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Malaria, Intestinal Parasitic Infection, Anemia, and Malnourishment in Rural Cameroonian Villages with an Assessment of Early Interventions

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Abstract

Malaria, water-borne diarrheal diseases, and geohelminth infections, combined with severe malnutrition ravage entire villages throughout sub-Saharan Africa. The Bawa Health Initiative (BHI) is a 501c(3) non-profit organization with the goal of implementing a comprehensive public health program in an attempt to address these problems in a series of rural villages located in the West Province of Cameroon, Africa. Interventions include the provision of permethrin-treated bed nets to reduce the transmission of malaria, the installation of biosand water filters to reduce the prevalence of water-borne diseases, and a geohelminth control program utilizing mass treatment with albendazole. This study details the results of surveys conducted to monitor the success of the interventions. Since implementation of interventions, the number of clinical cases of malaria, diarrheal disease and typhoid has decreased, the prevalence of water-borne protozoan parasites has decreased, the prevalence and intensities of geohelminth infections has significantly decreased, and the prevalence of anemia has significantly decreased. When viewed in its entirety, these data show that the comprehensive approach to public health challenges in these villages initiated by BHI has been extremely successful. However, much work remains to be done. The primary purpose of this paper is to further inform academicians, students, and the general public about the continuing problems associated with these diseases and to describe and assess the effectiveness of some current interventions being used to combat them.

Introduction

Malaria, water-borne diarrheal diseases, and helminth infections combined with severe malnutrition is the perfect formula for extreme human suffering. More than 2 billion people are infected with worms, more than 500 million suffer from malaria, 884 million people lack access to improved water, and 2.6 billion do not use improved sanitation (Breman et al. 2006, Hotez et al. 2006, World Health Organization (WHO) and United Nations Children's Fund (UNICEF) 2010). Millions die because of intestinal infection in combination with malnutrition. Undernutrition itself is directly or indirectly responsible for at least 35% of global deaths in children less than five years of age. Due to the disastrous effects of malnutrition, approximately 186 million (32%) children under five in developing countries are stunted, and about 55 million (10%) are wasted (WHO 2010a).

Malaria, arguably the most important human disease that has ever existed, is caused by infection of red blood cells with apicomplexan parasites of the genus *Plasmodium*, transmitted to humans through the "bite" of female mosquitoes of the genus *Anopheles*. Approximately 732,000 children under 5 die each year of malaria (Bryce et al. 2005, Breman et al. 2006, Black et al. 2010). Sub-Saharan Africa accounts for 90% of all cases of malaria that occur in the world. Treatment of malaria consumes 40% of sub-Saharan Africa's public health expenditure, and accounts for 30-50% of hospital admissions (Akande and Musa 2005, WHO 2010b). In Africa, a child under five dies of malaria every 45 seconds (WHO 2010b). Additionally, malaria infection during pregnancy causes up to 10,000 maternal deaths each year, 8-14% of low birth-weight babies, and 3-8% of all infant mortality (Steketee et al. 2001, Marchesini and Crawley 2004). Furthermore, malaria inflicts a heavy burden on the economies of Africa and the general well-being of her people by placing a heavy burden on both nutritional and human resources. If malaria had been eradicated as hoped in the early 1960s, it is estimated that Africa's gross domestic product would currently be $100 billion greater than it is now (Brundtland 2005).

A similarly vexing public health challenge in sub-Saharan Africa is that of water-borne diseases. Unsafe drinking water and inadequate sanitation has been implicated as the source of 2.5 billion cases of diarrhea in children under 5 each year, leading to 1.5 million deaths (UNICEF and WHO 2009). Most of these deaths are of children in developing countries, and the majority is in sub-Saharan Africa. Water-borne diarrheal diseases include bacterial diseases such as typhoid and cholera, and diseases such amebiasis and giardiasis caused by the parasitic protozoans *Entamoeba histolytica* and *Giardia lamblia*, respectively. It is estimated that *E. histolytica* and *G. lamblia* infect nearly 500 million and 200 million at any given time, respectively (WHO 1990). Callahan (2010) provided a summary of reports of water-borne protozoal diseases in Africa.

A third party in this "unholy trinity," a phrase coined by Hotez (2008), is infection with geohelminths. These soil-borne nematodes include whipworms (*Trichuris trichiura*), the large human roundworm (*Ascaris lumbricoides*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*). An estimated 300 million people suffer severe morbidity as a result of heavy infection with goehelminths and 150,000 die each year as a result (Crompton 1999, Montresor et al. 2002, Hotez et al. 2006). While, relatively speaking, geohelminth infections cause few deaths, they have profound and insidious effects on the health and nutritional status of millions. Hotez (2008) pointed out that geohelminth infections comprise the most common infections of the world's poorest people, may be the leading cause of growth retardation and stunting, and are "a major cause of economic underdevelopment, as they presently block the escape from poverty." This observation becomes particularly profound when one considers that these infections are often most prevalent alongside malnutrition from a severe lack of food.

The global statistics related to geohelminth infections demonstrate that they have a crippling effect on society. Whipworms infect over 1 billion people worldwide (Crompton 1999). World-wide 21% of preschool-age children (114 million) and 25% of school-age children (233 million) are infected with whipworms (Stephenson 2002). Chronic whipworm infection contributes to stunted growth and anemia in children (Cooper and Bundy 1988, Bundy and Cooper 1989, Despommier et al. 2005). *Ascaris lumbricoides* infects 1 in 4 people worldwide. As of 1990, 158 million (29%) of the world's preschool age children and 320 million (35%) of school-age children were estimated to be infected (Stephenson 2002). Approximately 1.3 billion people are infected with either *A. duodenale* or *N. americanus*, leading directly to 65,000 deaths per year, mostly children. Because of their hematophagous zeal, hookworms are a primary cause of anemia. Despommier et al. (2005) pointed out that children heavily infected with hookworms are likely to develop deficits in both physical and cognitive development and that hookworm infection during pregnancy may lead to low birth weight, premature birth, and increased risk of maternal mortality.

The Bawa Health Initiative (BHI) is a 501(c)3 with the goal of initiating a comprehensive public health care program in the village of Bawa and surrounding areas located in the Menoua Divison, West Province of Cameroon, Africa that lies along a volcanic line in the western Cameroon highlands (Tchuinkam et al. 2010). These Bamiléké villages are a homogenous assemblage of remote rural villages populated almost exclusively by subsistence level farmers. There is no electricity, running water, or improved sanitation in the villages. See Smith (2007), Callahan (2010), and Richardson et al. (2011a) for more complete descriptions of the villages and Tchuinkam et al. (2010) for a more complete geographic characterization of the region. A broader goal of BHI is to educate the general public, particularly young people, in the developed world about the heavy toll taken on humanity by infectious diseases in the developing world.

This study details the results of surveys of intestinal parasites, nutritional status, and prevalence of clinical diseases conducted in Bawa-Nka (a quarter within the village of Nka), Bawa, and Nloh. This facilitates the assessment of the early effectiveness of interventions initiated by BHI.

During the summer of 2006, an oral survey was conducted of each family compound in the village of Bawa to assess knowledge and attitudes concerning infectious diseases and matters of basic hygiene and sanitation. The survey revealed malaria and diarrheal diseases to be among the greatest health problems in the village. Over 50% of parents responding to the survey indicated that at least one of their children had been afflicted with malaria within the preceding two weeks. Records at the nearest government-operated health clinic indicated that more than 80% of all hospitalizations of residents from Bawa were a result of malaria or diarrheal disease.

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Tchuinkam et al. (2010) pointed out that although the highland areas of Africa are known to be malaria hypoendemic, because climatic conditions are not ideal for development of *Anopheles* mosquitoes, the probability of transmission of malaria by a single mosquito encounter in these regions is actually higher than in holoendemic areas (Beier et al. 1994, Snow et al. 1997). Tchuinkam et al. (2010) further suggested that the recent increase in the number of malaria epidemics with a spread of endemic malaria into the highland fringes (Adjuik et al. 1998) may be a consequence of global warming (Martens et al. 1995, Jetten et al. 1996), as anophelid vectors extend their normal range or exhibit local increases in abundance in response to changing climate.

The proper use of insecticide-treated bed nets substantially reduces morbidity and mortality associated with malaria (Breman et al. 2006, Lengler 2009). Additionally, the greatest efficacy is being realized in hypoendemic zones (Lengeler 2009) such as in the western Cameroonian highlands (Tchuinkam 2010). Insecticide-treated nets typically provide more than 50% efficacy in preventing episodes of malaria, a 29% reduction in the number of cases of malaria, and they may reduce childhood mortality by 18% (Breman et al. 2006).

Materials and Methods

In June 2007, a survey was conducted in Bawa and Nloh in which the prevalence of intestinal protozoans (*E. histolytica/dispar*, *G. lamblia*, and *C. parvum*) and prevalence and intensity of geohelminths was ascertained. The results of the geohelminth and protozoological surveys conducted in 2007 were reported by Richardson et al. (2011a) and Richardson et al. (2011b), respectively. In addition, morphometric data and hemoglobin concentrations determined in 2007 are reported herein.

In June 2010, a survey was conducted in which the prevalence of intestinal protozoans was determined in Nloh and Bawa-Nka, and the prevalence and intensity of geohelminths along with morphometric data and hemoglobin concentrations were determined for all available residents in Nloh, Bawa, and Bawa-Nka. Bawa-Nka lies directly adjacent to Bawa and is a quarter of the village of Nka. The results of the 2010 survey are reported herein.

Table 1 presents a summary of all surveys conducted and interventions initiated in Bawa, Nloh, and Bawa-Nka between 2006 and 2010.

Table 1. Summary of interventions and surveys conducted by the Bawa Health Initiative, 2006-2010.

	Malaria	
	Bed nets Installed	Assessment
Bawa	2007	Ongoing, Fig. 1 herein
Nloh	2008	--
Bawa-Nka	2010	--

	Waterborne Disease		
	Biosand Filters Installed	Baseline	Follow-up
Bawa	2006	Figs. 2 and 3 herein	Ongoing, Figs. 2 and 3 herein
Nloh	2007	Table 1 in Richardson et al., 2011b	2010, Reported herein
Bawa-Nka	Projected 2012	Reported herein	--

	Helminth Disease		
	Treatment Initiated	Baseline	Follow-up
Bawa*	2008 2x/year	Table 4 in Richardson et al., 2011a	2010, Table 4 herein
Nloh*	2008 3x/year	Table 3 in Richardson et al., 2011a	2010, Table 5, herein
Bawa-Nka	2010 1x/year	Table 3 herein	--

*Prevalence and intensities of geohelminth infections between 2007 and 2010 for Bawa and Nloh combined are given in Figures 5 and 6, herein. In June 2010, treatment frequency in Nloh was changed to 2x/year.

	Nutritional Status (Morphometrics)	
	Baseline	Follow-up
Bawa	2007,Reported herein	2010, Reported herein
Nloh	2007,Reported herein	2010, Reported herein
Bawa-Nka	2010,Reported herein	--

	Hemoglobin Concentration	
	Baseline	Follow-up
Bawa	2007, Table 6 herein	2010, Table 6 herein
Nloh	2007, Table 6 herein	2010, Table 6 herein
Bawa-Nka	2010, Table 6 herein	--

This study protocol was approved by the Human Experimentation Committee, Quinnipiac University, Hamden, Connecticut (HEC/IRB Protocol#5905) and the Cameroonian Ministry of Health. After the study was explained, written consent was obtained from all adult participants and parents or guardians of minors.

Installation of Insecticide-treated Bed Nets

Bed nets treated with permethrin were distributed to all residents of Bawa and Nloh during the summers of 2007 and 2008, respectively. In all, more than 600 bed nets were distributed. Bed nets were installed by members of the Village Health Committee under the direct supervision of the BHI field coordinator. The Village Health Committees and BHI maintain an ongoing program of education stressing the importance of continual proper usage of bed nets, including inspection and replacement of nets as necessary.

Installation of Biosand Water Filters (BSFs)

In order to remediate health-related problems associated with fecally contaminated drinking water, the BHI installed and implemented the use of 100 biosand water filters (BSFs) in each household in the villages of Bawa and Nloh. The BSF is an in-home filtration unit that is a modification of traditional slow-sand filtration that has been used for centuries (Richardson et al. 2011b). When used properly the BSF has been reported to be extremely effective in removing water-borne pathogens and in reducing the probability of diarrheal disease (see Richardson et al. (2011b) and references therein). Biosand water filters were installed in each compound in Bawa during the summer and fall of 2006 and BSFs were installed in each compound in Nloh during the summer and fall of 2007, subsequent to the 2007 survey of waterborne protozoan parasites (Richardson et al. 2011b). In addition to the use of BSFs, the village health committees of Bawa and Nloh conduct on-going education for the villagers concerning the proper use of BSFs and basic matters of hygiene and sanitation.

Universal Helminth Treatment

Anthelmintic drug treatment is aimed at reducing morbidity by decreasing worm burden; however, there is no one-size-fits-all approach to optimizing a deworming program. There are 3 primary considerations that must be taken into account when establishing a helminth control program: 1) who will be treated? 2) what anthelmintic will be used? and 3) what will be the frequency of treatment (Hotez et al. 2006)?

There are 3 primary approaches to treatment: universal, targeted, and selective. These approaches were described by Anderson (1989) and summarized by Richardson et al. (2011a). Many factors, such as the amount of labor required and the cost, contribute to the final choice of treatment strategy to be employed. The merits and disadvantages of these approaches were discussed by Richardson et al. (2011a). A universal treatment strategy was initiated in the villages of Bawa and Nloh beginning in 2008. The drug used for treatment was albendazole because it is a broad spectrum anthelmintic that in a previous study in Cameroon demonstrated high efficacy, excellent tolerance, and considerable reduction of egg output in the case of residual infections (Raccurt et al. 1990).

Beginning January 2008 in Bawa and Nloh, albendazole was administered as a single dose of 400 mg at each treatment cycle to each individual in the community over the age of 2 years, excepting pregnant women. Individuals in Nloh were treated 3 times per year, specifically in February and June 2008, January, May, and September 2009, and January 2010. Individuals in Bawa were treated twice per year, specifically in January and June 2008, January and June 2009, and January 2010. Treatment was suspended following the January 2010 cycle until completion of the helminth survey described herein. All treatment was supervised by Dr. Pierre Tsekeng, Chief Medical Officer of the Bawa Health Initiative.

Assessment of the Prevalence of Malaria, Diarrheal Disease, and Typhoid in Bawa

Medical records were obtained from the government-operated health clinic in the village of Nka documenting all visits of residents from the village of Bawa beginning in 2006. The head nurse at the clinic records each visit of any individual from Bawa and reports directly to the BHI field coordinator. Additionally, detailed mortality records have been kept for the village of Bawa since 2006. Clincal data should be viewed conservatively because records of clinical visits are not a highly effective means of assessing prevalence of disease. Many people who are ill may elect not to seek treatment, seek treatment from traditional healers, or to seek treatment at a clinic in a distant village. Although clinical records do not provide a comprehensive account of the occurrences of clinical diseases in Bawa, they serve as a relative indication in trends of the occurrence of various diseases, particularly considering that the population of Bawa has remained consistent at about 340 individuals.

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***Survey of* E. histolytica/dispar, G. lamblia, *and* C. parvum**

During June 2010, 36 stool samples from Nloh and 64 stool samples from Bawa-Nka were tested for the presence of *E. histolytica/dispar*, *G. lamblia,* and *C. parvum* utilizing the Triage Micro Parasite Panel® (Biosite Diagnostics, San Diego, California), a rapid enzyme-linked immunoassay used for the detection of these protozoans in human fecal specimens, as described by Richardson et al. (2011b). Garcia et al. (2000) and Sharp et al. (2001) provided a detailed description and clinical assessment of the assay, respectively. Although the Triage Micro Parasite Panel® does not differentiate between *E. histolytica* and the non-pathogenic congener *E. dispar*, the two are epidemiologically similar. Prevalences between villages were statistically compared using contingency table analyses as described by Zar (1999). Prevalence data from this survey were also compared to those from 2007 (Richardson et al. 2011b) using contingency table analyses. All significant differences assume $P \leq 0.05$.

Individuals testing positive for *E. histolytica/dispar* or *G. lamblia* were treated with metronidazole according to Despommier et al. (2005). Treatment was supervised by Dr. Pierre Tsekeng, Chief Medical Officer of the Bawa Health Initiative.

Geohelminth Survey

The geohelminth survey was conducted according to Richardson et al. (2011a) as follows. Between 10 June and 24 June 2010, 376 stool samples from Nloh, Bawa, and Bawa-Nka were examined for the presence of the geohelminths *A. lumbricoides*, *T. trichiura,* and hookworms as described by Richardson et al. (2011a), using the Kato-Katz technique and when sample volume permitted, fecal flotation as described by Richardson et al. (2008). All slides examined using the Kato-Katz technique were examined within one hour of preparation. Thirty six, 213, and 127 samples were examined from Nloh, Bawa, and Bawa-Nka, respectively. The data were divided by gender and among age groups as follows: 0-5 years (pre-school children), 6-15 (school-aged children), 16-59 (adults), and ≥ 60 (senior adults). For Nloh, Bawa, and Bawa-Nka, 30, 197, and 94 samples were examined both by Kato-Katz and fecal flotation respectively and 6, 16, and 33 samples were examined only by Kato-Katz, respectively. Prevalences between villages, among demographic groups within villages, and between this survey and that of Richardson et al. (2011a) were compared using contingency table analyses. Intensities of infection determined by egg output recorded as egg per gram of feces (epg) were based on Kato-Katz analyses and compared using either analysis of variance (ANOVA), Student's two-tailed t-tests, or paired two-tailed t-tests as appropriate. Specimens testing negative by Kato-Katz but positive by fecal flotation were recorded as having an intensity of 24 epg, the minimum value detectable by Kato-Katz, as described by Richardson et al. (2011). Infection intensity was categorized as light, moderate or heavy based on Montresor et al. (2002) (Table 2).

Table 2. Classes of intensity given as eggs per gram of feces for geohelminth infections proposed for use by a WHO Expert Committee in 1987 (after Montresor et al. 2002). From Richardson et al. (2011a).

Helminth	Light	Moderate	Heavy
Ascaris lumbricoides	≤ 5,000	5,001-49,999	≥ 50,000
Trichuris trichiura	≤ 1,000	1,001- 9,999	≥ 10,000
Hookworm	≤ 2,000	2,001- 3,999	≥ 4,000

Morphometrics and Hemoglobin Concentration

To assess the general health and nutritional status of individuals in Bawa, Nloh, and Bawa-Nka, morphometric data (height, weight, and body mass index (BMI)) and hemoglobin levels were recorded. As the geohelminth control program proceeds, it is predicted that growth-retardation and stunting will become less pronounced. Additionally, BHI is considering implementation of a program of nutritional supplements and a nutrition education program. The morphometric data presented herein will provide a baseline to assess the effectiveness of such programs.

For each individual included in the survey, height was recorded in cm using a stadiometer and weight was recorded in kg using a Detecto® mechanical weigh-beam scale. Body Mass Index (BMI) was calculated according to WHO (2006) using the formula BMI=mass (kg) ÷ [height (m)]^2. Individuals with a BMI ≤ 2SD from the WHO growth standards were considered malnourished and individuals with a BMI ≤ 3SD were categorized as exhibiting severe acute malnutrition following universal standards calculated by WHO for children ≤ 5 years (WHO 2006; WHO and UNICEF 2009), adolescents 6-19 years (de Onis et al. 2007), and adults (WHO 1995). Children and adolescents ≤ 19 years exhibiting height for age ≤ 2SD and ≤ 3 SD from the WHO standards were considered stunted and severely stunted, respectively (Caulfield et al. 2006). Children exhibiting weight for age ≤ 2SD and ≤ 3SD from the WHO standards were considered underweight and severely underweight, respectively. Children whose weight for height was ≤ 2SD from the WHO standards were considered wasted (Caulfield et al. 2006).

Hemoglobin concentration was recorded in g/dl using a STAT-Site® MHgb hemoglobin test meter from a drop of blood procured by dermal puncture. Individuals were categorized as not anemic, anemic or severely anemic according to standards established by Stoltzfus and Dreyfuss (1998). The hemoglobin concentration (g/dl) cut-off for being considered anemic for children under 5 years and pregnant women was 11.0. For children 5-11 years and pregnant women the cutoff was 11.5, for non-pregnant women the cutoff was 12.0, and for men the cutoff was 13.0. Individuals with a hemoglobin concentration ≤ 7 were considered severely anemic. These represent conservative estimates because the standards were established assuming hemoglobin concentration at sea level (Stoltzfus and Dreyfuss 1998) and the numbers were not adjusted for altitude (Nestle et al. 1999).

Results

Assessment of the Prevalence of Malaria, Diarrheal disease, and Typhoid in Bawa

The number of cases of malaria, diarrheal disease, and typhoid based on visits of residents of Bawa to the government clinic in the neighboring village of Nka are given in Figures 1-3 respectively.

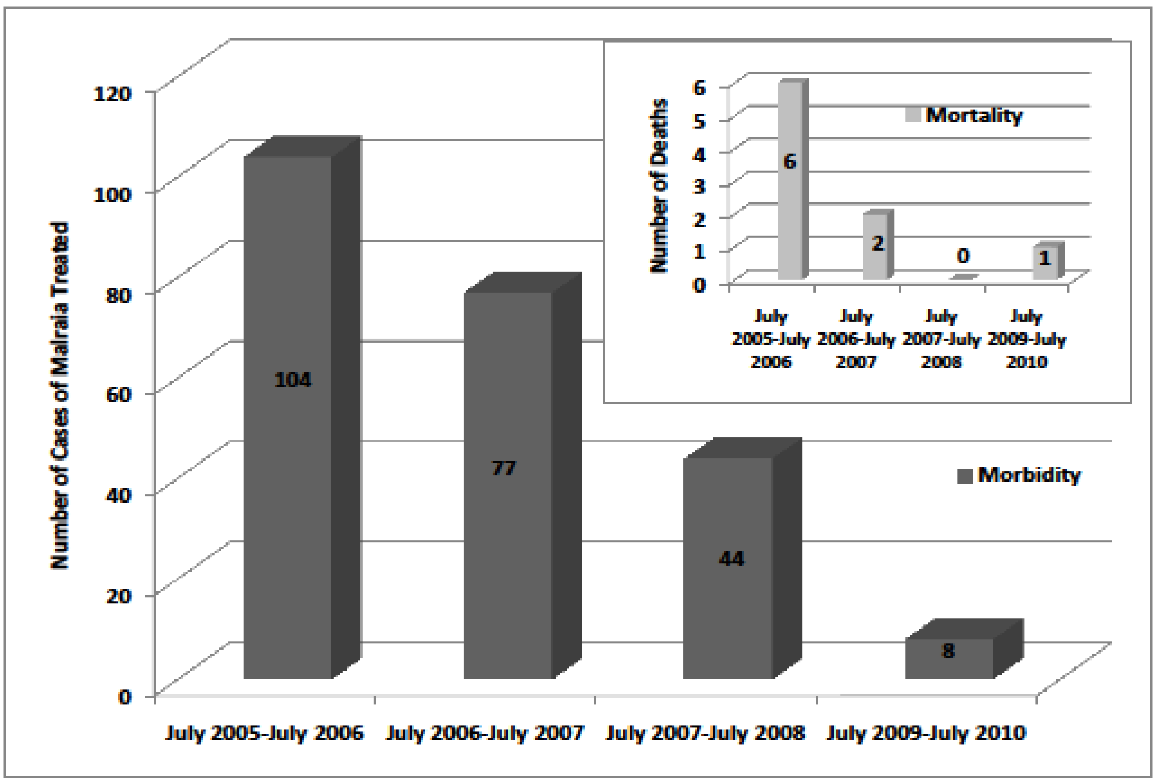

Figure 1. Mortality and morbidity of malaria in the village of Bawa for the various time periods indicated. Data are based on visits made by residents of Bawa to a government operated health clinic, in the village of Nka which lies adjacent to Bawa.

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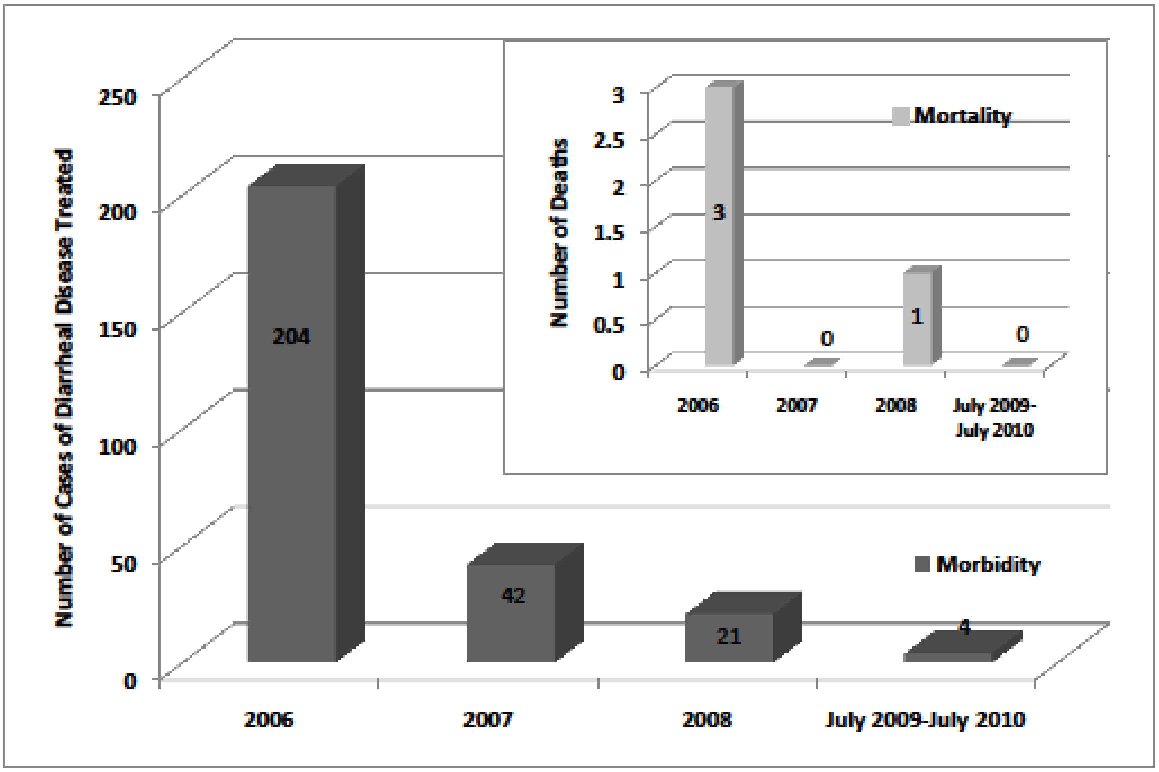

Figure 2. Mortality and morbidity of diarrheal disease in the village of Bawa for the various time periods indicated. Data are based on visits made by residents of Bawa to a government operated health clinic, in the village of Nka which lies adjacent to Bawa.

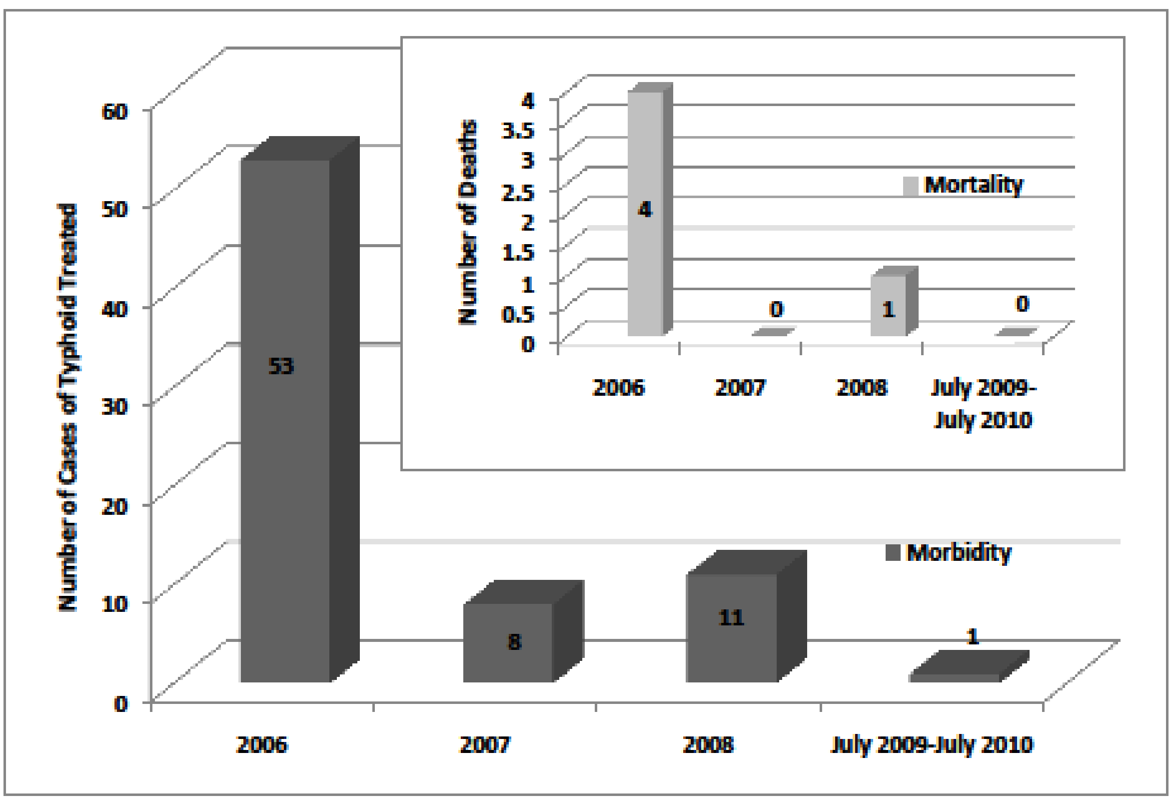

Figure 3. Mortality and morbidity of typhoid in the village of Bawa for the various time periods indicated. Data are based on visits made by residents of Bawa to a government operated health clinic, in the village of Nka which lies adjacent to Bawa.

***Survey of* E. histolytica/dispar, G. lamblia*, and* C. parvum**

In Nloh for 2010, 5 (13.9%) of 36 individuals tested were infected with *E. histolytica/dispar* or *G. lamblia*. Four of 36 (11.1%) individuals were infected with *E. histolytica/dispar*, 1 of 36 (2.8%) with *G. lamblia*, and no infection with *C. parvum* was detected. For 2007, Richardson et al. (2011b) reported that 22 (25.9%) of 85 individuals surveyed from Nloh were infected with *E. histolytica/dispar*, *G. lamblia*, *C. parvum*, or some combination thereof. Thirteen of 83 (15.7%) individuals were infected with *E. histolytica/dispar*, 12 of 83 (14.5%) with *G. lamblia*, and 3 of 83 (3.6%) with *C. parvum*. Contingency table analysis revealed no significant difference in prevalence for protozoan infection in general (X^2=2.083; 1 d.f.; P=0.149), or for *E. histolytica/dispar* (X^2=0.358; 1 d.f.; P=0.550) and *G. lamblia* (X^2=3.385; 1 d.f.; P=0.066), individually between 2007 and 2010.

In the 2010 Bawa-Nka survey, 14 (21.9%) of 64 individuals tested were infected with *E. histolytica/dispar* or *G. lamblia*. Ten (15.6%) individuals were infected with *E. histolytica/dispar*, and 4 (6.3%) were infected with *G. lamblia*. No individual was infected with multiple species and none of the 64 individuals tested for *C. parvum* were infected.

Helminth Survey

Bawa-Nka

In 2010, stool samples from 127 individuals were examined. Sixty-eight (53.5%) individuals were infected with *A. lumbricoides*, *T. trichiura*, hookworms, or some combination thereof. Sixteen (12.6%) were infected with 2 species, and 8 (6.3%) were infected with all 3 helminth species. Twenty-seven (21.3%) individuals were infected with *A. lumbricoides*, 56 (44.1%) individuals were infected with *T. trichiura*, and 17 (13%) individuals were infected with hookworm. The mean intensity (±SE) of *A. lumbricoides, T. trichiura*, and hookworm infection was 10,946 (±5,895), 182 (±35), and 134 (±53) epg respectively. The relative abundance (±SE) of *A. lumbricoides, T. trichiura*, and hookworm infection was 2,327 (± 1,298), 79 (±17), and 18 (± 8). Data are summarized in Table 3.

Contingency table analysis revealed no significant differences in prevalence between males and females in Bawa-Nka for any helminth, although females exhibited markedly higher prevalence of infection with all species than did males. T-tests revealed no significant differences in intensity or relative abundance between males and females. Genders were combined for subsequent analyses.

Contingency table analysis did reveal a significant difference in the prevalence of hookworm infection among age groups (X^2=10.29; 3 d.f.; p=0.016). Chi square analyses revealed that adults exhibited a significantly higher prevalence than did school-age children (X^2=5.35; 1 d.f.; p<0.025). Sample sizes were not adequate to make comparisons among other age groups.

The populations of *A. lumbricoides, T. trichiura*, and hookworms were each highly aggregated, exhibiting the characteristic negative binomial distribution (Figure 4). For *A. lumbricoides*, the 2 (1.6%) most heavily infected individuals of the 127 sampled were responsible for 76.7% of the total egg production. The variance to mean ratio was 91,913.0:1. For *T. trichiura*, the 7 (5.5%) most heavily infected individuals of the 127 sampled were responsible for 56.0% of the total egg production and the eighteen (14.2%) most heavily infected individuals were responsible for 77.6% of the total egg output. The variance to mean ratio was 475.7:1. For hookworms, the 2 (1.6%) most heavily infected individuals of the 127 sampled were responsible for 57.9% of the total egg production and the 6 (4.7%) most heavily infected individuals were responsible for 83.2% of the total egg output. The variance to mean ratio was 459.6:1.

For *A. lumbricoides* there were 2 heavy infections (a 3-yr-old female and an adult male) and 4 moderate infections (a school-aged male and female and an adult male and female). For *T. trichiura* there was 1 moderate infection (a 7-yr- old female) and no heavy infections. For hookworms, there were no heavy or moderate infections. Among the 7 moderate to heavy geohelminth infections, no individual was moderately or heavily infected with multiple species.

Bawa

Forty-five (21.1%) of the 213 individuals examined were determined to be infected with *A. lumbricoides, T. trichiura*, hookworms, or some combination thereof. This was significantly lower than the 142 (51.6%) of 275 individuals infected with geohelminths in 2007 (X^2=46.053; 1 d.f.; p=1.15 x 10^{11}) reported by Richardson et al. (2011a). Seven (3.3%) were infected with 2 species, and 2 (0.9%) were infected with all three helminth species. Eleven (5.2%)

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Table 3. Prevalence (number infected/number sampled (%), mean intensity (+SE) given in eggs per gram of feces (epg), and range of infection intensity (epg) of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and combined geohelminths in Bawa-Nka, West Province, Cameroon for various demographic cohorts, in 2010.

Cohort		*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworm	Overall Geohelminths
♂ Preschool	# infected/# sampled (%)	1/6 (16.7%)	4/6 (66.7%)	0/6 (0.0%)	4/6 (66.7%)
	Mean intensity (+SE)	476±0	168±71	---	
	Range	---	24-360	---	
♀ Preschool	# infected/# sampled (%)	2/11 (18.2%)	4/11 (47.1%)	0/11 (0.0%)	5/11 (45.5%)
	Mean intensity (+SE)	67,176±67,152	66±28	---	
	Range	24-134,328	24-144	---	
Overall Preschool	# infected/# sampled (%)	3/17 (17.6%)	8/17 (47.1%)	0/17 (0.0%)	9/17 (52.9%)
	Mean intensity (+SE)	44,943±44,693	117±40	---	
	Range	24-134,328	24-360	---	
♂ School-aged	# infected/# sampled (%)	3/17 (17.6%)	10/17 (58.8%)	1/17 (5.9%)	10/17 (58.8%)
	Mean intensity (+SE)	10,328±9,361	156±69	456±0	
	Range	24-29,016	24-600	---	
♀ School-aged	# infected/# sampled (%)	4/25 (16.0%)	12/25 (36.4%)	2/25 (8.0%)	13/25 (52.0%)
	Mean intensity (+SE)	3,384±2,556	348±129	96±0	
	Range	24-10,872	24-1,320	96-96	
Overall School-aged	# infected/# sampled (%)	7/42 (16.7%)	22/42 (52.4%)	3/42 (7.1%)	23/42 (54.8%)
	Mean intensity (+SE)	6,360±4,044	261±78	216±120	
	Range	24-29,016	24-1,320	96-456	
♂ Adult	# infected/# sampled (%)	3/11 (27.3%)	4/11 (36.14%)	1/11 (9.1%)	5/11 (45.5%)
	Mean intensity (+SE)	32,672±29,826	114±34	48±0	
	Range	24-92,232	24-192	---	
♀ Adult	# infected/# sampled (%)	10/36 (27.8%)	13/36 (36.1%)	11/36 (30.6%)	20/36 (55.6%)
	Mean intensity (+SE)	1,598±760	109±22	109±77	
	Range	24-7,368	24-312	24-864	
Overall Adult	# infected/# sampled (%)	13/47 (27.7%)	17/47 (36.2%)	12/47 (25.5%)	25/47 (53.2%)
	Mean intensity (+SE)	8,769±6,988	110±18	126±70	
	Range	24-92,232	24-312	24-864	
♂ Senior	# infected/# sampled (%)	0/7 (0.0%)	5/7 (71.4%)	0/7 (0.0%)	5/7 (71.4%)
	Mean intensity (+SE)	---	226±144	---	
	Range	---	24-792	---	
♀ Senior	# infected/# sampled (%)	4/14 (28.6%)	4/14 (28.6%)	2/14 (14.3%)	6/14 (42.9%)
	Mean intensity (+SE)	552±389	78±32	60±36	
	Range	24-1,680	24-168	24-96	
Overall Senior	# infected/# sampled (%)	4/21 (19.0)	9/21 (42.9%)	2/21 (9.5%)	11/21 (52.4%)
	Mean intensity (+SE)	552±389	160±81	60±36	
	Range	24-1,680	24-792	24-96	
♂ Overall	# infected/# sampled (%)	7/41 (17.1%)	24/41 (58.5%)	2/41 (4.9%)	24/41 (58.5%)
	Mean intensity (+SE)	18,496±12,902	8,304±6,663	252±204	
	Range	24-92,232	24-134,328	48-456	
♀Overall	# infected/# sampled (%)	20/86 (23.3%)	32/86 (37.2%)	15/86 (17.4%)	44/86 (51.2%)
	Mean intensity (+SE)	8,304±6,662	187±51	118±56	
	Range	24-134,328	24-1,320	24-864	
OVERALL	# infected/# sampled (%)	27/127 (21.3%)	56/127 (44.1%)	17/127 (13.4%)	68/127 (53.5%)
	Mean intensity (+SE)	10,946±5,895	182±35	134±53	
	Range	24-134,328	24-1,320	24-864	

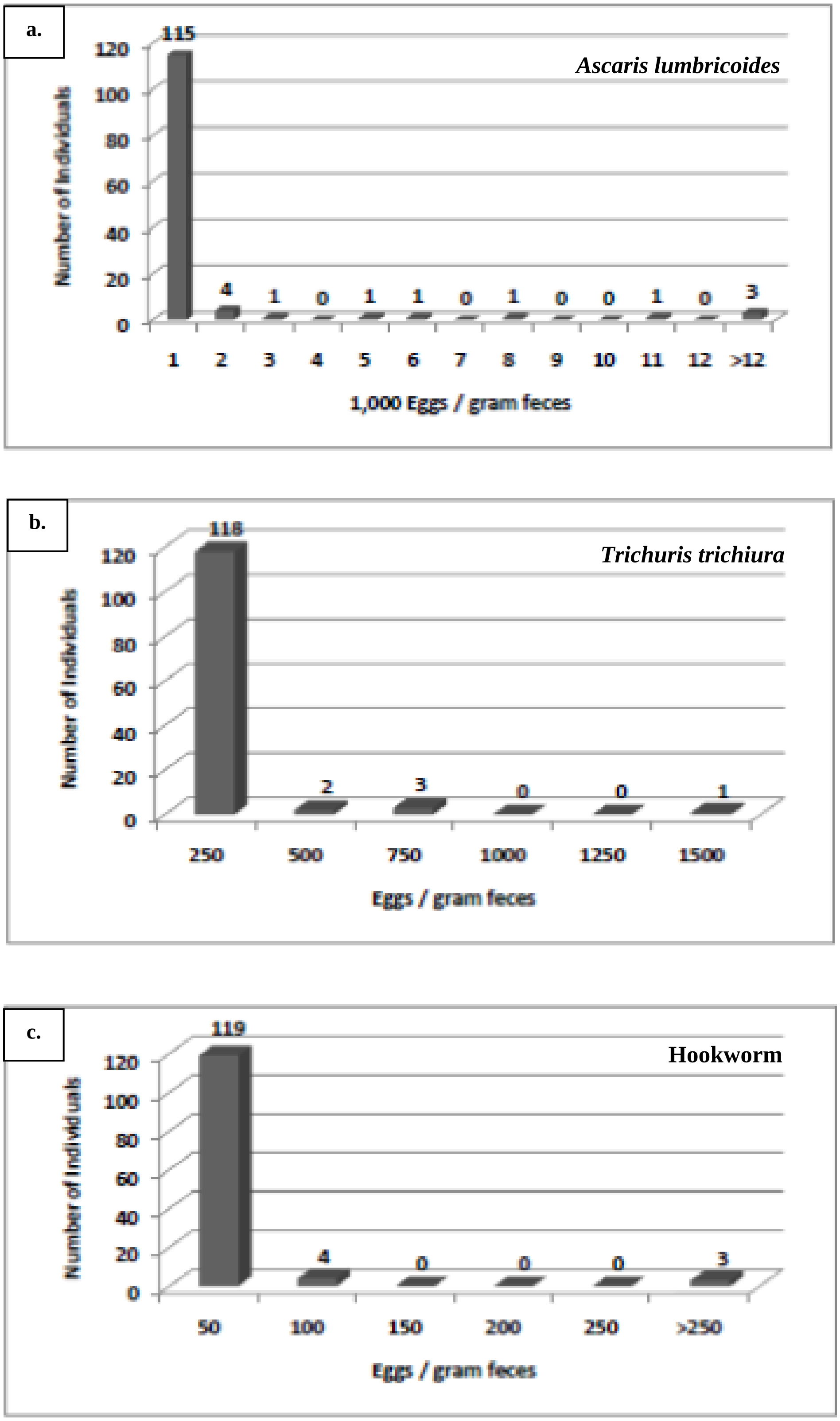

Figure 4. Frequency distributions of geohelminth intensity for Bawa-Nka, West Province, Cameroon. Infection intensities are reported as maximum eggs per gram feces for each class. **a.** *Ascaris lumbricoides*. **b.** *Trichuris trichiura.* **c.** Hookworm.

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individuals were infected with *A. lumbricoides*. This was significantly lower than the 42 (15.3%) of 275 individuals infected with *A. lumbricoides* in 2007 (X^2=12.349; 1 d.f.; p=4.41x10^{-4}). Thirty-four (16.0%) individuals were infected with *T. trichiura*. This was significantly lower than the 114 (41.5%) individuals infected in 2007 (X^2=35.514; 1 d.f.; p=2.53 x 10^{-9}). Twelve (5.6%) individuals were infected with hookworm, which was significantly lower than the 39 (18.7%) of 275 individuals infected in 2007 (X^2=14.542; 1 d.f.; p=1.37 x 10^{-4}). The mean intensity (±SE) of *A. lumbricoides, T. trichiura,* and hookworm infection was 1,898 (±935), 68 (±15), and 60 (±22) epg respectively. These values were not significantly different than the mean intensities (±SE) of *A. lumbricoides, T. trichiura,* and hookworm infection of 18,904 (±6,995), 346 (±80), and 57 (±13) epg reported for Bawa in 2007 by Richardson et al. (2011a). The relative abundances (±SE) of *A. lumbricoides, T. trichiura*, and hookworm infection were 98 (± 54), 11 (±3), and 3 (±2). These were each significantly lower than the values of 2,887 (±1,134) (t=2.158; 482 d.f.; p=0.031), 144 (±35) (t=3.219; 482 d.f.; p=0.001), and 10 (±3) (t=1.991; 482 d.f.; p=0.047), respectively, reported for Bawa in 2007 by Richardson et al. (2011a). Data are summarized in Table 4.

Chi square analysis revealed no significant difference in prevalence between males and females in Bawa for any helminth. T-tests revealed no significant difference in intensity or relative abundance between males and females. Genders were combined for subsequent analyses.

Contingency table analyses and ANOVA revealed no significant difference in the prevalence or intensity, respectively of infection among age groups for any helminth species.

The populations of *A. lumbricoides*, *T. trichiura*, and hookworms were each aggregated. For *A. lumbricoides*, the 2 (0.9%) most heavily infected individuals of the 213 sampled were responsible for 69.1% of the total egg production and the 3 (1.4%) most heavily infected individuals were responsible for 88.7% of the total egg production. The variance to mean ratio was 6,438.2:1. For *T. trichiura*, the 5 (2.3%) most heavily infected individuals of the 213 sampled were responsible for 55.7% of the total egg production and the 14 (6.6%) most heavily infected individuals were responsible for 79.4% of the total egg production. The variance to mean ratio was 171.6:1. For hookworms, the 2 (0.9%) most heavily infected individuals of the 213 sampled were responsible for 53.3% of the total egg production. The variance to mean ratio was 164.7:1.

For *A. lumbricoides* there was 1 moderate infection, a 57-yr-old male, and no heavy infection. For *T. trichiura* there were no heavy or moderate infections. For hookworms, there were no heavy or moderate infections. The single moderate infection representing 0.5% of the population is significantly lower than the 27 moderate to heavy geohelminth infections recorded by Richardson et al. (2011a) for 2007 (X^2=18.663; 1 d.f.; p<0.001).

Nloh

Thirteen (36.1%) of the 36 individuals examined were determined to be infected with *A. lumbricoides, T. trichiura*, hookworms, or some combination thereof. This was significantly lower than the 68 (72.3%) of 94 individuals infected with geohelminths in 2007 (X^2=11.961; 1 d.f.; p=0.001) reported by Richardson et al. (2010a). Three individuals (8.3%) were infected with 2 species. No individual was infected with all 3 species. Six (16.7%) individuals were infected with *A. lumbricoides*. This was significantly lower than the 31 (33.0%) of 94 individuals infected with *A. lumbricoides* in 2007 (X^2=5.99; 1 d.f.; p=0.014). Six (16.7%) individuals were infected with *T. trichiura*. This was significantly lower than the 51 (54.3%) individuals infected in 2007 (X^2=19.097; 1 d.f.; p=1.24 X 10^{-5}). Five (13.9%) individuals were infected with hookworm which was not significantly lower than the 25 (26.6%) of 94 individuals infected in 2007. The mean intensity (±SE) of *A. lumbricoides*, *T. trichiura*, and hookworm infection was 82 (±27), 28 (±4), and 29 (±5) epg respectively. These values, although lower, were not significantly different than the mean intensities (±SE) of *A. lumbricoides*, *T. trichiura*, and hookworm infection of 2,490 (±1,216), 246 (±92), and 293 (±172) epg reported for Nloh in 2007 by Richardson et al. (2011a). Likewise, although substantially lower, the relative abundance (±SE) of *A. lumbricoides, T. trichiura*, and hookworm infection of 11 (± 6), 5 (±2), and 4 (± 2) were not significantly lower than the values of 821 (±415), 133 (±51), and 78 (±47), respectively, reported for Nloh in 2007 by Richardson (2011a). Data are summarized in Table 5.

Table 4. Prevalence [number infected/number sampled (%)], mean intensity±SE given in eggs per gram of feces (epg) of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and combined geohelminths in the village of Bawa, West Province, Cameroon for various demographic cohorts in 2010.

Cohort		*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworm	Overall Geohelminths
♂ Preschool	# infected/# sampled (%) Mean intensity (±SE) Range	0/11 (0.0%) --- ---	2/11 (18.2%) 24±0 24-24	0/11 (0.0%) --- ---	2/11 (18.2%)
♀ Preschool	# infected/# sampled (%) Mean intensity (±SE) Range	0/9 (0.0%) --- ---	1/9 (11.1%) 48±0 48-48	0/9 (0.0%) --- ---	1/9 (11.1%)
Overall Preschool	# infected/# sampled (%) Mean intensity (±SE) Range	0/20 (0.0%) --- ---	3/20 (15.0%) 32±8 24-48	0/20 (0.0%) --- ---	3/20 (15.0%)
♂ School-aged	# infected/# sampled (%) Mean intensity (±SE) Range	2/48 (4.2%) 288±264 24-522	9/48 (18.8%) 59±21 24-216	2/48 (4.2%) 60±36 24-96	12/48 (25.0%)
♀ School-aged	# infected/# sampled (%) Mean intensity (±SE) Range	3/42 (7.1%) 360±300 48-960	6/42 (14.3%) 36±8 24-72	2/42 (4.8%) 24±0 24-24	8/42 (19.0%)
Overall School-aged	# infected/# sampled (%) Mean intensity (±SE) Range	5/90 331±185 24-960	15/90 (16.7%) 50±13 24-216	4/38 (10.5%) 42±18 24-96	20/90 (22.2%)
♂ Adult	# infected/# sampled (%) Mean intensity (±SE) Range	2/19 (10.5%) 6,948±240 4,104-9,792	2/19 (10.5%) 240±168 72-408	3/19 (15.8%) 24±0 24-24	5/19 (26.3%)
♀ Adult	# infected/# sampled (%) Mean intensity (±SE) Range	3/46 (6.5%) 1,768±1,442 48-4,632	7/46 (15.2%) 86±41 24-312	1/46 (2.2%) 24 ---	7/45 (15.6%)
Overall Adult	# infected/# sampled (%) Mean intensity (±SE) Range	5/65 (7.7%) 3,840±1,744 48-9,792	9/65 (13.8%) 120±48 24-408	4/38 (10.5%) 24±0 24-24	12/64 (18.8%)
♂ Senior	# infected/# sampled (%) Mean intensity (±SE) Range	0/14 (0.0%) --- ---	5/14 (35.7%) 72±37 24-216	1/14 (7.1%) 72 ---	6/14 (42.9%)
♀ Senior	# infected/# sampled (%) Mean intensity (±SE) Range	1/24 (4.2%) 24±0 24-24	2/24 (8.3%) 24±0 24-24	3/24 (12.5%) 128±81 24-288	4/24 (16.7%)
Overall senior	# infected/# sampled (%) Mean intensity (±SE) Range	1/38 (2.6%) 24±0 24-24	7/38 (18.4%) 58±27 24-216	4/38 (10.5%) 114±59 24-288	10/38 (26.3%)
♂ Overall	# infected/# sampled (%) Mean intensity (±SE) Range	4/92 (4.3%) 18,496±12,902 24-92,232	18/92(19.6%) 79±24 24-408	6/92 (6.5%) 44±13 24-96	20/92 (21.7%)
♀Overall	# infected/# sampled (%) Mean intensity (±SE) Range	7/121 (5.8%) 915±635 24-4,632	16/121 (13.2%) 57±19 24-312	6/121 (5.0%) 76±43 24-288	25/121 (20.7%)
OVERALL	# infected/# sampled (%) Mean intensity (±SE) Range	11/213 (5.2%) 1,898±935 24-9,792	34/213 (16.0%) 68±15 24-408	12/213 (5.6%) 60±22 24-288	45/213 (21.1%)

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Table 5. Prevalence (number infected/number sampled (%)), mean intensity±SE given in eggs per gram of feces (epg), and range of infection intensity (epg) of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and combined geohelminths in the village of Nloh, West Province, Cameroon for various demographic cohorts in 2010.

Cohort		***Ascaris lumbricoides***	***Trichuris trichiura***	**Hookworm**	**Overall Geohelminths**
♂ Preschool	# infected/# sampled (%)	0/0	0/0	0/0	0/0
	Mean intensity (±SE)	---	---	---	
	Range	---	---	---	
♀ Preschool	# infected/# sampled (%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)
	Mean intensity (±SE)	---	---	24	
	Range	---	---	---	
Overall Preschool	# infected/# sampled (%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)
	Mean intensity (±SE)	---	---	24	
	Range	---	---	---	
♂ School-aged	# infected/# sampled (%)	0/7 (0.0%)	1/7 (14.3%)	1/17 (14.3%)	2/7 (28.6%)
	Mean intensity (±SE)	---	24	48	
	Range	---	---		
♀ School-aged	# infected/# sampled (%)	1/6 (16.7%)	1/6 (16.7%)	1/6 (16.7%)	2/6 (33.3%)
	Mean intensity (±SE)	24	24	24	
	Range	---	---	---	
Overall School-aged	# infected/# sampled (%)	1/13 (7.7%)	2/13 (15.4%)	2/13 (15.4%)	4/13 (30.8%)
	Mean intensity (±SE)	24	24	36±12	
	Range	---	24-24	24-48	
♂ Adult	# infected/# sampled (%)	0/4 (0.0%)	0/4 (0.0%)	0/4 (0.0%)	0/4 (0.0%)
	Mean intensity (±SE)	---	---	---	
	Range	---	---	---	
♀ Adult	# infected/# sampled (%)	2/12 (16.7%)	2/12 (16.7%)	2/12 (16.7%)	5/12 (41.7%)
	Mean intensity (±SE)	72±0	36±12	24±0	
	Range	72-72	24-48	24-24	
Overall Adult	# infected/# sampled (%)	2/16 (12.5%)	2/16(12.5%)	2/16 (12.5%)	5/16 (31.3%)
	Mean intensity (±SE)	72±0	36±12	24±0	
	Range	72-72	24-48	24-24	
♂ Senior	# infected/# sampled (%)	1/2 (50.0%)	1/2 (50.0%)	0/0 (0.0%)	2/2 (100.0%)
	Mean intensity (±SE)	144	24	---	
	Range	---	---	---	
♀ Senior	# infected/# sampled (%)	1/4 (25.0%)	1/4 (25.0%)	0/4 (0.0%)	1/4 (25.0%)
	Mean intensity (±SE)	24	24	---	
	Range	---	---	---	
Overall senior	# infected/# sampled (%)	2/6 (33.3%)	2/6 (33.3%)	0/6 (0.0%)	3/6 (50.0%)
	Mean intensity (±SE)	84±60	24±0	---	
	Range	24-144	24-24	---	
♂ Overall	# infected/# sampled (%)	1/13 (7.7%)	2/13 (15.4%)	1/13 (7.7%)	4/13 (30.8%)
	Mean intensity (±SE)	144	24±0	48	
	Range	---	24-24	---	
♀Overall	# infected/# sampled (%)	5/23 (21.7%)	4/23 (17.4%)	4/23 (17.4%)	9/23(39.1%)
	Mean intensity (±SE)	53±12	30±6	24±0	
	Range	24-72	24-48	24-24	
OVERALL	# infected/# sampled (%)	6/36 (16.7%)	6/36 (16.7%)	5/36 (13.9%)	13/36 (36.1%)
	Mean intensity (±SE)	82±27	28±4	29±5	
	Range	24-144	24-48	24-48	

Chi square analysis revealed no significant difference in prevalence between males and females in Nloh for any helminth. Because of the small sample sizes, prevalence, intensity and relative abundance was not analyzed among age groups in Nloh.

The populations of *A. lumbricoides*, *T. trichiura*, and hookworms were each aggregated. For *A. lumbricoides*, the 2 (5.6%) most heavily infected individuals of the 36 sampled were responsible for 70.6% of the total egg production and the 3 (8.3%) most heavily infected individuals were responsible for 88.2% of the total egg production. The variance to mean ratio was 109.2:1. For *T. trichiura,* the single (2.8%) most heavily infected individual of the 36 sampled was responsible for 28.6% of the total egg production. The variance to mean ratio was 26.8:1. For hookworms, the single (2.8%) most heavily infected individual of the 36 sampled was responsible for 33.3% of the total egg production. The variance to mean ratio was 28.8:1.

In Nloh there were no moderate or heavy infections for any of the 3 geohelminths. Richardson et al. (2011a) reported 9 moderate infections for Nloh in 2007, 4 for *A. lumbricoides*, 3 for *T. trichiura,* and 2 for hookworms.

Comparison of Geohelminth Infections Among Villages

Chi square analysis revealed that the prevalence of 16.7% of *A. lumbricoides* in Nloh was significantly higher than the prevalence of 5.2% in Bawa (X^2=3.90; 1 d.f.; p=0.048). No significant differences in prevalence were detected between villages for *T. trichiura*, hookworms, or geohelminths overall. A comparison of intensities of *A. lumbricoides, T. trichiura*, hookworms, and geohelminths overall by t-tests revealed no significant differences between Bawa and Nloh. Combined data for Bawa and Nloh are summarized in Figures 5 and 6.

Contingency table analysis revealed that prevalences of *A. lumbricoides, T. trichiura,* hookworms, and overall geohelminths in Bawa-Nka were significantly higher than those for Bawa and Nloh combined. T-tests analysis revealed the intensity ($\pm$SE) of 51 ($\pm$ 76) of *T. trichiura* for Bawa and Nloh combined was significantly lower than the intensity ($\pm$SE) of 182 ($\pm$ 265) for Bawa-Nka (t=-3.078; 95 d.f.; p=0.003). No significant differences were detected in the mean intensities of *A. lumbricoides* or hookworm between Bawa and Nloh combined and Bawa-Nka. T-tests analysis did reveal that the relative abundances of all 3 geohelminths were significantly lower in Bawa and Nloh combined than in Bawa-Nka.

Nutrition

Nloh

In 2010, in Nloh, 2 (4.9%) of 41 individuals were categorized as malnourished based on BMI. Both were adult females. No individual was categorized as severely malnourished based on BMI. In 2007, 3 (3.4%) of 87 individuals examined were categorized as malnourished based on BMI, a senior male and 2 adult females. No individual was categorized as severely malnourished based on BMI.

Among children and adolescents in 2010, 4 (26.7%) of 15 individuals were categorized as stunted based on WHO height for age standards. No individual was found to be severely stunted. In 2007, 8 (25.8%) of 31 children were found to be stunted or severely stunted; with 6 being categorized as stunted and 2 being severely stunted. In 2010, among children 2 to 10 years of age, 2 of 10 (20%) were categorized as underweight based on WHO weight for age standards. No child was found to be severely underweight. In 2007, 5 (26.3%) of 19 children were underweight or severely underweight. Two of these, constituting 10.5% of the childhood population were categorized as severely underweight. In 2007, 1 (20.0%) of 5 children under 5 examined, a 3-year-old female, was categorized as wasted based on WHO standards of weight for height. In 2010, none of the 3 children under 5 years of age examined were categorized as wasted.

Bawa

In 2010, in Bawa, overall 11 (5.0%) of the 220 individuals examined were categorized as malnourished based on BMI, 1 adolescent male, 3 adolescent females, 2 adult females, 3 senior males, and 2 senior females. No individual was categorized as severely malnourished based on BMI. In 2007, 23 (8.6%) of 269 individuals examined were categorized as malnourished based on BMI, 3 senior males, 4 senior females, 4 adult females, 2 adolescent males, 4 adolescent females, 4 male children (under 5 years old) and 2 female children. Of these, 4 individuals constituting 1.8% of the population, 3 males under 5 years old and one 14-year-old male, were categorized as severely malnourished. Chi square analysis revealed no significant difference in the relative number of malnourished individuals between 2007 and 2010.

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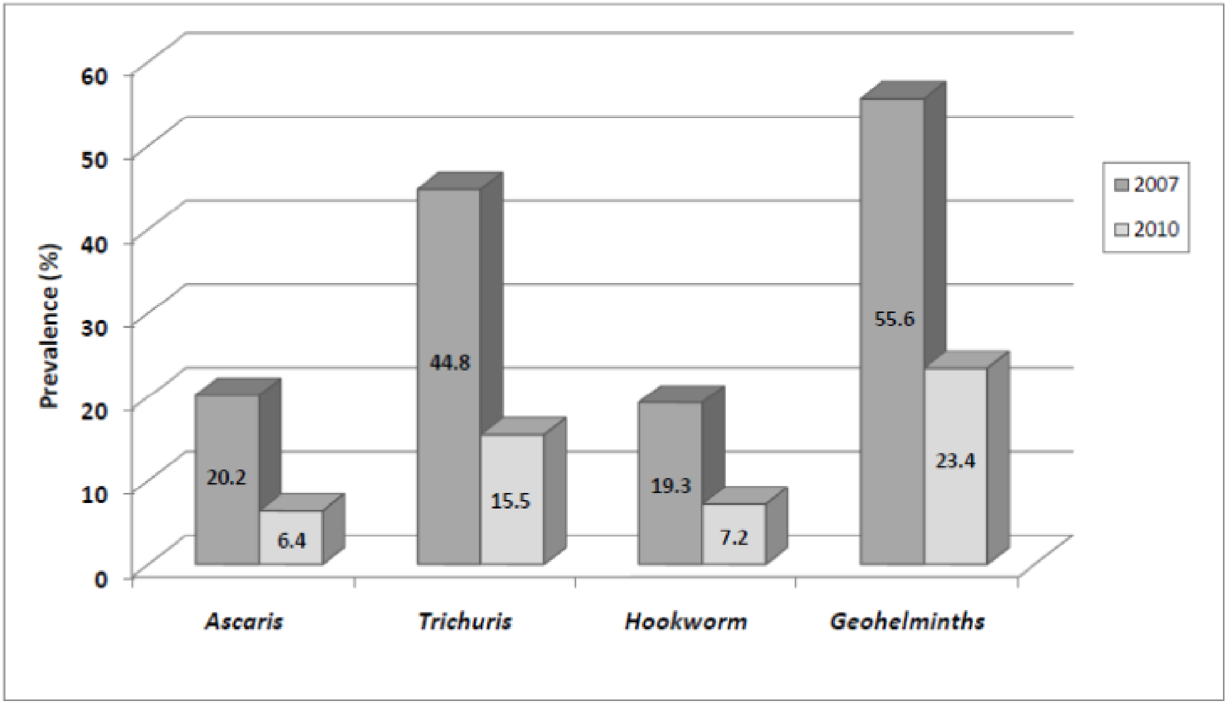

Figure 5. Comparison of prevalences of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, and combined geohelminth between 2007 and 2010 for the combined population of the villages of Bawa and Nloh, West Province, Cameroon.

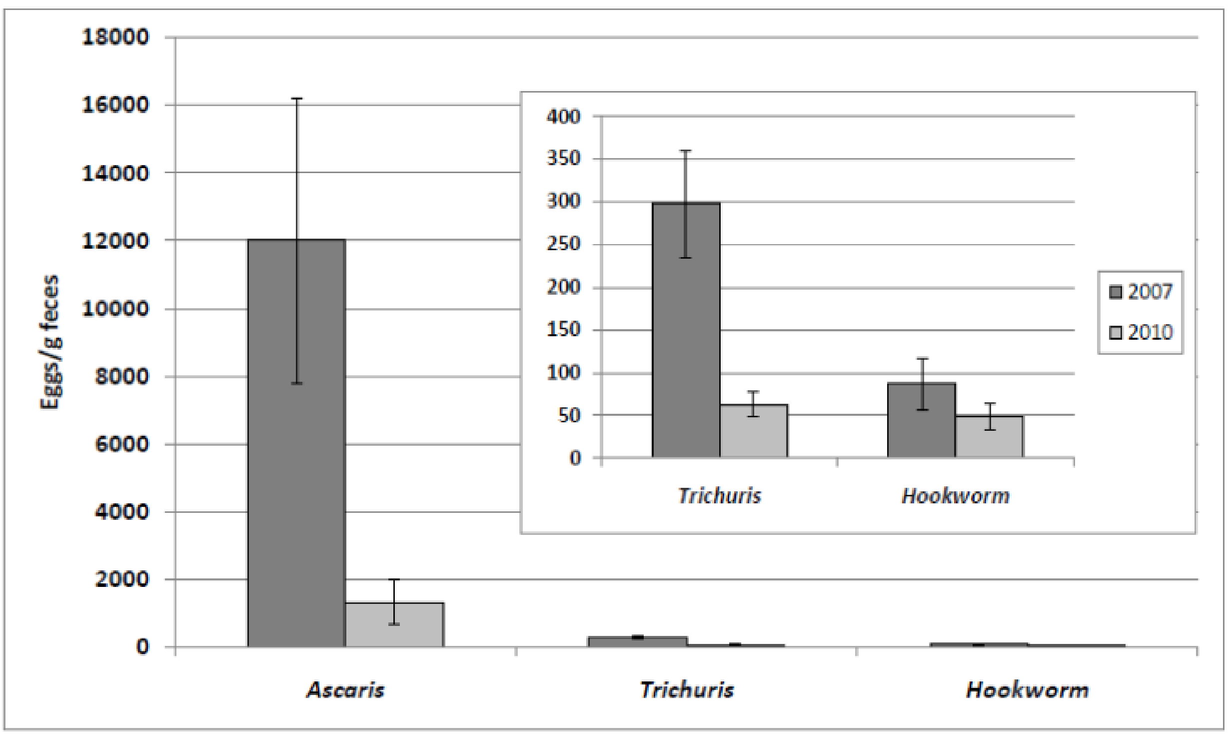

Figure 6. Comparison of mean intensities of *Ascaris lumbricoides*, *Trichuris trichiura,* and hookworms given as eggs per gram feces between 2007 and 2010 for the combined population of the villages of Bawa and Nloh, West Province, Cameroon. Error bars show standard error about the mean.

Among children and adolescents examined from Bawa in 2010, 41 (36.3%) of 113 individuals were categorized as stunted based on WHO height for age standards. Of these, 20 constituting 17.7% of the sample were severely stunted. Chi-square analysis revealed a significant difference in the relative number of stunted individuals between genders (X^2=8.20; 1 d.f.; p=0.004) with 28 (49.1%) of 57 males and 13 (23.2%) of 56 females sampled stunted or severely stunted. In 2007, 52 (43.0%) of 121 children and adolescents were stunted or severely stunted; with 33 categorized as stunted and 20 (16.3%) severely stunted. Chi-square analysis revealed no significant difference in the relative number of stunted children and adolescents between 2007 and 2010. In 2010, among children 2 to 10 years of age, 23 of 56 (41.1%) were underweight or severely underweight based on WHO weight for age standards. Nine of the 23 (39.1%) were severely underweight. Chi-square analysis revealed no significant difference between genders in weight for age indices. In 2007, 31 (35.6%) of 87 children were underweight or severely underweight. Chi-square analysis revealed no significant difference in the relative number of underweight or severely underweight individuals between 2007 and 2010. In 2007, 2 (5.9%) of the 34 children under 5 years of age were categorized as wasted based on WHO standards of weight for height. None of the 13 children under 5 examined in 2010 were categorized as wasted.

Bawa-Nka

In 2010 in Bawa-Nka, overall 10 (6.8%) of the 148 individuals examined were categorized as malnourished or severely malnourished based on BMI, 4 adolescent males, 2 adolescent females, and 4 adult females. Among these, 3 of the adolescent males, constituting 2.0% of the population, were categorized as severely malnourished based on BMI. Among children and adolescents examined from Bawa-Nka in 2010, 27 (42.9%) of 63 individuals were categorized as stunted based on WHO height for age standards. Of these, 15 (23.8%) were severely stunted. Among children 2 to 10 years of age, 19 of 39 (48.7%) were underweight or severely underweight based on WHO weight for age standards. Eight of the 19 (20.5%) were severely underweight. Chi-square analysis revealed no significant difference between genders in weight for age indices. Three (25.0%) of 12 children, ages 2 to 4 years, were categorized as wasted. Chi-square analyses revealed no significant differences between genders in height for age or weight for age.

Chi-square analyses revealed no significant differences in 2010 between Bawa and Bawa-Nka in nutritional status determined by BMI, height for age, and weight for age.

Hemoglobin Concentrations

Nloh

In Nloh for 2010, 25 (69.4%) of 36 individuals tested were anemic with 1 (2.8%) individual, an 81-year-old female, being severely anemic. Chi-square analysis revealed no significant difference in prevalence of anemia measured in Nloh for 2007 when 51 (63.8%) of 80 individuals were determined to be anemic, with 1 (1.3%) individual, a 16-year-old female being severely anemic. Data are summarized in Table 6.

Bawa

In Bawa for 2010, 105 (49.1%) of 214 individuals tested were anemic with 1 (0.5%) severely anemic individual, a 14-year-old female. This was significantly lower than the prevalence of anemia in Bawa for 2007 when 194 (75.8%) of 256 individuals were anemic, with 9 (3.5%) severely anemic individuals (X^2=35.94; 1 d.f.; p=2.03 x 10^{-9}). Chi-square analysis revealed no significant differences in the prevalence of anemia between genders in Bawa for 2007 or 2010. Chi-square analyses did reveal significant differences among age groups in the prevalence of anemia in Bawa for 2007 (X^2=19.46; 3 d.f.; p=2.19 x 10^{-4}). In 2007, the prevalence of anemia among children 5 and under was 86.7% which was significantly higher than that of adults (34.9%) (X^2=7.473; 1 d.f.; p=0.006) and senior adults (61.0%) (X^2=8.12; 1 d.f.; p=0.004). Likewise the prevalence of anemia in school-aged children (86.3%) was significantly higher than that of adults (X^2=11.101; 1 d.f.; p=0.001) and senior adults (X^2=10.962; 1 d.f.; p=0.001). Chi-square analyses in Bawa for 2010 revealed that the prevalence of anemia in children under 5 was 76.2% which remained significantly higher than that of adults (42.4%) (X^2=7.267; 1 d.f.; p=0.007) and of senior adults (46.2%) (X^2=5.015; 1 d.f.; p=0.025). Data are summarized in Table 6.

Bawa-Nka

In Bawa-Nka for 2010, 55 (39.6%) of 139 individuals were anemic with no individual being severely anemic. Chi-square analysis revealed no significant difference in the prevalence of anemia between Bawa-Nka and Bawa in 2010. Chi-square analysis revealed no significant differences in the

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Table 6. Incidence (number anemic/number sampled (%)), mean intensity (±SE) given as hemoglobin concentration as g hemoglobin/dl of whole blood, and incidence of severe anemia (≤7 g hemoglobin/dl) in the villages of Bawa and Nloh and in Bawa-Nka, West Province, Cameroon for various demographic cohorts.

Age (years)		*Bawa*		*Nloh*		*Bawa-Nka*
		2007	*2010*	*2007*	*2010*	*2010*
<5	# anemic/# sampled	26/30 (86.7%)	9/13 (69.2%)	3/4 (75.0%)	1/2 (50.0%)	11/17 (64.7%)
	Mean Hbg (±SE)	9.4±0.3	9.5±0.8	9.9±0.5	12.3±2.6	10.3±0.3
	# Severely anemic (%)	3 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
5-11	# anemic/# sampled	60/67 (89.6%)	36/69 (52.2%)	7/15 (46.7%)	6/8 (75.0%)	15/28 (53.6%)
	Mean Hbg (±SE)	9.6±0.2	11.4±0.2	11.9±0.6	10.7±0.5	11.6±0.2
	# Severely anemic (%)	4 (6.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
>11	# anemic/# sampled	108/159 (68.0%)	60/132 (45.5%)	41/61(67.2%)	18/26 (69.2%)	29/94 (30.9%)
	Mean Hbg (±SE)	11.0±0.2	11.4 ±2	11.3±0.3	11.7±0.4	12.9±0.2
	# Severely anemic (%)	2 (1.2%)	1 (0.8%)	1 (1.6%)	1 (3.8%)	0 (0.0%)
Overall	# anemic/# sampled	194/256 (75.8%)	105/214 (49.1%)	51/80(63.8%)	25/36 (69.4%)	55/139 (39.6%)
	Mean Hbg (±SE)	10.5±0.1	11.9±0.1	11.4±0.2	12.3±0.1	12.3±0.1
	# Severely anemic (%)	9 (3.5%)	1 (0.5%)	1 (1.3%)	1 (2.8%)	0 (0.0%)

prevalence of anemia between genders. Contingency table analyses did reveal a significant difference among age groups in Bawa-Nka (X^2=15.81; 3 d.f.; p=0.001). The prevalence of anemia among children 5 and under was 64.7% which was significantly higher than that of adults (22.2%) (X^2=10.669; 1 d.f.; p<0.005) and senior adults (33.3%) (X^2=3.938; 1 d.f.; p<0.05). Likewise, the prevalence of anemia in school-aged children (54.5%) was significantly higher than that of adults (X^2=10.902; 1 d.f.; p<0.001). Data are summarized in Table 6.

Discussion

In the villages of Bawa and Nloh where BHI has implemented intervening modalities, the number of clinical cases of malaria, diarrheal disease, and typhoid has decreased, the prevalence of water-borne protozoan parasites has decreased, the prevalence and intensities of geohelminth infections has significantly decreased, and the prevalence of anemia has significantly decreased. When viewed in its entirety, these data clearly demonstrate that the comprehensive approach of intervention to public health challenges facing Bawa, Cameroon and surrounding villages has been extremely effective. Following is an independent assessment of each modality.

Clinical Data

Clinical data presented were based on visits by residents of Bawa to the government clinic in Nka, which is the nearest available healthcare for residents of Bawa. Although the clinical data are encouraging in that the number of cases of malaria, diarrheal disease, and typhoid treated have dropped substantially over the 4 years subsequent to commencement of implementation of modalities, these data are anecdotal and should be assessed conservatively. Although it is tempting to attribute the decline in clinic visits for these diseases directly to interventions implemented by BHI, many socio-demographic factors could influence such clinical data. For instance, the nursing staff has changed during this time period and periods of time have passed that the clinic was not open for patients. Nevertheless, these data, when viewed in lieu of other data assessing the efficacy of specific modalities, support the assertion that insecticide treated bed-nets have been effective at reducing the burden of malaria and that biosand water filters (BSFs) have been effective in reducing the occurrence of diarrheal disease, including typhoid.

Prevalence of Water-Borne Protozoan Parasites

Although the prevalences of *E. histolytica/dispar*, *G. lamblia.* and *C. parvum* in Nloh were substantially lower in the summer of 2010 than they were in the summer of 2007, no significant differences were detected. In view of the small sample size for the village of Nloh, these data should be viewed as suggestive that the BSF may be effective in reducing the prevalence of water-borne protozoan parasites.

One potential explanation for the small sample size recorded for the village of Nloh was that many of the villagers, particularly the household patriarchs, were away working at their farms in the nearby village of Santchou. In addition to presenting an interference with compliance in follow-up surveys, the water in Santchou is reported by local residents and physicians to be notoriously contaminated. Several villagers from Nloh and Bawa indicated that although they always use their BSFs when at "home" they are unable to do so while they work at their farms, where they may spend several days at a time. This presents the likelihood of individuals becoming infected with water-borne protozoans while traveling. The urgency to *always* consume only filtered water is being stressed in the continuing education concerning proper use and maintenance of BSFs by the Village Health Committees.

The clinical data obtained from the government clinic in Nka support the assertion of the effectiveness of BSFs in reduction of diarrheal disease, as does the observation of a similarly low prevalence of *E. histolytica/dispar* and *G. lamblia* infection observed by Richardson et al. (2011b) in Bawa, 1 year subsequent to installation of BSFs. In addition, both Nloh and Bawa, 3 years and 1 year respectively following implementation of BSF use, exhibited lower prevalences of water-borne protozoans than did Bawa-Nka, where no intervention or education program had been implemented.

In Nloh, the prevalence of *G. lamblia* exhibited a greater reduction (14.5% to 2.8%) from 2007 to 2010 than did *E. histolytica/dispar* (15.7% to 11.1%). Similarly, one year following installation of BSFs in Bawa, the prevalence of *G. lamblia* was 1.8% whereas that of *E. histolytica/dispar* was 7.1%. Comparatively, in Nloh, prior to installation of BSFs, the prevalences of *G. lamblia* and *E. histolytica/dispar* were 14.5% and 15.7% respectively (Richardson et al. 2011b). These data suggest that BSFs may be more effective in reducing the prevalence of *G. lamblia* than that of *E. histolytica*. Both *E. histolytica/dispar* and *G. lamblia* may be transmitted to humans in a variety of ways; however, it has been suggested that *G. lamblia* is primarily a water-borne parasite (Pung 2003); whereas *E. histolytica/dispar*, in addition to being transmitted by fecally contaminated water, is also commonly transmitted by a variety of other pathways (Marquardt et al. 2000). Transmission through contaminated food may be an important source of infection with *E. histolytica*. For instance, identification and treatment of infected food handlers has been shown to result in a 12-fold reduction in the occurrence of infection with *E. histolytica* (Marquardt et al. 2000). Roberts and Janovy (2009) suggested that the manner of human waste disposal is the most important factor in the epidemiology of *E. histolytica*. While BSFs appear to be having an impact on the control of giardiasis, control of amebiasis in Bawa and surrounding areas may depend more heavily upon improved sanitation than water filtration.

Another piece of anecdotal evidence supporting the effectiveness of the BSF at preventing intestinal disease is the significant differences in the prevalences of geohelminths reported by Richardson et al. (2011a) between Nloh prior to implementation of interventions, and Bawa 1 yr subsequent to installation of BSFs alongside the implementation of a sanitation and hygiene education program. Nloh exhibited *A. lumbricoides* and *T. trichiura* prevalences of 33.0% and 54.3%, respectively, while Bawa exhibited prevalences of 15.3% and 41.5%, respectively. Although *A. lumbricoides* and *T. trichiura* are primarily soil-borne, there is a body of evidence supporting the assertion that the provision of "clean" water may have a substantial impact on their prevalence (Esrey et al. 1991, Schliessmann et al. 1958, Henry 1981, Hotez et al. 2006, Jombo et al. 2007, Richardson et al. 2011a).

When used properly, the BSF is remarkably effective in removing water-borne pathogens, including protozoan cysts and helminth eggs (Palmateer et al. 1999, Duke et al. 2006, Stauber et al. 2006, Baumgartner et al. 2007, Center for Affordable Water and Sanitation Technology (CAWST) (2008a,b)). Nevertheless, effectiveness of the BSF in removing water-borne pathogens does not necessarily equate to a reduction in the prevalence of water-borne disease in a community served by BSFs, although anecdotal clinical data are supportive of this assertion (CAWST 2008b, Stauber et al. 2009, present study).

A preponderance of circumstantial data gathered in this study is supportive of the effectiveness of the BSF at reducing water-borne disease. However, we emphasize the point that these are anecdotal data and more robust empirical evidence that demonstrates the effectiveness of BSFs in actually reducing the prevalence of water-borne parasites in a community is urgently needed in view of the phenomenal resources being expended in implementation of BSF projects. Hopefully, the data gathered in Nloh and Bawa-Nka will provide the baseline for more robust definitive tests of the ultimate effectiveness of the BSF at reducing the prevalence of water-borne parasites.

Geohelminth Survey

The universal helminth treatment program implemented by BHI in 2007 has been successful in reducing the prevalence, intensity, and relative

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abundance of geohelminth infections in Bawa and Nloh. Overall, the prevalence of geohelminth infections in Bawa and Nloh has been reduced from 56.9% in 2007 (Richardson et al. 2011a) to 23.3% in 2010. The finding that there were no significant differences in the prevalences or intensities of geohelminths between the two villages suggests that 2 treatments per year are as effective as 3 treatments per year in the control of geohelminths. Because of the possible development of albendazole resistance by worms, the number of treatments in Nloh is being reduced from 3 per year to 2 per year.

Hotez et al. (1996) pointed out that repeated chemotherapy at regular intervals (periodic deworming) in high-risk groups can ensure that the levels of infection are kept below those associated with morbidity, resulting in rapid improvement in child health and development and may reduce transmission over time. Hotez et al. (1996) also pointed out some disadvantages to periodic deworming including the lower efficacy of a single-dose of anthelmintic (Albonico et al. 1994, Adams et al. 2004), high rates of post-treatment reinfection (Albonico et al. 1995, 2003), and most concerning, the possibility of the appearance of anthelmintic resistance by the worms (Albonico et al. 2003, Hotez et al. 1996).

The ultimate goal of most geohelminth control programs is reduction in the number of moderate and severe infections and thus reduction in morbidity and potential mortality. In Bawa and Nloh collectively, in 2007, 36 (9.8%) of 369 individuals examined exhibited moderate or severe infections (Richardson et al. 2011a). In the present study, only 1 of 249 individuals exhibited a moderate infection in 2010.

The secondary goal of most geohelminth control programs is reduction of the prevalence of helminth infections with the ideal of eradication. Richardson et al. (2011a) postulated that the relative effectiveness of a universal helminth control program was contingent upon disruption of the negative binomial distribution exhibited by worm populations. Universal or mass treatment undermines the infrastructural stability of the helminth metapopulation by attacking the two components of overdispersion that underlie the negative binomial distribution, the many lightly infected individuals to the "left," and the few heavily infected individuals to the "right." Figures 7-9 show the comparative frequency distributions of geohelminths between 2007 and 2010 for the villages of Bawa and Nloh combined. Hilbe (2007) gave a detailed explanation of the negative binomial distribution and its derivation. Bliss and Fisher (1953), Crofton (1971), and Schmid and Robinson (1972) provided detailed explanations and examples of fitting the negative binomial distribution to biological and parasitological data. The degree of overdispersion of all 3 geohelminths, has been reduced in the course of the universal control program carried out in Bawa and Nloh, evidenced by the reduction in variance to mean ratios in all instances. Likewise, the overdispersion parameter k (Bliss and Fisher 1953) is expected to increase because the overdispersion parameter varies inversely with the degree of overdispersion, or aggregation, such that as k approaches 0 the worms are more highly aggregated and as k approaches ≥ 5 the worms are randomly distributed among the hosts (Anderson 1989). Reduction in mean intensity will coincide with reduction in the number of heavily infected individuals, while reduction in prevalence will lead primarily to reduction in the number of lightly infected individuals. As the intervention progresses and intensities are reduced the data will become less heteroskedastic (skewed to the right); therefore, the mean intensity will be expected to decrease. As the mean intensity decreases, the variance is expected to decrease concomitantly. Likewise, as prevalence decreases, the size of classes representing lightly infected individuals to the left will decrease, while the "0 class" representing uninfected individuals will increase. A decrease in the degree of heteroskedasticity along with concomitant decrease of intensity in classes that represent lightly infected individuals will result in a distribution that fits the negative binomial less efficiently as the overdispersion parameter k increases and the variance to mean ratio approaches unity. The data must be 0-truncated because an increase in the number of uninfected individuals would give the illusion of overdispersion. The insets in Figures 7-9 show the 0-truncated distributions of *A. lumbricoides*, *T. trichiura*, and hookworm, respectively for Bawa and Nloh combined. Analysis of the truncated distributions reveal that for *A. lumbricoides* between 2007 and 2010 the mean intensity was reduced from 12,344 to 1,281 and that the sample variances decreased from 1.31 x 10^9 to 7,245,130. This results in a decrease of the variance to mean ratio for the 0-truncated distribution of *A. lumbricoides* by 95.7%, from 106,125:1 in 2007 to 5,656:1 in 2010. Analysis of the truncated distributions reveals that for *T. trichiura* between 2007 and 2010 the mean intensity was reduced from 299 to 52 and that the sample variances decreased from 623,557 to 5,925. This results in a decrease of the variance to mean ratio for the 0-truncated distribution

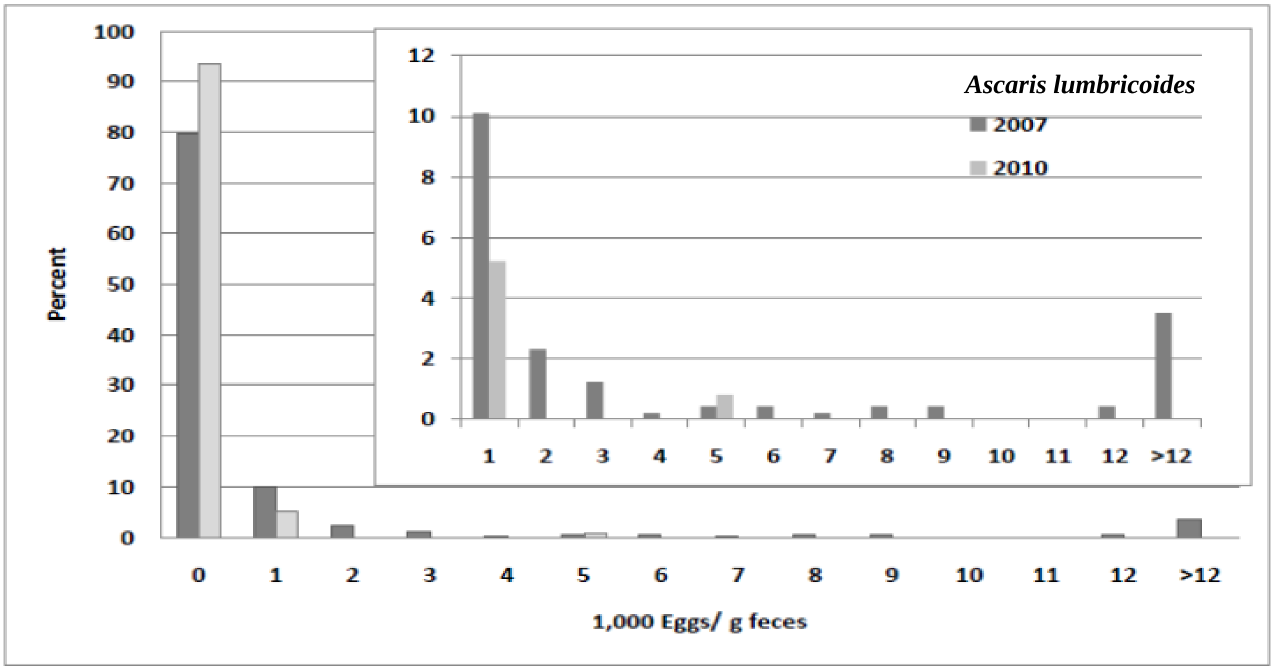

Figure 7. Frequency distribution of intensities of *Ascaris lumbricoides* for the combined populations of Bawa and Nloh, West Province, Cameroon comparing intensities in 2007 and 2010. Infection intensities are reported as maximum eggs per gram feces for each class. Insets show 0-truncated frequency distributions (i.e. exclude the 0 class).

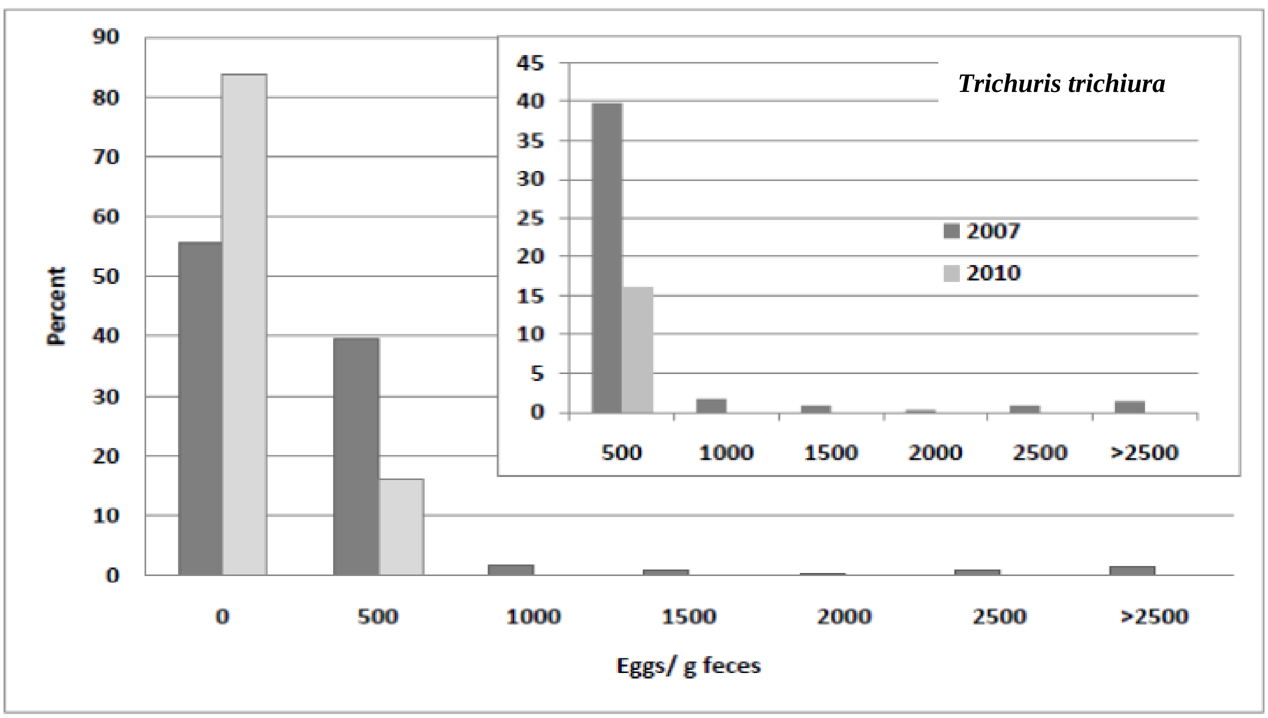

Figure 8. Frequency distributions of intensities of *Trichuris trichiura* for the combined populations of Bawa and Nloh, West Province, Cameroon comparing intensities in 2007 and 2010. Infection intensities are reported as maximum eggs per gram feces for each class. Insets show 0-truncated frequency distributions (i.e. exclude the 0 class).

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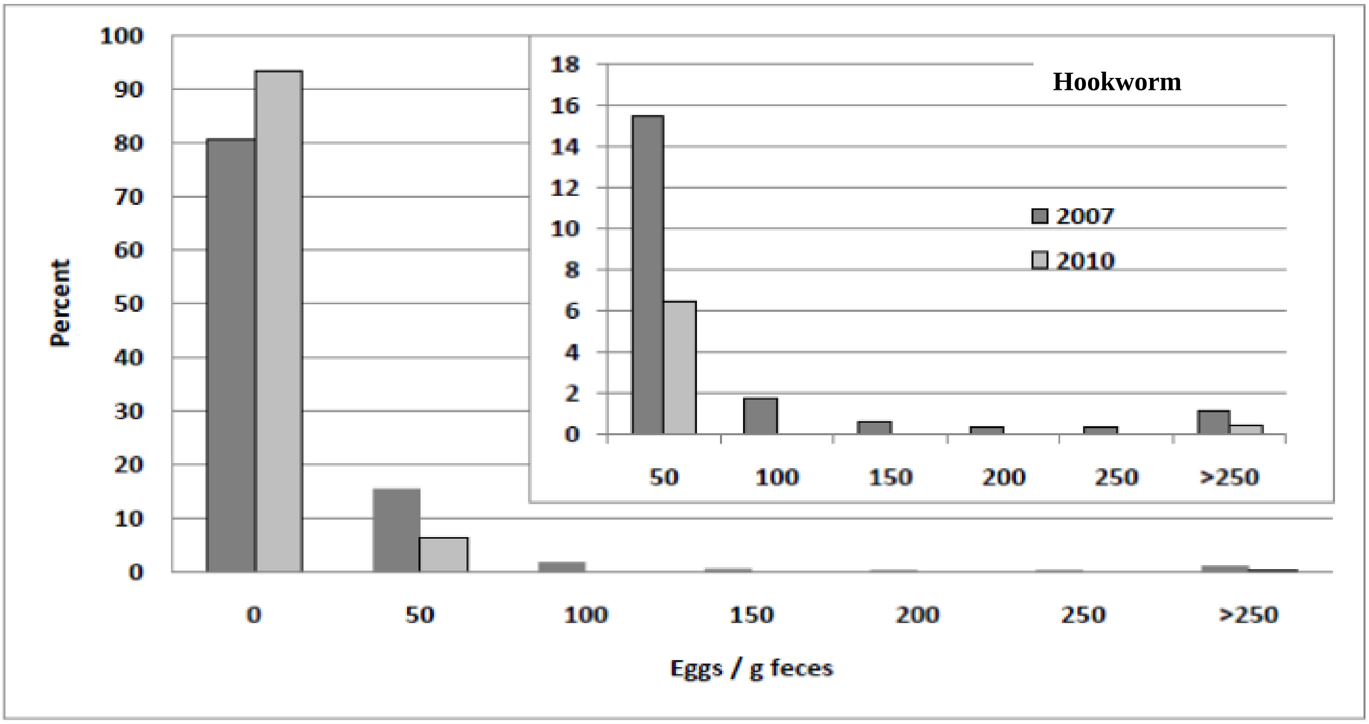

Figure 9. Frequency distributions of intensities of hookworm for the combined populations of Bawa and Nloh, West Province, Cameroon comparing intensities in 2007 and 2010. Infection intensities are reported as maximum eggs per gram feces for each class. Insets show 0-truncated frequency distributions (i.e. exclude the 0 class).

of *T. trichiura* by 94.5%, from 2,088:1 in 2007 to 114:1 in 2010. Analysis of the truncated distributions reveals that for hookworm between 2007 and 2010 the mean intensity was reduced from 86 to 43 and that the sample variances decreased from 60,647 to 4,044. This results in a decrease of the variance to mean ratio for the 0-truncated distribution of hookworm by 98.7%, from 7,487:1 in 2007 to 95:1 in 2010.

Although the geohelminth populations in Bawa and Nloh continued to exhibit overdispersion in 2010, the degree of overdispersion, and thus the negative binomial distribution, has been heavily impacted by chemotherapeutic intervention, evidenced by the profound decreases in variance to mean ratios. The relatively small sample sizes, particularly regarding the heavy infection classes for 2010, preclude robust estimation of the overdispersion parameter k for these populations. Although these preliminary data are compelling, the distribution of infection intensities for large populations comprised of thousands of individuals should be compared before and after treatment to further assess the impact that population-level chemotherapy has on reducing overdispersion. Review of the literature failed to provide data sets reported in such a way as to lend themselves to retrospective analysis. Laboratory models that may be utilized in detailed study of the negative binomial distribution of parasite populations and their response to population-level treatment are being sought.

The general recommendations of the World Health Organization (2002) regarding frequency of treatment are as follows: In areas where the prevalence of geohelminth infection is greater than 70% and more than 10% moderate and heavy infections, 2-3 treatments per year are recommended. When the prevalence is between 40-60% and the incidence of moderate and heavy infections is less than 10%, treatment is recommended once per year.

The prevalence of geohelminth infection in Bawa-Nka is 53.5% and 5.5% of the population exhibits moderate or heavy infections. This offers an ideal scenario with which to assess the efficacy of the single annual treatment approach in comparison with 2 or 3 treatments per year. The treatment strategy of single annual treatments in Bawa-Nka and 2 treatments annually in Bawa and Nloh that has been adopted by BHI will facilitate such comparison.

Over the next 7 years the prevalence and intensity of geohelminth infections will be monitored in all 3 villages to assess effectiveness of the different approaches in periodicity of treatment. Changes in metapopulation structuring as illustrated by the negative negative binomial distribution will be monitored to test the hypothesis of Richardson et al. (2011a), which predicts that disruption of the negative binomial distribution is essential to success of geohelminth control programs.

Although many studies report the prevalence of geohelminths and many report the short-term results of treatment programs, few provide well-controlled long term detailed data concerning the effectiveness of community helminth treatment programs. The goal of BHI is to track the effectiveness of community control over a period of decades. This approach will facilitate long-term assessment of various strategies and provide early detection of possible failure of treatment due to drug resistance.

Nutrition

Three different measures of nutritional status were considered: BMI, height-for-age (stunting), and weight-for-age. It is important, especially in developing countries, to use multiple measures of nutritional status to gain a clear picture of a situation. One of the most commonly used indices for nutritional status is BMI (de Onis et al. 2007). While BMI is generally adequate for assessing nutritional status of adults, it can be misleading when applied to children. Because BMI is based upon weight relative to height, an individual that is concomitantly underweight or severely underweight and stunted or severely stunted may exhibit a BMI within the normal range. For instance, in Bawa and Nloh BMI measurements suggested that 5.0% (13/261) of the overall population was malnourished or undernourished, 9 adults and 4 children. These data suggest that there are no serious widespread nutritional issues in the village. However, height-for-age indices revealed that 35.2% (45/128) of children and adolescents were stunted, 15.6% (20/128) were severely stunted and 37.9% (25/66) of children 2 to 10 years of age exhibited low weight-for age. Thus it is important to collectively consider a variety of indices when assessing the extent of malnutrition and undernutrition in a population. Stunting tends to result from chronic undernutrition (Caulfield et al. 2006) and/or geohelminth infection (Hotez 2008), whereas wasting is more indicative of acute undernutrition or disease (Caulfield et al. 2006). Low weight-for-age encompasses both stunting and wasting (Caulfield et al. 2006).

Growth retardation may result from malnutrition, undernutrition, and/or infection with geohelminths. Hotez (2008) pointed out that geohelminth infections may represent the world's leading cause of growth retardation and stunting. Caulfield et al. (2006) pointed out that childhood malnutrition diminishes adult intellectual ability and work capacity. This further diminishes the socioeconomic integrity of a community thereby creating a vicious cycle by setting the stage for the likelihood of more malnutrition. Furthermore, Caulfield et al. (2006) pointed out that malnourished women are more likely to deliver premature and small birth-weight babies that are likely to exhibit suboptimal growth and development. Caulfield et al. (2006) projects if malnutrition and undernutrition were eliminated, childhood mortality could be reduced by 53% because undernutrition and malnutrition increases the risk that a child will die as a result of a given disease.

Anemia

Hemoglobin concentration is an indicator of anemia which as pointed out by Crawley (2004) is multi-factorial in origin and may result from malaria, geohelminth infections, and/or malnutrition or undernutrition. Thus, the frequency of anemia in a population may be the most important single measure of the overall health status of a population regarding malaria, geohelminth infection and mal- or undernutrition. Between 2007 and 2010, the prevalence of anemia in Bawa was reduced from 75.8% to 49.1% with a concomitant decrease in prevalence of clinical malaria and decrease in the prevalence and intensity of geohelminth infection. Additionally, the frequency of severe anemia in Bawa has significantly decreased from 3.5% to 0.5%. Although the frequency of anemia actually exhibited a slight increase in Nloh between 2007 and 2010, from 63.8% to 69.4% and the frequency of severe anemia increased from 1.3% to 2.8%, these increases were not significant.

Interventions put in place by BHI have alleviated a substantial degree of the burden of disease among these Cameroonian villages including malaria, diarrheal disease, and geohelminth infection. Although cause for cautious optimism, these data also reflect the degree of work that remains to be done.

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