
<td>100</td>
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<tr>
<td>18-23 months</td>
<td>25,146</td>
<td>64</td>
<td>6 (9.4)</td>
<td>535</td>
<td>46 (8.6)</td>
<td>52</td>
</tr>
<tr>
<td>24-29 months</td>
<td>30,859</td>
<td>40</td>
<td>4 (10.0)</td>
<td>399</td>
<td>22 (5.5)</td>
<td>26</td>
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<tr>
<td>30-36 months</td>
<td>27,353</td>
<td>18</td>
<td>1 (5.5)</td>
<td>269</td>
<td>13 (4.8)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>256,366</strong></td>
<td><strong>459</strong></td>
<td><strong>41 (8.9)</strong></td>
<td><strong>2965</strong></td>
<td><strong>278 (9.4)</strong></td>
<td><strong>319</strong></td>
</tr>
</tbody>
</table>
Table 3. Associations of *Campylobacter* infection with age group during follow-up, mixed logistic regression model, Moramanga, 2010-2012

<table>
<thead>
<tr>
<th>Confounding variables</th>
<th>Presence of <em>Campylobacter</em></th>
<th>Crude OR</th>
<th>Adjusted OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>≤ 6 months</td>
<td>16 (3.0)§</td>
<td>521 (97.0)</td>
<td>Reference</td>
</tr>
<tr>
<td>6-11 months</td>
<td>94 (13.1)</td>
<td>619 (86.9)</td>
<td>4.9 (2.7-8.9)†</td>
</tr>
<tr>
<td>12-17 months</td>
<td>90 (15.0)</td>
<td>509 (85.0)</td>
<td>6.0(3.3-10.9)</td>
</tr>
<tr>
<td>18-23 months</td>
<td>51 (9.0)</td>
<td>514 (91.0)</td>
<td>3.3(1.8-5.9)</td>
</tr>
<tr>
<td>24-29 months</td>
<td>25 (6.0)</td>
<td>396 (94.0)</td>
<td>2.2(1.2-4.0)</td>
</tr>
<tr>
<td>30-36 months</td>
<td>14 (4.9)</td>
<td>272 (95.1)</td>
<td>1.8(0.8-3.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>290</strong></td>
<td><strong>2831</strong></td>
<td></td>
</tr>
</tbody>
</table>

† Numbers in brackets, 95% confidence interval; OR: odds ratio

§ Numbers in brackets, percentage

*Adjusted for the ownership of livestock, mixed models were generated from 3121 observations
Figure 1: Survival curves for *Campylobacter* infections in children with primary and multiple infections, Moramanga 2010-2012, log-rank test $p<0.01$
Figure 1

Probability of Campylobacter Infection

- Green line: Children with primary infection
- Blue line: Children with multiple infection

Days

0 100 200 300 400 500