Aus der Medizinischen Universitätsklinik und Poliklinik Tübingen
Abteilung VII, Tropenmedizin
(Schwerpunkt: Institut für Tropenmedizin, Reisemedizin,
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Prevalence of parasitic co-infections and their association with the Hb-level and the anthropometric status in children aged 1-5 years in Lambaréné, Gabon

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Tausend, Johannes

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Dekan:	Professor Dr. I. B. Autenrieth
1. Berichterstatter:	Professor Dr. P.G. Kremsner
2. Berichterstatter:	Professor Dr. W. Bethge
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Abbreviations

A. lumbricoides	*Ascaris lumbricoides*
AIDS	Acquired Immune Deficiency Syndrome
CI	Confidence interval
DALY	Disability-adjusted life year
Dl	Decilitre
EDTA	Ethylenediaminetetraacetic acid
EPI	Expanded Program on Immunization
Fig	Figure
g	Gram
h	Hour
HAZ	Height-for-age Z-Score
Hb	Hemoglobin
HIB	Haemophilus influenzae Type b
HIV	Human immunodeficiency virus
IDA	Iron deficiency anaemia
µl	Microliter
mm	Millimetre
n	Number
OR	Odds Ratio
P. falciparum	*Plasmodium falciparum*
P. falc.	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*
S. guineensis	*Schistosoma guineensis*
S. haematobium	*Schistosoma haematobium*
S. haem.	*Schistosoma haematobium*
S. intercalatum	*Schistosoma intercalatum*
S. japonicum	*Schistosoma japonicum*
S. mansoni	*Schistosoma mansoni*
SD	Standard deviation
SSA	Sub-Saharan Africa
STH	Soil-transmitted helminth
T. trichiura	*Trichuris trichiura*
UNSCN	United Nations Standing Committee on Nutrition
WHO	World Health Organization
WHZ	Weigh-for-height Z-Score

1 Introduction

1.1 Soil-transmitted helminths

Summarized as soil-transmitted helminths (STHs), *Ascaris lumbricoides* (roundworm)*, Trichuris trichiura* (whipworm)*, Ancylostoma duodenale* and *Necator americanus* represent the most prevalent nematodes. According to recent estimates, infections with STHs concern one-sixth to one-fourth of the global population while *A. lumbricoides* is the most frequent species with about 1 billion infections [1, 2]. STHs are responsible for about 40% of tropical diseases in children if malaria is not taken into account [3]. The distribution of STHs varies throughout the world. The greatest number of infections is detected in the Americas, China, East Asia and Sub-Saharan Africa [4]. Single infections play a minor role, whereas co-infections with two or more species are more common [5]. Further, for some parasites, parasite loads are higher in subjects carrying multiple infections than those of subjects with a single infection [6, 7]. This is especially important, as the morbidity level is determined by the intensity of the infection [8]. In addition, subjects infected with STHs, may show a higher vulnerability for other illnesses such as tuberculosis, malaria and HIV infection [9, 10]. Due to the high number of infections worldwide, STHs remain a major burden for public health, especially in poor areas with low standard of life and hygiene [11]. Therefore, the WHO recommends periodic treatment of people living in endemic areas without diagnostics prior to treatment. However, helminth infections remain neglected diseases whose importance has only recently been emphasized [12, 13]. Therefore, the WHO established the global target to eradicate morbidity caused by STHs by 2020 [14].

1.2 Schistosomiasis

Schistosomiasis, also known as bilharzia, is a tropical disease caused by Schistosoma, a parasite belonging to the genus of trematodes. For humans, there are several species relevant for causing diseases: *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. guineensis* and *S. intercalatum*. They differ from each other regarding the geographical distribution. *S. haematobium* can be found in Africa, parts of Arabia, the Middle East, Madagascar and Mauritius. *S. mansoni* is endemic in Africa and has been distributed to parts of South America, the Caribbean and Arabia. *S japonicum* is scattered in China, the Phillipines, Sulawesi and Thailand [15]. *S. mekongi* can be found in Cambodia and Laos, whereas *S. guineensis* and *S. intercalatum* appear in rainforests of central Africa [16]. *S. haematobium* causes urinary schistosomiasis, whereas the others species account for intestinal schistosomiasis, the other major form of schistosomiasis.

Adult schistosomes are worms of 7-20 mm in length. Males embrace females permanently. They are located in perivesical (*S. haematobium*) or mesenteric (other species) venous plexus. Every fertilized adult female produces hundreds to thousands eggs a day, every egg contains one miracidium. The eggs produce proteolytic enzymes that allow them to migrate to the lumen of the bladder (*S. haematobium*) or the bowel. After having been excreted with urine or faeces, the ciliated miracidium larva leaves the egg. Guided by light and chemical stimuli, it searches for a suitable intermediate host. Such appropriate hosts are different genus of fresh water snails. After penetrating the host, the miracidium starts an asexual replication cycle. Via sporocysts, it transforms into cercariae within 4-6 weeks. Provoked by light and therefore mainly during the day, the characteristically fork-tailed cercariae of about 200-500 µm in length are released into the water. They can survive in the water up to 72 h. Then the cercariae penetrate the human skin, shed their tails and thus, transform into schistosomules. They enter the circulation, pass the lungs and arrive at the liver. In intrahepatic portal veins, they mature within 4-6 weeks. After coupling with an appropriate mature worm, they migrate to their final location, the perivesical or mesenteric venous plexus, respectively, where the life cycle

restarts. The prepatent period, the time between larval penetration and excretion of eggs, varies from about 4 weeks (*S. mansoni*) to 12 weeks (*S. haematobium*). Based on the life cycle already mentioned, schistosomiasis flourishes wherever natural or man-made surface water is contaminated with human excreta, susceptible snail hosts are present, environmental conditions allow development in the intermediate host and humans are exposed to the contaminated water. Regarding the three most frequent forms of schistosomes, humans are the main reservoir. For *S. mansoni*, there are further hosts like baboons and rodents; with respect to *S. japonicum*, many animals are susceptible. [15, 17].

According to the WHO, there were at least 261 million people requiring preventive treatment and over 40 million people were treated against schistosomiasis in 2013. The estimation of the number of casualties due to schistosomiasis is subject to huge variations and ranges from 20 000 to 200 000 per year [16]. However, a big proportion is allotted to Africa [18].

Clinical features of schistosomiasis depend on the underlying species. They all have in common, that cercarial penetration can evoke an itchy skin rash, especially in primary infections. An infection with Schistosoma can cause acute schistosomiasis. The underlying pathomechanism is a systemic hypersensitivity reaction to the migrating schistosomules about 4 weeks after infection [19-22]. It can occur as a complex of symptoms called Katayama fever including fever, urticaria, eosinophilia, diarrhea, hepato-splenomegaly and cough.

The pathomechanism of chronic infection is induced by schistosome eggs causing eosinophilic and granulomatous inflammation and subsequently, fibrotic replacement [23]. Regarding chronic effects of the infection, the particular species differ. *S. haematobium* causes urinary schistosomiasis. Chronic inflammation of the bladder and ureters lead to ulceration and pseudopapillomas [24]. Pollakisuria, dysuria, proteinuria and haematuria are signs for these processes [25]. In the course of infection, inflammation can cause fibrosis and calcification of the bladder and ureters resulting in hydroureter and hydronephrosis and, in severe cases, kidney failure [15].

Further, urinary schistosomiasis is associated with squamous bladder cancer [26]. Schistosoma eggs of other species can evoke granulomatous inflammation leading to ulcerations and pseudopapillomas [27]. Symptoms as abdominal pain and discomfort, (bloody) diarrhea and loss of appetite can be the result [25]. *S. mansoni* infection can present as hepatic schistosomiasis. Granulomas, due to Schistosoma eggs in periportal regions, lead to the development of the "Symmer's pipestem fibrosis", a characteristic liver disease [27].

1.3 Malaria

Malaria is an infectious disease caused by Plasmodium parasites. There are several relevant species for humans, namely *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi.* The parasites are transmitted by *Anopheles*, a genus of mosquitoes. In non-immune patients, infections with Plasmodium manifest themselves in fever, chill, headache and malaise. All symptoms appear at least 7 days after the infection. If not treated in time, malaria can cause severe anaemia, respiratory distress, acidosis, cerebral malaria, multi-organ failure and, in the worst case, lead to death [28].

The burden of malaria has recently decreased. However, the WHO estimated 212 million malaria episodes due to the disease, resulting in 429 000 deaths, in 2015. Ninety-two percent of these deaths were attributable to the WHO African Region. Children aged less than 5 years bear the biggest burden as they account for 70% of all deaths caused by malaria, and 15% of all deaths among children aged younger than 5 years are due to malaria [29-31]. Other authors come to different conclusions. Murray et al estimate that 24% of casualties among children in Africa can be attributed to malaria, a considerably higher number than the 16% stated by Black and colleagues, on whose methods the WHO estimates are based [32, 33], whereas the methods of Murray et al are controversial.

Almost 3% of the worldwide DALYs (disability adjusted life years) are caused by malaria, in Africa as much as 10% [34]. However, other authors suggest that the DALY approach does not take effects like chronic anaemia, low birth weight and

enhancement of the severity of other childhood diseases into account sufficiently. In the underlying study of this hypothesis, the total mortality in children under 5 years of age was more than doubled by the presence of *P. falciparum* parasites. These results imply that the burden of malaria in children under 5 years is underestimated by the common DALY approach [35].

1.4 Co-Infections

The global distribution, as well as the age groups suffering from specific parasitic infections, raise the question of co-infections and their effects on public health. Surprisingly, there is a lack of data, especially for children under five years of age, although this age group is heavily affected by parasitic infections.

Regarding geographical patterns of helminth and plasmodia infections, the highest absolute case numbers are for Asia [36, 37]. Sub-Saharan Africa (SSA) bears the greatest burden of clinical disease [36-38]. Hookworm is the widest spread of the three main STH species [39]. Considering the whole continent, Ascaris and Trichuris play a minor role regarding the numbers of co-infections with malaria as they concentrate in equatorial regions [39]. Ascaris and Trichuris have been shown to be highly prevalent and co-infections with Plasmodium are common [40]. Schistosomiasis shows a wide distribution throughout the continent with a focal concentration in southern and SSA [41]. Thus, geographical distribution of hookworm and Plasmodium infections coincide the most [41, 42].

Considering the age patterns of helminth infections and malaria, there are some differences. While African preschool children bear the greatest burden of malaria infections [43], helminth infections show the highest prevalence and intensity in school-aged children [44]. Preschool children show a considerable prevalence of helminth infection, however intensities of infections are relatively low [44]. The prevalence of helminths in Kenyan preschool children is as high as 42%. Thirty-four percent of the same group of children showed *P. falciparum* [44]. However, these mild infections might still be clinically relevant, as even relatively low egg counts of 200 per gram were shown to be associated with a reduction in hemoglobin and with anaemia. [44]. However, results are

controversial. Other studies showed no association of hookworm infections with hemoglobin concentration among preschool children [45, 46].

Data regarding the effect of helminth infection on the severity of malaria episodes is inconsistent as well, e.g. mild malaria episodes and asymptomatic Plasmodium infections mostly affect schoolchildren, an age at which highest prevalence rates as well as intensities of helminth infections are found [47-49].

Murray and colleagues examined the association of helminth infection and malaria. Comparing two Comorro islands, the island with higher prevalence rates for *A. lumbricoides* (93%) showed low malaria prevalence rates (2%). The island with higher prevalence rates for malaria showed lower prevalence rates for *A. lumbricoides* [50]. Anthelminthic treatment of highly intensive Ascaris infections lead to an increase of clinical malaria episodes [51]. However, other studies found STH infections to increase the risk of infection with Plasmodium [52].

The global significance of parasitic co-infections should not be underestimated as, according to Brooker et al, about a quarter of school-aged children are likely to be at risk of co-infection with *P. falciparum* and hookworm [42].

1.5 Anaemia

Anaemia is a public health subject of exceptional relevance, as it is affecting 25% of the world's population (1.62 billion people in absolute numbers) [53]. It is a problem for both industrialized and non-industrialized countries, yet prevalence rates, age patterns and underlying causes may differ. In absolute numbers, non-pregnant women are most commonly afflicted (468.4 million). Regarding the prevalence, it is highest among preschool children (47%). The highest number of affected people can be found in Asia, whereas Africa shows the highest prevalence for the main population groups: Pregnant women (56%), non-pregnant women (44%) and preschool children (65%) [53]. Although anaemia concerns the whole world's population, it is important to remark that the prevalence rates depend considerably on the stage of the development of the country. Therefore, the prevalence is 9%, 26% and 43% of the population in high, medium and less developed countries, respectively. This amounts to 111 million, 1.1 billion and 367 million affected individuals in these countries [53]. Worldwide, anaemia has a considerable share of the burden of disease [54].

According to the United Nations, the prevalence rate of anaemia in children in Africa reaches 60% [55]. With regards to Gabon, the WHO estimates 45% of preschool children to be afflicted by anaemia [56].

The underlying causes of anaemia are manifold. Globally, the most important reason seems to be iron deficiency. As a result, the terms iron deficiency anaemia (IDA) and anaemia are often used synonymously. According to the WHO, IDA constitutes 50% of global anaemia cases [57].

Further causes for anaemia can be haemolysis or acute or chronic blood loss, for example due to menstruation or parasitic infections like hookworms, *P. falciparum* or *T. trichiura* [58].

1.6 The anthropometric status

The importance of understanding the processes of under- and malnutrition is emphasized by the first Millennium Development Goal, which aims at halving the proportion of people suffering from hunger to “eradicate extreme poverty and hunger” [55].

To distinguish between different types of malnutrition, the terms wasting and stunting were established. Wasting describes deficits in weight-for-height, whereas stunting represents low height-for-age. Although stunting and wasting often concern the same individuals, several analyses have shown that there is no significant correlation [59]. Wasting can rapidly evolve and, when the underlying causes are successfully addressed, vanish quickly [59]. In contrast to that, stunting is a lack of gaining height and thus develops over months or years. To speak in scientifically correct terms, malnutrition summarizes both of them and in addition, also comprises overnutrition. In this work, when malnutrition is mentioned, it refers to wasting and stunting, respectively.

Malnutrition in children younger than 5 years decreased from 33% in 1990 to 26% in 2006 [55]. However, it remains a great burden for public health. The WHO estimates that 115 millions of children aged less than five years are underweight [60].

Stunting in Eastern Africa affects about 48% of all preschool children. From 1980 to 2000, the number of stunted Eastern African children increased from 12.9 million to 22 million [61]. In contrast, the prevalence rate of stunted preschool children in Northern Africa decreased from 33% in 1980 to 20% in 2000. For Western Africa, the proportion of stunted people has only changed little.

The most important factors associated with a decrease in stunting are a high gross domestic product, wide spread availability of energy and a high female literacy rate [62]. Crucial factors associated with a decrease in wasting are a high immunization rate and, for Asia, widespread availability of energy [62]. Genetic issues play a minor role in variability of child growth [63].

1.7 Parasitic infections, hemoglobin levels and the anthropometric status

1.7.1 Soil-transmitted helminths

Infections with STHs can stay asymptomatic for a long time before they trigger general gastrointestinal symptoms such as constipation, diarrhea, indigestion, vomiting and abdominal discomfort. Heavy infections can result in a bolus of adult worms leading to intestinal obstruction, volvulus or perforation and peritonitis. Hookworm infections may initially cause pruritus and cutaneous eruptions at the site of penetration of the larvae. Due to migration of STHs in the human body, pulmonary symptoms like cough, dyspnoea or wheeze can be observed.

Although most of the infections with STHs proceed asymptomatically, they remain a major cause for morbidity and mortality among children in tropical countries [64]. The extent of morbidity depends on the particular parasite species, intensity of infection, the nutritional and immunological status of the subjects and several socio-economic factors. The exact measurement of the aforementioned factors is of great importance for a correct clinical assessment [65].

One of the greatest burdens for children's health seems to be the insidious effects on child growth and physical development. Severe infections with round- or whipworms are associated with protein energy malnutrition [66]. Whipworms can trigger the Trichuris dysentery syndrome and thus iron deficiency anaemia and growth retardation [67]. Several studies as well as a review of numerous studies regarding the effect of anthelminthic treatment showed a positive effect on child growth. The effect on cognitive performance was not apparent in all studies [68-70]. The positive effect on physical growth has also been shown for malnourished children presenting mainly light infections [71-73]. For example, Burmese children aged 2-10 years showed a significant gain of height after 6 months and of weight after 24 months of treatment with anthelminthic drugs [74]. Furthermore, a significant improvement of physical fitness by a single dose treatment with Albendazole was shown in Kenyan schoolboys [75]. Concerning long-term effects, helminthiasis also seems to impair physical growth in the long

term. One longitudinal study lasting 9 years found Brazilian children suffering from early-childhood helminthiasis to show a deficit of 4.6 cm at the age of 7 years compared to non-infected children [76].

Concerning anaemia, STHs have repeatedly been shown to lower Hb-levels in infected children. Severe hookworm infections are known to be a major cause for iron deficiency anaemia [67, 77, 78]. Feeding habits of hookworms are an important factor [67, 78]. Blood loss due to Trichuris infection is estimated to 0.005 ml per day and worm [58]. This amount of blood loss accounts for an increase of the daily iron requirement considerably. Taking into account that iron deficiency is common in many areas where Trichuris is endemic, the additional iron needs due to infection may be important [79]. Consequently, school performance is lowered in children suffering from chronic helminth infections [80]. These effects seem to be mediated by iron deficiency anaemia and malnutrition [81].

The underlying mechanisms for impairment of height and weight remain uncertain. One major cause seems to be reduced food intake. This has been supported in animal models [82]. Food intake of infected children increased after anthelminthic treatment. Results of self-evaluation of their appetite were corresponding [83]. Another explanation for the effects on physical growth seems to be an abnormal structure of the jejunal wall like broader and shorter villi and elongated crypts as observed in Ascaris-infected children. These abnormalities were mostly reversible by de-worming [84].

For Ascaris, a negative effect on lactose tolerance could be shown [85]. Furthermore, an effect of lactose intolerance on food uptake must be assumed, because mothers of infected children reported discomfort after milk consumption [86]. Besides, lower absorption of Vitamin A by infected children compared to non-infected ones has been mentioned [87, 88].

However, other studies showed no association of the intensity of STH infection with the children’s growth or age, except for hookworms [89]. Trichuris has also been reported not to be related to stunting [89].

As stunting but not wasting is associated with slow mental development, it is important to recognize and understand the contributing factors [90, 91].

1.7.2 Urinary Schistosomiasis

Not the acute illness but the effects of chronic schistosomiasis have been shown to be a heavy load for public health. The impact of high-grade morbidity on disability adjusted life years (DALYs) is lower than the impact of low-grade illnesses [92]. These effects are malnutrition, anaemia, fatigue and impaired cognition [92]. The mechanisms of these effects remain unclear. One hypothesis are interdependencies of several socio-economic factors. Other reasons may be immunological interactions such as increased susceptibility for Schistosoma caused by malnutrition and anaemia [93]. Anaemia, as a result of Schistosoma infections, is not well-understood, either. Severe infections including hepatosplenic disease can lead to portal hypertension and oesophageal varices and thus, blood loss and haemostatic dysfunction [94]. Furthermore, anaemia can be observed regarding all intensities of infection, whereas occult blood is only detected in severe ones [95]. Hence, there must be further mechanisms that so far remain unknown.

1.7.3 Malaria

In 2015, the WHO estimated the number of deaths caused by malaria at 429 000 [31]. Seventy percent of these concern children aged less than 5 years. Malaria is a major cause of anaemia in African children [96]. The most frequent cause of death due to malaria is anaemia [34]. In addition, infections with *P. falciparum* seem to have long-term effects as well. As highlighted above, there is evidence that the DALY approach underestimates the mortality on account of malaria, because chronic anaemia, low birth weight and enhancement of other diseases are not taken into account [35]. For example, *P. falciparum* infection in asymptomatic children aged younger than 5 years is known to be a predictive factor for anaemia and chronic malnutrition [97]. Besides, infections with *P. falciparum* are associated with low birth weight and high premature mortality in infants [98].

1.7.4 Co-infections

Malnutrition, anaemia and parasitic infections are known to interact intensively [99]. Particular parasitic infections are known to impact blood counts and nutritional status. As geographical patterns of parasites such as STHs and *P. falciparum* infections match and co-infections are common, it is important to understand their interaction. Recent studies have shown that hookworm and *P. falciparum* infections have synergistic effects on anaemia [100]. There are several theories concerning the pathomechanism of these effects. For example, helminth infections have shown to modulate immune response to malaria by suppressing pro-inflammatory cytokines. Thus, they increase the association with severe malarial anaemia, one major manifestation of complicated malaria [101, 102]. However, it could not be shown that malaria and helminth co-infection are associated with malnutrition [103].

1.8 Study objectives

Parasitic infections - including malaria, STHs and urinary Schistosomiasis - affect a large share of the world's population, especially children. Surprisingly enough, there is little data about parasitic co-infections concerning the age group of children aged 1-5 years. It is known that they account for a big proportion of malaria deaths and morbidity due to parasitic infections, and growth retardation is known to originate in this particular age [43, 104].

This study aims at describing the patterns of the various parasitic (co-) infections and their contribution to anaemia and malnutrition in infants. The data will provide scientists groundwork for further investigations concerning the interaction between different parasite species and their clinical presentation and effects.

2 Methods

2.1 Study area

The study was conducted at the Centre de Recherches Médicales de Lambaréné, Gabon.

Gabon is a central African country which borders on Equatorial Guinea, Cameroon, the Republic of the Congo and Sao Tome and Principe [105]. Gabon counts 1.5 million inhabitants, 86% of which live in urban areas [106]. Its size is about 267 600 sq km [107]. The climate is tropical with an annual mean temperature of 26.6°C, two rain and dry seasons per year and an annual humidity of 83% [107]. The official language is French, however, there are more than 40 different languages spoken in the country [107, 108]. The gross national income per capita sizes up to $ 12 450 [106] and the national revenues are dominated by natural resources [108]. However, Gabon ranks 106th place of the Human Development Index [109].

Life expectancy at birth is 60 years for males and 64 years for females. The mortality rate for children under five years of age amounts to 69 per 1000 live births. The prevalence of HIV and tuberculosis among adults is 5% or 6%, respectively [106].

Lambaréné

The Centre de Recherches Médicales de Lambaréné is located about 6 km from the city centre of Lambaréné, which itself is situated about 250 km southeast of Libreville, the capital of Gabon [108]. Lambaréné is located in the province Moyen-Ogooué and counts roughly 25,000 inhabitants.

2.2 Study population

The underlying study of this analysis is “COPAR 01-The study about the prevalence of parasitic co-infections in infants from one to five years living in Lambaréné and surroundings”.

COPAR 01 was conducted in the context of the GMZ-2 study. This phase 2b clinical trial investigated a candidate vaccine against malaria and was carried out at the Centre de Recherches Médicales de Lambaréné from 2010. The parents of children participating in the GMZ-2 study were also instructed about the COPAR study. When they agreed to the participation of their children, they signed an informed consent form to confirm their agreement. Children participating in the GMZ-2 study had to meet the following criteria:

Subject inclusion criteria:

1. Children aged 12 -60 months at the time of first dose
2. Healthy by medical history, physical examination and laboratory investigation
3. Signed/thumb printed informed consent by guardian/parent
4. Resident in the study area villages and being available for vaccination and follow-up during the whole trial period

Subject exclusion criteria

1. Symptoms, physical signs of disease that could interfere with the interpretation of the trial results or compromise the health of the subjects if they were enrolled
2. Immunosuppressive therapy (steroids, immune modulators or immune suppressors) within 3 months prior recruitment. (For corticosteroids ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.)
3. Cannot be followed for any social, psychological or geographical reasons
4. Use of any investigational drug or vaccine other than the study vaccine within 30 days preceding the first dose of study vaccine, or planned use up to 30 days after the third dose
5. Suspected or known hypersensitivity to any of the vaccine components or to previous vaccine
6. Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality
7. Anemia associated with clinical signs or symptoms of decompensation or hemoglobin <7.0 g/dL

8. Planned administration of a vaccine not foreseen by the study protocol within 30 days before the first dose of vaccine. An exception is the receipt of an EPI or licensed vaccine (measles, oral polio, Hib, meningococcal and combined diphtheria/pertussis/tetanus vaccines) which may be given 14 days or more before or after vaccination
9. Presence of chronic illness that, in the judgement of the investigator, would interfere with the trial outcomes or pose a threat to the participant's health
10. Administration of immunoglobulin and/or any blood products within the three months preceding the first dose of study vaccine or planned administration during the study period
11. History of surgical splenectomy
12. Moderate or severe malnutrition at screening defined as weight-for-age Z-score less than -2 or -3 respectively

In addition, for the participation in de COPAR 01 study both urine and stool samples had to be obtainable.

Candidates for the GMZ-2 study that were excluded from participation in GMZ-2 due to exclusion criteria 7 or 12 were still enabled to participate in COPAR 01.

2.3 Methods of measurements

2.3.1 Soil-transmitted helminths diagnostics

Sample collection

A pot for stool samples was handed over to the parents or caretakers. They were instructed to fill it with the child's stool in the morning of their next visit. The samples were collected and immediately transferred to the laboratory. Stool and urine samples were also collected at participants' houses, if they were not returned during the scheduled visits.

For scatoscopy, a combination of Kato-Katz-technique and coproculture was used.

Kato-Katz technique

Cellophane strips were soaked in 50% Glycerol solution overnight. A small amount of faeces was put on a piece of scrap paper. Then a screen (60-105 mesh) was firmly pressed on the faecal sample. To sieve the specimen, it was scraped on the top of the screen using a wooden applicator stick. A stainless steel template with a hole in it was placed on a clean microscope slide. The hole was then filled with the sieved specimen. To guarantee a standardized amount of faeces, the template was removed in a manner that the whole cylinder of faeces rested on the microscope slide. Afterwards, the specimen was covered with the soaked glycerol strip. Surplus glycerol was removed with paper towels. The slide was firmly pressed against a flat surface to scatter the specimen. Finally, the slide was cautiously moved sideways. The slides were then read by trained laboratory technicians. A single Kato-Katz analysis was performed for each participant. When there were doubts or incomplete results, a second faecal sample was collected.

Coproculture

For coproculture, two microscope slides were wrapped with blotting paper. A few grams of the collected faecal sample were dispensed on them. The slides,

the paper and the stool specimen were then put in petri dishes containing sterile distilled water. After 72 hours at 25 C°, the water was filtered and the sediment was put on microscope slides. The slides were then read by trained laboratory technicians.

2.3.2 Schistosoma diagnostics

The collection of urine samples was performed in the same manner as the stool samples.

For the analysis of the specimen, a filter was placed in the filter holder. After having carefully agitated the urine, the syringe was filled with 10 ml. The filter holder was attached to the syringe. The urine was then passed through the filter while holding it over a suitable container. The filter holder was then unscrewed and the filter was placed on a glass slide. After having added a drop of physiological saline or methylene blue, it was covered with a cover glass. Subsequently, the whole filter was examined by experienced laboratory technicians. The results were documented as egg count per 10 ml of urine. Analogous to the faecal analysis, one urine examination was performed and in case of doubts or incomplete results, a second sample was collected.

2.3.3 Malaria diagnostics

The diagnostics of malaria were done with thick blood films to evaluate parasitaemia. The blood for thick blood films was either collected from a finger pulp or venous, for which an EDTA tube was used. EDTA tubes were collected according to the protocol of the GMZ2 study. Thick blood films were prepared according to the regulations of the WHO and - for backup – according to the Lambaréné method as described by Planche et al. [110]. In general, thick blood films done by the WHO method were analyzed. In case they were not available, for example due to illegibility, the ones obtained according to the Lambaréné method were used.

Lambaréné Method:

Ten µl of blood were evenly distributed on a 10x18mm area of a glass slide. Thereafter, the slides were dried and stained in Giemsa solution (20%, ph=7.2) for 20 minutes, washed with clean water and dried again in upright position.

The slides were read with a 100x microscope objective and immersion oil. Parasites were counted in at least 100 high power fields if the parasite count was ≤ 5 parasites per field, in 30 fields if the parasite count was between 5 and 50 parasites per field, and in 20 fields if the parasite count was ≥ 50 parasites per field. If no parasites were found after examining 100 fields, the slide was considered negative. The total number of parasites counted was divided by the total number of fields and multiplied with the microscope factor, a factor specific for each microscope, to obtain the parasite count per µl. Each slide was read twice by two independent investigators. If the ratio of densities from the higher to the lower count was > 2.0, or in case of discrepant opinions regarding positivity/negativity, a third reading was done. If both initial readings showed < 300 parasites/µl and the difference between the two was more than 100, a third reading was performed as well. The absolute differences between the results of each reading were calculated and the two results with the lowest difference were chosen to determine their arithmetic mean.

WHO method:

Blood collection, preparation and staining were the same as for Lambaréné Method.

The slides were read with a 100x microscope objective and immersion oil. Parasites were counted in 100 fields. If no parasites were found after 100 fields, the slide was considered negative. If, after counting 200 leukocytes, ≥ 10 parasites were found, the result was reported in parasites per 200 leukocytes. In case of ≤9 parasites found after having counted 200 leukocytes, examination was continued and results were reported in number of parasites per 500 leukocytes. Subsequently, parasite loads per µl were calculated as follows:

If a reliable estimation of the white blood count was not available, e.g. due to missing blood samples:

$$\frac{Parasite\ count\ x\ 8000}{counted\ Leukocytes} = Parasites/\mu l$$

If a reliable absolute white blood count was available:

$$\frac{Parasite\ count\ x\ Absolute\ white\ blood\ count}{counted\ leukocytes} = Parasites/\mu l$$

The absolute white blood count was determined with ADVIA® 120 Hematology System by Siemens Healthcare Diagnostics.
The rules for double reading and third readings were the same as for Lambaréné Method.

2.3.4 Measurement of hemoglobin level

An EDTA tube with venous blood was collected. The tubes were analyzed with the ADVIA® 120 Hematology System by Siemens Healthcare Diagnostics which was calibrated once a year or if technical dysfunctions or inaccuracies occurred. According to the recommendations of the WHO, anaemia was defined as a hemoglobin level lower than 11 g/dl [111]. Following Stoltzfus et al., anaemic participants were divided into moderately anemic (Hb 9-10.9 g/dl) and severely anemic (Hb < 9 g/dl) groups. [112].

2.3.5 Assessment of the anthropometric status

Shoes and heavy clothes were removed before measurement. Height was measured using a stadiometer with movable headboard. Weight was measured with a calibrated electronic balance.
In order to guarantee standardized results, all subjects performing the measurements received a detailed instruction and were familiar with the procedures. The anthropometric measurements were transformed into standardized sex-specific z-scores of height-for-age (HAZ) and weight-for-height (WHZ). According to the definition of the WHO, moderate stunting was

defined as HAZ < -2SD and ≥ -3SD, severe stunting as HAZ < -3SD. Definitions for wasting were the same using WHZ.

2.4 Study design

The study was designed cross-sectional. Stool, urine and blood samples were collected from the participants to determine their current hemoglobin status and whether they showed infections with intestinal helminths, Schistosoma or Plasmodium. In addition, the participants' weight and height were determined, which allowed an assessment of the anthropometric status. Every participant showing parasitic infections was treated. In case of bilharzia, subjects were treated with 40 mg/kg body weight praziquantel. When intestinal helminths were found, 200 mg albendazole for children younger than 2 years and 400 mg for children older than 2 years were administered. If *T. trichura* was detected, treatment with albendazole lasted for 3 days, because treatment with a single dose was proven to be less effective [113]. In case of infection with Plasmodium, the treatment consisted of Coartem® (artemether-lumefantrine) depending on the child's weight. In addition, the participant received supportive care if considered necessary.
When anaemia was observed, participants were treated according to their medical condition and symptoms.

2.5 Data management and statistics

Data entry was performed using Microsoft Office Excel 2007®. All data were double checked. Anthropometric measurements were transformed into standardized sex-specific z-scores by means of the WHO Child Growth Standards SPSS Syntax File which uses WHO child growth standards [114-116]. Statistical analysis was done using JMP® (JMP® 11.1.1, SAS Institute, Inc., NC, USA). Descriptive statistics were calculated for the aforementioned parameters. Comparisons were done using the chi^2 test and Fisher's exact test.

Odds ratios and their confidence intervals were calculated. The level of significance was defined as $p < 0.05$.

2.6 Ethical aspects

Informed consent was obtained by every participant's caregiver according to Good Clinical Practice, the Declaration of Helsinki and applicable regulations in Gabon. All collected data was treated confidentially

The study protocol was approved by the Ethics Committee of the International Foundation of the Albert Schweitzer Hospital.

3 Results

3.1 Participants' characteristics

A summary of the participants' characteristics is shown in table 1.

A total of 444 children were included in the study. Complete data including age, sex, weight, height, malaria, STH, Schistosoma and anaemia diagnostics could be obtained from 327 (73%) participants.

Median age was 37 months, the youngest subject being 12 months and the eldest being 60 months old. Mean age was 3.03 years.

The participants were divided into three age groups, 12-28, 29-44 and 45-60 months. Thirty-one percent of the subjects were in group 1, 33% in group 2 and 37% in group 3.

Two-hundred-forty-four (55%) of all participants were male, whereas 202 (46%) were female. Male subjects were more frequent in all age groups.

Table 1 Demographics

	Total	N (%)
Age group (months)		
12-28	444	139 (31%)
29-44	444	147 (33%)
45-60	444	158 (37%)
Sex		
Male	444	242 (55%)
Female	444	202 (46%)

3.2 Parasite infections

A brief description of detected parasitic infections and their prevalence can be found in table 2.

Table 2 Number of subjects with various parasite infections

	Total	N (%)
Any parasitic infection	339	166 (49%)
Soil-transmitted helminth infection	394	130 (33%)
T. trichiura	394	98 (25%)
A. lumbricoides	394	50 (13%)
Hookworms	394	10 (3%)
Others	394	13 (3%)
S. haematobium	407	39 (10%)
Co-infection with STH	375	20 (5%)
P. falciparum	401	59 (15%)
Co-infection with STH	358	16 (4%)
Co-infection with *S. haematobium*	365	3 (0.8%)
Co-infection with *S. haematobium* and STH	339	2 (0.6%)
Any parasitic co-infection	339	55 (16%)
Bi-infection	339	42 (12%)
Infection with three or more parasites	339	13 (4%)

3.2.1 Intestinal helminths

A complete examination of stool samples was performed for 394 participants. Regardless of the species, intestinal helminths were found in 130 of 394 participants (33%). The rate of positive subjects increased significantly with age. While 28/134 (23%) in age group 1 showed intestinal helminth infection, 47/133 (35%) in age group 2 and 55/137 (40%) in age group 3 were tested positive. Odds ratios (95% CI, p) for age group 2 and 3 compared to age group 1 were 1.9 (95% CI: 1.1–3.3; p=0.028) and 2.3 (95% CI: 1.3-4.0; p=0.0033), respectively.

Comparing the two sexes, 76/215 (35%) of male subjects were positive, whereas 54/179 (30%) of the females (OR 1.3, 95% CI: 0.8-1.9; p=0.28) were diagnosed with STH infections.

The most common species was *T. trichiura* with a prevalence of 25%, followed by *A. lumbricoides* with 13% and *Ancylostoma spp.* with 3%.

Seventy-five percent of the 130 participants showing helminth infection, were tested positive for *T. trichiura*, 38% for *A. lumbricoides*, 8% for *Ancylostoma spp.* and 10% for other, not otherwise specified intestinal helminths. Twenty-eight percent showed an infection with more than one STH.

Figure 1 shows the prevalence of different parasitic infections in different age groups.

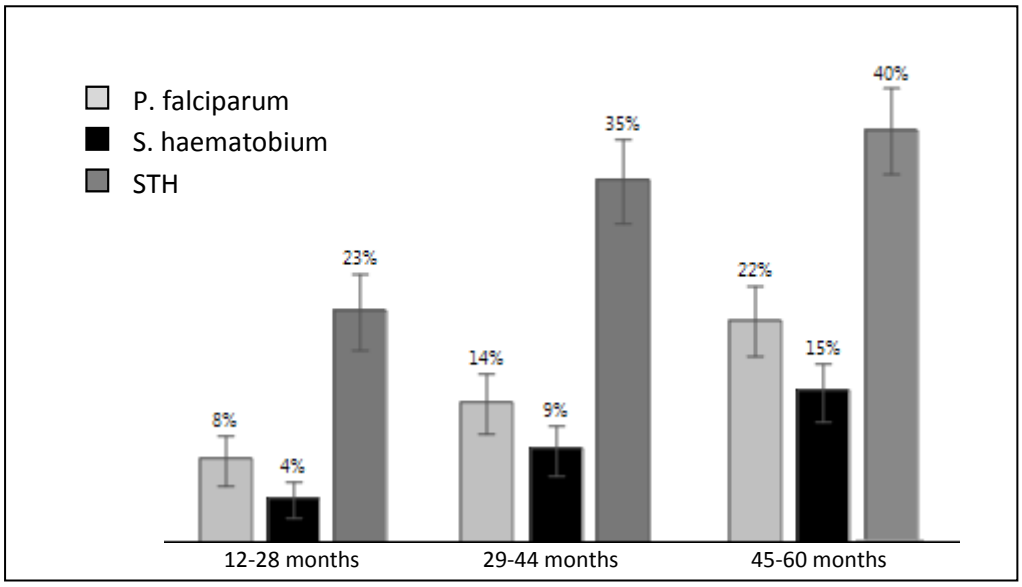

Fig 1: Prevalence of different parasites by age group and 95% CI

3.2.2 Schistosomiasis

Sufficient samples for complete Schistosoma diagnostics were obtainable for 407 subjects. Disregarding age group and sex, 39/407 (10%) were diagnosed with schistosomiasis.

The lowest age group showed the lowest infection rates. In age group 1 only 5/123 (4%) were tested positive, whereas 12/134 (9%) in age group 2 and 22/150 (15%) in age group 3 were infected. Odds ratios (95% CI; p) of age group 2 and 3 for infection with *S. haematobium* were 2.3 (0.8-6.8; p=0.1) and 4.1 (1.5-11.1; p=0.0038), respectively, when compared to the lowest age group.

Schistosoma was more common in male subjects. Out of 229, 25 (11%) were positive compared to 14/178 (8%) of females (OR 1.4, 95% CI: 0.7-2.9; p=0.31).

3.2.3 Malaria

Samples for malaria diagnostics were obtained from 401 participants, 59 of them (15%) were positive, whereas 342 were negative. Regarding different age groups, the prevalence of malaria infection increased with age. In age group 1 (12-28 months) only 10/125 (8%) were positive compared to 18/133 (14%) in age group 2 (29-44 months) and 31/143 (22%) in age group 3 (45-60 months). Odds ratios (95% CI; p) of age group 2 and 3 for infection with *P. falciparum* compared to age group 1 were 1.8 (0.8-4.1; p=0.2) and 3.2 (1.5-6.8; p=0.0021), respectively.

When comparing the two sexes, 16% of the male subjects were positive but only 13% of the females (OR 1.3, 95% CI: 0.7-2.2; p=0.5).

3.2.4 Co-infections

Of the 339 participants with complete parasitological analysis, 166 (49%) showed at least one parasite.

For 375 subjects both Schistosoma and STH diagnostics could be completed; 20 (5%) of these subjects were positive for both Schistosoma and STHs, 10 of them belonging to age group 3, 7 to age group 2 and only 3 to age group 1. The co-infection rate with Schistosoma and STHs was similar in both sexes (6% of male participants compared to 5% of female ones (OR 1.2, 95% CI: 0.5-3.0)). Participants infected with STH were significantly more likely to bear a *S. haematobium* infection (OR 2.9; 95% CI: 1.4-5.9; p=0.0042).

Regarding co-infection with STHs and malaria, complete diagnostics were carried out for 358 participants. Sixteen of 358 (5%) were positive for both of them. One of them belonged to age group 1, whereas 6 belonged to age group 2 and 9 to age group 3. Half of the double positives were male.

Complete diagnostics for Schistosoma and malaria were available for 365 subjects. Only 3/365 (0.8%) of them were positive for both of the parasites, 1 belonging to age group 2 and 2 of them belonging to age group 3.

Three-hundred-thirty-nine participants provided samples for diagnostics concerning STHs, malaria and Schistosoma. Only 2/339 (0.6%) showed a co-infection of all three of them, one male and one female subject.

3.3 Anaemia

Anaemia was very common in the study population as 334/436 (77%) were shown to be anaemic, thus showed Hb-levels lower than 11 g/dl. A majority of 284/436 (65%) of the children were moderately anaemic and 50/436 (11%) suffered from severe anaemia.

Regarding different age groups, anaemia was most common in age group 1 (12-28 months). Of 134 subjects 119 (89%) showed anaemia, compared to 101/145 (70%) in age group 2 (29-44 months) and only 114/157 (73%) in age group 3 (45-60 months). The median of Hb-level was 9.9 g/dl, 10.5 g/dl and 10.4 g/dl for the different age groups.

Rates of anaemia were similar in both sexes (77% of males compared to 76% of females with a corresponding median Hb-level of 10.3 g/dl for both sexes.)

Figure 2 shows the distribution of different anaemia grades in different age groups.

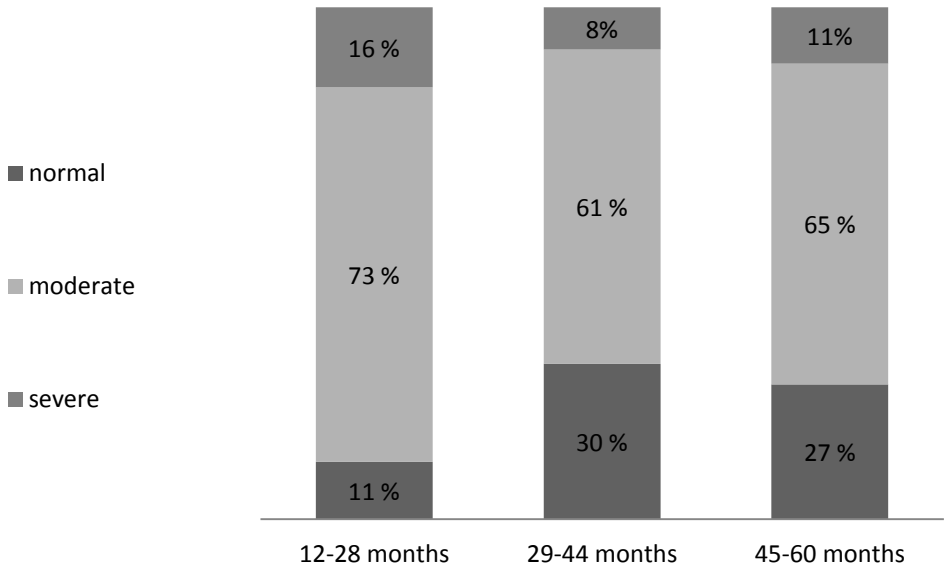

Fig 2 *Prevalence of different grades of anaemia by age group*

3.4 Nutritional status

Moderate wasting as an indicator for acute malnutrition was found in 6/429 subjects (0.9%), 2 of them belonging to age group 1 (12-28 months), 1 to age group 2 (29-44 months) and 3 to age group 3 (45-60 months). Severe wasting was not observed.

A total of 87/429 (20%) participants were found to be stunted. Moderate stunting was observed in 66/429 (15%) and severe stunting in 21/429 (5%) subjects, whereas 342 (80%) of them were classified as normally developed regarding height in relation to their age. It could be seen that (moderate and severe) stunting was more common in lower age groups (25% in age group 1, 26% in age group 2 and 11% in age group 3).

Stunting showed similar rates in boys (51/236; 22%) and girls (36/193; 19%) but the rates were still slightly higher in male participants (OR: 1.2, 95% CI: 0.8-1.9; p=0.5).

3.5 Parasitic infections and Hb-level

The association of different factors with the prevalence rates of anaemia is shown in table 3.

3.5.1 STH infection

Complete data concerning STH diagnostics and hemoglobin level could be collected for 387 participants. Three-hundred-one (79%) of them were anaemic. Comparing the subjects infected with helminths with the non-infected ones, rates of anaemia were the same (78%). Looking at infections with different species of STHs, none of them could be proven to be associated with higher rates of anaemia.

Concerning median hemoglobin concentrations, neither a statistically significant association with STH infection in general, nor with certain species of STHs was found.

Table 3 Risk factors for anaemia in children

	n	anaemic n (%)	OR	95% CI	p*
Total	436	334 (77)	-	-	-
Sex					
Male	239	184 (77)	1.1	0.7-1.6	0.9
Female	197	150 (76)	Ref.		
Age group (months)					
12-28	134	119 (89)	3.0	1.6-5.7	0.0006
29-44	145	101 (70)	0.9	0.5-1.4	0.6
45-60	157	114 (73)	Ref.		
T. trichiura					
Positive	96	75 (78)	1.0	0.6-1.8	1.0
Negative	291	226 (78)	Ref.		
A. lumbricoides					
Positive	48	36 (75)	0.8	0.4-1.7	0.6
Negative	339	265 (78)	Ref.		
Hookworm					
Positive	9	7 (78)	1	0.2-4.9	1.0
Negative	378	294 (78)	Ref.		
Other STH					
Positive	13	10 (77)	0.9	0.3-3.5	1.0
Negative	374	291 (78)	Ref.		
S. haematobium					
Positive	37	30 (81)	1.3	0.5-3.0	0.7
Negative	362	279 (77)	Ref.		
P. falciparum					
Positive	59	54 (92)	3.9	1.5-10.0	0.0025
Negative	336	247 (74)	Ref.		
Co-infections					
STH + *S. haem.*	19	15 (79)	1.1	0.3-3.3	1.0
STH + *P. falc.*	16	16 (100)	-	-	0.0279
P. falc. + *S.haem.*	3	3 (100)	-	-	1.0
Triple infection	2	2 (100)	-	-	1.0

* *two-tailed Fisher's exact test*

3.5.2 Schistosoma infection

Both Schistosoma and anaemia diagnostics were carried out for 399 participants. Subjects that were infected with Schistosoma showed non-significantly higher rates of anaemia compared to the negative ones (30/37 or 81% versus 279/362 or 77%). Looking only at male subjects, this effect was higher, but still not significant. Twenty-two of 24 (92%) of the male subjects tested positive were anaemic in comparison to 153/202 (76%) of the negatives.

Median hemoglobin concentrations of all participants were 10.4 g/dl with a range from 8-12.4 g/dl and 10.3 g/dl with a range from 4.7-13.6 g/dl in the positive and in the negative group, respectively. Looking at different grades of anaemia, severe anaemia was less present in the positive tested (2/37 or 5%) than in negative ones (45/362 or 12%), whereas moderate anaemia was more common among positive ones (28/37 or 76% compared to 234/362 or 65%).

3.5.3 Malaria

For 395 participants both malaria and anaemia diagnostics were completed. In total, 76% of them were anaemic. Subjects being positive for malaria were significantly more likely to be anaemic than those being negative (54/59 or 92% versus 247/336 or 74%, OR: 3.9, 95% CI: 1.5-10.4, p=0.0025).

Twenty-one of 59 (36%) tested positive were severely anaemic and 33/59 (56%) were moderately anaemic in comparison to 28/336 (8%) and 219/336 (65%) of the tested negative.

The median hemoglobin level among positive tested was 9.4 g/dl with a range from 6.2 to 11.5 g/dl. Regarding the negative tested, it was 10.3 g/dl and ranged from 4.7 to 13.6 g/dl. This difference could be detected in all age groups and was slightly more distinct among female participants. A median hemoglobin level of 9.35 g/dl for positive tested was measured in comparison to 9.5 g/dl in male subjects.

3.5.4 Co-infections

Considering different co-infections, they all showed higher rates of anaemia in the infected group. This effect was less distinct for the combined infection of STH and Schistosoma. Rates of anaemia were almost the same (15/19 or 79% versus 272/349 or 78% in the positive and the negative group, respectively).

Regarding the co-infection of STHs and malaria, positive tested persons were significantly more likely to be anaemic (p=0.0279). Sixteen of 16 (100%) of the positive tested were anaemic compared to 257/337 or 76% of the negatives. Median hemoglobin concentrations were 9.35 g/dl with a range from 7.3-10.9 g/dl in the infected group and 10.3 g/dl with a range from 6.2-13.6 g/dl in the non-infected one.

3.6 Parasitic infections and anthropometric status

The association of parasite infections and other risk factors regarding the anthropometric status is shown in table 4.

3.6.1 STH infection

Complete evaluation of the anthropometric status and diagnostics for STH infection was performed on 382 subjects.

Only 4 (1%) of them were wasted. None of them showed STH infection.

The rate of stunted participants was slightly higher in STH-positive than in STH-negative ones (30/126 or 24% compared to 54/256 or 21%, OR 1.2, 95% CI: 0.7-1.9, p=0.6).

3.6.2 Schistosoma infection

There were 395 participants with complete Schistosoma diagnostics and anthropometric evaluation. All 39 subjects, that were positive for Schistosoma, were classified as normal concerning their weight in relation to the height (wasting). Regarding the height in relation to their age (stunting), abnormal values could be found for 21% of both positive and negative tested for Schistosoma.

Table 4 Risk factors for stunting in children

	n	stunted n (%)	OR	95% CI	p*
Total	429	87 (20)	-	-	-
Sex					
Male	236	51 (22)	1.2	0.8-1.9	0.4
Female	193	36 (19)	Ref.		
Age group (months)					
12-28	134	33 (25)	2.6	1.4-4.9	0.0046
29-44	144	37 (26)	2.7	1.5-5.1	0.0015
45-60	151	17 (11)	Ref.		
T. trichiura					
Positive	94	27 (29)	1.6	1.0-2.8	0.08
Negative	288	57 (20)	Ref.		
A. lumbricoides					
Positive	48	10 (21)	0.9	0.4-1.9	1.0
Negative	334	74 (22)	Ref.		
Hookworm					
Positive	48	10 (21)	0.9	0.4-1.9	1.0
Negative	334	74 (22)	Ref.		
Other STH					
Positive	13	2 (15)	0.6	0.1-2.9	0.74
Negative	369	82 (22)	Ref.		
S. haematobium					
Positive	39	8 (21)	1.0	0.4-2.3	1.0
Negative	356	73 (21)	Ref.		
P. falciparum					
Positive	58	11 (19)	0.9	0.5-1.9	1.0
Negative	329	67 (20)	Ref.		
Co-infections					
STH + *S. haem.*	20	5 (25)	1.2	0.4-3.4	0.7
STH + *P. falc.*	16	5 (31)	1.7	0.6-5.0	0.35
P. falc + *S.haem.*	3	1 (33)	2.0	0.2-22.1	0.5
Triple infection	2	1 (50)	3.7	0.2-59.7	0.3

* *two-tailed Fisher's exact test*

3.6.3 Malaria

Anthropometric measurements and malaria diagnostics were performed on 387 participants.

All 6 wasted individuals were negative for malaria. The rate of stunted subjects was 11/58 (19%) in STH-positive tested individuals versus 67/329 (20%) in negative tested ones (p=1.0, OR 0.9, 95% CI: 0.5-1.9).

3.6.4 Co-infections

The influence of co-infections on the anthropometric status was not statistically significant. Most common were co-infections with soil-transmitted helminths and Schistosoma as well as soil-transmitted and *P. falciparum*. Stunting was more frequent in co-infected children compared to those not showing co-infections (25% vs. 22% and 31% vs. 21%). However, the difference was not significant (OR: 1.2; 95% CI: 0.42-3.41; p=0.7 and OR: 1.69; 95% CI: 0.57-5.04; p=0.3 respectively).

The influence of co-infections with Schistosoma and *P. falciparum* as well as the triple infection with STH, Schistosoma and *P. falciparum* on the anthropometric status was not calculated further due to the low number of cases.

4 Discussion

In this cross-sectional study, a total of 444 children aged 1-5 years were recruited and their blood, stool and urine samples were collected. They were then examined for infections with soil-transmitted helminths, Schistosoma and Plasmodium. An assessment of the anthropometric status and measurements of the hemoglobin level were performed in order to allow a detailed description of these parameters and an investigation of the association between them. Complete data was obtained from 327 subjects.

Infections with intestinal helminths were found in 33%, infections with Schistosoma in 10% and infections with malaria in 15% of all subjects.

Anaemia was also common in the examined population, as 77% were classified as anaemic with different patterns in the particular age groups.

Wasting as an indicator for acute malnutrition was found in 0.9%, stunting, reflecting chronic malnutrition, was detected in 20% of the participants.

Neither STH, nor Schistosoma infections were associated with significantly higher rates of anaemia or malnutrition. Merely subjects infected with malaria were significantly more likely to be anaemic (p=0.0025). Accordingly, the co-infection with malaria and soil-transmitted helminths showed a significantly higher rate of anaemic participants (p=0.028).

4.1 Methods

4.1.1 Recruitment

The study was conducted in the Centre de Recherches Médicales de Lambaréné, Gabon. The recruitment took place in both rural and urban areas, and is therefore comparable to former studies [117]. Sampling could not take place in local schools for pre-school aged children. This could be one explanation for the fact that only 73% of the participants provided complete data which included all parameters of interest. In addition, recruitment in schools would have biased the study population as well, since only children attending

schools would have been sampled. If all participants that took part in COPAR 01 had also participated in GMZ-2, the infants would have been seen more often during scheduled visits for the latter study and thus, return rates of samples could have been improved, analogously to the work of Brooker et al., in which pre-school children were recruited out of a large case-control study regarding malaria [44]. Nevertheless, adequate numbers of subjects were entered into the study for these particular analyses.

Recruiting from another study's population could have biased the results, as two of the exclusion criteria from GMZ-2 concerned issues of interest of COPAR 01. To prevent bias, children that were excluded from the GMZ-2 study due to these criteria, were nevertheless included in COPAR 01 as mentioned in the methods section.

Analogous to the above mentioned study of Brooker et al., the participants of our study were almost evenly distributed among age groups and sex [44]. In most of the other studies, older children were overrepresented [118].

4.1.2 Parasite diagnostics

Sampling

Samplings for stool and urine were collected by the parents or caretakers. When the samples arrived at the Research Unit, they were examined immediately. Nevertheless, time between sampling and examination could have reduced the sensitivity of the diagnosis. The WHO recommends examination of stool specimens within 1-4 hours after sampling [119]. Furthermore, it is advised to add formalin to every urine sample which cannot be analysed within one hour [120]. As geographical conditions in Gabon did not always allow us to follow these recommendations, the sensitivity of urine and stool examinations might have been affected.

Stool examination

For scatoscopy, we used a combination of Kato-Katz technique and coproculture. It was found that Kato-Katz has its limitations, especially

concerning low-intensity infections [121]. Another approach could have been sample examination using FLOTAC®, as this technique has been shown to be more sensitive than Kato-Katz-technique concerning infections with hookworms, *A. lumbricoides* and *T. trichiura* [122] [123, 124].

One could consider collecting multiple stool samplings as this approach was found to be more sensitive [125]. Knopp et al. were able to show that triple Kato-Katz analysis increase the prevalence compared to single analysis, especially with respect to hookworms and *T. trichiura*. For hookworms, this increase totaled up to 161%. Nevertheless, by combining two methods, we were able to assure sufficient sensitivity of specimen examination. Concerning urine sampling, the effect of multiple sampling has been shown as well [126]. Farooq et al. found that the examination of a single urine sample (compared to sampling three times) identifies 13% less of the *S. haematobium* infections.

Thick blood smears

Joanny et al. [127] found that malaria diagnostics, performed as described above by the research centre in Lambaréné, provide accurate results. Nevertheless, one could consider other techniques such as molecular assays to increase sensitivity of malaria diagnosis - especially for low parasitemia infections [128].

4.1.3 Anaemia diagnostics

Measurements of the hemoglobin level have only been conducted by trained laboratory technicians using the ADVIA® 120 Hematology System by Siemens Healthcare Diagnostics, which is regularly calibrated. One can thus expect exact values.

4.2 Results

4.2.1 Soil-transmitted helminths

Thirty-three percent of all participants providing sufficient samples for diagnostics of STHs were found to be positive for at least one species. This rate is lower than described in most other studies on the topic. Compared to studies with comparable participant characteristics concerning age, we had similar findings. For instance, in the work of Brooker et al. [44], an overall prevalence of STHs was found in 42% of preschool children. Another comparable result is the work of Davis et al., which showed a prevalence of 40% in preschool children [129]. The majority of studies concerning the prevalence of STHs in children were conducted in schools. Furthermore, the rate of infection with soil-transmitted helminths was found to increase with age during the first years of life [118]. Hence, one explanation for our results deviating from most of the other studies is the different age patterns. However, the rate of positive tested subjects in our study was higher than in some other publications, e.g. Mbuh et al., who found 20% of children aged 1-5 years to be infected with STHs [130]. Their study was conducted in Buea, Cameroon, a neighbouring country of Gabon. However, the number of participants in this age group was relatively low (n=31).

The most frequent STH in our study was *T. trichiura* with a prevalence of 25%. The second most common species was *A. lumbricoides* showing a prevalence of 13%, followed by hookworms with 3%. This result is consistent with other studies like the one of Tchuem Tchuente, a big cross-sectional study which included 12486 schoolchildren in Cameroon [40]. They found a prevalence of 19% regarding *T. trichiura*, 12% with respect to *A. lumbricoides* and 2% for

hookworms - similar rates to our study. However, the different age patterns of the studies should be taken into account, as they had also examined schoolchildren whereas our participants were pre-school children.

Among the 130 participants tested positive for STH, we found co-infections with other STHs (two or more species) in 28%, which is similar to the results of Mbuh et al., who found a rate of STH co-infections of 37% [130].

According to our findings, the probability of STH infection increased with age. Comparing the different age groups, we found significantly higher rates of infection in higher age groups ($p=0.0083$), an observation that was previously made by Dana et al., who also found a significant increase of STH-infection from 1-3 years of age [118]. In our results, males showed non-significantly higher rates of STH infection than females (35% versus 30%). This was also observed by Mbuh et al., but contradictory findings can be found as well [118, 130].

4.2.2 Urinary Schistosomiasis

Our population showed an overall prevalence for Schistosoma infection of 10%. Ekpo et al. found a higher prevalence of 17% in a comparable age group [131]. In their study, mean age was 4.36 years (compared to 3.03 years in our study) with children aged 5-6 years showing the highest prevalence and being the largest proportion of participants. Hence, regarding only children aged 1-5 years, as in our study, the prevalence found by Ekpo et al. would probably have been lower. Other works, such as the one from Amuta et al., showed a much higher prevalence of 45% in a - concerning the age range - comparable population [132]. As they used the same analyzing methods, the discrepancy in results cannot be explained by methodological factors. But according to the WHO, Nigeria accounted for more than 10% of all people requiring preventive chemotherapy for schistosomiasis worldwide in 2013, and is thus known to bear a high burden regarding this disease [133].

Comparing the two sexes, we found a non-significantly higher rate in male subjects. This effect was observed in other studies as well and seems to be

associated with number and type of water contacts [132, 134]. In our study, the rate of Schistosoma infection was shown to rise with age (4%, 9% and 15% in age group 1, 2 and 3, respectively). This observation was also made by Ekpo et al. and van den Bigelaar et al. for preschool and school children [131, 135]. Nevertheless, some studies have not found this increase. Dabo et al. found the highest rates of infection in their highest age group, which was children of 4 years, and the lowest rate in 2-year-olds. However, these results were non-significant [136].

4.2.3 Malaria infection

For malaria infection, we found a prevalence of 59/401 (15%). In contrast, Nkoghe et al. found a prevalence of 5% among 399 asymptomatic Gabonese children aged younger than 15 years [137]. Comparing these different results, one has to consider the different age patterns as they do not mention an additional sub-analysis for children in our age range. Furthermore, they only included asymptomatic participants, which is also different from our study. Other studies like the one of Bouyou-Akotet showed a much higher prevalence of malaria in febrile children [138]. They detected a positivity rate of 43% among 8195 febrile children aged 0-10 years. In contrast to the latter study, only febrile children were enrolled, which is one explanation for the high prevalence of Plasmodium found in this work.

As with the other infections, we found the prevalence of malaria infection to increase with age. Age group 1, 2 and 3 showed a prevalence of 8%, 14% and 22%, respectively. This result is contradictory to findings of Nkoghe et al.. These authors could not show a correlation between the prevalence and the age of children [137], however, there were different age patterns among the studies.

4.2.4 Parasitic co-infections

Concerning the co-infections with different parasites, we found the co-infection with Schistosoma and STH to be most common with an overall prevalence of 6%, followed by STH and malaria, which was found in 4% of the cases. There is few data concerning an analysis of co-infections with STHs, *S. haematobium*, as well as *P. falciparum*. A comparable study focusing on co-infections in pregnant women has recently been published by Adegnika et al. [139]. In contrast to their findings, in our population the odds ratio for children positive for STHs or *S. haematobium* to be co-infected with *P. falciparum* was below 1 for all species.

One explanation for the high rate of participants co-infected with *P. falciparum* and STHs could be the fact that helminth infection has been shown to increase the risk of infection with *P. falciparum* [52]. However, this data has been obtained in a different population, as merely Thai adults were included. Thus, comparability is limited. Furthermore, the rate of co-infection with STH and *P. falciparum* could just be caused by the high prevalence of both single infections.

Other co-infections such as schistosomiasis and malaria or triple infection with schistosomiasis, malaria and any STH showed negligible rates of 0.8% and 0.6%, respectively. Looking at co-infection with Schistosoma and *P. falciparum*, a negative association between *S. haematobium* infection and *P. falciparum* densities has been shown by Briand et al. [134]. They interpreted their findings as negative interactions between the parasites. This could be one explanation for the low rate of this co-infection in our analysis. Nevertheless, they examined children aged 3-15 years and did not describe the prevalence of co-infections for other age groups. Another explanation could be inverse associations of malaria infection and schistosomiasis with age. Schistosomiasis is more common in older children [131], whereas malaria cases were found to decrease with age [140]. More comparable data on co-infection with STHs, *S. haematobium* and *P. falciparum* is missing so far and could be the content of further studies.

4.2.5 Anaemia

In our study, 334 of 436 (77%) children were classified as anaemic. This rate is higher than in data provided by the WHO concerning the worldwide prevalence of anaemia in 2011 [141]. They classified 62% of the children aged 6-59 months living in the African Region as anaemic. Another worldwide systematic analysis showed 80% of the children aged less than 5 years in central and west Africa to be anaemic [142]. The latter is even better comparable to our study, as the relatively low rates of anaemic children in northern and southern Africa are not included- as it is the case in the report of the WHO. Stevens et al. found 5% of these children to suffer from severe anaemia, a rate which is lower than the one we found (11%). However, different thresholds of hemoglobin concentration were set in these studies. Stevens et al. chose 7 g/dl, whereas our cutoff was 9 g/dl, thus, with the same thresholds, our rate would have been considerably lower. Another explanation is the way of recruitment. As hemoglobin concentrations of 7 g/dl or lower were an exclusion criterion for the GMZ-2 study, the participation rate in COPAR 01 might have been lower as well.

The mean hemoglobin concentration in our study was 10.18 g/dl (95% CI: 10.08-10.28 g/dl) compared to 10.0 g/dl (95% CI: 99-10.2 g/dl) in the work of Stevens et al. - thus, slightly higher. In contrast to our study, their study included children from 6-11 months as well, who are known to show the highest rates of anaemia [44, 142]. Thus, our result of mean Hb concentration would possibly have been lower if we had included this age group as well. Unfortunately, they did not describe the amount of children aged 6-11 months in their study. Therefore, the effect on mean Hb concentration cannot be quantified.

According to studies like those of Brooker et al., rates of anaemia can be expected to be highest in the lowest age group, as in our study, where 89% of them were classified as anaemic. However, in age group 2, prevalence of anaemia was 70% and therefore, higher than in age group 3 (in which 73% of the participants were found to be anaemic). It is thus in line with other studies, which showed the prevalence of anaemia in children living in areas with intense and stable malaria transmission to reach its peak at the end of the first year of life [143].

One approach to improve data concerning hemoglobin levels in further studies could be a recruitment of participants independent from other studies and their inclusion and exclusion criteria.

4.2.6 Nutritional status

Regarding the nutritional status, we found 20% of our participants to be stunted. Compared to the 6th Report on the World Nutritional Situation by UNSCN, this is a rather low rate. They found a prevalence of 39% for stunting in African children aged under the age of 5 [144]. Further data from the WHO state that the percentage of Gabonese children between 0 and 5 years who are 2SD or more below the median regarding height for age ("stunting" in our study) to be 26% [145], a rate which is similar to our findings. The data provided by the WHO was collected in 2000 and 2001, whereas our sampling was done 2010 and 2011. Onis et al found that childhood stunting in Africa is slowly but steadily decreasing. Therefore, we can assume that current data of the WHO would show lower rates of stunting [146]. A further explanation for the discrepancy could - analogously to the rate of anaemia – be due to the way of recruitment. Like severe anaemia, severe malnutrition was an exclusion criterion of the GMZ-2 study which might, assuming a lower participation rate of stunted children, have influenced our findings.

The WHO found that 4 % of Gabonese children from 0-5 years show a weight-for-height which was 2 SD or more below the median ("wasting" in our study). Corresponding to the results concerning stunting, one has to note the different period of time during sampling as well as varying modes of recruitment.

Analogous to data on hemoglobin levels, information about malnutrition may be improved by recruitment independent from other studies and their inclusion and exclusion criteria.

Corresponding to the WHO Database on child growth and malnutrition for Gabon, we showed the prevalence of stunting to be lower in higher age groups and to be non-significantly higher in males than in females [145].

4.2.7 Parasitic (co-)infections and Hb-level

The association between STH infection and Hb-levels was not significant. High density hookworm infections (>200 egg counts) have shown to be associated with lower hemoglobin levels in preschool children [44]. In our population, hookworm prevalence was only 3%. Low intensity infections are more common and effects on the Hb-level have only been shown for high density infections as well as polyparasite low-intensity infections. This could explain that not even hookworm infections could be shown to be associated with lower hemoglobin levels in our study [44, 89].

In our analysis, we did not take the intensity of infection into account. If further studies with more participants are conducted, one could also consider egg loads and create different polyinfection profiles to enable a more detailed description of associations of these profiles with anaemia.

Regarding the association of Schistosoma infection with Hb-level, there were higher rates of anaemic participants among infected compared to non-infected ones. However, the effect was not significant. This result is consistent with the work of King et al., a meta-analysis concerning disability-related outcomes in schistosomiasis [92]. They showed an association of *S. haematobium* infection with lower Hb-levels merely for high intensity infections. Not taking the intensity of infection into account, they could not show this effect. As recently mentioned, we did not cover an analysis of intensity of infection in our study, which could be one explanation for the non-significance of the association between Schistosoma infection and anaemia.

Regarding malaria infection, we showed a significantly higher probability for infected participants to be anaemic ($p=0.0025$). This is a frequently recorded finding and the mechanisms of the interaction are well described [96, 147]. In our study, 92% of the positive tested participants showed hemoglobin concentrations lower than 11 g/dl, and thus, were anaemic. Bouyou-Akotet et al. also found an anaemia rate of 91% in Gabonese children aged 1-5 years if they were positive for *P. falciparum* [138]. As for the other parasite parameters, we did not take the parasite density of *P. falciparum* into account. In further studies

one could take this into account as the prevalence of anaemic children is known to increase with parasite density [138].

Analogous to single infection with malaria, children co-infected with *P. falciparum* and STHs were more likely to be anaemic. With respect to that, 100% of the positive tested were anaemic, but only 76% of the negatively tested subjects. Kinung'hi et al. had similar findings. They found this effect for children co-infected with more than one parasite [148]. Yet in our study, one has to have to consider the low case numbers of co-infected children. In further studies, one could target a larger number of participants to allow a quantification of the effect of co-infections on anaemia.

4.2.8 Parasitic (co-)infections and anthropometric status

When analyzing the association of particular STH infections with the anthropometric status, we were not able to show a contribution of an infection to the prevalence of wasting. In contrast to that, Mupfasoni et al [117] demonstrated an infection with at least 2 parasites at high intensity to increase the odds of being wasted significantly. However, they could not show this effect on stunting. One explanation for the discrepancy of our results could be the low prevalence of wasting recorded in our population (0.9%). Furthermore, as repeatedly mentioned, infection intensity was not considered in our analysis and the association has only been shown for high intensities by Mupfasoni et al..

In our findings, there was no association between infection with *S. haematobium* and a higher prevalence of malnutrition, a result that is consistent with former studies [149].

Concerning malaria infection, we did not find a significant association with an impaired physical development as shown by Alexandre et al. [150]. However, this observation could not be made for children aged less than 5 years in the latter study, which covers exactly the age range of our study. In our findings, the prevalence of stunting in the infected group was 31% compared to 21% in non-infected children (OR: 1.7; 95% CI: 0.6-5.0; p= 0.3). However, these associations were non-significant, possibly due to low case numbers. Other studies, such as the one of Maketa et al. showed *P. falciparum* infection to be a

predictive parameter for chronic malnutrition [97]. They observed 700 asymptomatic children aged younger than five years and compared rates of anaemia and chronic and severe malnutrition in infected and non-infected children. Analogous to our study, they could not show an effect of infection with *P. falciparum* on acute malnutrition. Maketa et al. evaluated acute malnutrition by measuring the mid upper arm circumference, whereas in our study, weight-for-height was chosen.

In our findings, the particular parasitic co-infections didn't influence the anthropometric status significantly. However, there were tendencies to a higher prevalence of stunting in affected children. There are – to our knowledge – no publications concerning these co-infections and their association to nutritional status in children. Nevertheless, assuming that each single infection influences the anthropometric status, there might be synergistic effects as is the case for anaemia. A quantification of these effects could be the issue of interest in further studies.

In summary, our data provide in-depth information about the prevalence of parasitic (co-) infections in Gabonese preschool children.

A description of the anthropometric status, as well as a detailed survey of the presence of anaemia was accomplished. In addition, we showed the impacts of parasitic co-infections in African preschool children.

The high prevalence of parasites and their impact on physical growth and anaemia imply a stepping up of efforts concerning educational campaigns addressing parents and caretakers.

Regardless of the underlying causes, anaemia has shown to be an issue of great importance in African preschool children, which should be targeted by the international public health community.

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6 Summary

6.1 Summary

In 2000, the Millenium Committee, convened by the United Nations, established the 8 Millenium Development Goals that should be addressed by the year 2015. Among others, these goals concern the reduction of child mortality and the struggle against HIV/AIDS, malaria and other diseases. To achieve a decline in child mortality, it is indispensable to focus on parasitic (co-) infections as these are known to account for a great burden in children. The WHO therefore recommends preventive chemotherapy in endemic areas.

This study aims at filling the gap of information concerning parasitic (co-) infections in preschool children. Furthermore, the impact of parasitic infections on physical development of children is assessed to better understand their burden.

In this cross-sectional study, faecal, urine and blood samples were obtained from children aged 1-5 years in Lambaréné, Gabon. The samples were analyzed to identify (co-) infections with soil-transmitted helminths, *S. haematobium* and *P. falciparum* and to determine the level of hemoglobin concentrations in these children. In addition, anthropometric parameters were collected and compared to sex- and age-specific reference values. The anthropometric and haematological data was then analyzed and described in dependence on the observed (co-) infections.

A total of 444 participants were included of which 327 provided complete data allowing parasitological, anthropometric and haematological analysis.
At least one species of parasites was found in 49% (166/339), whereas soil-transmitted helminths, *S. haematobium* and *P. falciparum* were present in 33% (130/394), 10% (39/407) and 15% (59/401), respectively. Co-infections were detected in 16% (55/339) of all participants.

Seventy-seven percent (334/436) showed hemoglobin levels lower than 11 g/dl and were therefore classified as anaemic. Age and infection with *P. falciparum* (p=0.0025) were determined as significant risk factors for anaemia.
Stunting, as defined by low height-for-age, was present in 20% (87/429) while 0.9% (6/429) of them were wasted, thus showed low weight-for-height.
Age was identified as significant risk factor for stunting. The prevalence of stunting was higher in co-infected children, but these effects were non-significant.

The high rates of parasite infections and anaemia and the negative impact of parasitic co-infections on the nutritional condition highlight the necessity of stepping up efforts concerning preventive chemotherapy and education of parents and caretakers.

6.2 Zusammenfassung

Im Jahr 2000 haben die Mitgliedsstaaten der Vereinten Nationen auf dem sogenannten Milleniumsgipfel acht Milleniums-Entwicklungsziele für 2015 beschlossen. Unter anderem beinhalten diese Ziele die Reduktion der Kindersterblichkeit, sowie den Kampf gegen HIV, AIDS, Malaria und andere schwere Krankheiten. Um eine Senkung der Kindersterblichkeit zu erreichen, ist es unerlässlich, auch parasitische Koinfektionen in den Fokus zu rücken, da diese einen erheblichen Anteil daran haben. Die WHO empfiehlt daher die präventive Chemotherapie in endemischen Gebieten.

Diese Studie hat zum Ziel, den Mangel an Informationen über parasitische Koinfektionen von Kindern im Vorschulalter zu reduzieren, um diesen Empfehlungen folgen zu können.
Weiterhin wird der Einfluss von Parasiteninfektionen auf die kindliche Entwicklung untersucht, um deren Auswirkungen besser zu verstehen.

In dieser Querschnittstudie wurden in Lambaréné (Gabun) Stuhl-, Urin- und Blutproben von Kindern im Alter von 1-5 Jahren entnommen. Die Proben wurden analysiert, um parasitische (Ko-) Infektionen mit Geohelminthen*, S. haematobium* und *P. falciparum* zu entdecken und um den Hämoglobinwert zu bestimmen. Weiterhin wurden anthropometrische Parameter gemessen und mit alters- und geschlechtsspezifischen Referenzwerten verglichen. Die anthropometrischen und hämatologischen Daten wurden im Anschluss in Abhängigkeit von den aufgetretenen (Ko-) Infektionen analysiert und beschrieben.

Insgesamt wurden 444 Probanden eingeschlossen, von denen bei 327 eine vollständige parasitologische, anthropometrische und hämatologische Analyse durchgeführt werden konnte. Mindestens eine Spezies von Parasiten wurde in 49% (166/339) entdeckt, wobei Geohelminthen, *S. haematobium* und *P. falciparum* in 33% (130/394), 10% (39/407) bzw. 15% (59/401) präsent waren. Koinfektionen traten in 16% (55/339) auf.
Siebenundsiebzig Prozent (334/436) der Teilnehmer zeigten einen Hämoglobinwert unter 11 g/dl und wurden entsprechend als anämisch klassifiziert. Das Alter, sowie die Infektion mit *P. falciparum* wurden als signifikante Risikofaktoren, eine Anämie zu entwickeln, identifiziert.
Stunting (am ehesten als Verkümmerung zu bezeichnen) wurde definiert als zu geringe Körperlänge in Relation zum Alter und wurde in 20% (87/429) beobachtet. Wasting (am ehesten als Abmagerung zu übersetzen) wurde als zu geringes Gewicht in Relation zur Körperlänge definiert und wurde in 0.9% (6/429) beobachtet. Das Alter war ein signifikanter Risikofaktor für Stunting. In koinfizierten Kindern war die Prävalenz von Stunting höher, dieser Effekt erwies sich jedoch als statistisch nicht signifikant.

Die hohe Rate an Parasiteninfektionen und anämischen Kindern, sowie die Hinweise auf einen negativen Einfluss von parasitischen Koinfektionen auf den Ernährungszustand von Kindern lassen eine Intensivierung der präventiven

Behandlung sowie die Ausweitung der Schulung und Aufklärung von Eltern bzw. Betreuern notwendig erscheinen.

7 Erklärung zum Eigenanteil

Die Konzeption der Studie erfolgte in Zusammenarbeit mit Dr. med., M. sc. Bertrand Lell, Kodirektor des Centre de Recherches Médicales de Lambaréné und Dr. med., rer. nat. Ayola Akim Adegnika, Kodirektor des Centre de Recherches Médicales de Lambaréné.

Die Versuche wurden durch die Labortechniker des Centre de Recherches Médicales de Lambaréné durchgeführt.

Die statistische Auswertung erfolgte nach Beratung durch das Institut für Biometrie durch mich.

Ich versichere, das Manuskript selbstständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Zug, den 13.07.2018

Johannes Tausend

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9 Curriculum vitae

Name:	Johannes Tausend
Date of birth:	26th November 1986
Place of birth:	Schwäbisch Hall, Germany
Nationality:	German

Secondary school:	1997-2006 Parler-Gymnasium Schwäbisch Gmünd,
	2006 Abitur
University:	2007-2015 Medical studies at the University of Tübingen, 13th May 2015 third State examination
	2010-2011 Research work at the Centre de Recherches Médicales de Lambaréné, Gabon
Work :	2015-2017 General surgery at Spital Männedorf, Switzerland
	Since 2017 Gynaecology at Spital Muri, Switzerland
Additional Training	Good Clinical Practice ICH-GCP Workshop 2018