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ABSTRACT

Background: **Polyparasitism seems to be a common feature in human populations in sub-Saharan Africa. However, very little is known about its epidemiological significance, its long term impact on human health or the types of interactions that occur between the different parasite species involved.**
Objectives: **To determine the prevalence and co-occurrence of intestinal parasites in a rural community in the Kibwezi, Makueni district, Kenya.**
Design: **A cross sectional study.**
Setting: **Kiteng'ei village, Kibwezi, Makueni district, between May and September 2006.**
Subjects: **One thousand and forty five who comprised of 263 adult males, 271 adult females> 15 years of age and 232 boys, and 279 girls < 15 years of age.**
Interventions: **All infected members of the community were offered Praziquantel (at dosages of 40 mg/ kg body weight) for Schistosomiasis and Albendazole (600 mg) for soil transmitted helminths.**
Results: **A total of ten intestinal parasite species (five protozoan and five helminth parasite species) were present in this community and polyparasitsm was common in individuals 5- 24 years of age with no gendar related differences. Most of the infections were mild. The protozoan parasites of public health significance present were *Entamoeba histolytica* and *Giardia lamblia* with prevalence of 12.6% and 4.2%, respectively. The helminth parasites of public health significance in the locality were *Schistosoma mansoni* with a prevalence of 28%, and hookworms prevalence of 10%. About 53% of the study population harboured intestinal parasite infections, with 31 % of the infected population carrying single parasite species infections, and 22% harbouring two or more intestinal parasite species per individual. Significant positive associations (p values <0.05) were observed between *S. mansoni* and hookworms, hookworms and *Hymenolepis. nana* and *Entamoeba histolytica* and *Entamoeba coli.***
Conclusion: **Intestinal polyparasitism was common in the Kiteng'ei community, particularly in individuals aged of 5-24 years old. An integrated control programme of approach would be recommended for the control of *S. mansoni,* hookworms and *Entamoeba histolytica* for this community.**

INTRODUCTION

Polyparasitism is a common phenomenon worldwide, and while its effects are often clinically inapparent, multiple parasite species infections may exacerbate clinical manifestations (1). For example, malaria parasites, intestinal helminths and protozoan may co-exist in an individual causing micro-nutrient deficiencies, anaemia, and contribute to retarded growth and development (2). Co-occurrence of schistosomiasis and filariasis in an individual may also result in retarded growth and development (2). Overall, individuals with multiple parasite infections are often at an elevated risk of morbidity (3). Intestinal parasitic infections are common in sub-Saharan Africa and often multiple parasite species involving both helminths and protozoans may occur in an individual (4-7). Children are the most commonly infected and the parasite species involved include *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidia* (all protozoans) and hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* (helminths), (4,5,8,9).

The global burden of disease caused by the three major intestinal nematodes (hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*) is estimated at 22.1 million disability adjusted life years (DALYs) lost to hookworm, 10.5 million for *A. lumbricoides* and 6.4 million for *T. trichiura* (2). The combined burden of infection is estimated to exceed 1000 million infected persons with *Ascaris lumbricoides*, hookworm (*Ancylostomum duodenale* and *Necator americanus*), and *T. trichiura* (7).

Three major intestinal helminths namely, hookworms, *Trichuris* and *Ascaris*, hookworm is the most important public health problem with an estimated 1.3 billion people infected worldwide (10) and often associated with iron deficiency anaemia (11). Hookworm infections frequently co-occur with *A. lumbricoides* and *T. trichiura* and comprise the major soil transmitted helminths (7). The World Health Organization (WHO) has estimated that *schistosomiasis* and soil-transmitted helminth infections represent 40% of the disease burden due to all tropical diseases excluding malaria (12). Giardiasis, with an estimated 200 million people infected, is most common in children aged one to five years old, and in severe cases may be associated with acute and persistent diarrhoea, malabsorption of nutrients and impairment of children's growth and development (8).

Intestinal polyparasitism is common in Kenya (8,13-15). The effects of polyparasitism in children may include wasting, stunting, decreased physical fitness and activity, decreased cognitive development and performance, decreased work performance capacity and productivity, and increased school and work absenteeism; and in adults its effects include decreased fitness, cognition, activity, performance, work capacity and productivity, increased maternal and foetal morbidity and death (3). The purpose of the present study was to assess the extent of intestinal polyparasitism as a measure of disease burden in a rural community based in Kiteng'ei village, Kibwezi area, Kenya, in preparation for an integrated intervention programme targeting schistosomiasis and intestinal parasitic infections. In addition, gender and age were investigated as factors in polyparasitism, and the association between the different parasite species involved determined.

MATERIALS AND METHODS

Study area and study population: This study was conducted in Kiteng'ei village, Kambu location in the Kibwezi, Makueni District which lies between longitude 38 ° -38.2° East and latitude 2.1° -2.3° South of the Equator, and located approximatel 250 km south east of Nairobi. The village is located along the Kambu river, a seasonal stream which is a tributary of the Athi River complex. The demographic information obtained for the study village included number of households, number of persons in each homestead and age/ sex of each study individual. Each study individual was given a unique identification number.

Faecal sample collection and laboratory procedures: This was a cross- sectional study, carried out from May to September 2006, and involved the entire population of Kiteng'ei. A single faecal sample was collected from each enrolled and consenting individual in an individual specimen container and processed for parasitological examination by microscopy. Faecal specimens were examined for both protozoan and helminth parasite species. A single Kato-Katz thick faecal smear was prepared from each stool sample essentially as described by Carter and Lema (16) for the detection of schistosomes and helminth ova, and the formol-ether wet faecal smear was prepared for detection of intestinal protozoan cysts as described by Carter and Lema (16). The smears were examined for presence of parasite ova or cysts by microscopy, but only *Schistosoma mansoni* ova were quantified.

Treatment of infected individuals: All the individuals diagnosed to have intestinal protozoan infections were given metronidiazole (Flagyl^{R}), standard adult dose 750 mg (by oral administration) three times a day (tid) for five days and standard paediatric dose of 10-15 mg/kg body weight tid for five days, and those found to have schistosome infection received a standard oral dose of praziquantel (40 mg/kg body weight). Those infected with intestinal helminth infections received albendazole (600 mg) as recommended by the WHO (12).

Data analyses: Demographic and parasitological data were entered into a computer for storage and subsequent analysis. The SPSS-PC version 11.0 (SPSS Chicago Illinois) computer software was used for data handling and analysis. Frequency distribution of variables was performed to illustrate the prevalence of the intestinal protozoan and helminth parasite species. Chi squared test (X^2) was done to compare proportions. Univariate statistics were applied to assess the influence of age or sex on infection status. Cross tabulations were performed to compare (prevalence or intensity) between males and females for *Entamoeba coli*, *Entamoeba histolytica*, *I.butschlii*, *S. mansoni* and hookworms. The association between parasite species was examined by using 2 x 2 contingency tables. The Chi- Squared test at 95% confidence interval with one degree of freedom and 5% significance level were done to show the differences in parasite pairs. In a second step, logistic regression modeling techniques were applied to assess significant association between a particular intestinal parasite and sex, age group and another intestinal parasite. For each parasite, a baseline model was established with infected study participants defined as cases. Sex, age group and infection status with intestinal parasites were all incorporated into the model. Non significant associations ($p > 0.05$) were removed. The odds ratio (OR) including 95% confidence interval (CI) and corresponding P- values were calculated for the associations that were significant. Measurements of association between the different parasite species was performed using Pearson's Correlation (17). Multivariate analysis was performed to show associations between the different species of parasites.

Study approvals: This study was undertaken with the approval of both the scientific and ethical review committees of the Kenya Medical Research Institute (KEMRI). The project was presented and explained to the community through a Chiefs *baraza* and informed consent was obtained from the adults (18 years of age and above) and from parents / guardians of participating children (<18 years old), before stool specimens were obtained from individuals for parasitological examination.

RESULTS

Intestinal parasite infections in the Kiteng 'ei Community: There were 295 households in the Kiteng'ei village with a total population of 1785 individuals (comprising 898 males and 887 females). A total of 1045 individuals who included 511 children and 534 adults were examined for intestinal parasites. Both protozoan and helminth parasites were present in the community, and a total of ten parasite species were identified under a microscope based on presence of characteristic cysts (for protozoa) or ova (for helminths) in the faecal specimens (Table 1).

Table 1

Sex-related differences in the prevalence of intestinal protozoan parasites, Schistosoma mansoni and soil-transmitted helminths in the Kiteng'ei Community of Kambu, Kibwezi, Makueni District, Kenya

Intestinal parasite species encountered	Overall prevalence (n=1045)		Male (n=508)		Females (n=537)		P-value *
	No.	(%)	No.	(%)	No.	(%)	
A Protozoan parasite							
Entamoeba coli	174	16.7	87	17.1	87	162	>0.001
Entamoeba histolytica/ Entamoeba dispar	132	12.6	76	15.0	56	10.4	<0.01
Iodamoeba butschlii	63	6.0	25	4.9	38	7.1	<0.025
Giardia lamblia	44	4.2	18	3.5	26	4.8	>0.001
Endolimax nana	17	1.6	10	2.0	7	1.3	<0.025
B Helminth parasites							
Schistosoma mansoni	291	28	167	32.9	124	23.1	<0.025
Hookworm	108	10.3	55	10.2	53	9.9	>0.005
Hymenolepis nana	3	0.3	1	0.2	2	0.4	<0.005
Trichiuris trichiura	2	0.2	0	0	2	0.4	>0.005
Ascaris lumbricoides	1	0.1	0	0	1	0.2	>0.005

* Tested using Chi-squared (X^2), P-values of 0.05 and below were considered statistically significant.

Of the 1045 individuals examined, 53.4% harboured at least one parasite species, with 41% harbouring protozoan infections and 39 helminth infections. The most common protozoa present in the Kiteng'ei community were *Entamoeba coli* with a prevalence of 17% and *Entamoeba histolytica/ Entamoeba dispar* a pathogen that causes diarrhoea (prevalence 13%), and *Giardia lamblia* common intestinal flagellate (prevalence 4%). Other protozoan parasites present in the community were *Iodamoeba butschlii* (6%) and *Endolimax nana* (2%). Of the parasitic protozoans present, *Entamoeba histolytica* and *G. lamblia* are considered to be of public health significance. Interestingly, significantly more males (7.3%) than females (5.4%) harboured *Entamoeba histolytica*, infections whereas no gender related differences in prevalence were observed for *Entamoeba coli*. Protozoan infections were present across all age groups, with peak prevalences occurring in individuals in the 5-14 years and 15-24 year age groups (Table 2).

The most common helminth infections in this community were *Schistosoma mansoni* (prevalence 28%) and hookworms (10.3%) (Table 1). Other helminth parasites present were *Hymenolepis nana*, *Trichuris trichiura* and *Ascaris lumbricoides*, but their prevalences were less than 0.5%. With respect to *S. mansoni* infection, the proportion of males (16%) infected was significantly greater than the females (12%), $P<0.025$. However, no gender related difference in prevalence was observed in hookworm infections. Overall, *S. mansoni* infections were most common in individuals aged 24 years and below, whereas hookworm infections were most common in older individuals (Table 2).

Multiple parasite infections: Multiple parasite infections (Polyparasitism) were recorded in 22% of the study population. Majority of those with multiple parasite infections (21.4%) had two to three parasite species per individual, and only 0.6% harboured four parasites. Of the 173 individuals harbouring two parasite species, 114 (65.9%) had both protozoan and helminth parasite species while 48 (27.7%) had protozoan parasite species, and 11 (6%) had helminth parasite infections only. Of the 43 individuals habouring three parasite species infections, 24 (55.8%) of the individuals harboured two protozoan and one helminth parasite species, 14 (32.6%) of the individuals had two; one protozoan and two helminth parasite species, and only five (11.6%) of the individuals harboured three protozoan parasite species. Interestingly, none of the study individuals harboured three helminth parasites concurrently. In general, intestinal protozoan parasite infections occurred across all the age groups in the study population, but most of the helminth parasite infections occurred in the younger age groups (<24 years old). The prevalence of polyparasitism was apparently not gender related (Figure 1) but was age-related, being most prevalent in individuals in the 5-14 year age group (Figure 2).

Table 2

Prevalence of intestinal parasite infections by host age in the Kiteng'ei Community of Kambu, Kibwezi area in Makueni District, Kenya

Parasite Species	Age group in years											
	<5 (n=173)		5-14 (n=338)		15-24 (n=199)		25-34 (n=92)		35-44 n=69		≥45 (n=174)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
E. coli	14	8.1	43	12.7	41	20.6	17	18.5	12	17.4	47	27.0
E. histolytical	10	5.8	51	15.1	30	15.1	12	13.0	7	10.1	22	12.6
E. dispar												
I. butschlii	3	1.7	21	6.2	20	10.0	4	4.3	6	8.6	9	5.2
G. lamblia	8	4.6	14	4.1	10	5.0	3	3.3	3	4.3	6	3.4
E. nana	4	2.3	3	0.9	4	2.0	3	3.3	0	0	3	1.7
S. mansoni	8	24.6	112	33.1	103	51.8	23	25.0	15	21.4	30	17.2
Hookworm	3	1.7	25	7.4	31	15.6	7	7.6	13	18.8	29	16.7
H. nana	0	0	2	0.6	1	0.5	0	0	0	0	0	0
Tr. Trichiura	1	0.6	1	0.3	0	0	0	0	0	0	0	0
A. lumbricoides	1	100	0	0	0	0	0	0	0	0	0	0

Figure 1

Prevalence of single and multiple parasite species infections in the Kiteng'ei community of Kambu, Kibwezi, Makueni district, Kenya by gender

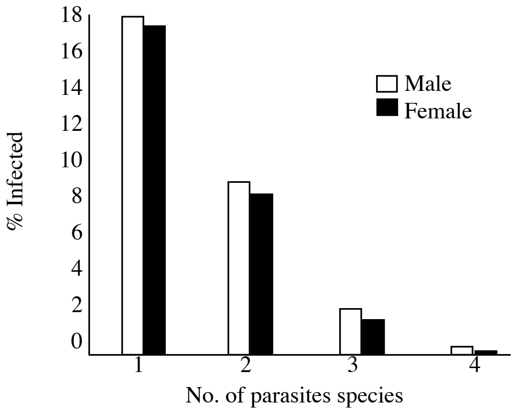
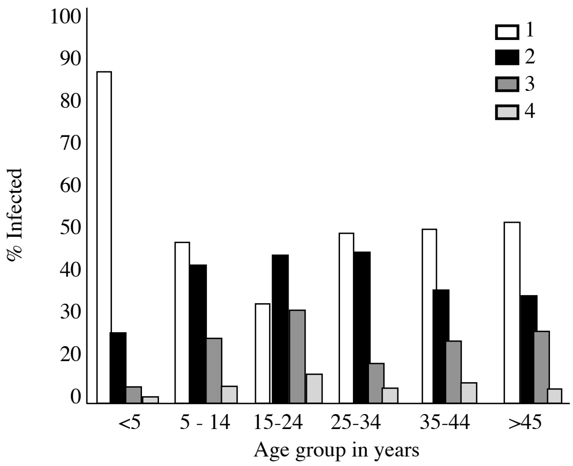


Figure 2

Prevalence of polyparasitism in Kiteng'ei community of Kambu, Kibwezi, district, Kenya in relation to age groups



Association between parasite species: Both positive and negative associations were observed for parasite species present in the Kiteng'ei community. Significant positive associations were observed between protozoan species, between helminth and protozoa species, and between helminth species (Table 3). Although *Schistosoma mansoni* was the most common parasite species in the community, it was not associated with the occurrence of other parasites, and only a marginal association was observed between *Schistosoma mansoni* and hookworm infections. Statistically significant pairwise interactions were observed between *Entamoeba histolytica/ Entamoeba coli* and hookworms/ *Entamoeba coli*. Similarly, negative associations were observed between protozoan and helminth species. However, these associations were not statistically significant by Pearson's correlation coefficient (r) analysis.

The risk estimator (Odds Ratio): Given one parasite species, the estimation of the measure of the odds of being infected to non-infected by the particular parasite species was calculated at 95% confidence interval and 5% significant for the different parasite pairs. The presence of hookworm was a risk for the occurrence of *Hymenolepis nana*, with odds ratio of 17.66. Other parasite pairs which had high odds ratio were: *Endolimax nana* with *Iodomoeba butschlii* (6.968), *Entamoeba coli* with *Iodomoeba butshclii* (6.106), and *Entamoeba coli* with *Endolimax nana* (5.884). The presence of *S. mansoni* which was the most prevalent parasite was not associated with the occurrence of other parasites.

Table 3

Positive associations between intestinal parasite species present in the Kiteng'ei Community of Kambu, Kibwezi area in Makueni District, Kenya

Parasite pair	Pearson's R-value	Significance test	Odds ratio (95% CI)	P-value*
S. mansoni versus Hookworm	0.021	0.0507	1.56 (0.75, 1.79)	0.046
A. lumbricoides versus *E. coli*	0.069	0.025	0.17 (0.15, 0.99)	0.035
Hookworm versus *H. nana*	0.099	0.001	17.7 (1.6, 196.4)	0.001
Hookworm versus *E.coli*	0.152	0.000	2.9 (1.9, 4.5)	<0.001
E. histolytica	0.108	0.000	2.1 (1.4, 3.2)	<0.001

versus *E. coli*				
E.coli versus *E. nana*	0.125	0.000	5.9 (2.2, 15.50	<0.001
E. coli versus *I. butschlii*	0.232	0.000	6.1 (3.6, 10.3)	<0.001
E. nana versus *I. butschlii*	0.126	0.000	7.0 (2.4, 20.4)	<0.001
Hookworm versus *I. butschlii*	0.083	0.055	2.0 (1.0, 3.8)	0.055

* P- values of 0.05 and below were considered statistically significant

DISCUSSION

In the present study, the prevalence of intestinal parasites was examined in a rural community living in Kiteng'ei village in the Kibwezi area. This information is important for a rationale design and implementation of a sound control programme. Intestinal protozoan parasites, *Schistosoma mansoni*, and soil transmitted helminths are endemic in the Kiteng'ei community. Polyparasitism was common in the community and accounted for 22% of the parasite infections. Previous studies on intestinal parasites in Kenya considered specific groups mostly school children and focused primarily on helminth parasite infections (14,15). The current study investigated both helminth and protozoan parasites, across a community comprising both children and adults. The results of the current study are comparable with those of previous studies with respect to intestinal parasite infections in Kenya (18).

The results of the present study were obtained from a cross-sectional survey of a population of 1045 individuals. Diagnosis of the intestinal parasites was based on both the Kato-Katz and formol-ether faecal processing techniques on a single faecal sample per individual. The combined faecal processing techniques improved the detection of hookworms in particular, whose detection becomes difficult beyond 30 to 60 minutes on Kato - Katz slide preparation. Detection of intestinal protozoan parasites relied on a single formol-ether processed faecal samples and parasites were scored as present or absent. The weakness of relying on single faecal sample versus the conventional three stool samples is that intestinal parasites are shed in stools intermittently, hence reliance on single sample may miss some parasites and hence lead to underestimation of parasite prevalence (4,8). However, a stochastic model for egg count variation which incorporates the distribution of worms and worm pairs in the population has been developed (19). A pocket chart for estimating the true *Schistosoma mansoni* prevalence for each combination of observed prevalence and geometrical mean of positive egg counts has been developed and validated (20). Using this model, and our observed single stool survey prevalence of 28%, the predicted true prevalence from the pocket chart is 50%. The cost of collection and processing of three stool samples per individual in a community surveillance may be too high hence prohibitive in terms of resources. The drop out rates in experiments involving communities may be as high as 30% hence a very large community may need to be sampled to ensure the correct sample size.

In our study, we observed presence of five protozoan and five helminth parasite species with 22% of the study population harbouring two or more parasite species and was most common in individuals below 24 years of age. For parasites of medical significance, intestinal protozoan parasites (primarily *Entamoeba histolytica*), *S. mansoni*, and geohelminths (primarily hookworms) were fairly common in the study population. Our observations are in agreement with other studies carried out in Kenya (8,13) and other parts of sub-Saharan Africa (6,20) that have demonstrated that the multiple parasite species infections are the norm rather than the exception. Presence of multiple parasite species in an individual may be associated with severe morbidity and complication (4,11). Therefore, any control initiative that targets only a single parasite species may not be effective in reducing morbidity due to intestinal parasitic infections. An integrated approach targeting the major parasite species present is recommended for this community.

The current study confirms a significant positive association found between *S. mansoni* and hookworm infections (21). The 5 -14 year age group with higher infection intensities of *S. mansoni* are at higher risk of concurrent hookworm infection. The interactions are likely to be ecological in nature, largely explained by lack of water and sanitation (21). *S. mansoni*, though the most commonly occurring helminth did not have significant positive associations with other parasite species. In the present study, there was consistent evidence for strong positive association between certain parasite species especially between the various species of *amoeba*. For example *Entamoeba histolytica* and *Entamoeba coli* were associated, consistently. This is consistent with findings in other studies (1, 4). In the current study, there were positive associations between *Entamoeba coli* and hookworms, *E. histolytica* and *E. nana, I. Butschlii* and *A. lumbricoides*. These associations suggest that factors that increase the likelihood of infection, for one' species are shared by others. Positive associations observed among different pathogenic parasites may be of clinical significance and may result in aggravated morbidity in the infected individual. Although *E. coli* is a non pathogenic protozoan, its co-infection could influence morbidity caused by the other parasites (1). The positive association between *E. coli* and *E. histolytica* is in agreement with previous studies conducted in Kenya (8) and other tropical countries (4).

Negative parasite associations were observed between different intestinal parasites with *Giardia lamblia* indicating that *G. lamblia* is negatively correlated with the occurrence of other intestinal parasites. This has also been observed in other studies (8,22). Clinical *giardiasis* is usually most prevalent in young age groups, which was confirmed in this study.

In conclusion, intestinal polyparasite infections were common in Kiteng'ei community affecting 22% of the study population and most common in individuals in the 5- 24 year age group. These estimates were based on a single stool examination, so the actual prevalence will be even higher. There were no gender related differences in prevalence of multiple parasite infections in the study population. From public health view point, the parasites that deserve attention in this community are *Entamoeba histolytica* and *S. mansoni* and an intervention strategy that targets these parasites is recommended for this community.

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