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Chefarzt: Professor Dr. med. August Stich

Prevalence of *Strongyloides* infection and other intestinal parasites in paediatric patients in a referral hospital in Northern Tanzania

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Anita Janzen-Maaser

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Referent: Prof. Dr. med. August Stich

Koreferent: Prof. Dr. rer. nat. Klaus Brehm

Dekan: Prof. Dr. med. Matthias Frosch

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Die Promovendin ist Ärztin

Dedication

In remembrance of Martin Fries

Contents

1 Introduction

In 1876 french soldiers in Vietnam were found to be infected by a worm. They had severe diarrhoea that was known for many years as Choin-China diarrhoea. This was the first report of *Strongyloides stercoralis*. It took many years to trace the understanding of the biology of this nematode with its extravagant life cycle and ability to provoke a variety of clinical symptoms. Even nowadays there is a lack of knowledge about this parasite which is estimated to infect 30-100 million people (Bethony, Brooker et al. 2006, Khieu, Schar et al. 2013).

Helminthic infections as well as protozoan infections are common in tropical and subtropical countries. Poverty and poor hygiene are the key determinants of the high prevalence of parasitic infections in childhood. Although some progress has been made in the meantime, the health impact of parasitic diseases remains tremendous in Sub-Saharan Africa (WHO 2013).

Diarrhoea is a leading symptom in infectious diseases due to several causes with a high estimated mortality rate of 3-4 million per year worldwide. It is still a major life-threatening problem for children, mainly in developing countries (Santosham, Chandran et al. 2010). Insufficiently hygienic living conditions such as poor water access, poor sanitation as well as inadequate nutrition have an increasing impact on infections with intestinal protozoa (Speich, Marti et al. 2013). The United Nations aimed in the Millennium Development Goal 4 for a reduction of two-thirds in child mortality rate between 1990 and 2015. Goals of this type will remain unachievable if morbidity and mortality caused through diarrhoea is not enormously decreased (Bado, Susuman et al. 2016).

One of the WHO 2030 targets for soil-transmitted helminthiases control programmes is to establish an efficient strongyloidiasis control programme in SAC and the coverage with effective medicine of children at risk (Montresor, Mupfasoni et al. 2020).

Since there are no reliable data available for the Victoria Lake region in Tanzania, a study named StrongPaed was conducted with the main objective of

providing data on the prevalence of *S. stercoralis* and other intestinal parasitic infections in paediatric patients attending the BMC in Mwanza, Tanzania. Information about the prevalence of intestinal parasites, the risk factors and the feasibility of diagnostic methods can help improve diagnostic and treatment procedures in case of diarrhoeal diseases in children.

In the following introduction an overview is given about the parasites upon which this study mainly focuses. This shows the current scientific knowledge and gives an insight into the complexity of the characteristics of the parasites, their epidemiology and diagnostic methods.

1.1 **Parasites**

In recent years it has been clearly shown that chronic helminthic infections impair the physical and intellectual growth of children (Hotez, Brindley et al. 2008). Cryptosporidiasis and giardiasis occur in high association with water conditions and correlate with low socio-economic circumstances (Savioli, Smith et al. 2006). They have been included in the WHO Neglected Disease Initiative since 2004 along with strongyloidiasis. *E. histolytica* was also considered to be one of the most common pathogens causing diarrhoea in poor sanitary environments. Discovering the distinction between the pathogenic *E. histolytica* and the morphologically identical commensal *E. dispar* changed the estimation of its true prevalence (Markell 1999).

Concerning the intestinal parasites in this study, in addition to *S. stercoralis* there was a focus on *G. lamblia* and *E. histolytica*. These protozoa cause diarrhoeal diseases, posing major threat to the health of children in low-resource countries. There was one new rapid test recently available including both protozoa and *C. parvum*, so the diagnostic methods in this study focussed on these protozoa, but minor priority was given to *C. parvum.*

1.1.1 *Strongyloides stercoralis*

Strongyloides is a helminthic soil-transmitted parasite, adapted to the moist tropics and subtropics. It is found in primates and dogs. Three species were found to occur in the human host within the *Strongyloidoidae* superfamily: *S.*

stercoralis, S. fuelleborni fuelleborni and *S. fuelleborni kellyi*. Among them *S. stercoralis* is the most common and globally distributed pathogen of clinical importance (Siddiqui and Berk 2001). *S. fuelleborni fuelleborni* and *S. fuelleborni kellyi* are mainly found as parasite of primates and in restricted geographical distribution (Dorris, Viney et al. 2002)

Generally, *Strongyloides* is more prevalent in the tropics although it is globally distributed. Soil in warm and moist areas like the tropics is an appropriate environment for the reproduction of the larvae outside the host (Ash 1990).

Among the *Strongyloides* species hosted by humans, other than *S. stercoralis, S. fuelleborni* is prevalent in central and east Africa in monkeys and infants under six months. *S. fuelleborni kellyi* has been found to infect young infants in Papua New Guinea. *S. fuelleborni* and *S. fuelleborni kellyi* larvae have similar morphology to *S. stercoralis* larvae, but eggs and not larvae are excreted in the stool, enabling the differentiation to *S. stercoralis* infection. (Markell 1999)

Although *Strongyloides* is formally a soil-transmitted helminth, the WHO separates this species and its disease from the general use of the term soil-transmitted helminthiasis. The term of STH includes generally *Ascaris lumbricoides*, *Trichuris trichiura*, and the two hookworm species, *Necator americanus* and *Ancylostoma duodenale* (Montresor, Mupfasoni et al. 2020). The separation developed from the different diagnostic and treatment in *Strongyloides* compared to other STH. In this work, the term soil-transmitted helminth includes *Strongyloides stercoralis.*

The WHO recommends administering albendazole 400 mg or mebendazole 500mg as a single dose once a year for populations at risk of STH disease (WHO 2012). In such administration, both drugs have no effect on *S. stercoralis* infection and only limited effect on hookworm infection.

1.1.1.1 Life cycle

The special characteristic of *S. stercoralis* is an endogenous autoinfective life cycle *(Figure 1: Life cycle of S. stercoralis).* Infective filariform larvae L3 from contaminated soil penetrate the skin and enter the circulatory system by various

routes until they stay in the small intestine. The larvae can migrate directly to the intestine or via bloodstream to the lungs where they penetrate the alveoli and are coughed up and swallowed. In the small intestine they moult twice and develop into parthenogenetic females. Those are able to propagate asexually and to place thin-walled eggs in the mucosal epithelium. Rhabditiform larvae L1 hatch within one month and migrate to the intestinal lumen where they may moult into rhabditiform larvae L2 and L3. When the larvae are excreted in stool, they enter either a free-living cycle in the soil or initiate autoinfection. In the soil they can moult twice into infective filariform larvae L3 in 1-3 days, or they moult one more time and transform into free-living male and female adult worms L4 in 2-3 days. Sexual reproduction is only seen in free-living adults. In warm and humid soil as favourable condition, fertilised female worms produce eggs. Depending on the temperature, rhabditiform larvae hatching from the eggs develop within 7-10 days into infective filariform larvae, ready to enter the parasitic cycle again. They remain viable in the humid soil for many weeks. The free living adults die after one generation and do not persist in the soil. In debilitated or immunosuppressed patients, the rhabditiform L2 larvae may develop directly into filariform larvae L3 in the intestinal tract and initiate autoinfection. Thereby the larvae penetrate the mucosa in the large intestine and enter the life cycle again. Another way of autoinfection may occur when larvae are excreted and penetrate the anal skin. The autoinfection is perpetuates a potentially life-long infection if not treated appropriately. (Ash 1990, Gillespie 1995, Mehlhorn 1995, Markell 1999, Lucius, Loos-Frank et al. 2017)[1]

[1] The whole paragraph is based on these references

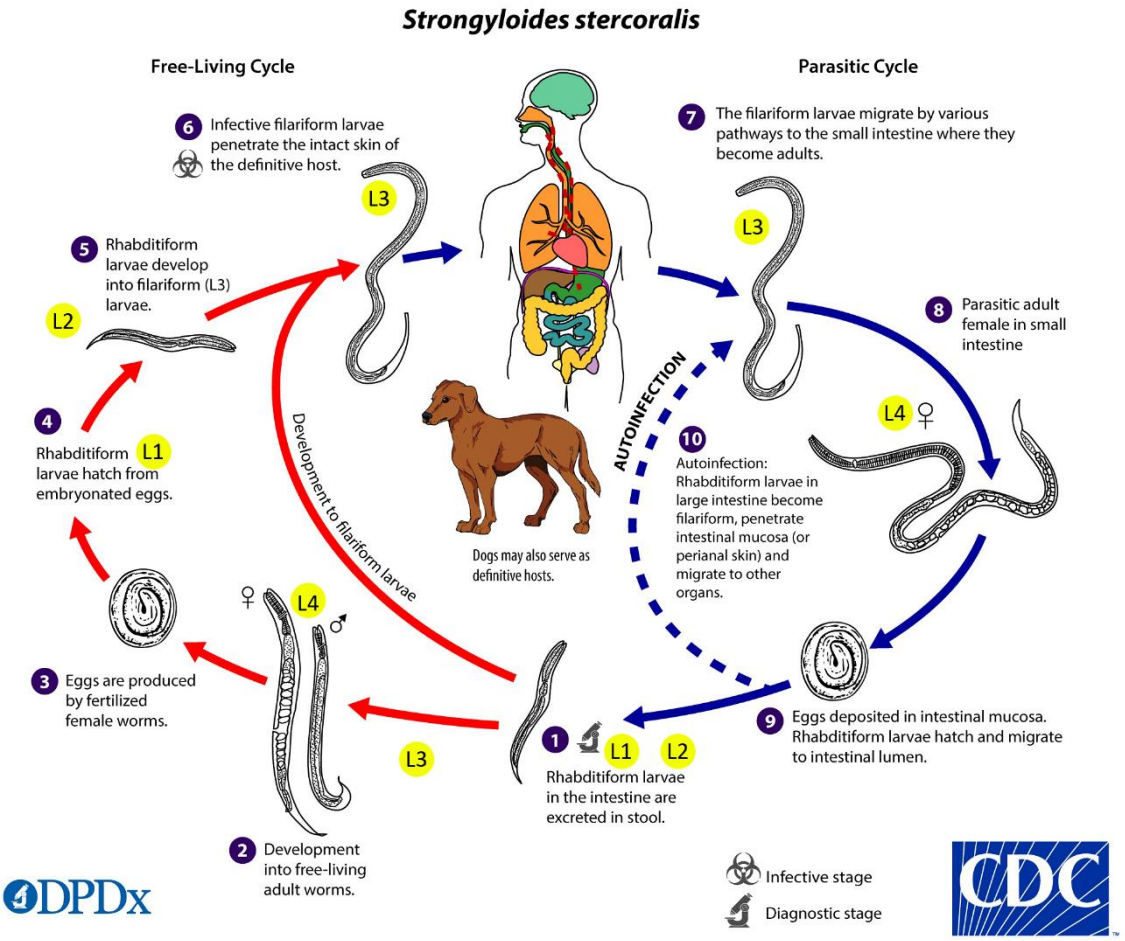

Figure 1: Life cycle of S. stercoralis (Reproduced and modified from https://phil.cdc.gov/Details.aspx?pid=3419 on 03.10.2022 with permission from Centers for Disease Control and Prevention; Content providers: CDC/ Alexander J. da Silva, Melanie Moser; this image is in the public domain of the Public Health Image Library and thus free of any copyright restrictions)

1.1.1.2 Morphology

The parthenogenetic ovoviviparous female larvae range from 2.1-2.7 mm in length. They have a slender, pointed tail, a small buccal capsule and a filariform pharynx. The free-living females are 1 mm long and have a pointed tail. The vulva is in the midpoint of the body. The free-living males are shorter than the females and are characterised by a short curved and pointed tail and visible genitals *(Figure 2: Larvae stadium of S. stercoralis)*. The thin-shelled eggs range in size of about 54x32 µm and may be present in severe diarrhoeal stool, even with developing

larvae inside. The rhabditiform larvae L1 and L2 are 180-380 µm long. They have a short buccal canal with a prominent oesophagus, and the genital primordium is located in the middle of the body length. In contrast, the hookworm rhabdiform larvae have a long buccal capsule *(Figure 3: Rhabditiform larvae)*. The *S. stercoralis* filariform larva L3 is 490-630 µm long and has a notched tail. The size proportion of the oesophagus to the intestine is 1:1. In the hookworm filariform larvae this proportion is in contrast 1:4, and so the oesophagus is shorter *(Figure 4: Filariform larvae).* (Ash 1990, Gillespie 1995, Mehlhorn 1995, Markell 1999, Lucius and Loos-Frank 2008, Lucius, Loos-Frank et al. 2017)[2]

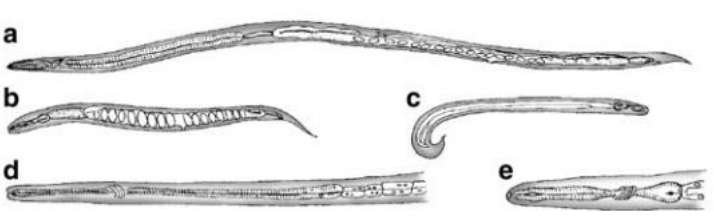

Abb. 6.15a–e Entwicklungszyklus von *Strongyloides stercoralis*. (L = Larvenstadien). **a** Parasitisches Weibchen. **b** Frei lebendes Weibchen. **c** Frei lebendes Männchen. **d** Vorderende der filariformen Larve. **e** Vorderende der rhabditiformen Larve

Figure 2: Larvae stadium of S. stercoralis (Reproduced from Springer Verlag, Biologie von Parasiten by Richard Lucius and Brigitte Loos-Frank, 2. Auflage, 2008 with permission from Springer Nature Customer Service Centre GmbH)

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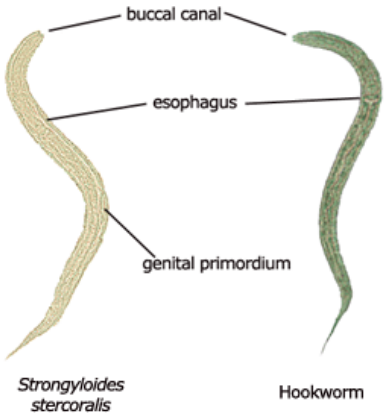

Figure 3: Rhabditiform larvae (Reproduced from https://www.cdc.gov/dpdx/diagnosticprocedures/stool/morphcomp.html on 20.12.2020 with permission from Centers for Disease Control and Prevention; Content provider: CDC/ Dr. Mae Melvin; these images are in the public domain of the Public Health Image Library and thus free of any copyright restrictions)

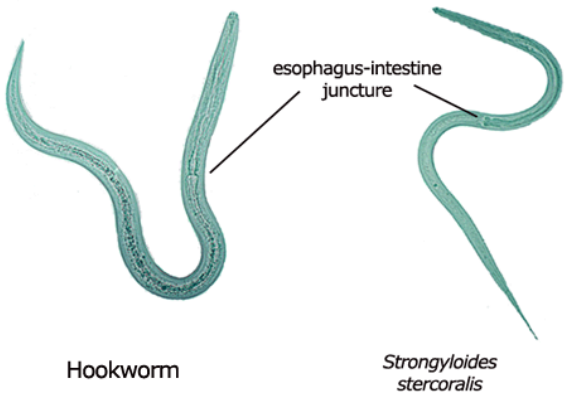

Figure 4: Filarifom larvae (Reproduced from https://www.cdc.gov/dpdx/diagnosticprocedures/stool/morphcomp.html on 20.12.2020 with permission from Centers for Disease Control and Prevention; Content provider: CDC/ Dr. Mae Melvin; these images are in the public domain of the Public Health Image Library and thus free of any copyright restrictions)

1.1.1.3 Pathology

The clinical features of strongyloidiasis are very variable, ranging from asymptomatic carrier state to hyperinfection syndrome and disseminated disease with high mortality (Ramanathan and Nutman 2008). However, the morbidity and mortality related to *S. stercoralis* is still not completely clear (Bisoffi, Buonfrate et al. 2013).

Manifestations of acute strongyloidiasis are common gastrointestinal symptoms such as abdominal pain, diarrhoea and bowel obstruction. The local inflammatory reaction of the intestinal mucosa may induce a malabsorption syndrome. Pulmonary symptoms may occur during lung passage of filariform larvae presenting as cough, bronchopneumonia and dyspnoea. Dermatological manifestations may occur as petechial haemorrhages at the port of entry of infective larvae, accompanied by intense pruritus, congestion and oedema. (Page and Speare 2016)[3]

Chronic infection with *S. stercoralis* often remains clinically asymptomatic. Patients may show elevated IgE levels and helminthic infection should be considered as differential diagnose in patients from endemic areas with peripheral eosinophilia and gastrointestinal symptoms. Symptoms may occur as dermatological manifestations such as urticaria and “larva currens” caused by migration of *S. stercoralis* in the skin (Pelletier 1984). Gastrointestinal symptoms such as malabsorption, constipation, diarrhoea and vomiting also appear in chronic infection. Unusual manifestations include arthritis (Richter, Muller-Stover et al. 2006), nephrotic syndrome (Miyazaki, Tamura et al. 2010) or hepatic lesions (Gulbas, Kebapci et al. 2004).

Alteration in immune status may lead to an accelerated autoinfection with increased number of larvae. Patients may develop a rare but potentially fatal hyperinfection syndrome (Qu, Yang et al. 2016). The hyperinfection syndrome is not finally defined but means a massively increased larvae count compared to regularly autoinfection. Organs involved are still those of the pulmonary autoinfection cycle but may result in high mortality rates (Siddiqui and Berk

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2001). It might be also presented as an acute shock-like illness with gram-negative septicaemia due to bacteria carried by the migrating filariform larvae (Geri, Rabbat et al. 2015).

Disseminated infection involves affected organs other than in the pulmonary autoinfection cycle. Manifestation can be seen in mesenteric lymph nodes, heart, brain, kidneys, pancreas and muscles (Keiser and Nutman 2004). That does not necessarily mean a more severe disease compared to the hyperinfection syndrome. However, it may lead to a life-threatening course of disease (Siddiqui and Berk 2001). Conditions associated with disseminated infection are diseases or therapies resulting in immunosuppression. The autoinfection cycle of *S. stercoralis* is especially a threat to severely immunosuppressed individuals, e.g. due to HTLV-1 infection (Carvalho and Da Fonseca Porto 2004), HIV infection (Mascarello, Gobbi et al. 2011), antineoplastic chemotherapy, received organ transplantations (Mokaddas, Shati et al. 2009) or prolonged high-dose corticosteroid treatment (Ramanathan and Nutman 2008). In particular corticosteroid therapy is responsible for hyperinfection and disseminated strongyloidiasis, since the pharmaceutics are similar to moult-regulating substances and induce faster development of the larvae than under normal conditions (Lucius, Loos-Frank et al. 2017). Corticosteroids have a strong effect on the immune system but even short period treatment with moderate dosage in immunocompetent patients has been reported to increase the risk of disseminated strongyloidiasis (Ghosh and Ghosh 2007).

1.1.1.4 Treatment

The complete eradication of the helminth is recommended for effective treatment to avoid autoinfection of the larvae which may cause relapse later. (Luvira, Watthanakulpanich et al. 2014). The WHO recommended treatment for acute and chronic *Strongyloides* infection is ivermectin, tiabendazole or albendazole.

Ivermectin shows a higher cure rate than albendazole and similar cure rate as thiabendazole (Henriquez-Camacho, Gotuzzo et al. 2016). For uncomplicated

infection, ivermectin 200 µg/kg p.o. for 1-2 days is favored over tiabendazole 25 mg/kg p.o. for three days because of fewer gastrointestinal side effects. Both drugs are often not available and not licensed in all endemic countries (Gann, Neva et al. 1994). At the time of the study, ivermectin was not available in Mwanza but at the time of finishing this dissertation, ivermectin is available at local pharmacies in Mwanza for a reasonable price with the cost of about 25 Eurocent for a recommended dosage of one therapy. In Germany, ivermectin was licensed in April 2016 and recommended by the AWMF but only for scabies infection (AWMF 2016). For children less than 15 kg, the usage of ivermectin is not proven and studies concerning dosage are ongoing. Therefore reduced dosage with 100 µg/kg p.o. for two days is recommended (Khieu, Schar et al. 2013).

Albendazole 400 mg p.o. BID for seven days is frequently used, although it is considered the least effective of these antihelminthics but is available in many resource-poor countries (Henriquez-Camacho, Gotuzzo et al. 2016).

Moxidectin is a well-established drug in veterinary medicine for the treatment of onchocerciasis and might be used as an alternative to ivermectin. Trials up to now have considered it to be as safe and effective as ivermectin, but lager trials have to be conducted, and the drug is not registered yet (Barda, Sayasone et al. 2017).

For disseminated infection and hyperinfection syndrome, longer treatment regime and special symptomatic therapy is required (Nutman 2017).

1.1.2 *Giardia lamblia*

Giardia lamblia is a flagellated protozoan parasite, also known as *G. intestinalis* and *G. duodenalis*. Different *Giardia* spp. are detectable in humans, domestic animals and wildlife, but *G. lamblia* is the only one found in humans. This parasite is characterised by genetic diversity with division into eight assemblages A-H and further sub-assemblages based on protein or DNA polymorphisms. Two of them, group A and B, are hosted both by humans and animals, whereas the other species are rarely or not at all found in humans.

This species complex leads to different names and synonyms for the species, and confusion may arise from the terminology. (Ryan and Caccio 2013)[4]

Giardia is a common human enteric protozoan pathogen with worldwide distribution and higher prevalence rates in warm climates and low-sanitation areas. Waterborne outbreaks of the infection are of public health relevance particularly for infants and children in endemic areas with poor sanitary conditions (Ouattara, Silue et al. 2008).

1.1.2.1 Life cycle and morphology

The infection is caused by faecal-oral transmission of the cysts in faecal contaminated water and food or by person-to-person transmission. The ovoid quadrinucleated cysts have a robust cell wall. They are very resistant and can persist in suitable environment for a long time, even for several months in cold water. They range in size from 8-14 µm by 7-10 µm and can be detected microscopically in the faeces. (Markell 1999)[5]

After ingestion, the infective cysts undergo excystation in the proximal small intestine. Each cyst releases two trophozoites that are 10-20 µm long. The pear-shaped trophozoites are the vegetative form with two nuclei and the ability to move with attached flagella. Trophozoites multiply by binary fission and transit towards the colon. In the jejunum they form cysts that are passed in the faeces. Those cysts contain initially two nuclei but rapidly develop four. Sometimes trophozoites may also be passed in faeces and can be detected microscopically but cannot survive in the environment for long time. They are typically found in acute infection stadium in diarrhoeal stool but degenerate within a few hours. (Mehlhorn 1995, Lucius and Loos-Frank 2008, Lucius, Loos-Frank et al. 2017)[6]

1.1.2.2 Pathology

Giardiasis often remains asymptomatic but may also cause irritation of the mucosa in the small intestine. After an incubation period of 3-20 days, the main

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[6] The whole paragraph is based on these references

symptom is watery diarrhoea that lasts for about one week. During this time, millions of cysts are excreted. Steatorrhoea is a possible symptom after an acute phase of diarrhoea. According to textbooks, Giardia may colonize the gall bladder, although the sources of this information and the clinical impact remain unclear (Lucius and Loos-Frank 2008). Associated symptoms occur as vomiting, nausea, abdominal cramps, epigastric tenderness and malabsorption syndrome with fatigue and weight loss. Mostly, the infection is self-limited and patients recover after a while (Gillespie 1995).

In children the infection may cause abdominal pain without diarrhoea. Sometimes the symptoms persist and lead to chronic infection. Chronic giardiasis may last for several months or even years and can occur with allergic and inflammatory conditions or gastrointestinal complications (Lucius, Loos-Frank et al. 2017). Severe chronic giardiasis with extreme malabsorption syndrome may cause wasting syndrome, intelligence deficiencies and growth retardation (Ajjampur, Koshy et al. 2011).

Lactose intolerance may be precipitated by *G. lamblia* infection and may remain even after eradication (Pond 2004). A previous infection may reduce the risk of reinfection or the severity of symptoms (Lucius, Loos-Frank et al. 2017).

1.1.2.3 Treatment

Nitroimidazole drugs such as metronidazole, tinidazole and ornidazole are therapeutically highly effective. Metronidazole 500 mg TID as a 5-7 day course is recommended by the AWMF as first-line therapy and is commonly used as therapy regime. A prolonged or combined course of treatment is necessary in case of resistant infection (Gardner and Hill 2001, Escobedo and Cimerman 2007). The effectiveness of albenazole is reported to be comparable to metronidazole but with fewer side effects and a simplified regime. It might be used as alternative therapy regime, but a sufficient therapy protocol is not approved at present (Solaymani-Mohammadi, Genkinger et al. 2010, Granados, Reveiz et al. 2012).

1.1.3 *Entamoeba histolytica*

Entamoeba histolytica is a potentially pathogenic invasive protozoan associated with intestinal and extraintestinal infections. Primates and humans are the only reservoir of *E. histolytica,* and in most cases the parasite stays non-invasive and infection remains asymptomatic (Lucius and Loos-Frank 2008). There are several other *Entamoeba* species infecting humans, but one is of special interest because of confusion in diagnostic interventions. In 1992, formal separation of *E. histolytica* from the morphologically indistinguishable but nonpathogenic *Entamoeba dispar* was described (Diamond and Clark 1993). The genetic difference in these two species involves 5 % of the gene coding regions. This makes *E. dispar* incapable of tissue-invasion but better adapted to its host (Lucius, Loos-Frank et al. 2017).

1.1.3.1 Life cycle and morphology

Entamoeba is an enteric parasite that exists as cyst and as trophozoite. Transmissions of the infective cysts occur through contaminated water, food or direct faecal-oral contact. They may also be inhaled by dust and swallowed up. In acute dysentery mainly trophozoites are passed whereas in the asymptomatic stage, mainly cysts and in chronic infection both are passed. The cysts are quite resistant and therefore important for transmission whereas trophozoites degenerate rapidly. This means that infected asymptomatic persons are the major source for spreading the infection. (Markell 1999, Lucius and Loos-Frank 2008)[7]

When the cysts are ingested and passed into the small intestine, they develop into trophozoites. These trophozoits range in size from 10-60 µm with an average size of 25 µm and contain a single nucleus and central karyosome. They multiply in 12-24 h through fission and may live as commensals without invading the host tissue (Lucius, Loos-Frank et al. 2017).

Tissue-invading trophozoits have an active metabolism and are typically larger. The active trophozoites move by means of pseudopodia and invade the

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intestinal mucosa. They cause ulcerating lesions by a lyzation process with hyaluronidase and proteases. One of the main differences compared to *E. dispar* is the presence of genes for proteases enabling the invasion process. The invasive *E. histolytica* trophozoites may also enter extraintestinal sides like brain, liver and lungs via bloodstream transport after invading the capillaries. (Gillespie 1995, Markell 1999)[8]

Trophozoites are typically found in diarrhoeal stool and may contain ingested erythrocytes from the invasion process. This can be seen rarely in chronic infection and is confined to this *Entamoeba* species (Ravdin 1995, Lucius and Loos-Frank 2008). As trophozoites pass the colon, they may also undergo a maturation process and divide into cysts that are passed in the faeces. The cysts are typically found in formed stool. They are characterised by a hyaline cyst wall and one to four nuclei, depending on their maturity. The size of the cysts rage from 10-20 µm and is in average 12 µm. They may remain viable in appropriate moist environment for several weeks while the vulnerable trophozoites rapidly degenerate outside the body and also do not survive the gastric low pH and enzymes (Ash 1990, Mandell, Bennett et al. 2000). The *E. histolytica* cysts survive chlorination in drinking water as well as temperatures up to 55 °C whereas other *Entamoeba spp*. like *E. dispar* and *E. moshkovskii*, the so-called Laredo strain, survive temperatures from 0-41 °C (Markell 1999, Lucius and Loos-Frank 2008, Lucius, Loos-Frank et al. 2017).

1.1.3.2 Pathology

Clinical symptoms depend on tissue damage and location of the trophozoite persistence. After an incubation period of 2-4 weeks, the *Entamoeba* infection occurs predominantly as colitis. The symptomatic intestinal form usually begins with abdominal discomfort, cramps and first mucous and then bloody diarrhoea with increasing severity (Farrar 2013). High grade tenesmus and tenderness is associated with an acute infection as a sign of rectosigmoidal involvement. Necrotic and hyperaemic lesions in cecum and colon may be detected in coloscopy. The most common extraintestinal form is a disseminated infection

[8] The whole paragraph is based on these references

through portal circulation resulting in amoebic liver abscess (Ralston and Petri 2011). This complication usually presents after months or years after the initial infection which may occur without diarrheal symptoms. Fever, pain and liver swelling are common accompanying symptoms, and the process may expand into perforation, pleural effusion and lung abscess if untreated (Mehlhorn 1995).

Other extraintestinal involved organs via direct or haematogenous spread are spleen, brain and skin. Immunosuppression like by corticosteroid therapy along with *E. histolytica* infection can lead into intestinal complications like toxic megacolon or bowel perforation with peritoneal amoebiasis. Repeated infections may result in amoebic granuloma presenting as palpable mass. (Eddleston 2008)[9]

The entire genetic and environmental factors of the host and the genetic factors of the pathogen that determine an intestinal, extraintestinal or asymptmatic infection process are still not clear, but health conditions such as malnutrition are associated with increased disease susceptibility (Begum, Quach et al. 2015). Pregnant women, young children, malnourished people and immunocompromised patients are at high risk of severe infection with fulminant colitis. Children are a vulnerable group, especially when they are malnourished because of fluid loss and electrolyte imbalance due to diarrhoea. But the infection may also present as rectal bleeding without diarrhoea (Mandell, Bennett et al. 2000).

1.1.3.3 Treatment

As a preventive disease management strategy, prophylactic measure should be strictly followed. Asymptomatic infections may be treated with paromomycin or diloxanide fuorate. In case of symptomatic infection, the treatment of choice is a nitroimadozole drug such as metronidazole or tinidazole in appropriate dosage according to actual guidelines. The AWMF recommends metronidazole 10 mg/kg TID for ten days, followed by paromomycin 500 mg TID for 9-10 days. Tinidazole is better tolerated with shorter treatment regime but is limited available. Amoebiasis usually responds well to metronidazole which is just as

[9] The whole paragraph is based on this reference

effective against the trophozoites but with little effect on the cysts remaining in the intestinal lumen (Haque, Huston et al. 2003). Therefore, treatment should be completed with paromomycin or second-line diloxanide but not given at the same time as metronidazole, since diarrhoea is one of the side effects of paromomycin (Blessmann and Tannich 2002).

1.1.4 *Cryptosporidium parvum*

Cryptosporidium is a coccidian protozoon. Several species are hosted by animals, but the human pathogenic species is *C. parvum* (Lucius, Loos-Frank et al. 2017). This species is also known as a major cause of severe diarrhoea in calves. The intracellular parasite infects the gastrointestinal tract with the potential of autoinfection and causes diarrhoea in children and adults with increased severity and duration in immunocompromised people (Markell 1999, Abeywardena, Jex et al. 2015).

1.1.4.1 Life cycle

The infective oocysts with a size of 4–6 µm are environmentally robust and are able to survive in suitable conditions for months. Ingested cysts start a new life cycle in the host, beginning in the stomach with excystation into four sporozoites. They attach to epithelial cells of the small intestine and envelope into parasitophorous vacuoles. The sporozoites may enter sexual and asexual reproduction cycle. They mature into trophozoites and divide into type I meronts (in some literature called “schizont”) which release merozoites. Merozoites perpetuate the infection asexually when they attach and infect other epithelial cells. They mature into trophozoites, and the cycle starts again. In the sexual reproduction cycle, the sporozoites develop into type II meronts which differentiate into gametocytes and then into either macrogametes or microgametes. Both fuse and form a zygote. Zygotes as the product of fertilisation mature into infective oocysts and start a new life cycle within the host or are excreted with the faeces. Those excreted oocysts are thick-walled with several wall layers making them resistant to environmental influences. The oocysts remaining in the host are thin-walled and more vulnerable but may provoke massive autoinfection if not limited by the immune system. (Gillespie

1995, Lucius and Loos-Frank 2008, Farrar 2013, Lucius, Loos-Frank et al. 2017)[10]

The transmission happens via faecal contaminated water or food, direct person-to-person contact or through domestic animals (Gillespie 1995). Environments such as lakes and streams or even surface water in rainy seasons enhance the spread of oocysts. Common water disinfectant and filtration techniques are often not efficient in eradicating oocysts and therefore *C. parvum* is a persistent threat to safe water supply (Kosek, Alcantara et al. 2001).

[10] The whole paragraph is based on these references

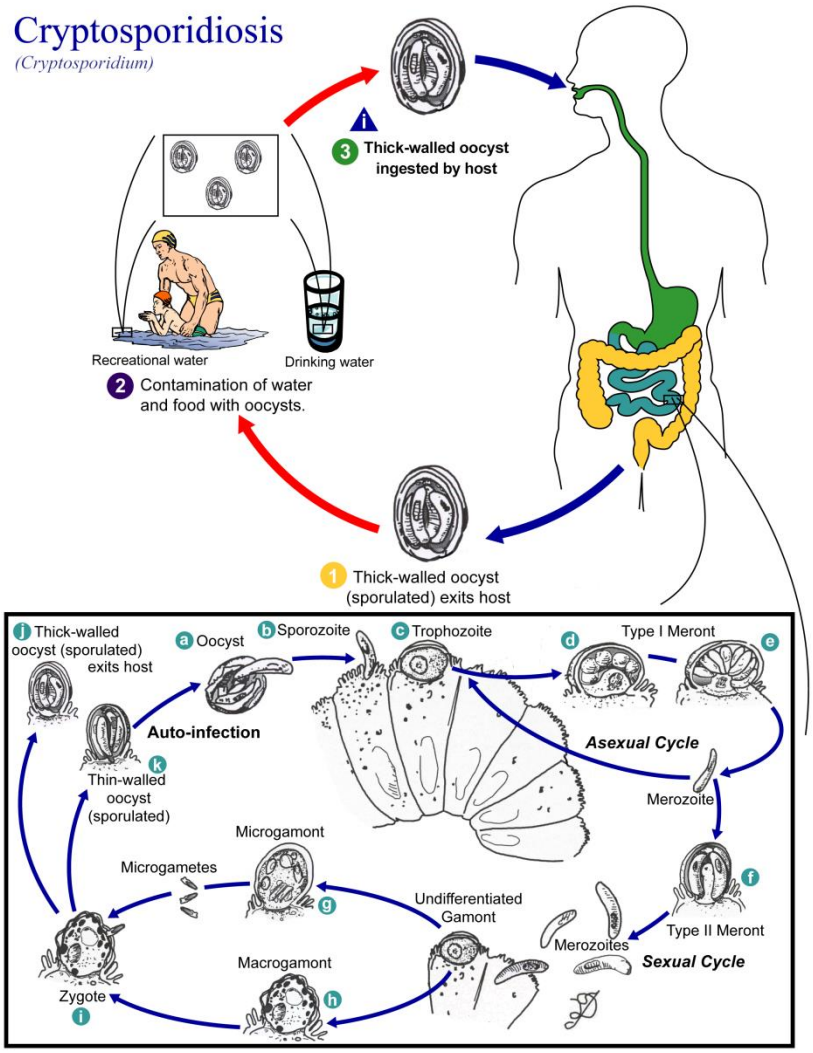

Figure 5: Cryptosporidium life cycle (Reproduced from https://phil.cdc.gov/Details.aspx?pid=3386 on 03.10.2022 with permission from Centers for Disease Control and Prevention; Content providers: CDC/ Alexander J. da Silva, Melanie Moser; this image is in the public domain of the Public Health Image Library and thus free of any copyright restrictions)

1.1.4.2 Pathology

Waterborne outbreaks are more frequent in regions with poor water access and lack of sanitation. The three main host populations are immunocomprimised patients, individuals in outbreaks in industrialised areas and children in tropical and resource-poor regions (Kosek, Alcantara et al. 2001). The protozoon is more prevalent in children and may cause diarrhoea also in immunocompetent patients (Lucius, Loos-Frank et al. 2017). Symptoms occur after an incubation period of 1-14 days with a mean duration of a week. Watery or mucous

diarrhoea in variable intensity is the leading symptom and may be associated with other gastrointestinal problems such as abdominal cramps, nausea and loss of appetite (Farrar 2013). Fever and malaise are also common symptoms, and frequently cholecystitis, hepatitis and arthritis may occur (Pond 2004). In immunocompetent persons, the infection is generally self-limited, but relapses are common, even after treatment with nitazoxanide (Ali, Mumar et al. 2014). For elimination of *C. parvum*, T-cell response and IFN-γ production play an essential role (Lucius and Loos-Frank 2008). In immunocompromised patients the infection typically occurs with severe, prolonged or chronic diarrhoea with a high mortality rate if untreated (Markell 1999). Malnutrition and stunting at birth are associated with *C. parvum* infection, eventually with even prolonged diarrhoea and unfavourable outcome (Tumwine, Kekitiinwa et al. 2003, Mondal, Minak et al. 2012). An acute dehydrating diarrhoeal syndrome complicates the course of disease and may become life-threatening more likely for children (Kosek, Alcantara et al. 2001). In young and malnourished children, the infection contributes additionally growth deficits, shortfalls in weight gain and cognitive deficits that can be persistent (Guerrant, Moore et al. 1999, Pond 2004). Even in asymptomatic patients, the infection is associated with reduced weight gain (Checkley, Gilman et al. 1997).

1.1.4.3 Treatment

The treatment of chronic cryptosporidiosis in immunocompromised patients was a problem in the past (Cabada and White 2010). One of the newer antiparasitic drugs, nitazoxanide, has been approved for use for cryptosporidiosis with a reduction in mortality in malnourished children but has limited effect in advanced HIV AIDS patients and is also limited by high cost and availability (Amadi, Mwiya et al. 2002). Symptomatic therapy with rehydration and optimization of antiretroviral therapy is one of the main principles in treatment of this infection in HIV patients (Checkley, White et al. 2015). Additionally, therapy with nitazoxanide or paromomycin should be considered (Cabada and White 2010).

1.2 Epidemiology and Risk factors

1.2.1 *Strongyloides*

1.2.1.1 Global distribution

Strongyloides was first described in 1876 when French soldiers returned from Vietnam. The infection is still prevalent mainly in tropical and subtropical environments. According to WHO 30-100 million people are estimated to be infected with this parasite and underestimation of this number is conceded (Olsen, van Lieshout et al. 2009). The global prevalence is diverse with an increase especially in low-income countries. In tropical and subtropical countries, the estimated prevalence ranges from 10-40 %. However, infection occurs also in temperate regions such as the USA, Japan and Australia. In Australia, prevalence ranges from 15-29 %, in China from 14-17 %, in the USA from 3-49 %. Thailand and Brazil are countries with the most reports of *S. stercoralis* (Schar, Trostdorf et al. 2013). Those prevalence data show the carriage of the helminth but probably rarely mean a symptomatic infection.

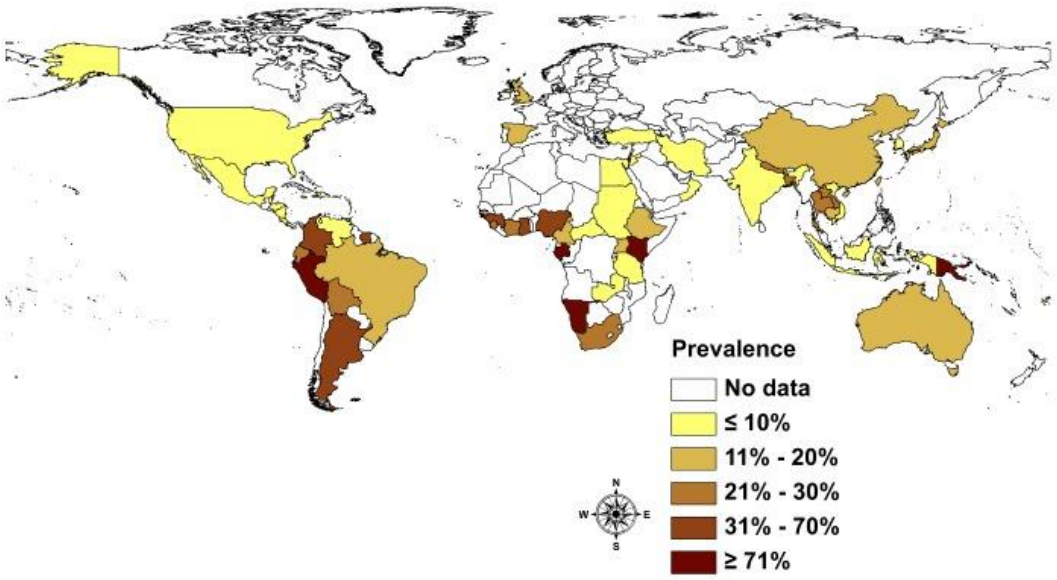

Figure 6: Prevalence of S. stercoralis infection by country based on community-based studies (Reproduced from Schar, Trostdorf et al. 2013 with permission from Schaer et al. under the terms of the Creative Commons Attribution License CC-BY)

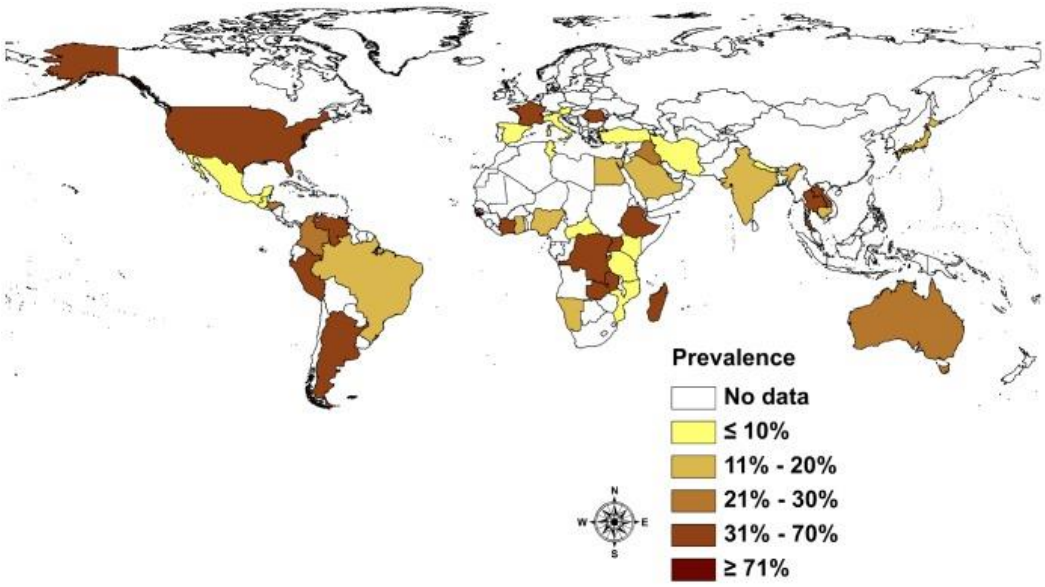

Figure 7: Prevalence of S. stercoralis infection by country based on health services studies (Reproduced from Schar, Trostdorf et al. 2013 with permission from Schaer et al. under the terms of the Creative Commons Attribution License CC-BY)

Data depend on the study population and setting. Community-based or health service based studies differ in prevalence, and the diagnostic methods also vary enormously. European studies mainly focus on prevalence in migrants, refugees and tourists who are coming from endemic countries. A prevalence rate of 12 % was found in farm workers in eastern Spain (Roman-Sanchez, Pastor-Guzman et al. 2003). In veterans with deployment in Southeast Asia, the prevalence rate was 12 % (Gill, Welch et al. 2004). In a multicentre case-control study in Northern Italy, strongyloidiasis was nine times more frequent in immigrants and Italians with eosinophilia than in those with normal eosinophil count. Overall, the prevalence of *S. stercoralis* was 5 %, confirmed as positive with two serological tests and one positive stool culture (Buonfrate, Baldissera et al. 2016). In Germany, the infection is recognised as an occupational disease in miners (Arbeitsmedizin 2014). However, published case reports in Germany are mainly about migrants with immunosuppressive health conditions.

In Africa, environmental and socioeconomic conditions are ideal for transmissions of *S. stercoralis* infection, but prevalence data are rare. Information about infection prevalence untill 2011 is available in 20 of the 46 African countries. Study data from Tanzania showed an average prevalence of

7.9 % in community-based surveys and an average prevalence of 9.3 % in hospital based surveys. Data of four studies in each of the two settings that were published from 1989 till 2011 were analysed. (Schar, Trostdorf et al. 2013)

1.2.1.2 Children

Particularly high risk groups for *Strongyloides* infection in endemic regions are HIV/AIDS patients, alcoholics, HTLV-1 patients, persons with other immunocompromised conditions and children. (Schar, Trostdorf et al. 2013)

In Indonesian children aged 0-14 years, a prevalence rate of 4.4 % was detected. Among 4-15 year-old children in Côte d'Ivoire 12.2 % were infected. In Thailand, a prevalence rate of 2 % in children was found. (Schar, Trostdorf et al. 2013)

A study in western Uganda showed a prevalence rate of 2 % in preschool children, tested with different methods but not PCR (Stothard, Pleasant et al. 2008).

In Zanzibar, *S. stercoralis* infection was detected in 10.3-12.7 % of the villagers, using a combination of Baermann method and Koga agar plate. Among the study population, the prevalence of *S. stercoralis* in children was 8-18 % depending on the region (Knopp, Mohammed et al. 2010). Another study in Zanzibar about soil-transmitted helminthiasis among schoolchildren showed a *S. stercoralis* prevalence rate of 10.2 %. (Knopp, Mohammed et al. 2009).

1.2.1.3 Risk factors

Generally people living in endemic countries as well as immigrants and travelers are at higher risk of *S. stercoralis* infection. Furthermore, immunosuppressive conditions like chronic malnutrition, usage of corticosteroids, HTLV-1, HIV, alcoholism, malignancies and received organ transplantation as well as chronic diseases like diabetes mellitus, COPD and chronic renal failure are a risk for strongyloidiasis. (Henriquez-Camacho, Gotuzzo et al. 2016)

In a study in a rural and a peri-urban community of Zanzibar in Tanzania, detected risk factors for helminth infections were age, sex, consumption of raw vegetables, recent travel history and socioeconomic status. (Knopp, Mohammed et al. 2010)

One big study in Africa, in northern Ghana, included 20.250 people and detected a higher infection rate in men than in women with 12.7 % and 10.6 % respectively (Yelifari, Bloch et al. 2005). Male gender is also reported in other studies to be associated with a higher infection prevalence (Jongwutiwes, Waywa et al. 2014, Khieu, Schar et al. 2014).

In a 2013-2015 conducted study in Tanzania with tuberculosis patients and household contacts in an urban setting, a *S. stercoralis* prevalence of 16.6 % was detected with Baermann, FLOTAC© and Kato-Katz method (Mhimbira, Hella et al. 2017).

The mortality rate of disseminated infections in immunocompromised patients is estimated to be as high as 87 % (Siddiqui and Berk 2001).

1.2.2 *Giardia*, *Entamoeba* and *Cryptosporidium*

Infections with intestinal protozoa in children are widespread in countries with insufficient hygiene, low socioeconomic levels and rare access to sanitation. The soil and climatic conditions influence the rate of contamination with microorganisms and result in spatial distribution (Ouattara, Silue et al. 2008). Three important diarrhoea causing protozoa are *Giardia, Entamoeba* and *Cryptosporidium,* but data on the prevalence of these parasites are scanty for Tanzania (Speich, Marti et al. 2013).

A prevalence rate of 74.4 % for any microscopically detected protozoa in schoolchildren was found in a study in 2011 on Pemba Island in Tanzania. The prevalence of *Giardia* was 16.4 %, and the prevalence of *Entamoeba* spp. was 18 % (Speich, Marti et al. 2013).

A study in Côte d'Ivoire in schoolchildren about the polyparasitism and spatial distribution of *E. histolytica*, *E. dispar* and *G. lamblia* showed that the

microscopically detected prevalence of *E. histolytica/ dispar* and *G. lamblia* was 11 % and 17.5 % respectively. The prevalence of polyparasitism was 80 % (Ouattara, Silue et al. 2008).

1.2.2.1 *G. lamblia*

G. lamblia is distributed worldwide and is considered to be one of the main pathogens causing diarrhoeal outbreaks from contaminated water supplies. The first recorded waterborne outbreak was in travellers to St. Petersburg in Russia. In the USA, several water supply connected outbreaks have been reported. Since beavers and muskrats can be infected with *Giardia* cysts from humans, the cross-species transmission is the reason for infection in remote places. In this way, the infection occurs also in climbers and hikers drinking from mountain streams like in the Colorado Rocky Mountains (Markell 1999).

A review of worldwide waterborne parasitic protozoan outbreaks that occurred with reports published between 2011 and 2016 showed *Cryptosporidium* spp. as the most common etiologic agent in 63 % and *Giardia* spp. in 37 % of the outbreaks (Efstratiou, Ongerth et al. 2017).

A Global Enteric Multicenter Study in sub-Saharan Africa and south Asia was conducted to identify the aetiology and burden of diarrhoeal diseases in children younger than five years. Among the detected pathogens, *Giardia* was not positively associated with moderate-to-severe diarrhoea but was even more frequent in controls without diarrhoeal symptoms. (Kotloff, Nataro et al. 2013)

In Ifakara in Tanzania, *G. lamblia* prevalence of 14 % in preschool children in rainy season and of 1 % in dry season was detected (Vargas, Gascon et al. 2004). The method used was a stool microscopy technique, and the data are not up to date since the study was done in 1997.

1.2.2.2 *E. histolytica* and *E. dispar*

In 1875 *E. histolytica* was first described in a Russian peasant suffering from diarrhoea in a port close to the Arctic Circle. Nowadays prevalence rates are high, particularly in the tropics where crowding, poor sanitation and warm climate increase the risk of transmission. (Markell 1999)

The nonpathogenic but morphologically identical species *E. dispar* was first described as a separate species in 1992. Therefore epidemiological literature contributed to *E. histolytica* prior to this date must be carefully interpreted. *E. dispar* is far more common worldwide with a nine-fold higher estimated prevalence than *E. histolytica* (Lucius, Loos-Frank et al. 2017).

Although mostly asymptomatic or light infection occurs, amoebiasis is estimated to kill 40.000 -100.000 people per year (Farrar 2013). Differentiation among acute, chronic and asymptomatic stages of the infection is important, focusing on the transmission of the amoeba. Infected asymptomatic persons mainly spread the infection via defecated cysts. Contaminated water plays a big role in regional outbreaks. Sanitation, contaminated foodstuff and use of human faeces for fertilizer have an important effect on prevalence rates (Markell 1999).

In northern Ghana, an *E. histolytica/dispar* prevalence of 39.8 % was shown, detected by microscopy. The performed qPCR detections identified just one of the 78 microscopic *Entamoeba* positive cases as positive for *E. histolytica*. 87 additional cases of E. dispar were found by qPCR in the former microscopy negative samples. (Verweij, Oostvogel et al. 2003)

A study performed in 1999-2001 in the Kilimanjaro region in Tanzania showed a prevalence of only 1 % for *E. histolytica* but of 7.3 % for *E. dispar*, detected by a specific ELISA (Nesbitt, Mosha et al. 2004).

1.2.2.3 *C. parvum*

The first cases of cryptosporidiosis in humans were described in 1976. In the 80s and 90s, the infection was a prominent cause of severe diarrhoea in AIDS patients. Nowadays it is still an important pathogen in immunocompromised patients and has long-term developmental effects on children in impoverished areas. In industrial nations regional water-source outbreaks are additionally recognised. (Kosek, Alcantara et al. 2001)

In Europe, the largest proportion of infected people are children younger than six years (Lucius, Loos-Frank et al. 2017).

A study in Burkina Faso showed a prevalence of 7.8 % for *Cryptosporidium* in paediatric patients less than three years with diarrhoeal symptoms. The overall prevalence in the children with and without diarrhoea was 5.2 %, detected by phenicated fuchsin technique microscopy. A correlation with malnutrition was found, but just one HIV-positive case. (Nacro, Bonkoungou et al. 1998)

The Global Enteric Multicentre Study in sub-Saharan Africa and south Asia identified *Cryptosporidium* as one of the main causative pathogens for moderate-to-severe diarrhoea during the first two years of life. Furthermore, cryptosporidiosis was associated with a higher risk of mortality among children aged 1-2 years with diarrhoea than in controls without diarrhoea. (Kotloff, Nataro et al. 2013)

In a case-control study among children less than two years of age admitted to hospitals in Dar es Salaam in Tanzania, the prevalence of *C. parvum*/*hominis* was 10.4 % (Tellevik, Moyo et al. 2015).

1.3 **Diagnostic tests**

1.3.1 ***Strongyloides***

For detection of *S. stercoralis* several techniques are common, but no gold standard has been set up. Stool concentration techniques are commonly used for the microscopic analysis of intestinal parasites in faecal material. Occasionally *S. stercoralis* larvae can also be detected, but the sensitivity for this method is low, and microscopy is cumbersome (Kitvatanachai S 1999, Buonfrate, Formenti et al. 2015). Faecal culture techniques are instead used which provide a higher yield. Among them, the Baermann-Moraes method, the Harada-Mori culture and the Koga agar plate culture are the most commonly applied culture techniques. The Koga agar plate culture showed the highest sensitivity for the detection of *S. stercoralis* larvae (Kitvatanachai S 1999, Glinz, Silue et al. 2010, Ines Ede, Souza et al. 2011) whereas the Harada-Mori culture proved to give the highest yield in hookworm infections (Kitvatanachai S 1999). The agar culture method has been recommended for epidemiological research questions (Arakaki, Iwanaga et al. 1990) and is preferred due to its high

sensitivity in standard microbiological laboratories (Puthiyakunnon, Boddu et al. 2014). Compared to direct smear procedures for detection of *S. stercoralis* larvae, agar plate culture is more than four times effective (Sato, Kobayashi et al. 1995). The Baermann-Moraes technique shows lower sensitivity and is even more laborious and time-consuming (Ines Ede, Souza et al. 2011). Sensitivity for other techniques such as Kato-Katz and FLOTAC has been described as low for detection of *S. stercoralis* (Glinz, Silue et al. 2010).

All the aforementioned culture tests focus on the detection of soil-transmitted helminths. Due to personnel, time and financial restraints, they are rarely used outside of specialized parasitological laboratories or research questions. Duodenal aspirate, duodenal biopsy and BAL may reveal *S. stercoralis* larvae or even ova but require much time and technical equipment. (Siddiqui and Berk 2001, Cringoli, Rinaldi et al. 2010, Glinz, Silue et al. 2010, Knopp, Salim et al. 2014)

Available immunological tests include ELISA, IFAT and LIPS but are of limited use in endemic countries and are restricted to reference laboratories (Silva, Barcelos et al. 2003, Corral, Paula et al. 2017). The assays differ in antigen preparation and used immunoglobulin isotypes with variable sensitivity and specificity. In European and American countries, serological testing is often used as a screening method in risk populations such as migrants from endemic countries or in cases of suspected infection, as its sensitivity is higher than in other diagnostic tests (Requena-Mendez, Chiodini et al. 2013, Buonfrate, Perandin et al. 2017). There are two ELISA commercially available, the Bordier ELISA (Bordier Affinity Products, Switzerland) and the IVD ELISA (SeroELISA *Strongyloides* IgG, IVD Research Carlsbad, CA) (Buonfrate, Sequi et al. 2015). They are quite sensitive (73-100 %) but show lower specificity (29-93 %) due to cross-reactivity with other nematodes. Furthermore, sensitivity is decreased in immunosuppressed patients and travelers (Requena-Mendez, Chiodini et al. 2013).

Serological testing in endemic countries is a problem, as antibodies can persist for a long time, and distinguishing between active and historical infections is

difficult (Buonfrate, Sequi et al. 2015). However, serology was found to be useful as a follow-up method because antibody titres reverse after successful treatment (Olsen, van Lieshout et al. 2009).

S. stercoralis DNA detection in stool samples with molecular diagnostic methods is an alternative diagnostic approach that is currently available as an in-house technique (Buonfrate, Formenti et al. 2015). Its potential use is still limited by cost and by the low availability apart from research laboratories. It has a role in the confirmation of serological positive cases, since its sensitivity is lower than serology (Buonfrate, Perandin et al. 2017). The reported sensitivity and specificity of the qPCR differs a lot in literature. The specificity ranges from 85.7-100 % but is mostly described as close to 100 %. The reported sensitivity shows a wide spectrum from 14.7-90 % (Verweij, Canales et al. 2009, Schar, Odermatt et al. 2013, Sultana, Jeoffreys et al. 2013, Becker, Piraisoody et al. 2015, Paula, Malta Fde et al. 2015). Still, the results should be interpreted with caution, as they are influenced by the used reference diagnostic method. Verweij showed a sensitivity of 72-92 % when compared with direct smear, baerman method and Coproculture (Verweij, Canales et al. 2009). The highest sensitivity was seen in samples compared with direct smear. A sensitivity of 27-91 % was seen in samples compared with the Baermann technique or two duplicated coprocultures. A lower sensitivity of 23,8 % was seen in samples compared with samples in which larvae were detected in one of the duplicated coprocultures (Verweij, Canales et al. 2009). Becker showed a sensitivity of 76.8 % when compared to a combination of all diagnostic techniques which were PCR, Koga agar plate and the Baermann method (Becker, Piraisoody et al. 2015). Paula showed a sensitivity of 90 % for qPCR in probes that were definded as positive or negative prior to testing. The positivity of the stool sample was defined by using a microscopic method such as a Koga agar plate or the method of Lutz or Rugai (Paula, Malta Fde et al. 2015). Sultana showed a sensitivity of 34.1 % when compared to triplicate Harada-Mori technique. They showed a lower sensitivity of 15 % in samples with lower larvae burden (Sultana, Jeoffreys et al. 2013). Schär showed a sensitivity of 88.9 % when compared to the combination of Koga agar plate and the Baermann method

(Schar, Odermatt et al. 2013). Buonfrate showed a sensitivity of 57 % when compared with serology and the agar plate method as a composite reference standard (Buonfrate, Perandin et al. 2017).

1.3.2 *Giardia*, *Entamoeba* and *Cryptosporidium*

The basic diagnostic method for the detection of *Giardia lamblia, E. histolytica* and *C. parvum* is microscopy of fresh or formalin-fixed faecal material using concentration methods. Concentration techniques combined with microscopy are still the most commonly used screening methods although they show a rather low sensitivity and specificity and high dependence on examining personnel. They allow demonstrating various amoebic cysts, *Giardia* cysts and several other intestinal parasites. For the detection of *C. parvum,* staining techniques such as a modified Ziehl-Neelsen acid fast staining method are commonly used. However, these show a low sensitivity and specificity and depend strongly on the skills of the personnel (Verweij, Blange et al. 2004, ten Hove, Schuurman et al. 2007).

Several faecal antigen tests are available for detection of *Giardia*, *Entamoeba* and *Cryptosporidium*. These immunochromatographic assays are commercially available even as a combined rapid test. They have shown an increased sensitivity compared to microscopy (Weitzel, Dittrich et al. 2006, Van den Bossche, Cnops et al. 2015).

Microscopy and faecal antigen ELISA do not allow the differentiation of *E. histolytica* from the non-pathogenic *E. dispar*. This can be achieved by faecal real-time PCR with high sensitivity and specificity (Verweij, Blange et al. 2004).

There are real-time PCR available with different primers and probes for each of these protozoa separately or as a combined multiplex real-time PCR (Verweij, Oostvogel et al. 2003, Haque, Roy et al. 2007, ten Hove, Schuurman et al. 2007).

Serological ELISA tests for these intestinal parasites are available but are not commonly used. The ELISA is useful for surveillance purposes, discrimination

of past infection and therapy follow-up, but it does not discriminate acute versus historical infection (Checkley, White et al. 2015).

1.4 **Study Objective**

According to the WHO, the global target is to eliminate morbidity due to soil-transmitted helminthiasis in children by 2020. This aim is intended to be obtained by the regular treatment of the children in endemic areas. 30–100 million people are estimated to be infected by *S. stercoralis* worldwide (Bethony, Brooker et al. 2006), but since precise data on the prevalence of *Strongyloides* infections in endemic countries are rare, fulfilling the global target of eliminating morbidity is difficult.

Current strategies of the soil-transmitted helminthiasis program of the WHO focus on ascariasis, trichuriasis and hookworm disease. Other programs also deal with schistosomiasis, but no public health strategies for controlling strongyloidiasis was active at the global level until the recently published WHO 2030 targets for soil-transmitted helminthiases control programmes. Apart from that, *Strongyloides* infection is not among the research priorities according to WHO programs although it is on the list of neglected tropical diseases. Further assessment of the prevalence data and associated socio-demographic factors is needed (Olsen, van Lieshout et al. 2009, Schar, Trostdorf et al. 2013) .

Prevalence data of *S. stercoralis* infection in children are scarce for Tanzania (Schar, Trostdorf et al. 2013).

A prevalence study was planned to show the burden of S. stercoralis infections and other parasites in children in the Mwanza region in Tanzania. This study was named StrongPaed study and serves as basis for this doctoral thesis. Before the beginning of the StrongyPaed study, sufficient data on the prevalence of *S. stercoralis* infection among children in the lake region of Tanzania were not available.

In general, strongyloidiasis is a commonly underdiagnosed parasitic infection due to its difficult diagnosis and the lack of sensitive diagnostic methods (Olsen, van Lieshout et al. 2009). Experienced personnel and laboratory facilities as

well as time consuming or expensive techniques are necessary. These are often not available for routine use in hospital settings, especially in sub-Saharan countries. Low sensitivity in commonly used diagnostic tests likely result in underestimated prevalence (Schar, Trostdorf et al. 2013).

Furthermore, there is a wide range of symptoms in strongyloidiasis, and many cases are asymptomatic. The clinical impact of this parasite is not fully clear, but it may be severe and even life-threatening in cases of immunodeficiency (Nutman 2017). Latent infections in children can become a problem when they develop an immunosuppressant disease. A *S. stercoralis* hyperinfection syndrome can easily be overlooked or misinterpreted as the symptoms overlap widely with sepsis or severe IRIS.

Common deworming strategies do not affect *S. stercoralis* infection effectively since substances and dosage of deworming treatment do not cure strongyloidiasis. Without appropriate treatment, the infection may persist and become a problem when children undergo corticosteroid therapy or other conditions of immunosuppression (Ramanathan and Nutman 2008). For treatment, ivermectin is the drug of choice but was not easily accessable as medication for strongyloidiasis in Tanzania at the time of the study, although it is recommended in national guidelines.

In general, helminthic infections show a spatial distribution with influence of climate and living conditions, and available data are regionally evident, but cannot be generalised (Schar, Trostdorf et al. 2013). In urban settings, a lower prevalence was shown than in rural areas (Knopp, Mohammed et al. 2008). Improvement of sanitation would have an additional impact on the transmission of STH but is still difficult to achieve in many poor tropical countries (Ziegelbauer, Speich et al. 2012). Children are at special risk for infection since they often are exposed to poor sanitary conditions. They play outside on the ground and do not necessarily wear shoes at all times.

Warm climates and poor sanitary conditions are also strong drivers for infections with other intestinal parasites such as *Giardia, Entamoeba* and *Cryptosporidium* (Ouattara, Silue et al. 2008). These are mainly causing agents

of diarrhoeal diseases with high mortality rates. The unspecific symptoms impede correct and fast diagnostic procedures.

Giardiasis and other intestinal protozoan infections have an impact on the physical development of children (Ajjampur, Koshy et al. 2011). Preventive mass treatment campaigns mostly have no sufficient effect on the infection incidence rate of these parasites. Common preventive chemotherapy campaigns focus mainly on soil-transmitted helminth infections. The WHO recommends an annual or biannual single-dose treatment of 400 mg albendazole or 500 mg mebendazole (WHO 2012). Even in the case of widespread deworming campaigns, many children still remain infected with chronic Giardiasis because the drugs used are not effective against *G. lamblia*.

Actually there are no effective control programs established, and actual data about the burden of these diseases are scarce. For the detection of *Entamoeba* species, *Giardia* and *Cryptosporidium*, there are different tests available, which vary in feasibility and price as well as specificity and sensitivity. Most of them are limited by costs, expertise or sensitivity. The available information on the prevalence of *E. histolytica* is outdated in many sub-Saharan countries, as it was mostly obtained by methods which could not differentiate between pathogenic and nonpathogenic species.

Infections with intestinal parasites have an enormous health relevance in the Victoria Lake region, especially concerning the health of children. Valid data are necessary to assess the situation and are the basis for further research. The data of this study may have an impact on future treatment and diagnostic strategies.

The aim of the conducted study was to provide prevalence data on *S. stercoralis* infections in children. Furthermore it included other intestinal parasites, namely *G. lamblia* and *E. dispar/ histolytica* in the same study group. A panel of diagnostic methods of varying sensitivity and specificity was used and compared.

Prevalence data about the infections were compared concerning the socio-demographic and clinical factors of the children. These associated parameters were surveyed by a standardised questionnaire.

1.4.1 Specific Objectives

1. Determination of the prevalence of *S. stercoralis* in children admitted to the paediatric wards of BMC, tested by FPC® technique and microscopy, qPCR, ELISA, modified Harada-Mori culture and Agar plate culture.

2. Determination of the prevalence of protozoa, namely *Giardia, Entamoeba* and *Cryptosporidium* in children admitted to the paediatric wards of BMC, tested by immunochromatographic rapid assay as well as by FPC® concentration technique based microscopy, and for *Giardia* and *Entamoeba* species also by qPCR.

3. Comparison of the applied techniques in sensitivity and specificity as well as feasibility.

4. Determination of the prevalence of other helminthic and protozoan infections as detectable by the applied techniques, namely the FPC® technique and microscopy.

5. Comparison of infection prevalence depending on age, living region, living conditions, clinical symptoms, diagnoses and previous treatment.

2 Material and Methods

2.1 Clinical Data

An exploratory healthcare-based cross-sectional study was conducted among children in the Mwanza region of the United Republic of Tanzania. The project was completed in cooperation with the Medical Mission Institute and Hospital in Würzburg, Germany, in connection with the University of Würzburg and the BMC in Mwanza, Tanzania, connected to the CUHAS in Tanzania. The study was named StrongPaed study.

2.1.1 Setting and timeframe

The study was conducted at the paediatric department of the Bugando Medical Centre in Mwanza in the United Republic of Tanzania. The BMC is the referral hospital of the Lake Region in Tanzania with a huge catchment area. Patients come from urban as well as from rural areas. Therefore, the study population was expected to have a mixed background concerning socio-economic status and living conditions.

The paediatric patients were included in the study in an enrolment period of two months from February 22 until April 23, 2013. At the time of enrolment, the patients and their parents or caretakers were approached by a study nurse and counselled about the purpose of the study and the personal benefit.

If the parents or caretaker gave their verbal and written informed consent, they were asked to provide some data according to the study questionnaire. Additional data were retrieved by study personnel from the patients' file concerning diagnoses, symptoms, previous treatment and follow up.

The patients were asked to provide a stool sample which was collected by study nurses and the author. Several tests were performed in the study laboratory at BMC, and the samples were preserved. Serum samples from the patients were also taken by the author.

2.1.1.1 Result information and treatment

After obtaining the results, the parents or caretakers were informed, and all positive results were listed in the patient file. Appropriate treatment was provided for free if results were positive and the patient was contactable. This was done in cooperation with the attending medical doctors and nurses at the wards. If the patients were already discharged at the time of positive results, they were contacted via phone for the opportunity of free treatment.

The results of the rapid test were available on the same day. In case of positive results in immunochromatographic rapid testing for *Giardia, Entamoeba* and *Cryptosporidium*, patients and their parents or caretakers were informed immediately and offered treatment according to actual guidelines by DTG and AWMF, availability of medication and after agreement of responsible doctors. In case of a positive result for *G. lamblia*, the patients were treated with Metronidazole 15 mg/kg, 8 hourly, for five days. In case of a positive result for *E. histolytica/ dispar*, the patients should be treated with Metronidazole 10 mg/kg, 8 hourly, for ten days, if clinical symptoms indicated the need for specific treatment. In case of a positive result for *C. parvum* and clinical symptoms, patients were encouraged to do a HIV-test if not present until then, and symptomatic treatment was offered after consultation with responsible doctors.

The results of culture techniques were available within at least 6 days after the stool sample was provided by the patient and laboratory tests were initiated. Positive results for *Strongyloides* infection were reported to the wards as well as to the patients and their parents or caretakers without delay, and they were offered free treatment with albendazole 400 mg bid for 7 days as ivermectin was not regularly available in Tanzania for *Strongyloides* infection at the time of the study.

2.1.2 Data collection

2.1.2.1 Study participants

The study group were patients 1-14 years of age, who were admitted to the paediatric wards at BMC regardless of their presumptive diagnoses. The

patients and the parents or caretakers of the study participants were informed about the study and gave their written informed consent. Patients had to be able to provide a stool sample. Study participants were excluded if no stool sample was returned or the amount was not enough for testing. Previous anthelminthic treatment was no exclusion criterion as the commonly used standard treatment regimen for deworming with a single dose of albendazole or praziquantel is assumed to have little impact on *S. stercoralis* infection (Olsen, van Lieshout et al. 2009) and also on *G. lamblia, E. histolytica* and *C. parvum* infection (Speich, Marti et al. 2013). The history of anthelminthic pre-treatment was recorded in the questionnaire.

2.1.2.2 Questionnaire

The parents or caretakers of the included patients were given a questionnaire to be filled with the assistance of the study nurse. Name and phone number were collected with the sole purpose of contact upon receiving results. For further work with the questionnaire as anonymised data, each patient was given a study number. Furthermore, the clinical file number was noted in order to enable the extension of information concerning the symptoms, diagnosis and treatment of the patient.

General information about age, gender, weight and height of the patient as well as information about profession of the parents or caretakers, area of residence, living conditions, water access, type of toilet and wearing of shoes was recorded in the questionnaire. Furthermore, the reason of admission, actual symptoms, diagnosis of asthma, deworming within the last 12 months, HIV testing and treatment with corticosteroids or other immunosuppressive drugs were registered.

Questions were written in English as well as in Swahili. Translation was done with the support of a Tanzanian medical doctor and a study nurse. Most questions were structured as checkbox questions to make data entry easier.

After the enrolment period of the study, the questionnaires were copied for further analysis without patient names. The originals were scheduled to stay at

the BMC for at least five years. The results of the questionnaire were digitalised and evaluated in Excel and SPSS.

2.1.2.3 Material collection

The parents or caretakers received two stool containers labelled with the study number. They were carefully instructed how to provide an appropriate faecal sample for the study and where to bring the containers or where to wait for the study personnel to take it. The stool sample was collected on the same or the next day and was taken to the study laboratory, where tests were done and the rest of the stool sample was preserved.

After tests with fresh stool samples had been performed, every fresh stool sample was divided and filled into two stool container tubes for preservation. One was filled with ethanol 70% and the other with formalin 10%. These containers were stored at room temperature and were transferred after the end of the study to the Medical Mission Institute in Würzburg, Germany.

The patients in consensus with the parents or caretakers were encouraged to provide a serum sample of about 4.5 ml, taken by the author. In case of an unfavourable clinical condition such as serious anaemia or other obstacles, this was omitted. A missing serum sample was no exclusion criterion for participation in the study. The blood sample was taken to the study laboratory and stored at -20 °C. One sample was shipped to the BNI laboratory in Hamburg, Germany and was analysed there with an ELISA for *Strongyloides* antibodies. One serum sample stayed at BMC as a backup sample. Remaining samples are intended to be destroyed at the latest five years after the end of the enrolment period.

2.1.3 Ethical considerations

The study proposal was submitted to the CUHAS/BMC Joint Research and Ethical Committee. Ethical clearance for the study was granted under certificate No. CREC/049/2013.

Participation in the study was voluntary, and no renumeration was paid for participation. Withdrawal was possible at any time without specific reasons and

with no disadvantages in clinical treatment. The patients and their parents or caretakers were informed about the study at the wards by a study nurse speaking English and Swahili and by the author when communication in English was possible. A written consent form in Swahili had to be signed by the parents or caretakers before enrolment.

All personal data were kept confidential and were accessible only to the staff directly involved in data entry and analyses. Personal data were only used to identify patients for treatment. After the end of the study, data were anonymised.

No others tests than thoses documented in the study proposal were done. Remaining samples will be destroyed at latest five years after the end of the enrolment period.

Shipment and use of specimens strictly followed the Material Transport Agreement of the National Institute for Medical Research which was signed prior to shipment.

The obtained data were presented at national and international conferences as an oral/ poster presentation. Any publications resulting from this study will be done in mutual agreement and according to the individual contribution of the investigators.

Data and results were entered into a database using Microsoft Excel on password protected computer systems and into a handwritten notebook which was kept locked up

After finishing the enrolment period, copies of the anonymized questionnaire, the list of results, the stool samples and the serum samples were transported to Germany for further testing and analysis. The original informed consent and questionnaire as well as the handwritten notebook are kept at BMC. All data will be kept for five years after the end of the enrolment period.

2.1.4 Statistical analysis

The sample size was calculated according to the Leslie Kish formula with assuming a prevalence of 10% for *S. stercoralis.*

Data entry was done using Microsoft Excel 2010, Microsoft Corporation, and analysed using IBM SPSS Statistics version 24.0. Data calculation was done with Odds Ratio for associated risk factors and with Cohen's kappa coefficient for method comparison.

2.1.4.1 Sample size calculation

S. stercoralis infection shows a very spatial distribution depending on living conditions, environment, climate and other factors. Estimating prevalence is therefore difficult. For Tanzania as a whole only scant data were available, and no data could be retrieved for the Mwanza or Victoria Lake regions in northern Tanzania. Therefore the study was designed as an exploratory cross-sectional study, and it was not possible to calculate an exact sample size. The sample size is calculated in assumption of data from Zanzibar, a different region in Tanzania. In Zanzibar a prevalence of 10,2 % for *S. stercoralis* infection in schoolchildren was shown (Knopp, Mohammed et al. 2009).

The sample size was calculated according to the following formula which was developed and published by Leslie Kish (Naing 2003).

$$n = \frac{Z^2 \times p \times (1-p)}{d^2}$$

Z = level of confidence: For the conventional level of confidence of 95 %, the Z value is 1.96

n = sample size

p = expected prevalence, in proportion of one = 0.1

d = precision, in proportion of one = 0.05

$n = 1.96^2 \times 0.10\ (1-0.1) / 0.05^2$

$n = (3.8416 \times 0.09) / 0.0025$

$n = 138.3$

Under the assumption that the prevalence would be 10 %, the required number of participants was calculated to be 139. The aim was to include 150 patients to ensure that the calculated minimum of evaluable patients was reached despite some eventual dropouts due to incomplete data sets.

It is well known that repeated sampling may significantly increase the yield of intestinal parasites. This applies especially to detection of S*trongyloides* (Khieu, Schar et al. 2013). As patients often stayed for a short period in hospital and did not defecate every day, practical circumstances were unsuitable to examine more than a single stool specimen per patient. Instead of multiple examinations, a combination of tests including stool PCR and serum ELISA for *Strongyloides* were performed.

The estimated prevalence of *Entamoeba*, *Giardia* and *Cryptosporidia* infection differ from the estimated prevalence of Strongyloides infection. However, due to the limited time frame for enrolment period, the sample size could not be increased.

2.1.4.2 Statistical methods

Data from the questionnaires were sorted and summed up in part to simplify the analysis. For some parameters such as weight and height, entries were frequently missing. They were therefore excluded from calculation. Diagnoses were grouped as gastrointestinal, other internal, neurological or surgical diagnosis.

Bivariate correlation analysis was done and correlation coefficients were calculated. Pearson product-moment correlation coefficient shows the significance of aspects in a 2x2 table. $P < 0.05$ was considered as significant at a 95 % confidence interval. Odds Ratio with 95 % confidence interval was calculated to show the strength of association between socio-demographic aspects of the questionnaire and sample test results. Kappa-coefficient was calculated to compare the concordance between the different test methods.

Odds ratio is an instrument in descriptive statistics for association effect between two binary data. It quantifies the occurrence of presence or absence in

one feature compared with presence or absence in another feature. The two odds are divided and obtain the odds ratio. An OR = 1 implies no difference between the surveyed aspects. If the OR is greater or smaller than 1 a positive or negative association can be assumed. Association does not necessarily mean a causal dependence but does show some correspondence. The confidence interval is the critical range for assumption of a parameter in the sample values. If the 95 % confidence interval includes OR = 1, random association must be assumed and the result does not refuse the background level, so it is not statistically significant. The p-value shows the exact statistic evidence (Gordis 2014).

The Cohen's kappa coefficient measures the interrater agreement among different raters or for repeated explorations for categorical items. It quantifies the chance-corrected agreement of the rating. In this study it is used as an intrarater agreement for repeated testing performed by one person but with different methods. Therefore the kappa coefficient calculates the measured concordance of the different test methods subtracted by the concordance by chance. A result of k = 1 means a total concordance and k = 0 means concordance by chance alone. The strength of concordance ranges from poor to almost perfect agreement as shown in *Table 1: Cohen's kappa agreement (Landis and Koch 1977)*.

Table 1: Cohen's kappa agreement (Landis and Koch 1977)

Cohen's kappa	Intensity of agreement
≤ 0,2	Poor agreement
0,21- 0,40	Slight agreement
0,41- 0,60	Moderate agreement
0,61- 0,80	Substantial agreement
0,81- 1,00	Almost perfect agreement

2.2 **Laboratory analysis**

In this study the Koga agar plate culture technique and a modified Harada-Mori culture technique for the direct detection of *S. stercoralis* larvae in faecal samples was used. In addition, a real-time PCR performed with faecal specimen and a serum ELISA was employed for the comparison of available methods. Furthermore, microscopy was done with stool samples undergoing a concentration method process. The FPC® stool concentration technique and microscopy were used mainly for the detection of *Giardia* and *Entamoeba* and for the differential diagnosis of other intestinal parasites. For *Cryptosporidia* microscopy, a modified Ziehl-Neelsen stain was performed additionally. A faecal antigen immunochromatographic rapid assay was used for the detection of *Giardia*, *Entamoeba* and *Cyptosporidium*. The differentiation of *Entamoeba* species and analysis of *Giardia* was done by faecal real-time PCR.

Depending on the accessability of methods and their feasibility, some laboratory analysis were done directly in the study laboratory at BMC in Mwanza, Tanzania, and some had to be done in Germany after transporting the specimen. All methods requiring fresh stool samples such as the Agar plate culture technique and the modified Harada-Mori culture technique, as well as the faecal ELISA cassette test were performed at the BMC study laboratory in Mwanza, Tanzania. Real-time PCR and further microscopy with FPC® concentration technique was conducted at the laboratory of the Medical Mission Institute in Wüzburg, Germany. The serum ELISA was done at the BNI laboratory in Hamburg, Germany.

2.2.1 Agar plate culture

An Agar plate culture was performed as described in the literature (Arakaki, Iwanaga et al. 1990, Koga, Kasuya et al. 1990, Koga, Kasuya et al. 1991). Petri dishes of 90 mm were prepared with Mueller-Hinton agar. The Mueller-Hinton agar contains 2.0 g beef extract, 17.5 g casein hydrolysate, 1.5 g starch, 17 g agar. This was suspended in one litre of distilled water and mixed. It was heated and boiled for one minute and sterilized at 120 °C for 15 minutes. Then it was cooled down to 40-45 °C and poured into the Petri dishes.

For each culture, about 3 g of fresh faeces was put in the centre of the agar plate, covered and sealed with Parafilm M *(Figure 8: Agar Plate culture).* The culture was kept at room temperature ranging from 26-28 °C, and after 24, 48 and 72 h it was evaluated by using a stereo microscope to detect furrows on the agar. Usual bacterial colonies were assessed as negative. Furrows left from crawling larvae and free living adults as well as detectable worms and larvae were further investigated. Larvae crawl over the agar carrying bacteria with them, and so they form visible tracks as can be seen in *Figure 9: Tracks of larvae at Agar plate culture.* Upon finding characteristic tracks, larvae were searched for and observed microscopically.

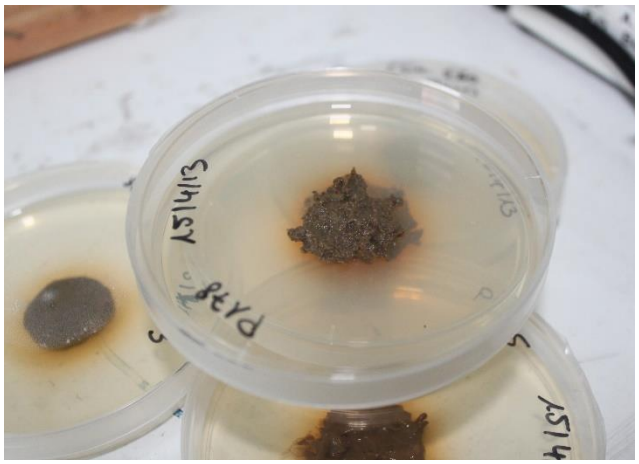

Figure 8: Agar Plate culture (Own source)

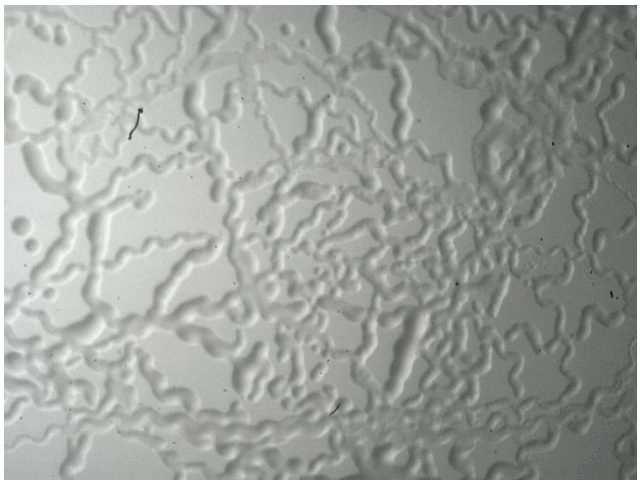

Figure 9: Tracks of larvae at Agar plate cultur (Own source, Stereo microscope, total magnification 10x)

When larvae were detected, the differentiation between *Strongyloides* and hookworm was done by analysing the size, buccal cavity, genital primordium and oesophagus *(Figure 10: Rhabditiform hookworm larvae, Agar plate culture)*. Collecting sediment with larvae was not possible in all positive samples due to practical reasons and loss of larvae. Therefore microscopic analysis at high magnification was not possible.

Figure 10: Rhabditiform hookworm larvae, Agar plate culture (Own source, transmitted-light microscope, total magnification 400x)

Observation was done three times to evaluate the time during which positive results can be seen. Since hookworms need about 1-2 days to hatch, positivity within the first day increases the likelihood that visible larvae are of *Strongyloides* and are not hookworm larvae. But just 24 h are often too short for the detection of crawling larvae. Some study groups evaluated agar plate cultures after 24 h (Koga, Kasuya et al. 1990), but most took a longer time for incubation of the agar plates: 48 h (Arakaki, Iwanaga et al. 1990, Marchi Blatt and Cantos 2003, Glinz, Silue et al. 2010) or even seven days (Kitvatanachai S

1999, Reiss, Harrison et al. 2007). Increased sensitivity has been reported by observing the agar plates every day (Jongwutiwes, Charoenkorn et al. 1999), with increased cumulative positivity until the third day of observation (Ines Ede, Souza et al. 2011). Therefore, in this study daily evaluation of the agar plates was done for three days and the result of the third day was taken as the final result.

2.2.2 Modified Harada-Mori culture technique

The conventional Harada-Mori culture technique as described 1955 by Harada and Mori was modified to allow the usage of a larger stool volume and to enable direct microscopy of the tubes. Instead of 15 ml tubes, 50 ml conical centrifuge tubes were used. The conical end of the tube was cut in a way that a 10 mm microscopic cover glass was able to be glued there. In this way, observation with an inverted microscope was possible. An amount of about 1-2 g of fresh faeces was put on one end of a 7x2 cm stripe of filter paper. This was carefully inserted into the tube filled with 20 ml of distilled water as can be seen in *Figure 11: Modified Harada Mori Culture*. The one end containing the stool sample stayed above the water level. Water soaks into the filter paper stripe up to the stool sample. Moisture and warm temperature create suitable conditions for the larvae and enable them to crawl towards the water in the tube (Ahmed 2014). The culture tubes were stored at room temperature and were observed with the microscope after 2-3 days and again after 5-6 days.

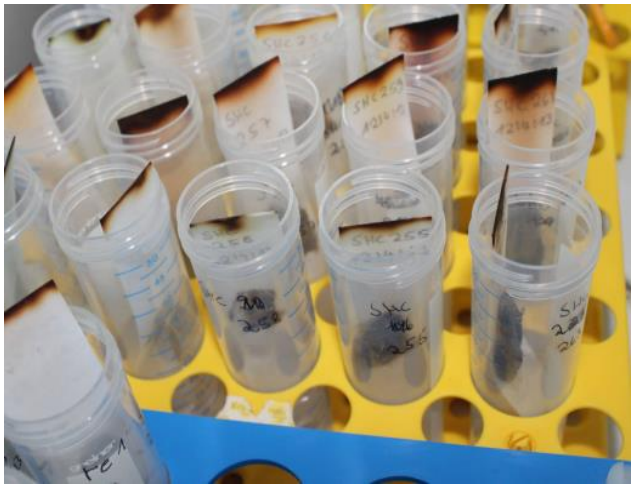

Figure 11: Modified Harada Mori Culture (Own source)

In cases where larvae were visible, the water was extracted after the observation period, and the larvae were fixed in ethanol, 70% solution. Then they were examined with a higher magnification microscope to differentiate between *Strongyloides* and hookworm larvae. The larvae were then pipetted on an object plate, covered with a cover slip and directly examined by microscopy in 100- and 400-fold magnification *(Figure 12: S. stercoralis, rhabditiform larva and adult female worm, modfied Harada-Mori culture).*

In cases where the tube was not observable because of stool-contaminated water, the sample was excluded from analysis by this method. The result of the sixth day was taken as the final result.

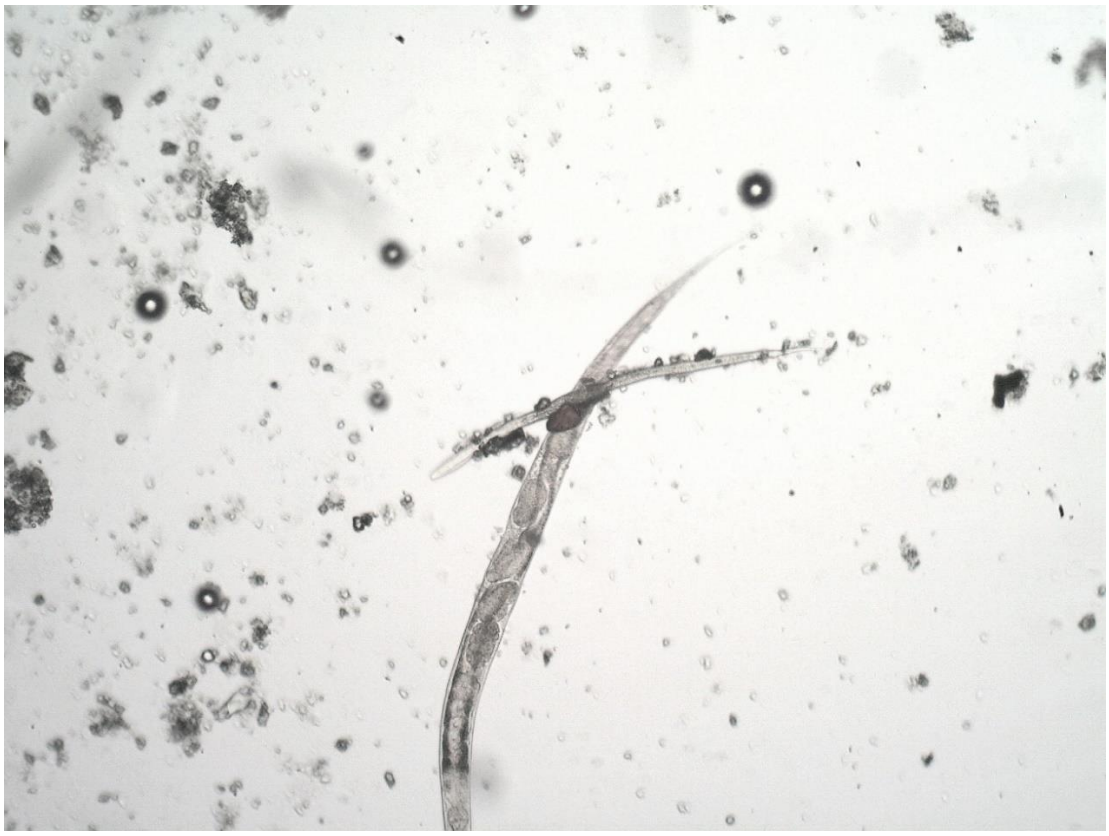

Figure 12: S. stercoralis, rhabditiform larva and adult female worm, modfied Harada-Mori culture (Own source, inverted transmitted-light microscope, total magnification 200x)

2.2.3 Immunochromatographic rapid assay

The commercially available faecal antigen immunochromatographic rapid assay RIDA ® Quick Combi in a single cassette format for *Cryptosporidium parvum* and / or *Giardia lamblia* and / or *Entamoeba histolytica* -sensu lato- was directly performed with fresh faeces according to the protocol provided by R-Biopharm.

In the single-step rapid assay, antibodies which are directed against the three pathogens attach to blue, green and red coloured latex particles.

1ml extraction buffer diluent was placed in a labelled test tube. When the stool sample was solid, 50 mg of the sample was placed in the buffer. When it was liquid, 100 µl was taken with a pipette. The sample was homogenised by repeated suction and ejection of the suspension using a disposable pipette. The suspension was left to precipitate for 3 min. 200-500 µl of the clear supernatant was transferred into another clean tube. The test strip from the test tube was removed, and the test was immersed into the prepared sample tube. After removing the test, the result was visible after 10 min with green, red and/ or blue bands and one purple crimson control band for quality control.

As stated by the company, none of the following parasites led to cross reactivity: *Entamoeba coli, Blastocystis hominis, Chilomastix mesnili, Endolimax nana, Entamoeba nana, Entamoeba hartmannii, Hymenolepsis nana, Isospora belli, Isospora felis* or *Jodamoeba buetschlii* (AG 2010).

2.2.4 Microscopy

About three spoonfuls, equivalent to about 0.5-1ml/ 0.5-1 ccm of every fresh stool sample was directly preserved with 10 % formalin solution. The faeces containers were shipped to the Medical Mission Institute in Würzburg and microscopically examined after processing with a concentration technique.

Concentration techniques as in the preparation of fresh stool samples are commonly used for microscopy of intestinal parasites. The FPC® Fecal Parasite Concentrator by Evergreen Scientific is a commercially available concentration method as a modification of the formalin-ether method of Ritchie (Knight, Hiatt

et al. 1976) and was developed at the National Institutes of Health in Bethesda, Maryland.

One spoonful (about 200 mg) of the formalin-preserved stool sample was added to a flat bottomed tube and mixed thoroughly. Three drops of Triton X and 3 ml of ethyl acetate were added. The FPC strainer and centrifuge tube wereattached and shaken vigorously. The tubes are connected with an interconnected strainer. The 0.6x0.6 mm holes allow parasites to pass but retain most of faecal debris. The conical end was pointed downward and the specimen was poured through the strainer into the centrifuge tube. This was capped and centrifuged at 500 xg for 10 min. The supernatant was extracted, and 2-3 drops of 10% formalin were added to the deposit and mixed. One microscope slide was prepared with about 0.2 ml from the sediment using 24x32 mm cover slips. (Evergreen , Knight, Hiatt et al. 1976, Zaglool, Mohamed et al. 2013)[11]

Microscopy with a binocular microscope was done at 100-fold and 400-fold magnification, and the whole slide was screened systematically. In cases of uncertainty of visible helminths and protozoa, additional expertise was sought from experienced laboratory assistants of the BMC in Tanzania and of the Medical Mission Institute in Germany.

The main focus of interest was the evaluation of *S. stercoralis* larvae, *G. lamblia* and *Entamoeba* cysts. Furthermore, detection of other helminths and protozoa was noted. The presence of intestinal parasites was assessed qualitatively (positive or negative) and not by number of larvae or cysts, as the applied method does not allow reliable quantification.

Microscopy was repeated if both the immunochromatographic rapid assay and the real-time PCR showed a positive result for *G. lamblia* or *E. histolytica/ dispar*. In cases where both faecal culture tests for *S. stercoralis* were positive, a second microscopic examination was performed unless larvae were detected during the first microscopy. In samples with positive *S. stercoralis* qPCR results,

[11] The whole paragraph is based on these references

up to three microscope slides from one FPC process were screened if no larvae were detected so far.

As sensitivity for the detection of *S. stercoralis* larvae with concentration techniques is known to be low (Anamnart, Intapan et al. 2013, Buonfrate, Formenti et al. 2015), repeated microscopy was used to increase the sensitivity.

The differentiation between hookworm and *S. stercoralis* larvae was eased by preserving fresh stool within a few hours after defecation. In freshly preserved specimen, hookworm larvae were still not hatched, and the eggs were visible, whereas *S. stercoralis* were already visible as larvae. Because of the known low sensitivity for *S. stercoralis* larvae, no effort was made to complete a quantitative analysis of larvae.

It is not possible to differentiate between *E. histolytica* and *E. dispar* by microscopy, so microscopy results were only intended to detect *Entamoeba* sensu latu.

2.2.4.1 *C. parvum*

C. parvum is usually missed by the microscopy of unstained stool samples. Therefore, additional laboratory tests were performed. In samples with positive faecal antigen tests for *C. parvum* the modified Ziehl-Neelsen acid fast staining method was additionally conducted as well as microscopy under high power magnification with oil immersion lenses applied. Faecal smears from stool sediment obtained by the FPC concentration method were prepared on microscope slides. These were dried in air, fixed with 95% methanol for 5 min and dried in air again. The slides were flooded with a diluted carbol fuchsin staining solution for 20-25 min and afterwards were carefully rinsed under tap water. Acid alcohol (3 % hydrochloric acid in 95 % ethyl alcohol) was added for 20-30 sec for decolourization, and the slides were then rinsed again under tap water. 1 % Methylene blue solution was added for 2-3 min for counter-staining. The slides were rinsed under tap water and then air dried. After this staining process, the slides were examined microscopically with a drop of immersion oil

at 1000-fold magnification. *C. parvum* oocytes are visible in bright red on a blue background. (Zaglool, Mohamed et al. 2013)[12]

2.2.5 Real-time PCR

Prior to DNA extraction, a washing procedure was performed. The ethanol-preserved stool samples were washed three times with PBS in the following manner: PBS buffer was blended with 9.55 g of PBS granulate per litre of water. Sample tubes were centrifuged for 1 min at 10.000 rpm. Then the ethanol supernatant was pipetted and discarded. 1 ml PBS was added, and the tube was vortexed and centrifuged again for 1 min at 10.000 rpm. This procedure was performed three times, and then an amount of about 200 mg of stool was used for DNA isolation.

The commercial QIAamp DNA stool Mini Kit was used for DNA isolation. The procedure was performed following the handbook protocol provided by Qiagen:

https://www.qiagen.com/us/search/qiaamp-dna-stool-mini-kit/#resources

About 200 mg of the stool sample was filled into to a tube and mixed with 2 ml of InhibitEX Buffer. After vortexing for 1 min, 1.2 ml of the stool lysate was pipetted into a 2 ml microcentrifuge tube and heated for 5 min at 70 °C. This heating is necessary for lysis of the parasites. After vortexing, the tube was centrifuged at 14.000 rpm for 1 min. 200 µl of the supernatant was added to a new tube filled with 15 µl of proteinase K. 200 µl of Buffer AL was added, and the tube was vortexed for 15 sec. This was followed by incubation at 70 °C for 10 min. 200 µl of ethanol was then added to the tube and was mixed by vortexing. 600 µl of the lysate was pipetted to the QIAamp spin column and centrifuged for 1 min. The spin column was placed into a new 2 ml collection tube and opened. 500 µl of Buffer AW1 was carefully added and centrifuged for 3 min. The spin column was again placed in a new collection tube, and the same procedure was done with Buffer AW2. The spin column was laced again into a new collection tube and centrifuged for 3 min. Then it was transferred into a new 1.5 ml microcentrifuge tube and 200 µl of Buffer ATE was pipetted onto

[12] The whole paragraph is based on this references

the QIAamp membrane. After incubation for 1 min at room temperature it was centrifuged at 14.000 rpm for 1 min to elute DNA.

As declared by Qiagen, DNA yield may range from 5-100 µg and is typically 10-50 µg. DNA concentration is typically 50-250 ng/µl, and the eluted DNA is up to 20 kb long. The eluted DNA is directly suitable for use in PCR. The samples were kept at -20 °C for storage.

DNA concentration in the DNA isolation samples was measured for one out of 8 samples, so in total for 20 samples.

Concentration was measured by the Qubit 2.0 Fluorometer with the Qubit ds DNA broad range assay. The procedure was performed according to the Qubit dsDNA BR Assay Kit protocol provided by Thermo Fisher Scientific (Scientific 2015). Primers and probes for all real-time PCR performed were produced by Eurofins Genomics GmbH, Ebersburg, Germany.

2.2.5.1 *S. stercoralis* qPCR

Real-time PCR for *S. stercoralis* was performed according to the method described by Verweij et al (Verweij, Canales et al. 2009).

The TaqMan™ Gene Expression Master Mix was used for PCR.

Each qPCR reaction tube contained 25 µl DNA elution:

- 12.5 µl TaqMan™ Gene Expression Master Mix
- 2.5 µl IPC Mix
- 0.5 µl IPC DNA
- 1.25 µl PPMix
- 5.75 µl A. dist.
- 2.5 µl sample DNA/ *S. stercoralis* control DNA/ A. dist./ IPC blocked

The TaqMan™ Gene Expression Master Mix contains AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure), Uracil-DNA Glycosylase (UDG), deoxyribonucleotide triphosphates (dNTPs) with deoxyuridine triphosphate (dUTP), ROX™ Passive Reference, and buffer components.

IPC Mix is the TaqMan™ Exogenous Internal Positive Control reagent, which is necessary to distinguish true target negatives from PCR inhibition and also contains an IPC blocking enzyme.

The PPMix contains two *Strongyloides* specific primers and one probe in between. The primers are 18S rRNA gene sequences from the cytochrome c oxidase subunit I gene (GenBank accession no. AF279916). DNA sequences and specifications of the *S. stercoralis* specific primers and probe are given in *Table 2: S. stercoralis primers and probe*.

Table 2: S. stercoralis primers and probe

	S. stercoralis 18S rRNA gene (AF279916)
Stro18S-1530F	5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3'
Stro18S-1630R	5'-TGCCTCTGGATATTGCTCAGTTC-3'
Stro18S-1586T	FAM-5'-ACACACCGGCCGTCGCTGC-3'-BHQ1

For 66.6 µl PPMix, 3.33 µl of each primer (13.32 pmol) and 1 µl of the probe (4 pmol) and 58.94 µl of A. dist. were mixed.

For each qPCR run with 48 wells, two internal positive controls, two negative controls and one *S. stercoralis* positive control were set up. For internal positive control, 2.5 µl of A. dist. was added, since every sample contains IPC Mix. For internal negative control, 2.5 µl of IPC blocked was added. For *S. stercoralis* positive control, 2.5 µl of *Strongyloides* DNA (Swiss TPH, Basel, Switzerland) in 100-fold dilution was added.

For study sample testing, 2.5 µl of the respective DNA isolation sample was added, so 43 samples were tested in every run.

The amplification process consisted of 30 sec at 60 °C, 2 min at 50 °C and 10 min of 95 °C followed by 50 cycles of 15 sec at 95 °C and 1 min at 60 °C. Amplification, detection and data analysis was performed using the Applied Biosystems StepOne Real-Time PCR system (Applied Biosystems, Life Technologies) with 48 wells and running the StepOne Software.

2.2.5.2 *G. lamblia*

Real-time PCR for Giardia was performed according to the method described by Haque et al (Haque, Roy et al. 2007).

Each qPCR reaction tube contained 25 µl DNA elution:

- 12.5 µl TaqMan™ Gene Expression Master Mix
- 2.5 µl IPC Mix
- 0.5 µl IPC DNA
- 1.25 µl PPMix
- 5.75 µl A. dist.
- 2.5 µl sample DNA/ *Giardia* control DNA/ A. dist./ IPC blocked

The PPMix contains two *G. lamblia* specific primers and one probe. The primers and probe were designed from the small subunit ribosomal RNA gene (GenBank accession no. M54878). DNA sequences and specifications of the *G. lamblia* specific primers and probe are given in *Table 3: G. lamblia primers and probe*.

Table 3: G. lamblia primers and probe

	G. intestinalis SSU **rRNA gene (M54878)**
Gd-80F	5'-GACGGCTCAGGACAACGGTT-3'
Gd-127R	5'-TTGCCAGCGGTGTCCG-3'
Gd-FT	FAM-5'-CCCGCGGCGGTCCCTGCTAG-3'-BHQ1

For 66.6 µl PPMix, 3.33 µl of each primer (13.32 pmol) and 1 µl of the probe (4 pmol) and 58.94 µl of A. dist. were mixed.

For each qPCR run with 48 wells, two internal positive controls, two negative controls and one *Giardia* positive control were set up. For internal positive control, 2.5 µl of A. dist. was added, since every sample contains IPC Mix. For internal negative control, 2.5 µl of IPC blocked was added. For *Giardia* positive control, 2.5 µl of *Giardia* DNA (BNI Hamburg, Germany) in 100-fold dilution was added.

Amplification was comprised of 30 sec at 60 °C, 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Finally, a Post-PCR-Read cycle was run for 30 sec at 60 °C. Amplification, detection and data analysis were performed using the Applied Biosystems StepOne Real-Time PCR system (Applied Biosystems, Life Technologies) with 48 wells running the StepOne Software.

2.2.5.3 *Entamoeba*

The Real-time PCR for *E. dispar* and *E. histolytica* was performed as described by Verweij et al (Verweij, Oostvogel et al. 2003).

The qPCR for *E. dispar* and *E. histolytica* differed only in probes. Beside this, qPCR for *E. dispar* and for *E. histolytica* were performed in the same way, and all procedures were done for both *Entamoeba* species.

Each qPCR reaction tube contained 25 µl DNA elution:

- 12.5 µl TaqMan™ Gene Expression Master Mix
- 2.5 µl IPC Mix
- 0.5 µl IPC DNA
- 1.25 µl PPMix
- 5.75 µl A. dist.
- 2.5 µl sample DNA/ *Entamoeba* control DNA (BNI Hamburg) / A. dist./ IPC blocked

The PPMix contains two *Entamoeba*-specific primers and one probe targeting a species-specific sequence of the PCR product in between. The primers are small subunit ribosomal RNA sequences (GenBank accession no. X64142). DNA sequences and specifications of the *Entamoeba* specific primers and probes are given in *Table 4: E. histolytica and E. dispar primers and probes.*

Table 4: E. histolytica and E. dispar primers and probes

	Entamoeba SSU rRNA gene (X64142)
Ehd-239F	5′-ATTGTCGTGGCATCCTAACTCA-3‘
Ehd-88R	5′-GCGGACGGCTCATTATAACA-3′
Eh-96T-Probe	FAM-5‘-TCATTGAATGAATTGGCCATTT-3‘-BHQ1
Ed-96T-Probe	FAM-5‘-TTACTTACATAAATTGGCCACTTTG-3‘-BHQ1

For 80 µl PPMix, 20 µl of each primer (1.25 pmol) and 6 µl of the probe (0.375 pmol) and 34 µl of A. dist. were mixed.

For each qPCR run with 48 wells, two internal positive controls, two negative controls and one *Entamoeba* positive control were set up. For internal positive control, 2.5 µl of A. dist. was added, since every sample contained IPC Mix. For internal negative control, 2.5 µl of IPC blocked was added. For *Entamoeba* positive control, 2.5 µl of *Entamoeba* DNA (BNI Hamburg, Germany) in 1000-fold dilution was added.

For study sample testing, 2.5 µl of respecting DNA isolation sample was added. 43 samples were tested in every run.

Amplification was comprised of 30 sec at 60 °C, 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Finally, a Post-PCR-Read cycle was run for 30 sec at 60 °C. Amplification, detection and data analysis were performed using the Applied Biosystems StepOne Real-Time PCR system (Applied Biosystems, Life Technologies) with 48 wells running the StepOne Software.

2.2.6 Serum ELISA

For the serum analysis, blood samples were taken with Sarsted safety multifly needles 23 G connected with a multi-adapter to S-Monovette. The blood samples were centrifugated at 3000 rpm for 10 minutes. Serum was removed with pipettes into two 2 ml Sarsted CryoPure tubes and stored in a refrigerator at -20 °C.

The ELISA was performed after arrival of the specimen at the collaborative laboratory of the Bernhard-Nocht-Institut in Hamburg, Germany as this analysis was not available in Tanzania or at the laboratory of the Medical Mission Institute in Würzburg.

According to personal communication with Prof. Dr. Dennis Tappe of BNI, serum analysis was done according to the following protocol:

“An in house ELISA developed by the Bernhard Nocht Institute for tropical medicine in Hamburg/ Germany was used. The in house ELISA is based on Strongyloides ratti antigens. Strongyloides ratti is hereby kept in a laboratory cycle.

The larvae gained from rats faeces are washed in PBS and afterwards lyophilized, grinded [sic] and re-suspended in distilled water. A sonication with ultrasound in pulse mode follows for eight times each for three seconds. This is in total repeated three times. In the next step an ultracentrifugation (30000 RPM) was done. The supernatant, an aqueous extract is used in a concentration of 0.5 µg per ELISA-plate. Annealing of the antigens takes place over night [sic] incubated at room temperature.

A washing with PBS/ 0.5% TWEEN and blockade over one hour with PBS/ 3% skim milk powder at room temperature followed. Afterwards a washing in PBS/ 0.05% TWEEN was done again.

The serum of the patients which was diluted with PBS/ 3% skim milk powder in a concentration of 1:100 was incubated for 45 min at room temperature and then washed with PBS/ 0,05% TWEEN.

The following step is an incubation process with anti human Ig G alkaline phosphatase-conjugate, which is obtained from goats in a concentration used in 1:10000, for 45 minutes at room temperature. Then P-nitro-phenyl phosphate is added and a photometric measurement at 410 nm with a reference filter at 490 nm is done.

Elevated antibody titres in the in house ELISA indicating a *Strongyloides stercoralis* infection were positive in 45 of 277 cases, as one sample was missing and therefore showed a percentage of 16,2%.“(van Eckert 2019)

ELISA results were considered slightly positive when 10-20 arb. units and positive when ≥ 20 arb. units. The cut-off was <10 arb. units.

2.3 **Material List**

Carbol fuchsin dilution	Merck & Co., Inc. Kenilworth, NJ, USA
Cardboard storage boxes	A. Hartenstein GmbH, Würzburg
Centrifuge tubes PP, conical, with screwed lid, 50 ml	Grainer GBO, Frickenhausen
Chromatographie Papier 195g/m²	A. Hartenstein GmbH, Würzburg
Cover slip 21 x 26 mm	Carl Roth, Karlsruhe
Cover slip, round, 10 mm	Carl Roth, Karlsruhe
CryoBox	A. Hartenstein GmbH, Würzburg
CryoPure tube 5.0 ml (PP) with QuickSeal screw cap	Sarstedt, Nürnbrecht
Disposable pasteur pipettes, graduated	Carl Roth, Karlsruhe
Disposable petri dishes 90mm	A. Hartenstein GmbH, Würzburg
Ethanol absolut	AppliChem, Darmstadt
Faeces container 107 x 25 mm with screw tab and spoon	Sarstedt, Nürnbrecht
Filter paper 580 x 580 mm	A. Hartenstein GmbH, Würzburg
Filter tip for Eppendorf pipette 10-100 µl, 0.5-10 µl	Eppendorf, Hamburg
FPC® Fecal Parasite Concentrator, Classic	Evergreen Scientific, Los Angeles, California
HCl-Alk	Merck & Co., Inc. Kenilworth, NJ, USA
Methanol 95%	AppliChem, Darmstadt
Methylene blue	Merck & Co., Inc. Kenilworth, NJ, USA
MicroAmp Fast Reaction Tube with cap	Applied Biosystems,

	Thermo Fisher Scientific
Mueller-Hinton agar	Sigma-Aldrich, Munich, Germany
Object plate 76 x 26 mm	Carl Roth, Karlsruhe
ParaFilm M, 1 role 4 inch x 250 feet	Bemis Company
Phosphate buffered saline	AppliChem, Darmstadt
Pipette tip for Eppendorf pipette 5-200 µl, 100-1000 µl	Eppendorf, Hamburg
Primers and probes for real-time PCR	Eurofins Genomics GmbH, Ebersberg, Germany
QIAamp DNA stool Mini Kit	Quiagen, Hilden
Qubit dsDNA BR Assay Kit	lifetechnologies, Thermo Fisher Scientific
Reaction tube with lid 1.5 ml, 2 ml	A. Hartenstein GmbH, Würzburg
RIDA®QUICK Cryptosporidium/Giardia/Entamoeba Combi dipsticks	R-Biopharm AG, Darmstadt
Safety-Multifly®-needle 23G with a long tube and assembled Multi-Adapter	Sarstedt, Nürnbrecht
S-Monovette® 7.5 ml, Clotting Activator/Serum, 92x15 mm	Sarstedt, Nürnbrecht
TaqMan™ Exogenous Internal Positive Control Reagents	lifetechnologies, Thermo Fisher Scientific
TaqMan™ Gene Expression Master Mix	lifetechnologies, Thermo Fisher Scientific
Va-Q-Tec® packaging system	Va-Q-Tec, Würzburg

2.4 **Instruments**

2.0 Mp USB Microscope camera in Tanzania	No-name product
Applied Biosystems StepOne Real-Time PCR system, 48 wells	Applied Biosystems, Thermo Fisher Scientific
Dry Block Heating Thermostat Bio TDB-100	Biosan, Riga, Latvia
Hettich Centrifuge	Hettich Lab Technology, Tuttlingen
Inverted microscope in Tanzania	Will / Hund, Wetzlar
Novex P20 Stereo microscope	Euromex Microsopes, Arnhem, The Netherlands
Olympus CH2 microscope	Olympus, Hamburg
Olympus CX31 microscope	Olympus, Hamburg
Olympus microscope camera CC12	Olympus, Hamburg
Primo Star Zeiss, LED microscope	Zeiss, Oberkochen
Qubit 2.0 Fluorometer	lifetechnologies, Thermo Fisher Scientific
Vortex Genie	Bender & Hobein AG, Munich

2.5 **Software**

Applied Biosystems StepOne Software	Applied Biosystems, Thermo Fisher Scientific
Endnote X8	Clarivate Analytics, Boston, Massachusetts, USA
IBM SPSS Statistics 24	IBM, Armonk, New York, USA
Microsoft Excel 2010	Microsoft Corporation, Redmond, Washington, USA
Microsoft Word 2010, Microsoft Word 365	Microsoft Corporation, Redmond, Washington, USA
GIMP 2.10.24	Free Software Foundation, Inc., Boston, USA

3 Results

Overall 191 children were enrolled during the study time. 153 (80.1 %) of these submitted a stool sample and questionnaire and were consequently included in the results of the study. Although some of the questions were not completed in all questionnaires, they were included in the analysis if the missing data did not exceed 10 %. Less than 90 % of the questionnaires contained information about weight and size. Therefore, these parameters were not included in the analysis.

3.1 Process and feasibility

After enrolment of the patients, the questionnaires were completed by a Swahili-speaking nurse. Information about the presumptive diagnosis and treatment of the patients was taken from the clinical file.

The parents of the patients submitted the stool samples and further diagnostic was done in a laboratory in Mwanza. The Harada-Mori culture, agar plate culture and rapid test were done locally. Preserved stool samples were also later shipped to Würzburg, Germany. Further work with the FPC® concentration method and microscopy as well as the real-time PCR was done there.

Blood samples were prepared and frozen. Shipment of the refrigerated specimens was done in special insulated containers (Va-Q-Tec®) with freeze packs, allowing maintenance of –20 °C for 72 h and transport to the the BNI in Hamburg, Germany for further diagnostics.

3.2 Population profile

The results of the questionnaire about sociodemographic and clinical data of the study group are shown in *Table 5: Descriptive analysis of sociodemographic and clinical data*.

The average age of the children was 4.9 years with a range from 1-14 years. 57.2 % (n=87) of the children were boys and 42.8 % (n=65) were girls.

Regarding the area of residence, 56.2 % reported to live in an urban region, while the remaining part lived in a rural region. Concerning domestic hygiene,

61 % used a latrine as a toilet and 3.3 % used the bush, whereas the rest used a flush toilet at home. This means that less than the half of the children had access to a flush toilet. Still, 65.8 % had access to tap water, and 60.5 % used a well. 78.9 % used the river, and 1.3 % used a spring as their water source. This means that many children had no access to safe and clean water. As many used several different water sources, many combinations of answers were possible and the sum is more than 100 %.

The profession of the parents was mainly listed as farmer, comprising 61.4 %. Other reported professions were housewife at 24.2 %, any kind of business at 6.5 % and other professions at 7.8 %. These other professions were all indoor professions such as pastor, teacher or nurse. Almost all parents reported their children always wore shoes, and only 1.4 % reported waring shoes often. This high number might be due to social' desired answers. Reality showed that many children often ran around without shoes especially when playing outside.

Questionnaire results and clinical files investigations revealed that fever (n=92; 62.2 %), abdominal pain (n=37; 25 %), cough (n=33, 22.3 %) and vomiting (n=32; 21.6 %) were the most common symptoms. The reason of admission or final diagnosis was not always reported by the parents and often had to be looked up in the clinical files. It was difficult to sum the reported symptoms and suspected diagnosis into one diagnosis since there were often several overlapping symptoms with unclear diagnose at the point of inclusion into the study. Even so, a diagnosis or leading symptom was documented in 94.1 % of the children. The results show a wide variety of symptoms and diagnoses in the included study population. In summary, 58.3 % can be classified as internal diagnose. This includes 14 children (9.4 %) with acute gastrointestinal diseases as well as diagnoses such as malaria (n=20; 13.1 %) and fever of unknown origin (n=15; 9.8 %). There were also diagnosed or suspected cases of sickle cell disease, cardiac diseases, malnutrition and tuberculosis, but less then ten cases of each. In 32.7 % (n=50) a surgical diagnosis was the reason of admission. This included a trauma, often with a fracture in 23 cases (15 %). Other surgical diagnoses included burn (n=8; 5.2 %) or urological diseases (n=9; 5.9 %). A neurological disease was the reason of admission in ten cases

(n=10; 6.5 %). This meant seizures or diagnosed epilepsy in five children, sometimes combined with fever or other symptoms.

23.5 % (n=36) of the children had received antihelminthic drugs within the previous twelve months either as regular deworming or as therapy. The drugs included albendazole (n=17), mebendazole (n=7), praziquantel (n=1) and metronidazole (n=8). In three cases, the parents did not know the name of the substance, and it was not documented in the files. The children did not receive antihelminthic drugs in 73.2 % (n=112) and in five cases it was unknown.

Concerning the immune status, the question of performed HIV-testing was not particularly helpful. Of those who answered the question, eight reported a testing, and this was positive in two children (2.1 %). In 93.8 % (n=135), no testing was ever done. The questions about corticosteroid therapy and other immunosuppressive drugs were rarely answered and were therefore excluded from the result analysis.

The parameters “weight“ and “height” had to be omitted from the analysis due to incomplete recording of the data.

Table 5: Descriptive analysis of sociodemographic and clinical data

Variables	**n**	**%**
Gender		
Male	87	57,2
Female	65	42,8
Age*		
1-5	91	59,9
6-14	61	40,1
*median: 4, mean: 4,9 ± 3,255		
Living region		
Urban	86	56,2
Rural	67	43,8
Access to toilets		
Flush toilet	54	36
Latrine	91	61
Bush	5	3,3
Access to water (multiple answers possible)		
Tab water	100	65,8
Spring	2	1,3
River	120	78,9
Well	92	60,5
Profession of parents		
Farmer	94	61,4
Fisher	0	0
Housewife	37	24,2
Business	10	6,5
Other	12	7,8
Wearing shoes		
Always	146	98,6
Often	2	1,4
Diagnosis		

Internal	84	58,3
GIT, acute	14	9,7
Neurological	10	6,9
Surgical	50	34,7
Symptoms		
Diarrhoea	17	11,5
Vomiting	32	21,6
Abdominal pain	37	25
Flatulence	18	12,2
Fever	92	62,2
Cough	33	22,3
Skin lesion	0	0
Antihelminthic therapy within last 12 months		
Mebendazole	7	4,7
Albendazole	17	11,5
Praziquantel	1	0,7
Metronidazole	8	5,4
Unknown substance	3	2
HIV-test		
positive	2	2,1
negative	6	4,2
no testing	135	93,8

3.3 **Prevalence of *S. stercoralis***

The prevalence of *S. stercoralis* was analysed with different methods: Koga agar plate, modified Harada-Mori-technique, faecal real-time PCR, serum ELISA and FPC® concentration method with microscopy. *Table 6: Prevalence S. stercoralis* and *Figure 13: S. stercoralis faecal diagnostics* show the results of detected prevalence with the different diagnostic techniques. Calculation was done based on a sample group of n=153 except when mentioned explicitly, such as in Harada-Mori culture and in serum ELISA.

Table 6: Prevalence S. stercoralis

	HM	AP	PCR	Microscopy	ELISA
valid cases (n)	138	153	153	153	51
positive (n)	8	11	3	2	10
positive (%)	5,8	7,2	2,0	1,3	19,6
missing (n)	15	0	0	0	102

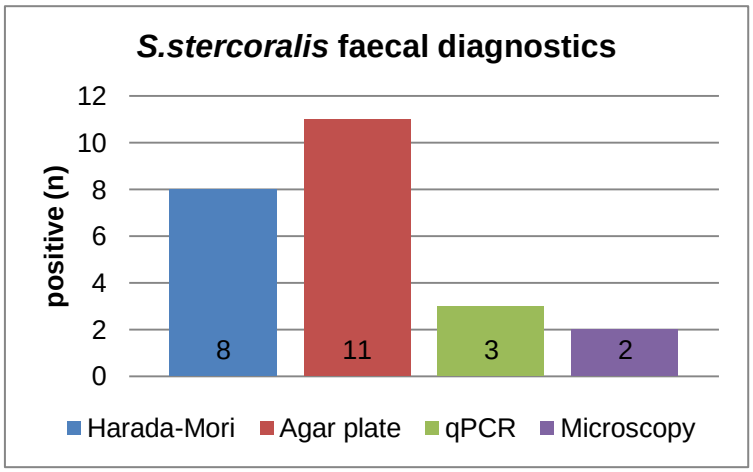

Figure 13: S. stercoralis faecal diagnostics

3.3.1 Harada-Mori culture

In the modified Harada-Mori culture technique, none of the samples was positive in the first examination after 2-3 days. Upon second examination after six days, eight samples (n=8/137, 5.2 %) showed a positive result. Adult *S. stercoralis* are shown in *Figure 14: Adult free living S. stercoralis with visible oesophagus, modified Harada-Mori Culture* and *Figure 15: Adult free living female S. stercoralis, modified Harada-Mori Culture.*

Eight of the cultures were dirty in the first examination and seven more cultures in the second examination. Thus it was not possible to evaluate these 15 contaminated samples, and the percentage was calculated based on n=137.

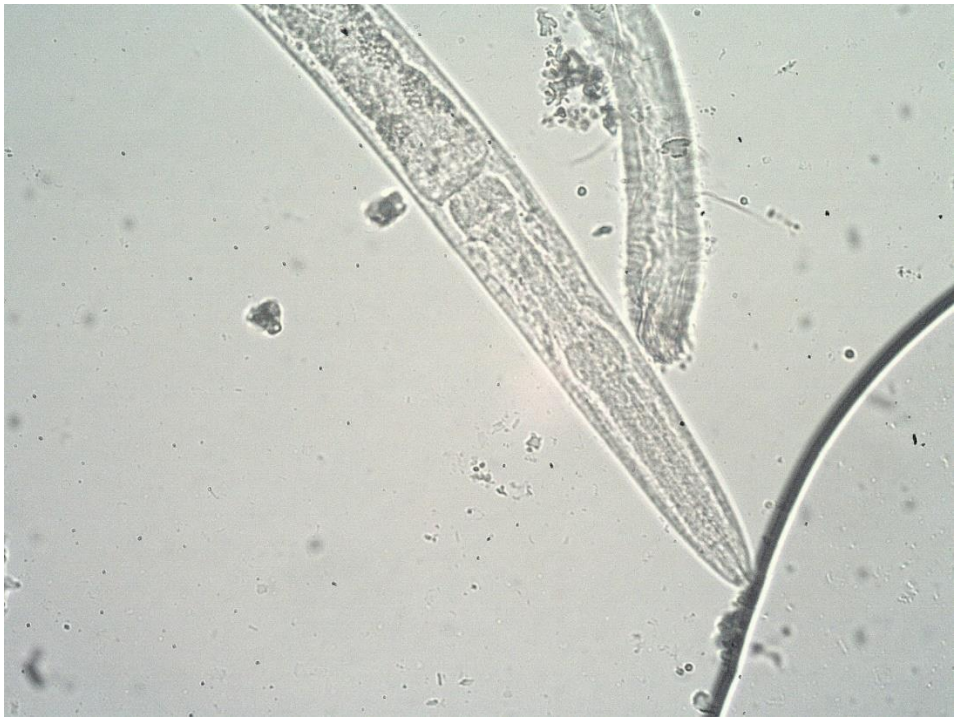

Figure 14: Adult free living S. stercoralis with visible oesophagus, modified Harada-Mori Culture (Own source, transmitted-light microscope, total magnification 1000x)

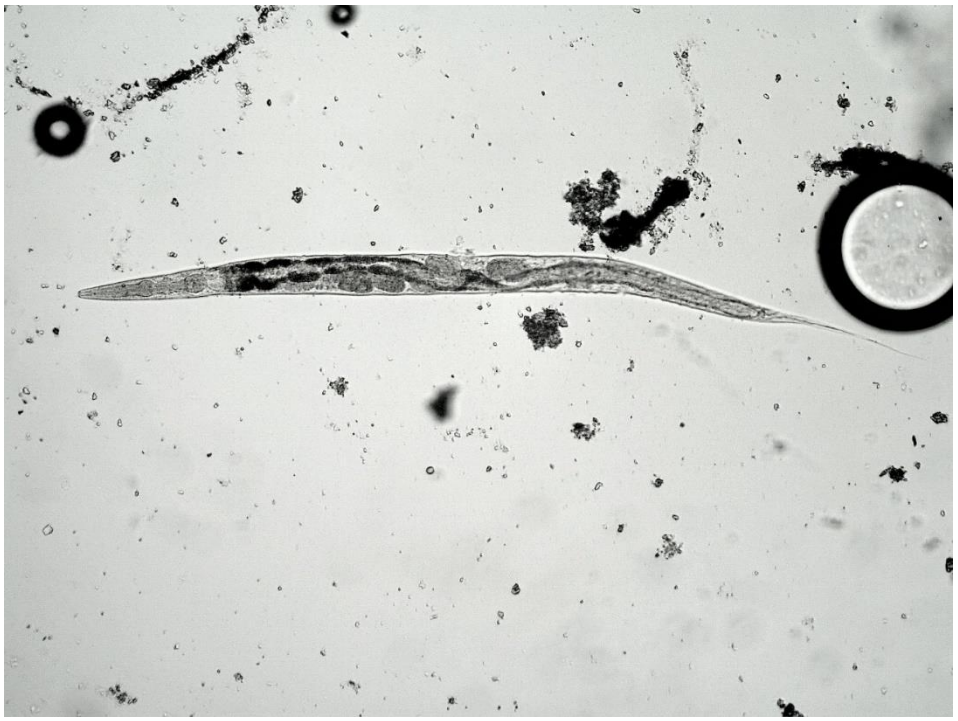

Figure 15: Adult free living female S. stercoralis, modified Harada-Mori Culture (Own source, inverted transmitted-light microscope, total magnification 200x)

3.3.2 Agar plate culture

Positive result in the agar plate cultures were visible after one day in two cultures, after two days in six cultures and after three days in eleven cultures (n=11, 7.2 %). Adult S. stercoralis on agar plate cultures can be seen in *Figure 16: Filariform S. stercoralis larvae, Agar plate culture*. The positive results were consistent, as none of the once positive cultures were negative on the following days.

As shown in *Table 7: Agar plate - Harada-Mori culture*, two samples among the positive samples in Agar plate culture were dirty, and four samples showed a negative result in Harada-Mori culture. Five samples were positive in Harada-Mori culture as well as in agar plate culture.

The two samples with positive results after 24 h showed no positive result in qPCR. One of the samples showed a hookworm egg in the FPC® material-based microscopy.

Table 7: Agar plate - Harada-Mori culture

		AP		
		neg	positive	sum
HM	neg	126	4	130
	positive	3	5	8
	dirty	13	2	15
sum		142	11	153

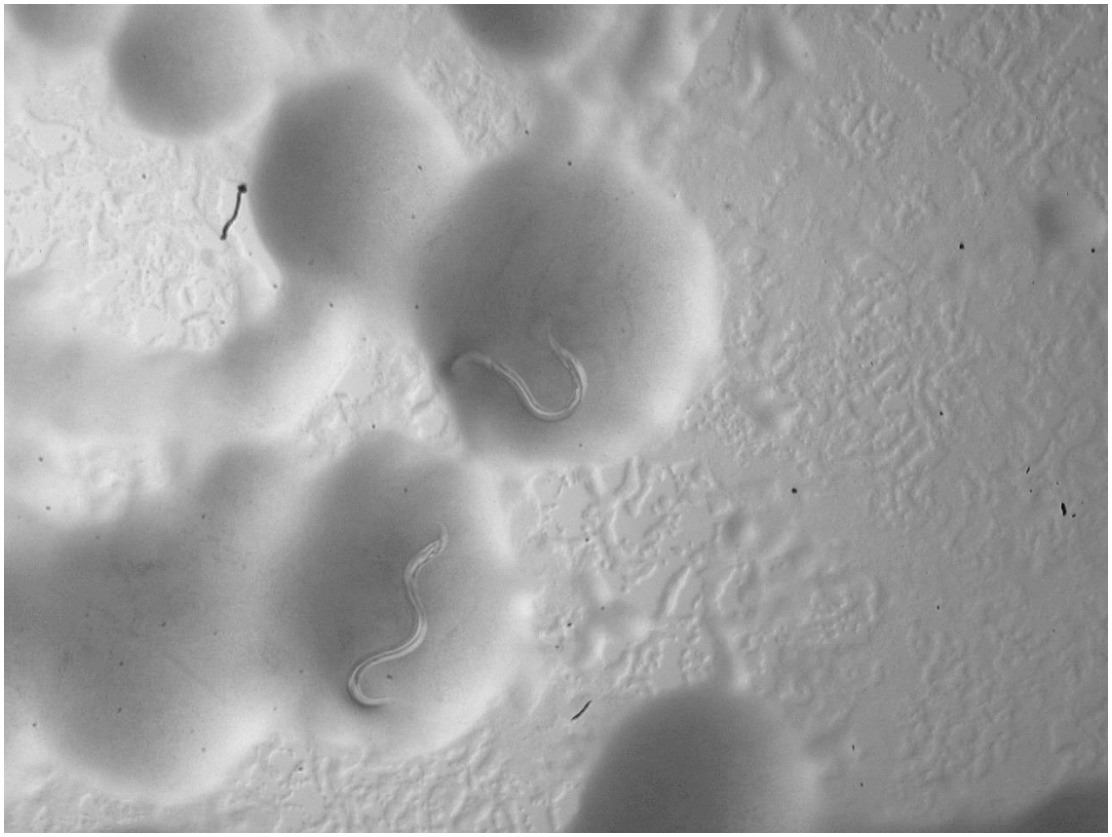

Figure 16: Filariform S. stercoralis larvae, Agar plate culture (Own source, Stereo microscope, total magnification 40x)

3.3.3 PCR

Three samples (n=3; 2 %) showed a positive result in the faecal real-time PCR. Two of them were also positive in Harada-Mori culture as well as in agar plate culture and microscopy. One of the qPCR positive samples showed no positive result in any of the other *S. stercoralis* detection methods. *Table 8: S. stercoralis PCR compared to HM, AP, microscopy* shows the distribution of PCR results and other *S. stercoralis* detection methods.

Table 8: S. stercoralis PCR compared to HM, AP, microscopy

Legend: - = negative
+ = positive

	Result in			
n	HM	AP	micro	PCR
2	+	+	+	+
3	+	+	-	-
3	+	-	-	-
6	-	+	-	-
1	-	-	-	+
138	-	-	-	-

3.3.4 Microscopy

One *S. stercoralis* larva was visible in the first round of FPC® material based microscopy of all samples. This sample was also positive in the qPCR as well as in Harada-Mori and agar plate culture.

A reassessment with additional slides was performed on positive samples in two of the other tests. No *S. stercoralis* larvae were visible in these five reexamined samples, but a hookworm egg was detected in one sample. Another reassessment was done with the two positive qPCR samples that had previously shown no *S. stercoralis*-positive result using microscopy. Even two larvae were visible in one of these samples. In that sample, a hookworm egg had also been previously detected microscopically. Both positive results under microscopy (n=2, 1.3 %) were also positive in qPCR, agar plate and Harada-Mori culture.

During microscopy, particular attention was given to hookworm eggs, since larvae in faecal cultures can be easily confused with *S. stercoralis* larvae. All six samples were detected positive for hookworm eggs using microscopoy. As shown in *Table 9: Hookworm positive,* three of the six hookworm egg-positive samples were also positive in *S. stercoralis* faecal culture. One of the

hookworm-positive samples was positive for *S. stercoralis* as well as for hookworm in microscopy. This shows not only the eventuality of mistaken larvae identification but also the possibility of polyhelminthic infection.

Table 9: Hookworm positive

Legend: *Strongy* = *S. stercoralis* positive
neg = negative
Hook = Hookworm positive
n.v. = not visible

Harada-Mori		Koga Agar Plate			Strongy	microscopy	microscopy 2	microscopy 3
2-3d	6d	24h	48h	72h	PCR			
neg	*Strongy*	neg	*Strongy*	*Strongy*	neg	**Hook**		
n.v.	n.v.	*Strongy*	*Strongy*	*Strongy*	neg	**Hook (many)**		
neg	*Strongy*	neg	neg	*Strongy*	*positive*	neg	**Hook**	*Strongy*
neg	neg	neg	neg	neg	neg	neg	**Hook**	
neg	n.v.	neg	neg	neg	neg	**Hook**		
neg	neg	neg	neg	neg	neg	**Hook**		

3.3.5 ELISA

Blood samples are necessary for the *S. stercoralis* serum ELISA. However, only 51 blood samples (n = 51/153, 33.3 %) could be obtained because many parents rejected a blood withdrawal. Therefore, a conclusion concerning this method is difficult. Calculation of percentage is based on n=51. Results ranged from 0-121 arb. units and positivity was assumed when the result was ≥ 20 arb. units. As shown in *Table 10: S. stercoralis ELISA*, ten (n=10, 19.6 %) samples showed a positive result, and six (n=6, 11.8 %) samples showed a borderline positive result. Out of the 15 children with a positive result in any of the other *Strongyloides* detection methods, a blood sample was available for only six children. Only two of those six ELISA were also assessed as positive. None of the other *Strongyloides* detection methods showed a consistent result among the other eight positive and six borderline positive ELISAs.

None of the children with qPCR positive result submitted a blood sample, so no information about the correlating serology is available.

This lack of data limits the results of the serologic testing in this study.

Table 10: S. stercoralis ELISA

ELISA	cases (n)	valid (%)
positive	10	19,6
borderline pos.	6	11,8
negative	35	68,6
no data	102	

3.4 **Comparison of diagnostic techniques for *S. stercoralis***

In regards to different diagnostic approaches, a comparison of methods can be done in terms of feasibility, of sensitivity and specificity and of intermethod agreement.

Table 11: Strongyloides positive - all methods gives an overview of positive diagnoses of *Strongyloides*. The possibility of mistaken interpretation in Harada-Mori culture as well as in agar plate culture must be considered. It should furthermore be mentioned that the ELISA test was not performed in every case because blood samples were not always available. Moreover, microscopy was performed with reassessment in several cases as mentioned before.

Calculating sensitivity and specifity for the choosen diagnostic tools is difficult in this study because the results are limited by the small number. Faecal PCR can be assumed as a reference standard diagnostic although there is no gold standard for *S. stercoralis* diagnostics. When using PCR as a gold standad, sensitivities for the modified Harada-Mori technique, agar plate culture and microscopy are all 66,7 % considering that two of the PCR positive samples were detected positive in all three other methods, and one positive PCR sample showed no positive result in the other methods.

Serum ELISA must be omitted in comparison, as no test was available for the PCR positive patients. Because of the limited number of valid tests in this study, calculating results for a comparison of methods was not reasonable.

Table 11: Strongyloides positive - all methods

Legend:

-	=	negative
+	=	positive
(+)	=	light positive
n.v.	=	not visible
no sign	=	no testing

Study-Nr.	HM 2-3d	HM 6d	AP 24h	AP 48h	AP 72h	PCR	ELISA	micro 1	micro 2	micro 3
9	n.v.	-	-	-	-	-	(+)	-		
25	-	+	-	-	-	-		-		
36	-	+	-	-	+	+		+		
43	-	-	-	-	-	-	(+)	-		
44	-	-	-	-	-	-	+	-		
53	-	-	-	-	-	-	+	-		
54	-	-	+	+	+	-	-	-		
57	-	n.v.	-	-	-	-	(+)	-	-	
63	-	-	-	-	-	+		-	-	-
77	-	+	-	-	-	-		-		
84	-	+	-	-	+	-	-	-	-	
87	-	-	-	-	-	-	+	-	-	
90	-	-	-	-	-	-	(+)	-		
94	-	+	-	-	+	+		-	-	+
98	-	+	-	+	+	-		-		
100	n.v.	-	+	+	+	-		-		
101	-	-	-	-	-	-	+	-		
102	-	-	-	-	-	-	(+)	-		
104	-	+	-	-	+	-	+	-	-	
107	n.v.	n.v.	-	+	+	-	-	-		
114	-	-	-	-	-	-	+	-		
127	-	-	-	-	-	-	+	-		
131	-	-	-	-	+	-	+	-	-	
134	-	-	-	+	+	-		-		
139	-	-	-	+	+	-		-		
150	-	-	-	-	-	-	+	-		
160	-	-	-	-	-	-	+	-		
162	-	-	-	-	-	-	(+)	-		
181	-	+	-	-	-	-	-	-		

When calculating specificity, microscopy shows the best result as is shown in *Table 12: S. stercoralis diagnostic with PCR as reference method*.

Table 12: S. stercoralis diagnostic with PCR as reference method

Technique	valid n	positive (n)	sensitivity (%)	specificity (%)
Harada-Mori	138	8	66,7	95,6
Agar plate	153	11	66,7	94
Microscopy	153	2	66,7	100
PCR	153	3	100	100

The Cohen's kappa interrater agreement, which in this study is actually an intermethod agreement, was calculated for the different methods separately from each other, since there is no gold standard set up for *S. stercoralis* diagnostic.

Results are shown in *Table 13: Cohen's kappa for S. stercoralis diagnostic methods* and in *Figure 17: Method agreement for S. stercoralis.* The best agreement was seen between qPCR and microscopy with κ=0.797. However, the number of positive samples in both diagnostic methods is very small. Slight and moderate agreement can be seen between the other methods. Among them, the agar plate and Harada-Mori culture methods show the best concordance with an almost substantial agreement of κ=0.561. The qPCR shows just slight agreement with the faecal culture methods and microscopy. ELISA is based on a minor range of samples and shows no agreement with the summed results of all the other methods. Consequently, further comparison and calculation of diagnostic techniques, study group analysis and risk factors was done based on the diagnostic results of the stool techniques for *S. stercoralis.*

Table 13: Cohen's kappa for S. stercoralis diagnostic methods

	PCR-micro	HM-AP	HM-micro	PCR-HM	PCR-AP	ELISA-other
kappa	0,797	0,561	0,386	0,343	0,263	-0,03

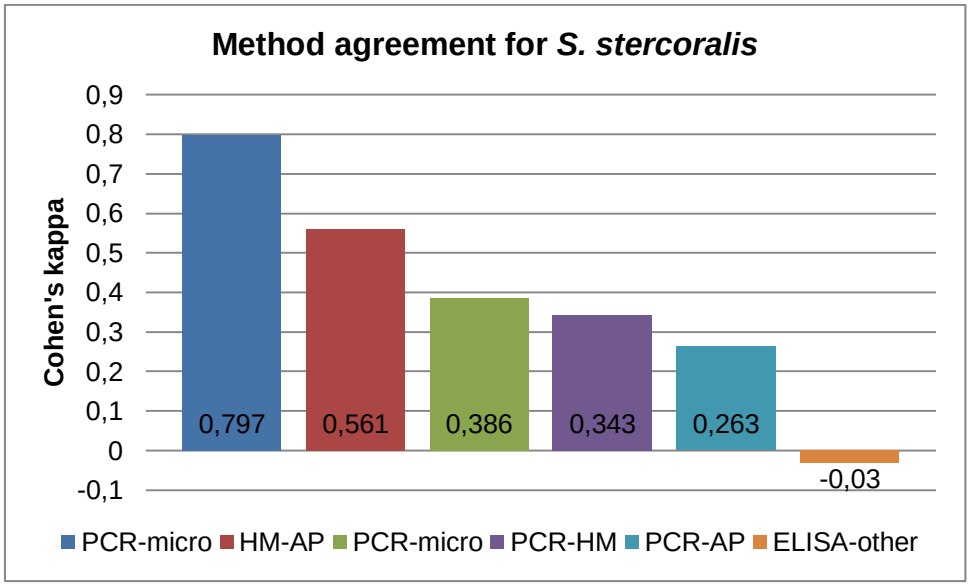

Figure 17: Method agreement for S. stercoralis

Regarding its feasibility, the performance of the modified Harada-Mori culture was both quite time consuming and material consuming, but it might be an alternative to the conventional Harada-Mori culture. Furthermore, although it takes more time than the agar plate culture, it enables direct microscopical analysis with the inverted microscope. Still, the risk of dirty tubes leads to the disfavour of this method. The necessity of an inverted microscope and specially prepared tubes complicate the method. The agar plate culture was quite convenient to perform. Daily analysis takes time but increases the sensitivity as well. Furthermore, the material and the microscope are standard laboratory equipment even in low-income countries.

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

Detection rates for protozoa vary depending on the diagnostic methods performed. The immunochromatographic rapid assay includes all three parasites in one test cassette. Microscopy with FPC® concentration material for *C. parvum* is limited in number, since staining was only performed for the samples with positive rapid assay results of that protozoon as a confirmatory test. This was done because of the known low sensitivity. Therefore, limited conclusion can be drawn for the prevalence of *C. parvum*. Real-time PCR as a

gold standard was performed for *G. lamblia* and for differentiation of *E. histolytica* and *E. dispar*.

Considering the respective method with highest sensitivity, the overall prevalence results for *G. lamblia, E. dispar* and *C. parvum* are shown in *Table 14: Giardia, Cryptosporidium, Entamoeba and Strongyloides prevalence* and *Figure 18: Overall prevalence Giardia, Cryptosporidium, Entamoeba and S. stercoralis*.

For the purpose of better comparison and overview, the prevalence rate of *S. stercoralis* detected by PCR is additionally shown in the table and figure.

A remarkably high *G. lamblia* prevalence rate of 27.5 % infected children in this study was detected by qPCR.

The prevalence rate of *E. dispar* detected by qPCR is 8.5 % in this study. No sample was positive for *E. histolytica* in qPCR. This also means that the results of the other methods rely only on the detection of *E. dispar* in this study group.

Limited diagnostics without qPCR were performed for *C. parvum.* A quite low prevalence rate of 3.9 % was detected with the immunochromatographic rapid test (n=6, 3.9 %).

Table 14: Giardia, Cryptosporidium, Entamoeba and Strongyloides prevalence

Parasites	prevalence (%)	positive (n)
G. lamblia PCR	27,5	42
E. dispar PCR	8,5	13
E. histolytia PCR	0	0
C. parvum rapid test	3,9	6
S. stercoralis PCR	1,9	3

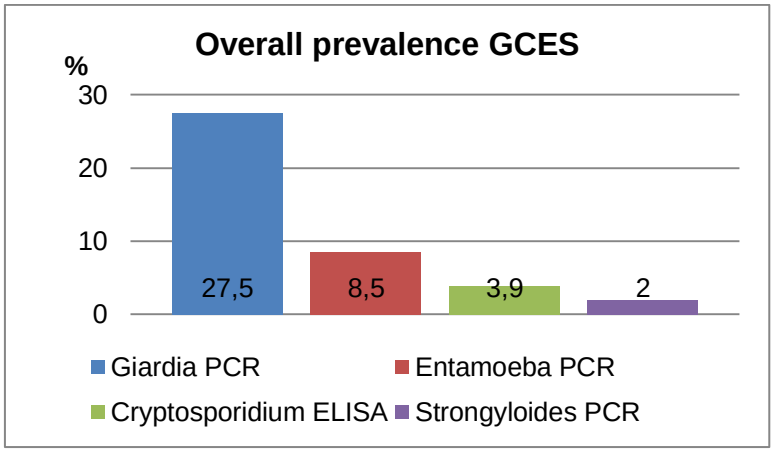

Figure 18: Overall prevalence Giardia, Cryptosporidium, Entamoeba and S. stercoralis

3.6 Comparison of diagnostic techniques for *G. lamblia, E. histolytica/ dispar* and *C. parvum*

3.6.1 *Giardia lamblia*

The prevalence rate of *G. lamblia* depended on the diagnostic methods. Results are shown in *Table 15: Giardia prevalence and method comparison* and *Figure 19: Giardia prevalence (%).*

FPC® concentration-based microscopy showed the lowest detection rate with 14 positive results among the 153 study participants (n=14, 9.2 %). In the immunochromatographic rapid assay, 19 samples were positive (n=19, 12.4 %). 42 positive samples were detected in the qPCR, more than twice as many as in the rapid test (n=42, 27.5 %). All positive samples in the rapid test were positive in the qPCR as well. In 12 of the 19 positive rapid test samples, microscopy also showed a positive result. This can be seen in *Table 16: Giardia Rapid test and microscopy.* None of the samples analysed as positive under microscopy were negative in the qPCR.

The sensitivity and specificity of the applied techniques was analysed related to the PCR as gold standard with an assumed sensitivity and specificity of 100%. Specificity of the rapid test and of the microscopy was 100 % respectively and the false positive rate (the error of the second kind) was 0 %. Sensitivity was

quite low for microscopy at 33.3 %. It was better for the rapid test but still only moderate at 45.2 %. False positive rate (the error of the first kind) was 66.7 % for microscopy and 54.8 % for the rapid test.

PCR is the gold standard as a diagnostic technique for *G. lamblia* and showed a much higher sensitivity than the rapid test or microscopy.

Table 15: Giardia prevalence and method comparison

Techniques	*G. lamblia*			
	positive (n)	positive (%)	sensitivity (%)	specificity (%)
Rapid test	19	12,4	45,3	100
Microscopy	14	9,2	33,3	100
PCR	42	27,5	100	100

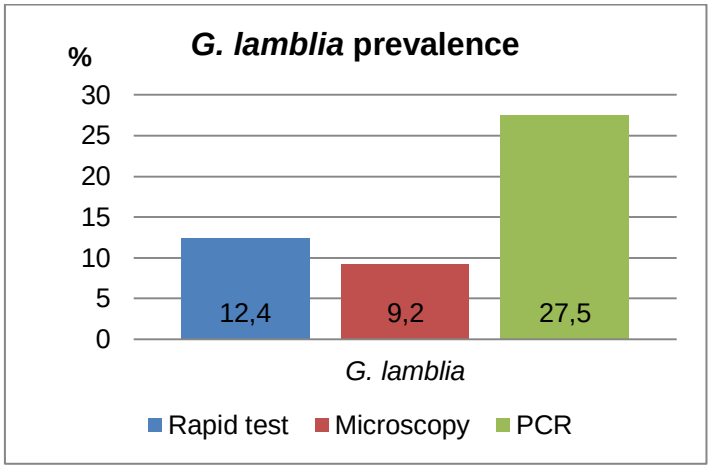

Figure 19: Giardia prevalence (%)

Table 16: Giardia Rapid test and microscopy

		Giardia Rapid test		
		neg	positive	sum
Giardia microscopy	neg	132	7	139
	positive	2	12	14
sum		134	19	153

3.6.2 *Entamoeba histolytica and Entamoeba dispar*

The problem in the detection of Entamoeba is the impossibility of morphologically distinguishing *E. histolytica* from *E. dispar*. This can be achieved with PCR which is the diagnostic gold standard for *Entamoeba*. Results of the different methods are shown in *Table 17: Entamoeba prevalence and method comparison*.

In the immunochromatographic rapid test, only three samples were positive for *Entamoeba* (n=3, 2 %). In microscopy with the FPC® concentration method, *Entamoeba* cysts were visible in six samples (n=6, 3.9 %). No positive results were detected for *E. histolytica* using qPCR, whereas 13 positive results (n=13, 8.5 %) were detected for *E. dispar*

As shown in *Table 18: Entamoeba Rapid test and PCR,* all three positive rapid test samples were also positive in the qPCR, but the results for microscopy were more diverse. This can be seen in

Table 19: Entamoeba Rapid test and microscopy. Three of the microscopically positive samples were also positive in qPCR, and two of them were additionally positive in the rapid test. Regarding sensitivity and specificity, the techniques are compared with qPCR as gold standard and under the assumption that all positive results are *E. dispar*. This means quite a low sensitivity of 23.1 % and a specificity of 97.9 % for microscopy. Specificity increased to 100 % in the rapid test, but sensitivity was also merely 23.1 %.

Table 17: Entamoeba prevalence and method comparison

Techniques	*E. dispar*			
	positive (n)	positive (%)	sensitivity (%)	specificity (%)
Rapid test	3	2	23,1	100
Microscopy	6	3,9	23,1	97,9
PCR	13	8,5	100	100

Table 18: Entamoeba Rapid test and PCR

Entamoeba	Rapid test	
PCR	negative	positive
negative	140	0
positive	10	3

Table 19: Entamoeba Rapid test and microscopy

Entamoeba	Rapid test	
microscopy	negative	positive
negative	146	1
positive	4	2

3.6.3 *Cryptosporidium parvum*

Results for *C. parvum* diagnostic tests are shown in *Table 20: Cryptosporidium prevalence*. In the microscopy that was performed for the six positive rapid test samples, *C. parvum* cysts were visible only in one sample (n=1, 16.7 % out of all n=6).

Regarding the practicability, the rapid test is quite easy to perform compared to microscopy, and it showed even more positive results. Staining requires even more time, material and personnel experience than regular unstained microscopy with stool concentration material. Therefore, the rapid test might be a good alternative if PCR is unavailable, as is the situation in many low-income countries. Still, the costs and availability of the rapid test must be considered although these might be acceptable.

Table 20: Cryptosporidium prevalence

Cryptosporidium	Rapid test (n=153)	Microscopy (n=6)
positive (n)	6	1
positive (%)	3,9	16,7
neg (n)	147	5

3.7 **Other intestinal parasites**

All samples were microscopically analysed at least one time with faecal material having been processed with the FPC® concentration method. Some of the samples were reassessed with additional slides from the same prepared FPC sample, as explained in 2.2.4. Microscopy was performed unstained except for *C. parvum* diagnostics in a few samples. Therefore the results in this chapter refer only to the results in unstained microscopy. The results of detected parasites in microscopy are shown in *Table 21: Microscopy - parasites prevalence*.

The highest detection rate was for *Blastoscystis hominis* (n=18, 11.8 %), followed by *Entamoeba coli* (n=16, 10.5 %), *G. lamblia* (n=14, 9.2 %), *Endolimax nana* (n=9, 5.9 %), *E. dispar/ histolytica* (n=6, 3.9 %), *Schistosoma mansoni* (n=6, 3.9 %), hookworm (n=6, 3.9 %), *Chilomastix mesnili* (n=4, 2.6%), *Ascaris lumbricoides* (n=2, 1.3 %), *Trichuris trichiura* (n=2, 1.3 %), *S. stercoralis* (n=2, 1.3 %) and *Enterobius vermicularis* (n=1, 0.7 %).

Table 21: Microscopy - parasites prevalence

microscopy	positive (n)	positive (%)
Blastocystis hominis	18	11,8
Giardia lamblia	14	9,2
Endolimax nana	9	5,9
Entamoeba histolytica/dispar	6	3,9
Ascaris lumbricoides	2	1,3
Chilomastix mesnili	4	2,6
Entamoeba coli	16	10,5
Trichuris trichiura	2	1,3
Strongyloides stercoralis	2	1,3
Schistosoma mansoni	6	3,9
Enterobius vermicularis	1	0,7
Hookworm	6	3,9

3.8 **Overall parasitological findings and polyparasitism**

The overall results for the prevalence of intestinal parasites including helminths and pathogenic and non-pathogenic protozoa as well as the corresponding diagnostic methods are shown in *Table 22: Overall parasitological findings*.

In summation of all faecal diagnostic methods, intestinal parasite of any kind was detected in 56.9 % of all samples (n=87, 56.9 %). Polyparasitism was detected in 35 samples (n=35, 22.9 %). This means polyparasitism in 40.2 % of the positive parasitic samples. The overall prevalence of helminths including *S. stercoralis*, hookworm (*Necator americanus* and *Ancylostoma duodenale*), *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis* and *Schistosoma mansoni* was 17.6 % (n=27, 17.6 %). Regarding only the soil-transmitted helminths including *S. stercoralis*, hookworm, *Ascaris lumbricoides* and *Trichuris trichiura,* the infection prevalence drops somewhat to 14.4 % (n=22, 14.4 %). The positivity rate of protozoa was 47.7 % (n=73, 47.7 %). The rate of nonpathogenic protozoa including *Chilomastix mesnili, Entamoeba coli, E. dispar* and *Endolimax nana* was 20.9 % (n=32, 20.9 %), whereas the proportion of potentially pathogenic protozoa including *G. lamblia, Blastocystis hominis* and *C. parvum* was 39.2 % (n=60, 39.2 %).

Blastocystis was considered to be a gastrointestinal commensal in the past, but in recent years it was discovered to be a pathogenic protozoan with clinical significance depending on the host's immune status. Consequently, it was included here in the pathogenic protozoa and pathogenic parasites because it is facultativly pathogenic.

The rate of pathogenic parasites of any kind in this study group was 48.4 % (n=74, 48.4 %), but the symptom of acute diarrhoea among the children was only mentioned in 11.5 %.

Table 22: Overall parasitological findings

	Cases (n) Total n=153	**Prevalence (%)**	**Diagnostic methods[13]**
Intestinal Parasites	87	56,9	
Helminth	27	17,6	
STH	22	14,4	HM, AP, micro, PCR
S. stercoralis (all methods)	15	9,8	HM, AP, micro, PCR
S. stercoralis (PCR)	3	1,9	PCR
Hookworm	6	3,9	microscopy
Ascaris lumbricoides	2	1,3	microscopy
Trichuris trichiura	2	1,3	microscopy
Enterobius vermicularis	1	0,7	microscopy
Schistosoma mansoni	6	3,9	microscopy
Protozoa	73	47,7	
Pathogenic protozoa	60	39,2	
Giardia lamblia	42	27,5	PCR
C. parvum	6	3,9	Rapid test
Blastocystis hominis	18	11,8	microscopy
Nonpathogenic protozoa	32	20,9	
Endolimax nana	9	5,9	microscopy
Chilomastix mesnili	4	2,6	microscopy
Entamoeba coli	16	10,5	microscopy
Entamoeba dispar	13	8,5	PCR
Polyparasitism	35	22,9	

[13] Diagnostic method with highest sensitivity is mentioned

3.9 **Risk factors of intestinal parasitic infections**

As shown in *Table 23: Sociodemographic risk factors in univariate analysis* and in *Table 24: Hygienic risk factors in univariate analysis*, the univariate analysis of risk factors associated with intestinal parasitic infection obtains some significant associations regarding hygienic status and sociodemographic characteristics. Interpretating the positive findings of *S. stercoralis* critically, the analysis of associated risk factors must be done with differentiation of the results. Counting the positive findings in all methods means significant association which is not seen if only PCR results are taken as true positives. Therefore, particular counting is noted as a positive result for *S. stercoralis* in all methods or only in PCR.

Residence in a rural region was significantly associated with intestinal parasites in general, *S. stercoralis* (all methods), STH, protozoans and non-pathogenic protozoans. Additionally, the profession of the parents as farmers was significantly associated again with intestinal parasites, STH and *S. stercoralis* (all methods). Access to flush toilet and tap water as a marker for hygienic standards show some significant association with infection prevalence. Toilet access including a latrine or no toilet at all at home is significantly associated with intestinal parasites in general, *G. lamblia* and pathogenic as well as non-pathogenic protozoa. Access to tap water is significantly associated with the prevalence of helminths as shown in *Table 25: Water access and Helminths* and in detail with *S. stercoralis* (all methods) but not with STH at all. This shows a greater importance of toilet access for the infection rate of protozoa, whereas water access seems to be more strongly associated with helminthic infection.

Table 23: Sociodemographic risk factors in univariate analysis

	No. Of cases positive (n)					
	Living region			Profession of parents		
	Urban	Rural	X^2/p	Farmer	Other	X^2/p
Giardia lamblia	19	23	0,092	29	13	0,234
Strongyloides stercoralis (all methods)	4	11	*0,015	14	1	*0,008
Strongyloides stercoralis (PCR)	1	2	0,420	2	1	0,756
Intestinal Parasites	41	46	*0,009	60	27	*0,028
Helminth	11	16	0,074	21	6	0,055
Soil-transmitted helminth	8	14	*0,043	18	4	*0,034
Protozoa	35	38	*0,049	49	24	0,168
Pathogenic protozoa	29	31	0,115	40	20	0,226
Nonpathogenic protozoa	12	20	*0,016	24	8	0,076

* $p < 0,05$

Table 24: Hygienic risk factors in univariate analysis

	No. Of cases positive (n)					
	Toilet			Water		
	Flush	No flush	X^2/p	Tab	No tab	X^2/p
Giardia lamblia	9	32	*0,028	26	16	0,533
Strongyloides stercoralis (all methods)	3	12	0,174	6	9	*0,027
Strongyloides stercoralis (PCR)	1	2	0,923	1	3	0,231
Intestinal Parasites	22	63	*0,003	54	32	0,374
Helminth	7	20	0,228	13	14	*0,033
Soil-transmitted helminth	7	15	0,658	11	11	0,091
Protozoa	17	54	*0,004	46	26	0,639
Pathogenic protozoa	14	45	*0,012	38	22	0,606
Nonpathogenic protozoa	5	26	*0,01	18	13	0,31

* $p < 0,05$

Table 25: Water access and Helminths

		Helminths (any method)		
		neg	positive	sum
Wateraccess	Tab	87	13	100
	no Tab	38	14	52
sum		125	27	152

Fewer significant associations can be seen when using a binary logistic regression model. The model was adjusted for the covariates gender, school age, living region, access to water, flush toilet and parents working as farmers. The logistic regression model was not significant for the prevalence of parasites in general, helminths, non-pathogenic protozoa or pathogenic protozoa.

The model only showed statistically significance for STH. In that model, school-age, no flush toilet and parents working as farmers were associated with an infection with STH, as can be seen in *Table 26: Risk factors for STH in bivariate logistic regression model*. This matches with the results in the univariate analysis for profession of the parents and access to water.

Parents were asked for the clinical symptoms of their children in the questionnaire. Symptoms included diarrhoea, abdominal pain, vomiting, fever, cough and flatulence as these are the common symptoms of intestinal parasitic infections. To analyse the correlation of symptoms with the prevalence rate of intestinal parasitic infection, a binary logistic regression model was used. The model was statistically significant only for positivity of intestinal parasites in general. However, none of the symptoms in that model showed significant correlation with the positive findings of parasites. Regarding helminths, STH, as well as pathogenic and non-pathogenic protozoa, the logistic regression model was not significant. Even in univariate analysis, no significant association was found between acute gastrointestinal symptoms such as diarrhoea, vomiting and abdominal pain and the prevalence of parasites generally, helminths or pathogenic protozoa.

Table 26: Risk factors for STH in bivariate logistic regression model

Variables	**Soil-transmitted helminths**		
	OR	95% CI	p value
Gender			
Female	1 (ref)	-	-
Male	0,835	0,309-2,256	0,723
Age			
6-14	1 (ref)	-	-
0-5	3,298	1,170-9,3	*0,024
Living region			
Urban	1 (ref)	-	-
Rural	0,265	0,068-1,026	0,055
Toiletaccess			
Flush	1 (ref)	-	-
Latrine/ Bush	9,106	1,599-51,849	*0,013
Wateraccess			
Tab water	1 (ref)	-	-
Spring/River/Well	0,563	0,161-1,969	0,369
Profession of parents			
Other	1 (ref)	-	-
Farmer	0,155	0,033-0,724	*0,018
* $p < 0,05$			

Deworming treatment is implemented regularly for children, especially those of school age. A statistically significant negative relation was found between previous antihelminthic treatment, regardless of the drugs used and their dosage, and the positivity of helminths in generals, as well as with STH infection. The results are shown in *Table 27: Deworming and helmiths/ STH infection prevalence, Table 28: Deworming and Helminths* and *Table 29: Deworming and STH*. Furthermore, none of the children tested positive with any stool diagnostic method for *S. stercoralis* and hookworm had received deworming drugs in previous twelve months. Even if they had received the

common deworming medication recommended by the WHO, no curing effect is to be expected (WHO 2012). Albendazole and mebendazole have no efficiency against *S. stercoralis* and lack in efficiency against hookworm (Schulz, Moser et al. 2018).

Table 27: Deworming and helmiths/ STH infection prevalence

	No. Of cases positive (%) Antihelminthic in last 12 month		
	no	yes	X^2/p
STH	21 (18,4)	1 (2,7)	0,019
Helminths	25 (21,9)	2 (5,4)	0,023

* $p < 0,05$

Table 28: Deworming and Helminths

		Antihelminthics		
		no	yes	sum
Helminths	neg	89	35	124
	positive	25	2	27
sum		114	37	151

Table 29: Deworming and STH

		Antihelminthics		
		no	yes	sum
STH	neg	93	36	129
	positive	21	1	22
sum		114	37	151

4 Discussion

4.1 Study group profile

The children included in the study were admitted to the Bugando hospital, a referral hospital in the second-largest city of Tanzania. This introduces a bias in the sociodemographic profile of the study group and does not represent the general situation of children in the northern region of Tanzania.

Going to a hospital is a financial issue and is more often used by economically well situated families. Consequently, the children in this study potentially might enjoy better hygienic living conditions than average children in Tanzania. However, less than half of the children had a flush toilet at home, but more than half had access to tap water at home. Having access to tap water and a flush toilet does not automatically mean that the children always use that kind of facility, since they also go to school, run around outside a lot and generally spend time outside the household of their parents. Hygienic conditions are also connected to the area of residence since tap water and flush toilets are more common in the city than in villages. 56 % of the children lived in an urban region. This differs from the general Tanzanian population, of which about 70 % is estimated to live in rural districts (Kabadi, Geubbels et al. 2015). In this study group, many families came from the Mwanza district. Travel distance is another issue for families bringing their children to a hospital. This might be another reason for any difference between this study group and the general Tanzanian population. Although many families lived in an urban district, the main profession of the parents was farmer. Work in a business or other indoor profession which is probably accompanied by better salary was only reported by 14 % of the parents. Often, only one of the parents accompanied the child to the hospital and answered the questionnaire. However, it can be assumed that the profession of the parents as farmer mostly includes both parents.

Gender was not homogenously distributed in this study group. There were more male patients than female patients. The Tanzanian population in general includes approximately the same number of women and men (Kabadi,

Geubbels et al. 2015). The group of infected children included more boys than girls, but this difference was not statistically significant.

4.2 *S. stercoralis*

4.2.1 Epidemiology

The overall prevalence of *S. stercoralis* in this study in children admitted to the BMC was 1.9 % (n=3), taking the results of qPCR and microscopy as true positive.

Higher prevalence rates can be found in literature. Between 1989 and 2011, four community-based surveys with an average prevalence of 7.9 % and four hospital-based surveys with an average prevalence of 9.3 % were conducted in Tanzania (Schar, Trostdorf et al. 2013).

These data rely on prevalence rates including adults in the study group. We focussed the StrongPaed study on children because they are a vulnerable group at risk for *S. stercoralis* infection and because prevalence data for children are rare for Tanzania.

A 2007 study in Zanzibar of soil-transmitted helminthiasis among schoolchildren (7-20 years) showed a *S. stercoralis* prevalence rate of 10.2 %. In this study, the diagnostic sensitivity increased enormously by about 50 % when two stool samples were examined rather than one stool sample. The diagnostic methods included the Kato-Katz technique, Koga agar plate and the Baermann method (Knopp, Mohammed et al. 2009). After collecting the stool samples and preparation, the Kaga agar plate cultures were incubated for two days. Then the sediment was transferred to a microscopic slide and examined under a microscope. The larvae were identified on the basis of morphologic characteristics (Knopp, Mgeni et al. 2008). Compared to the methods used in StrongPaed study, the agar plate culture was performed similarly but was incubated only for two days and was not assessed every 24h up to three days. The chance of detecting hookworm larvae rather than eggs in a longer incubation period is higher. Therefore the risk of misinterpretation of the larvae increases with longer incubation time. Still, the differentiation between

hookworm and *S. stercoralis* larvae was done in the aforementioned study in the same way as in StrongPaed, via morphologic characteristics seen in microscopy. An increased prevalence rate could be considered if two or even more stool samples had been examinded in StrongPaed study.

Children younger than school age were also included in the StrongPaed study population, but no significant differences in the prevalence of parasites in school age children and younger children were observed.

A cross-sectional study in the Bagamoyo district on the western coast of Tanzania was conducted in 2011/2012 with about 1000 children (0-10 years) using only the Baermann method for detection of *S. stercoralis*. They reported a prevalence rate of 5.8-7.5 % depending on the age, with the highest prevalence in preschool-aged children. Moreover, they observed co-infections with hookworm and *S. stercoralis* in all age groups (Salim, Schindler et al. 2014). In the StongPed study, no significant difference in the *S. stercoralis* infection rate depending on age was observed, even if all findings were to be counted as true positive. Here, a coinfection as well as the misinterpretation of hookworm larvae must be considered. The ages of the qPCR positive children were five, five and ten years, but having only three positive results is insufficient for more conclusions.

Significantly more school age children were infected with STH in logistics regression analysis. Generally, an increasing *S. stercoralis* infection rate can be considered, since infection may persist life-long without adequate therapy. On the other hand, a better immunologic status can be developed with age and that might mean better controlled larvae burdens in older children.

School age may mean an increased risk of STH infection since children run and play a lot outside when they grow up, especially when they go to school. Many of the primary and secondary schools have only basic sanitation with latrines and lack a constant water supply for hand washing, at least in rural areas. However, since data in the StrongPaed study are so small, only weak conclusions can be drawn.

A study in Zanzibar in 2007 showed a *S. stercoralis* prevalence rate of 2.2 % in children with the highest prevalence rate of 4 % in a humid forest region. The study included about 300 children of preschool and school age in rural and peri-urban settings. Diagnostic methods included Koga agar plate and Baermann method, but only one stool sample was analysed (Knopp, Mohammed et al. 2008). Higher true prevalence rates can be estimated due to the known insufficient sensitivity in these diagnostics, especially when stool samples are not repeated. However, the same problems can be considered in the StrongyPed study. By examining only one stool sample and counting qPCR as true positive result, a similar prevalence rate of about 2 % can be seen in the StrongPaed study.

A 2008 study in Zanzibar showed a *S. stercoralis* prevalence rate of 8-18 % in children, depending on the region and age. This was a community-based cross-sectional study in a rural and peri-urban setting and included about 80 schoolchildren (5-15 years old). The diagnostic methods for *S. stercoralis* detection included Koga agar plate and Baermann method (Knopp, Mohammed et al. 2010). In the StrongPaed study, a significant association between rural living region and intestinal parasites as well as with STH infection was seen. Since the study setting also included children from an urban living region, lower prevalence rates can be assumed compared to the rural population setting of the study in Zanzibar. However, the diagnostic spectrum was wider in the StrongyPed study, and higher detection rates can be expected for that reason. On the other hand, in the StrongPaed study, only one stool sample was examined compared to the Zanzibar study in with at least two stool samples were examined (Knopp, Mohammed et al. 2009).

There have been few recent studies on the prevalence of *S. stercoralis* in Tanzania, and those studies which have been mentioned are located in both a different geographical area and a different population setting than in the Mwanza region. The studies in Zanzibar included children from rural and peri-urban areas. In the StrongPaed study, the catchment area of the BMC also included many children from urban areas with often differing socioeconomic situations and sanitary conditions. Therefore, comparing prevalence data from

different study settings is difficult. However, underestimation of the prevalence has remained problematic for many years and still continues to be problematic because of the lack of awareness of *S. stercoralis* infection and the lack of sensitive diagnostics (Olsen, van Lieshout et al. 2009, Bisoffi, Buonfrate et al. 2013).

4.2.2 Diagnostics

The diagnostic methods used may have a large influence on the reported prevalence of *S. stercoralis* infection. The methodological problem in assessing test accuracy is the lack of a gold standard (Buonfrate, Formenti et al. 2015). Most studies in Africa were conducted with low-sensitivity diagnostic methods. Community-based studies mostly used fewer stool samples and low-sensitivity diagnostic methods whereas studies on health-based data often included only symptomatic patients with suspected helminthic infection and a high-sensitivity diagnostic approach. A meta-analysis study showed that adequate diagnostic tests such as Koga agar plate culture and the Baermann method had been performed in only 19 % of the studies in African countries by 2011 (Schar, Trostdorf et al. 2013). A low sensitivity has been reported for the Baermann method (Ines Ede, Souza et al. 2011).

In the StrongPaed study, positive *S. stercoralis* results differed a lot depending on the diagnostic methods. The observed prevalence was 1.3 % in FPC® concentrations method microscopy, 2 % in real-time PCR, 19.6 % in serum ELISA, 5.8 % in modified Harada-Mori culture and 7.2 % in Koga agar plate. The results of the different methods were not all concordant, and comparison is difficult without a proven gold standard.

The problem is that all of the available diagnostic methods are time consuming and require special equipment and dedicated, trained laboratory staff. All methods lack more or less in sensitivity especially in low-intensity infections, and they require repeated stool examinations for increased sensitivity (Siddiqui and Berk 2001, Cringoli, Rinaldi et al. 2010, Glinz, Silue et al. 2010, Knopp, Salim et al. 2014).

The larval burden is quite low in chronic infections, leading to problematic sensitivities in diagnostic methods. When there are only few larvae, either larger stool volume or repeated examinations are necessary for the detection. Different diagnostic methods require different stool volumes and this is one aspect of the differing sensitivities in the StrongPaed diagnostic methods. Repeated examinations not only mean increased costs and time, but also a study setting with patients who submit several stool samples. Serology has a higher sensitivity than both stool culture methods and molecular diagnostic methods, but it might overestimate the prevalence in endemic areas since antibodies persist up to a year after infection and because cross-reaction with other parasites is a problem in endemic regions (Buonfrate, Sequi et al. 2015). Also cross-reactivity can not be ruled out for the ELISA used in this study.

In this study no selection was done for children with specific symptoms. Instead, children with a wide variety of symptoms and diagnoses were included. Because of the large catchment area, the non-selected inclusion of children was meant to be representative of the population of children in the northwest region of Tanzania. Therefore, the infection intensity and consequently the larval burden in such a study group might probably be quite low, and diagnostic detection rate is a problem in such cases. Among the *S. stercoralis*-positive children (considering all results as true positive), none complained about diarrhoea, and larval burden was low. Likely, chronic infections of low intensity were observend in the study population.

On the other hand, the problem of specificity is caused by the risk of confusing the morphology of *S. stercoralis* larvae with hookworm larvae in diagnostic methods based on microscopic detection. In the modified Harada-Mori culture, no larvae were detected in the first examination after 2-3 days, but all were detected after six days of incubation. Therefore, the risk of hatched hookworms that can be misinterpretated as *Strongyloides* larvae has to be considered in all positive findings in Harada-Mori culture. In the agar plate cultures, most positive findings were detected after 48 h or even 72 h. Only two samples were detected with larvae visible after 24 h, but both samples remained negative in qPCR. Since the chance of hookworm detection rises with longer incubation times, the

misinterpretation of larvae must be considered in agar plate cultures as well. Coninfection might be a further problem, and the coexictence of the larvae in different stages increases the challenge of distinction between *Strongyloides* and hookworm larvae. Certain differentiation requires higher magnification in microscopic diagnostics. The modified Harada-Mori culture showed a new way to examine the culture during the incubation period. In agar plate culture, lower microscopic magnification was possible during cultivation time because of the culture plates. Finally, microscopic analysis at higher magnification was possible after 72 h.

Concerning other diagnostic methods such as serum ELISA, the risk of cross-reaction and the inability of discriminating active from previous infections lead to a deficit in specificity. Molecular methods show a satisfying level of specificity, but the protocols used are not standardized. Furthermore, the use of PCR is limited to a few laboratories and is mostly not available in low-income countries (Buonfrate, Formenti et al. 2015).

4.2.2.1 Agar plate culture

Taking a pseudo gold standard as the sum of all positive results, the highest sensitivity in the StrongPaed study was detected for the agar plate culture with 73.3 %. This matches the reportedly high sensitivity for the Koga agar plate in literature (Kitvatanachai S 1999, Glinz, Silue et al. 2010, Ines Ede, Souza et al. 2011).

A meta-analysis using data from 1980-2013 on the evaluation of conventional parasitological methods showed the highest sensitivity for Koga agar plate with 89%. The evaluation also included the Baermann technique in the observation but not the Harada-Mori culture which was used in the StrongyPed study. Since the results depend on the reference standard used, which were different faecal methods, and since no standard procedure has been established, comparison has to be taken carefully (Campo Polanco, Gutierrez et al. 2014).

Increased sensitivity for Koga agar plate and the Baermann method was reported when examining three stool samples. The combined prevalence rate increased from 5.1 % to 10.8 % (Knopp, Mgeni et al. 2008). We only performed

stool diagnostics on one stool sample, and more positive results could be assumed upon collecting and analysing two or more stool samples.

Distinguishing hookworm from *S. stercoralis* larvae according to their tracks seemed to be inconclusive. Therefore, final analysis with high microscopic magnification was performed. Different stages of the larvae were seen, and the final result was impaired by the chance of false interpretation of the larvae. Even reassessment through experienced personnel at the Bugando Hospital in Mwanza did not clear all doubts. Regular reassessment for every positive finding through experienced staff should be done in further assessment to improve the morphological interpretation of the larvae. Moreover further assessment should be done with hookworm PCR to evaluate morphological findings critically.

4.2.2.2 Harada-Mori culture

Harada-Mori culture is reported to be very sensitive for hookworm infection in addition to *S. stercoralis* detections (Kitvatanachai S 1999). We modified this method in order to use a larger stool volume and to enable direct microscopy with an inverted microscope. This worked quite well, and direct microscopy gave the chance to analyse the samples twice during the cultivation period. Still, the problem of stool contamination and therefore the impossibility of analysing those samples is a notable disadvantage of this method, and improvement of this diagnostic method is necessary.

Almost perfect intermethod agreement was seen in the StrongPaed study between agar plate and Harada-Mori techniques. Since most positive results were detected by agar plate culture, the diagnostic accuracy is predictably high.

Specificity is a problem concerning false positive results. Using the pseudo gold standard, calculations of technique comparison involve the problem of the absence of false negative results. Beside this, agar plate and Harada-Mori culture techniques show quite reasonable results in the StrongPaed study, but the numbers are small.

There is a substantial risk of confusing *S. stercoralis* with hookworm, although specific morphological characteristics for both species are known, and experienced laboratory personnel was available to support in cases of uncertainty. Obviously the problem of correctly diagnosing *S. stercoralis* in order to distinguish it from hookworm remains in the Harada-Mori and Koga agar plate culture, and experienced laboratory personnel is important (Bisoffi, Buonfrate et al. 2013, Knopp, Salim et al. 2014).

Furthermore, the stool cultures were observed at least two times over a period of some days. This increased the chance of detecting larvae, but time also increases the chance of mistaking hookworm larvae with *S. stercoralis* larvae since hookworm larvae hatch after some time. Harada-Mori culture was observed for a priod of six days while agar plate cultures were observed only for three days, and this might improve the chance of finding *S. stercoralis* larvae rather than hookworm. In the StrongPaed study, the correlation beween Harada-mori cuture and agar plate culture is high, and larvae were seen mostly after a while so that mainly hookworm larvae might have been found.

This high level of intermethod agreement might also show such a result because in both methods, hookworm larvae might have been interpreted as *Stongyloides* larvae, or at least some larvae might have been misinterpreted since most positive results were seen after more than 48 h. This aspect should be considered when interpreting the results of the comparison of methods.

An independent reassessment for every larva by different laboratory personnel might be an idea for further studies.

4.2.2.3 PCR and microscopy

The lowest amount of positive results was seen in qPCR and microscopy, but both techniques also include the least risk of false positive results. There is minimal risk of mixing up a positive *S. stercoralis* result with hookworm, since hookworm should be detectable microscopically as eggs and not as larvae in directly preserved samples. Patients and their parents were advised to bring the stool samples fresh without delay, and preservation of stool for later microscopic analysis was done daily after receiving the stool samples. Still,

some delay in receiving and preserving the stool sample was unavoidable. Perhaps a delay of even more than 24 h might have occurred in a few cases.

For qPCR, high specificity results have been published (Buonfrate, Formenti et al. 2015). However, both methods have a lower chance of detecting larvae because of the small stool volume that was analysed. Even in the positive culture samples, often only a few larvae were detected, sometimes even only one or two. Consequently, the more than five times lower stool volume of qPCR or concentration method microscopy (200 mg versus 1-3 g) also means a more than five times lower chance of including larvae.

Most studies in Tanzania were performed without using PCR as a diagnostic method. PCR is the gold standard diagnostic technique in industrialized countries for the detection of many pathogens. However, it is currently not a common practice for *S. stercoralis* detection in laboratories in low-income countries, nor even in industrialized countries. In the StrongPaed study, real-time PCR was included but did not substancially increase the detection rate.

The best diagnostic agreement exists for qPCR and concentration method microscopy. The problem of these two techniques might be far more the lack of sensitivity because of the low larval burden in chronic infections and the low stool volume. This means that both methods are not useful in ruling out a chronic infection in healthy or symptomless people.

There are not many qPCR protocols available, and the procedures are not standardised. Many differences in sample preservation, the primers and probes used, the mode of DNA extraction and in amplification affect the results of *S. stercoralis* detection with molecular methods (Buonfrate, Formenti et al. 2015). Furthermore, its potential use is still limited by cost and the availability outside of research laboratories in industrialized countries.

In the StrongPaed study, the sensitivity of qPCR was assumed to be quite high as reported in literature, and for calculation it was assumed to be 100 %. If compared to the other diagnostic methods (HM, AP and microscopy), sensitivity would drop to 20 %. That would presume a correct diagnosis of the larvae in the culture test and no misinterpretation of the morphology of hookworm versus *S.*

stercoralis larvae. As described before, this case of absolute correctness is implausible. In literature, the reported results in sensitivity and specificity of PCR differ a lot depending on the type of PCR, the reference methods and the study setting. When comparing the results with studies that used the gene sequence and qPCR primers and probes we used as described by Verweilj et al in 2009, specificity ranges from 85.7-100 % but is mostly described as close to 100 %. The reported sensitivity shows a wide spectrum ranging from 14.7-90 % (Verweij, Canales et al. 2009, Schar, Odermatt et al. 2013, Sultana, Jeoffreys et al. 2013, Becker, Piraisoody et al. 2015, Paula, Malta Fde et al. 2015). Still, the results should be interpreted with caution, as they are influenced by the reference diagnostic method used. A 2018 review and metaanalysis for molecular biology techniques showed a sensitivity of 64.4 % in real-time PCR when only compared to parasitological methods and of 56.5 % when including serology (Buonfrate, Requena-Mendez et al. 2018). The reference methods used were mostly agar plate culture or Baermann technique.

Lower sensitivity was reported for the PCR results in samples of asymptomatic patients or in samples with a low larval burden compared with moderate to high larval count samples (Schar, Odermatt et al. 2013, Sultana, Jeoffreys et al. 2013, Knopp, Salim et al. 2014). The children in the StrongPaed study with *S. stercoralis*-positive results were asymptomatic, or at least none complained of diarrhoea. Low-density infections in which the larval output is low and irregular can be presumed, and lower sensitivity in the qPCR for these cases can be expected. This may also explain the lower sensitivity of qPCR compared with other parasitologic diagnostics (Buonfrate, Requena-Mendez et al. 2018). Furthermore, in the stool culture methods, a larger volume of faeces was used, and therefore the chance of detecting larvae was higher. While for Koga agar plate and Harada-Mori culture about 3 g and 1-2 g of faeces was used, respectively 0.2 g was used in the concentration method for microscopy as well as in qPCR. That might result in detection rate some ten times lower. This might be one aspect of the difference in detection rate in the StrongPaed study. Taking a correction factor of ten times for the results of qPCR and microscopy probably exaggerates that bias. However, considering the sensitivity of about 60

% for qPCR, a correction factor of x2 might preestimate the true prevalence rate.

4.2.2.4 ELISA

While conventional faecal diagnostic methods as well as qPCR tend to miss chronic infections with low larval burdens, serum ELISA has been reported in literature to more likely give a positive result (Requena-Mendez, Chiodini et al. 2013, Buonfrate, Formenti et al. 2015, Buonfrate, Perandin et al. 2017).

Limited conclusions can be drawn from the results of the serological test in the StrongPaed study, since only 51 serum samples were collected because many children and parents rejected the blood sample. Of those 51 samples, 16 were positive or light positive results. Only two of those antibody positive participants showed a matching positive result in any of the other *S. stercoralis* diagnostic tests as shown in *Table 11: Strongyloides positive - all methods*. Among the other 14 positive and light positive ELISA samples, no other *S. stercoralis* diagnostic method showed positivity.

That many positive ELISA samples might mean an unknown amount of false positive results because of cross-reaction. Furthermore, low larval output because of good immune responses might be considered to be a reason for positive ELISA but negative stool culture results. That suggests the possibility of false positive as well as false negative results.

A meta-analysis including studies published from 2006-2014 showed a quite high sensitivity of 70.8-97.8 % and a specificity of 82.6-100 % for serological methods (Buonfrate, Formenti et al. 2015). The assays differ in antigen preparation and use immunoglobulin isotypes with variable sensitivity and specificity. Therefore, comparison with the StrongPaed study results has to be taken very carefully.

Ideally, a standardized Strongyloides-ELISA based on highly specific antigens should be used. A commercial ELISA is available by IVD Research inc. (Strongyloides Serology Microwell ELISA; IVD Research Inc, Carlsbad, CA, USA), but reliable data concerning sensitivity and specificity are hard to find. A

study using this kit calculated positive results in about 30 % of the patients. Since there was a large difference in the results of microscopy and PCR, they suspected ELISA positive results to be false positives (Ahmad, Hadip et al. 2013).

Taking the prevalence of the ELISA diagnostics when calculating the positive results > 20 arb. units, a prevalence of 19.6 % can be calculated in the StrongPaed study. That might show the dimension of the maximal expectable prevalence. Comparing the result of microscopy and qPCR with a prevalence of 2 %, a correction factor of ten times is introduced. As described before, this factor seems to overestimate the true prevalence.

Serology might be used in clinical work as a follow-up for auditing successful therapy or as a screening tool for *S. stercoralis* infection. A positive screening with ELISA should result in further faecal diagnosic tests and if available, enrichment culture techniques should be preferred. However, the ELISA is limited by cost and availability in low-resource countries.

4.3 *Giardia*, *Entamoeba* and *Cryptosporidium*

In this study additional focus was put on the intestinal protozoa *G. lamblia*, *E. dispar* and *E. histolytica* and to a lesser extends also on *C. parvum.* The performed diagnostic methods differ and consequently the comparison of the detected prevalence rates of the intestinal parasites gives an inhomogeneous picture.

The rate of any pathogenic parasite in this study group was quite high with 48.4 % (n=74, 48.4 %) but the burden of acute diarrhoea in the children was not that enormous with 11.5 %. This can be interpreted as a high rate of chronic but asymptomatic infections in the children of this study group.

4.3.1 *G. lamblia*

4.3.1.1 Epidemiology

The overall prevalence of *G. lamblia* in the StrongPaed study in children admitted to the BMC was 27.5 %, detected by qPCR as the gold standard

method. Both of the other performed diagnostic methods showed less positivity with a 12.4 % detection rate in the rapid test and 9.2 % in the FPC® concentration method microscopy.

Other recent studies in Tanzania showed prevalence rates with a wide spectrum between 4.6 % and 74.3 % using various diagnostic methods and different study populations.

In a large study about the determinants and the impact of *G. lamblia* infection in the first two years of life, a prevalence rate of 16.2 % was detected in Tanzania, using an enzyme immunoassay (Rogawski, Bartelt et al. 2017).

Among children younger than two years of age in Dar es Salaam in Tanzania, *G. lamblia* was detected by PCR in 4.6 % of the children. Those children in the control group without diarrhoea were significantly more often carriers of the protozoon than symptomatic children with diarrhoea (Tellevik, Moyo et al. 2015).

A *G. lamblia* prevalence rate of 13 % was seen in adults and children from villages in the Mwanza region in Tanzania who were analysed for intestinal parasites with direct smear, formol-ether concentration method and the Mini-FLOTAC technique (Barda, Ianniello et al. 2014).

G. lamblia was one of the most common parasites in a study population in Zanzibar, Tanzania, with a prevalence rate of 53.4 % identified by PCR. The positivity rate detected by microscopy was only 13 %. Among the symptomatic and asymptomatic study populations with broad age variability, *G. lamblia* was most prevalent with a 74.3 % carrier rate in children two to five years old. In the age group 15 years or older, it decreased to 46.7 %. There was no correlation found between diarrhoea and positivity of *G. lamblia* (Forsell, Granlund et al. 2016). The authors did not differentiate the definition of diarrhoea. The immunity response influenced by nutritional status, age and also repeated exposure to *Giardia* might explain that many asymptomatic infections (Forsell, Granlund et al. 2016). Definition of diarrhoea versus intermittent diarrhoea or loose stool was likewise not done in the StrongPaed study.

In a study among 550 school-aged children on Pemba Island in Tanzania, a prevalence rate of 16.4 % was found by microscopic identification before treatment and a prevalence rate of 17.3 % was found three weeks after various treatment regimes. The insufficient diagnostic method was discussed as an explanation for the unexpected positivity rate after treatment (Speich, Marti et al. 2013).

The aforementioned studies show very diverse prevalence rates although all were conducted in Tanzania and most even in a study population with children. There are many influencing factors such as age, socioeconomic status, diagnostic methods and the specific living region in Tanzania, making comparison quite difficult. The detected prevalence rate in the StrongPaed study lies somewhere in between these other published prevalence rates.

The study population differs in age, and high positivity was seen in preschool-aged children (Forsell, Granlund et al. 2016). In the StrongPaed study population positivity rates were also higher in preschool-aged children but not in statistically significant numbers.

The BMC in Mwanza has a broad catchment area, and people from rural and urban living regions were included in the StrongPaed study population, but many parents were farmers, and a low socioeconomic status can be expected. In other study populations with more people from the urban region such as in Dar es Salaam, higher hygienic standards and consequently lower prevalence rates of *G. lamblia* can be expected.

Even a study with people from the Mwanza region showed a quite different prevalence rate compared to this study. They included children and adults, and the diagnostic methods did not include PCR. Considering the StrongPaed study positivity rate of 9.2 % in microscopy, the detected prevalence rate is not far from the 13 % in the mentioned study (Barda, Ianniello et al. 2014). Many more positive samples were identified with the gold standard diagnostic method in the StrongPaed study, and higher true prevalence rates can be assumed in studies performed without PCR.

4.3.1.2 Diagnostics

Upon comparison of the performed diagnostic methods, qPCR showed the highest detection rate and best sensitivity. Specificity was 100 % in microscopy and in the rapid test compared with qPCR as the reference method. The immunochromatographic rapid test showed better sensitivity than microscopy.

Real-time PCR is considered as a reference method with a very good sensitivity of 91 % and specificity of 99 % for the used PCR primer and probes (Haque, Roy et al. 2007).

Molecular diagnostics is an expensive technique and requires specially equipped laboratories which are rare in low-income countries. Therefore it is not surrently considered a routine diagnostic method in Tanzania, but it worked well in the StrongPaed study as a reference diagnostic method.

The results in the StrongPaed study showed a lower sensitivity for the rapid test (45.3 %) and microscopy (33.3 %) than for qPCR. However, the rapid test showed more positive results than microscopy and no false-positive results compared with qPCR.

The rapid test showed even better results in other studies with a sensitivity of 80-83 % and specificity of 98-100 % for *G. lamblia* (Weitzel, Dittrich et al. 2006, Van den Bossche, Cnops et al. 2015). Weitzel at al. did not use PCR as a reference method but instead used microscopy, a fluorescent antibody test and different rapid tests. Van den Bossche at al. used PCR only for confirmation of samples that were positive in microscopy or ELISA. In both studies, PCR as the most sensitive method was not performed for all samples. This might be a reason for the lower sensitivity of the RIDA ® Quick Combi in the StrongPaed study.

Microscopy is now the standard diagnostic method for *G. lamblia* in low-income countries and is even standard in industrialised countries combined with an ELISA. This status may remain in the future in low-income countries, whereas molecular diagnostics is used more and more in industrialized countries. It must be considered that the rapid test is much easier to perform than microscopy or PCR. Potential problems are commercial availability and cost of the test, yet

otherwise it is worth considering it as an alternative to microscopy. Furthermore, dependence on experienced personnel in microscopy is a problematic factor.

4.3.2 *E. dispar* and *E. histolytica*

4.3.2.1 Epidemiology

The overall prevalence rate of *E. dispar* in the StrongPaed study in children admitted to the BMC was 8.5 % as detected by qPCR as gold standard method. No positive result in qPCR for *E. histolytica* was detected.

Because differentiation between *E. dispar* and *E. histolytica* was first described in 1992, epidemiological literature prior to this date will not be discussed here. Even in recent literature, not all studies are performed with diagnostic methods which are able to differentiate between these two *Entamoeba* species. This makes it more difficult to compare epidemiological data. Much higher prevalence rates are estimated worldwide for *E. dispar* than for *E. histolytica* (Lucius, Loos-Frank et al. 2017).

Verweij et al. found a prevalence rate in northern Ghana of about 40 % for *E. histolytica/E. dispar* with microscopy, but in PCR, only one *E. histolytica* case and a considerably higher prevalence of *E. dispar* at 82.2 % was detected (Verweij, Oostvogel et al. 2003). In the StrongPaed study, the prevalence rates were much lower, but the relation of *E. dispar* versus *E. histolytica* is similar.

One study in Tanzania in the Kilimanjaro region showed prevalence rates of 1 % for *E. histolytica* and 7.3 % for *E. dispar* detected by a specific ELISA (Nesbitt, Mosha et al. 2004). Another study with microscope diagnostics detected a prevalence rate of 18 % for *Entamoeba* spp. (Speich, Marti et al. 2013).

A study performed in Dar es Salaam detected no *E. histolytica* cases among young children with and without diarrhoea (Tellevik, Moyo et al. 2015). In this study, PCR was used, but only children younger than two years were included.

Limited data is available for *E. dispar/ histolytica* in Tanzania. Many study groups in Tanzania did not use PCR, so a conclusion concerning the

prevalence of *E. histolytica* versus *E. dispar* is not possible (Speich, Marti et al. 2013, Barda, Ianniello et al. 2014). Overall, literature results indicate that *E. histolytica* seems not to be very common among children in Tanzania, which matches with the findings of the StrongPaed study in Mwanza.

4.3.2.2 Diagnostics

Obviously, qPCR has the highest diagnostic sensitivity, since the prevalence rate increased more than four times compared with the rapid test and more than twice compared with microscopy. Massively increased prevalence rates with PCR diagnostics in comparison microscopy, as well as good sensitivity and specificity are also reported in literature (Verweij, Oostvogel et al. 2003, Verweij, Blange et al. 2004). Furthermore, a high rate of *Entamoeba* infections might be misdiagnosed even by experienced personnel (Speich, Marti et al. 2013).

The performed diagnostic methods other than microscopy showed less positivity with a 2 % detection rate in the rapid test and 3.9 % in concentration method microscopy. Therefore both detection methods are insufficiently sensitive and are furthermore unable to discern the pathogenic and non-pathogenic *Entamoeba* species.

However, the rapid test showed a better specificity than microscopy and is much more feasible, as it is very easy and quick to perform. The process of microscopy is more time-consuming and requires experienced personnel. Misdiagnosing *Entamoeba* cysts in microscopy is a problem even for experienced personnel (Speich, Marti et al. 2013). There is not only the problem of distinguishing *E. dispar* from *E. histolytica* but morphologically *E. moshkovskii* is also not differentiable and is non-pathogenic as well (Clark and Diamond 1991). This enhances the difficulty of interpretation microscopic detection of *Entamoeba* even more. Limited data is available on the prevalence of this commensal infection. A high prevalence of this species was found in children in Bangladesh. Furthermore, there was a high association seen between *E. moshkovskii* and *E. histolytica* or *E. dispar* (Ali, Hossain et al. 2003). In the Tanzanian HIV population, a higher prevalence rate of *E. moshkovskii* was detected with PCR than was of *E. dispar* (Beck, Dogan et al. 2008). In this

study, the exact analysis of *E. histolytica/ dispar* in microscopy is therefore complicated further by the potential availability of *E. moshkovskii.*

Since molecular diagnostic is expensive and often not available in low-income countries, the rapid test might be considered for future diagnostics.

4.3.3 *C. parvum*

Few positive findings and a limited diagnostic approach for *C. parvum* in the StrongPaed study result in limited conclusions concerning prevalence and a comparison of diagnostic methods. The detected prevalence rate of 3.9 % is based only on the rapid test results which is not the standard diagnostic method.

In a recent study in Tanzania, a prevalence rate of 10.4 % for *C. parvum* was detected with PCR technique (Tellevik, Moyo et al. 2015). Comparison with other published prevalence rates using different diagnostic methods is always difficult. Furthermore, the study population differs concerning age and clinical symptoms.

However, a good sensitivity of 82-100 % and specificity of 98-100 % was published for the rapid test for *C. parvum* which was used in this study (Weitzel, Dittrich et al. 2006, Van den Bossche, Cnops et al. 2015). The positive findings in the rapid test in the StrongPaed study were confirmed only in one case in microscopy. Since microscopy was performed only for the positive rapid test samples, a calculation of sensitivity and specificity is impossible or at least inadequate. Due to the known low sensitivity of microscopy in Ziehl-Neelsen stained stool smerars, this test was used as a confirmatory test only and for assessment of the infection intensity. Microscopy is often used as a diagnostic method for the detection of *C. parvum,* but different staining techniques are used. Diagnostic sensitivities vary depending on the staining used and the experience of personnel (Verweij, Blange et al. 2004, ten Hove, Schuurman et al. 2007). Low sensitivity in microscopy originates occasionally due to the tiny oocysts that can easily be mistaken as artefacts and can also be confused with other oocysts. A study in South Africa compared microscopy, PCR and ELISA diagnostics for *Cryptsporidium*. They showed a sensitivity of 32-46 % for Ziehl-

Neelsen stained microscopy and the highest sensitivity for PCR diagnostics (Omoruyi, Nwodo et al. 2014). Reference laboratories in the USA and in Europe often use immunofluorescence microscopy as a gold standard (Checkley, White et al. 2015).

PCR for *Cryptosporidia* could not be performed because of a limited study budget. Moreover, the focus of the StrongPaed study was not put on *C. parvum* so this is a limitation factor regarding the reported prevalence rate. However, molecular methods show the highest diagnostic sensitivities but are mostly limited to the research context.

4.4 Risk factors for IPI

4.4.1 Sociodemoraphic and hygienic risk factors

Sociodemographic aspects such as rural residence, parents working as farmers and no flush toilet at home were significantly associated with carriage of intestinal parasites in this study group upon univariate analysis. These results demonstrate that the living conditions are the key determinant for parasite carriage. However, these aspects were not confirmed in multivariate logistic regression. Since the hygienic and sociodemographic factors used in the model are related to each other, the binary logistic regression model shows that many associations of risk factors with infection prevalence may be attributed to confounders. The associations of parasitic infection and sociodemographic factors were significant only in univariate analysis but not in the logistic regression model except for the association of STH with school-age, toilet access and parents working as farmers.

The moist and warm African environment is ideal for the transmission and survival of helminths. Poor hygienic conditions with insufficient water access and limited sanitation perpetuate the cycle of excreted larvae, contaminated soil and percutaneous infection. A literature review and meta-analysis to assess the effect of sanitation on STH showed a reduced risk of helminthiasis transmission under proper sanitary living conditions. However, *S. stercoralis* infection was not assessed in that study (Ziegelbauer, Speich et al. 2012).

Toilet access seems to be of greater importance for the infection rate of protozoa, whereas limited access to clean water is more strongly associated with helminthic infection. However, lack of sanitation and hygiene are conditions that enable the transmission of protozoa as well as of helminths.

Obviously rural living and work in the fields increase the risk of intestinal parasitic infection, especially STH such as *S. stercoralis.* Although the children themselves do not work in the fields, they accompany their parents and are used walking outside in potentially faecal-contaminated soil. Rural living and farm work are linked to a lower socioeconomic status which might be associated with a lower hygienic standard.

4.4.2 Clinical symptoms

Upon analysing the correlation of clinical symptoms with infection prevalence, no significant association was found using a logistic regression model. This indicates the problem of symptom related implications to parasitic infections. The study group included children admitted to the paediatric ward due to several diagnoses, and no selection concerning the symptoms was done. Furthermore, parasitic colonisation is a common phenomenon in endemic areas and might become a problem in immune supressed situations. Symptomatic infections with intestinal parasites are just the “tip of the iceberg”.

One more aspect might be that children with uncomplicated acute gastrointestinal symptoms such as diarrhoea and vomiting are not brought to a hospital if the process is self-limited. Therefore, the typical gastrointestinal disease caused by intestinal parasitic infections might be a minor issue in this study group. At a referral hospital, patients might tend to come for more complicated health issues, and the detected parasitic infections were not the cause for admission.

The symptom profile of the StrongPaed study group showed diarrhoea in 11.5 % of the children, but no correlation was found between diarrhoea and positivity of *G. lamblia*. Among those children with diarrhoea, there were even fewer children with positive results for *G. lamblia* than with negative results. This matches with the results in other studies that showed either no correlation or

even a negative correlation between diarrhoea and positivity of *G. lamblia* (Kotloff, Nataro et al. 2013, Tellevik, Moyo et al. 2015, Forsell, Granlund et al. 2016).

The detected prevalence rate in the StrongPaed study population shows that *G. lamblia* is a noteworthy protozoon in children in the Mwanza region without significant association with age, symptoms and living situation. As a risk factor we only found the absence of a flush toilet at home to be significantly correlated with *G. lamblia* positivity. This shows the importance of improvement in hygienie at home. Limited access to appropriate toilets has already been shown in literature as a risk factor (Abate, Kibret et al. 2013, Choy, Al-Mekhlafi et al. 2014).

However, the numbers in the StrongPaed study are very small, and further investigations in a larger study group are necessary for significant conclusions concerning any correlation between symptomatology, age and socioeconomic profile of the children.

4.4.3 Deworming and preventive chemotherapy

The question of treating asymptomatic infections is not clear for all parasites. The relevance of *S. stercoralis* infection as a cause of morbidity and mortality in children is still an ongoing research question. Preventive chemotherapy treatment and mass deworming actions focussing on STH are implemented in many endemic regions in Africa (WHO 2013). However, the common deworming programmes do not include *S. stercoralis* therapy with ivermectin or albendazole in an adequate dose (WHO 2012).

Deworming treatments are implemented regularly for children, particularly those of school age. In this study group, no child testing positive in any diagnostic method for *S. stercoralis* or for hookworm had received deworming during the last 12 months, so there was no case of unsuccessful deworming. However, the numbers are small, and infections with helminths may occur even after deworming as a reinfection. Particularly *S. stercoralis* might be a problem since the common deworming medication recommended by the WHO (http://www.who.int/elena/titles/deworming/en/, 20.12.2020) with annual or

biannual single-doses of albendazole 400 mg or mebendazole 500 mg has no sufficient therapeutic effect on *S. stercoralis* but does on hookworm (WHO 2006).

In case in which deworming therapy was given in the last 12 months in this study group and parasites were still found, none of the parasites would have been eradicated by the given drugs. Due to the assumed high rate of re-infection, the need for treatment is debatable, at least in asymptomatic patients. In this study group, the negative relationship between previous antihelminthic treatment and the positivity of helmiths in general and also for STH infection was shown to be statistically significant.

Reinfection after treatment is a problem, especially for children who are exposed to contaminated soil regularly. In such cases reinfection with the parasites might be just a question of time. Generally, increasing numbers of resistances to various antiinfective drugs are a problem, but no proven resistances are known against anthelminthic drugs for human use to date. However, careful and critical usage of pharmaceutics is necessary in clinical work and in building treatment strategies or implementing deworming schemes for *S. stercoralis* infection in endemic areas.

4.5 **Limitations**

The results reported in this study are limited by the small number of included children and by the short enrolment time. The study group might also not be sufficiently representative since all children were admitted to a hospital.

It has to be considered that the observed prevalence rates taken for calculation are dependent on the performed diagnostic methods with limited sensitivity and specificity. The absence of a true gold standard for *S. stercoralis* diagnostics impedes the comparison of published results and implementation of a standard protocol.

Serologic diagnostics for *S. stercoralis* was only performed in about 1/3 of the study participants as only limited numbers of blood samples were provided. Taking blood samples in children for research purposes only is a sensitive

issue. To avoid parents refusing to take part in the study, provision of a blood sample had to be voluntary. Furthermore, serology was done by an in-house-ELISA with the assumed possibility of cross-reactions.

For *S. stercoralis* microscopy, only one stool sample was provided and not three as recommended in order to increase the sensitivity of microscopy (Knopp, Mgeni et al. 2008). This limitation was based on cost and time, as well on the compliance of the study participants.

Differentiation between hookworm and *S. stercoralis* larvae was performed on the basis of findings in literature, but longtime experience of the study personnel was not given. Support was given through laboratory personnel in the clinic, but not every sample was reassessed.

Results of the microscopy based on the FPC® concentration method were limited due to time. The sediment was only partially examined, and reassessment was only performed in selected samples.

No complete diagnostics for *C. parvum* were performed. The Ziehl-Neelsen acid fast staining method of microscopy was performed only for a limited number of samples, due to the known low sensitivity for Ziehl-Neelson stained microscopy and different study focus. No molecular diagnostics were done due to a limited study budget.

Information concerning the clinical and sociodemographic situation of the children and their family was dependent on correct answers in the questionnaire. The questionnaires were not always filled out completely or were only completed by one parent. Furthermore, social desirability bias has to be considered although the participants did not receive any consequences and protection of data privacy was given.

4.6 **Prospects**

The WHO global target was to eliminate morbidity due to soil-transmitted helminthiases in children by 2020 (WHO 2006, WHO 2012).

Using the term STH generally means infections with *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Necator americanus* or

Ancylostoma duodenale (hookworms). However, although *S. stercoralis* is soil-transmitted as well, it is not regularly included by WHO when building strategies to reach the global target. The WHO strategy in the 2012–2020 road map for neglected tropical diseases aims for regular treatment of at least 75 % of the children at risk of morbidity in endemic areas with preventive chemotherapy (WHO 2012, WHO 2019). That aim was yet still not been reached. In Africa, 268 million children needed preventive chemotherapy for STH in 2019, but only 57.9 % received treatment (WHO 2019).

The common deworming programmes do not include ivermectin or albendazole in a sufficient treatment regime for *S. stercoralis* therapy. Looking at the risk factors of parasitic infections, deworming must not remain the only way of reaching the WHO goal. Improvement in sanitation reduces the risk of transmission of helminthiases (Ziegelbauer, Speich et al. 2012). In highly endemic regions, health education strategies and improvements in access to WASH should be prioritized alongside chemotherapy (Jourdan, Montresor et al. 2017). Sustainable, long-term investments in sanitation-based approaches should be combined with preventive chemotherapy (Hawdon 2014, Coffeng, Bakker et al. 2015).

The potential of integrating ivermectin into deworming schedules has to be assessed further. This also means the necessity of improved availability of ivermectin, not only but especially in low-income countries. Possible scenarios of intervention concerning *S. stercoralis* diagnostics and treatment have to be developed.

Overall, there were not many positive cases of *S. stercoralis* infection detected in this study. Consequently, comparing the different methods is not sufficient for an exact conclusion. However, all the diagnostic methods had limitations and difficulties and were not completely satisfying in the end. Either they lack in sensitivity and specificity or in feasibility. Further research with larger study groups, long-term follow up and improved implementation of diagnostic methods is necessary to provide broad information about the prevalence of intestinal parasitic infections and particularly of *S. stercoralis* infection in

children in the Lake Victoria region. An improved diagnostic procedure for further studies in this setting might include the analysis of repeated stool samples. Another way of improvement of the Koga agar plate culture for *S. stercoralis* diagnostics was described by Pocaterra et al in 2017. They modified the agar plate by using cellophane paper in the centre to avoid bacterial colonisation (Pocaterra, Ferrara et al. 2017). This might be a notable modification for further diagnostics. However, the problem of distinguishing hookworm from *S. stercoralis* larvae is not overcome here. That needs further specialisation and training of laboratory personnel. The independent reassessment of positive findings might be a quality control but would mean higher workloads. Molecular diagnostics with PCR for hookworm and *S. stercoralis* should be implemented. Moreover, microscopy sensitivity for the FPC® concentration method might be improved when examining larger volumes of material.

The serodiagnosis of *S. stercoralis* infection by enzyme-linked immunosorbent assays showed an improvement when using a new recombinant antigen, termed NIE. A serological NIE-ELISA for *S. stercoralis* diagnostics that can be performed on dried blood spots was found to give comparable results to serum ELISA with venipuncture blood samples. The reported sensitivity was 85.7 %, and the specificity was 88.9 % (Mounsey, Kearns et al. 2014). This kind of ELISA might be an alternative approach for serological diagnostics in children to avoid the problem of taking blood samples and also to save time.

The results in this study may indicate the rapid test as a good alternative diagnostic method to standard microscopy, particularly for *G. lamblia*. There are not many results reported in literature for this exact rapid test. Therefore, it is difficult to give a clear recommendation and further evaluation of the test should be done (Weitzel, Dittrich et al. 2006, Van den Bossche, Cnops et al. 2015). The rapid test means fewer personnel and time constraints than with microscopy. Considering the overall costs, the rapid test might be favoured since personnel costs are quite high and are becoming higher even in low-income countries.

The same considerations apply to *E. dispar/histolytica* diagnostics. However, there is the problem of inability to discriminate between *E. dispar* and *E. histolytica.* Focussing on treatment relevance, clinical aspects have to be observed to determine the possibility of invasive amoebiasis. In case of academic focus on *Entamoeba* species, the qPCR is necessary.

5 Summary

5.1 Summary (English Version)

The StrongPaed study in the paediatric ward of a referral hospital in Mwanza in the lake region of Tanzania showed the prevalence of *S. stercoralis*, *G. lamblia*, *E. histolytica* and *E. dispar* as well as of other intestinal parasites with various diagnostic methods.

The prevalence of *S. stercoralis* was 2-10 % depending on the diagnostic methods used. There were no symptomatic infections but only carriage of the nematode. The positive results differed greatly depending on the performed diagnostic methods. None of the diagnostics showed satisfying results, neither in sensitivity and specificity nor in feasibility for this population in an endemic region in sub-Saharan Africa. PCR and microscopy were limited by the low amount of examined stool samples and by the resulting lack of sensitivity. Stool cultures were limited by time-consuming procedures and mainly by the problem of differentiation from hookworm and the resulting lack of specificity. ELISA was limited by the need of blood samples and also by poor specificity in the ELISA used.

The prevalence of *G. lamblia* was high, but mostly only carriage and not symptomatic infections was seen. No *E. histolytica* was detected, but 8.5 % samples were positive for *E. dispar*. Among the performed diagnostics, the rapid test showed sufficient results. It showed better sensitivity than microscopy and is cheaper and more feasible than PCR. Differentiation between *E. histolytica* and *E. dispar* was only possible with qPCR performed in Germany.

More children were positive for intestinal parasites from rural than from urban areas. The profession of the parents working as farmers was a risk factor for intestinal parasitic infections. Hygienic living conditions such as access to tap water and flush toilets at home were preventive for intestinal parasitic infections in children.

5.2 **Zusammenfassung (deutsche Version)**

Die StrongPaed Studie auf der pädiatrischen Station des Referenzkrankenhauses in Mwanza in der Seeregion von Tanzania zeigte die Prävelanz von *S. stercoralis*, *G. lamblia*, *E. histolytica* und *E. dispar* sowie weiteren Darmparasites mit verschiedenen Diagnostikmethoden.

Die Prävalenz von *S. stercoralis* betrug 2-10 %, abhängig von den gewählten diagnostischen Methoden. Es traten keine symptomatischen Infektionen auf, sondern lediglich Besiedelungen durch Würmer. Die positiven Ergebnisse unterschieden sich stark in Abhängigkeit von den durchgeführten diagnostischen Methoden. Keine dieser diagnostischen Methoden zeigte für die gewählte Population zufriedenstellende Ergebnisse, weder in Bezug auf Sensitivität und Spezifität noch in der Durchführbarkeit. PCR und Mikroskopie waren durch die geringe Menge an untersuchten Stuhlproben und die daraus resultierende fehlende Sensitivität limitiert. Die Stuhlkulturen waren durch zeitaufwändige Verfahren und vor allem durch das Problem der schwierigen Differenzierung zum Hakenwurm und der daraus resultierenden mangelnden Spezifität limitiert. Der ELISA war durch den Bedarf an Blutproben und in dieser Studie auch durch die geringe Spezifität limitiert.

Die Prävalenz von *G. lamblia* war hoch, meist handelte es sich jedoch nur um eine Besiedelung und nicht um eine symptomatische Infektion. Es wurde in keinem Fall *E. histolytica* nachgewiesen, aber 8,5 % der Proben waren positiv für *E. dispar*. In der durchgeführten Diagnostik zeigte der Schnelltest zufriedenstellende Ergebnisse. Er zeigte eine bessere Sensitivität als die Mikroskopie und ist billiger und praktikabler als die PCR. Eine Differenzierung zwischen *E. histolytica* und *E. dispar* war nur mit der in Deutschland durchgeführten qPCR möglich.

Mehr Kinder aus ländlichen als aus städtischen Gebieten waren positiv auf Darmparasiten. Der Beruf der als Landwirt arbeitenden Eltern war ein Risikofaktor für Darmparasiten. Hygienische Lebensbedingungen wie der Zugang zu Leitungswasser und Toilettenspülung im Haushalt korrelierten mit einer niedrigeren Rate an nachgewiesenen Darmparasiten bei Kindern.

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Appendix

I. Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
A. dist.	Aqua distilled
AP	Agar plate culture
arb. units	Arbitrary units
HTLV-1	Human T-cell Leukemia Virus Type 1
AWMF	Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V.
BAL	Broncho-alveolar Lavage
BID	Bis in Die, twice daily
BMC	Bugando Medical Centre
BNI	Bernhard-Nocht-Institut
C.	Cryptosporidium
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CUHAS	Catholic University of Health and Allied Sciences
d	Days
DNA	Deoxyribonucleic acid
DTG	Deutsche Gesellschaft für Tropenmedizin
E.	Entamoeba
e.g.	For example
ELISA	Enzyme-linked Immunosorbent Assay
G.	Giardia
GCES	Giardia, Cryptosporidium, Entamoeba, Strongyloides

GIT	Gastro-intestinal tract
h	Hours
HIV	Human Immunodeficiency Virus
HM	Harada-Mori culture
Hook	Hookworm
HTLV-1	Human T-cell Lymphotropic Virus
IFAT	Immunofluorescence Antibody Test
IFN	Interferon
IPI	Intestinal Parasitic Infection
IQ	Intelligence quotient
IRIS	Immune Reconstitution Inflammatory Syndrome
κ	kappa
LIPS	Luciferase Immunoprecipitation System
micro	Microscopy
min	Minutes
ml	Millilitre
mg	Milligramm
mm	Millimetre
MTA	Material Transport Agreement
nd	Not done
NPV	Negative predictive value
n.v.	Not visible
OD	Once a day
OR	Odds ratio
PBS	Phosphate buffered saline

PCR	Polymerase chain reaction
qPCR	Quantitative/ Real-time polymerase chain reaction
p.o.	Per os, orally
PPMix	Primer Probe Mix
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
rpm	Revolutions per minute
S.	Strongyloides
SAC	School-aged children
SAF	Sodium acetate-acetic acid formaldehyde
sec	Seconds
SSU	Small sub unit
STH	Soil-transmitted helminths
TID	Three times a day
TPH	Tropical and Public Health Institute
WASH	Water, Sanitation and Hygiene
WHO	World Health Organisation
µm	Mikrometre

II. List of figures

III. List of Tables

IV. Acknowledgements

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V. Curriculum Vitae

Name	Anita Janzen-Maaser, geb. Janzen
Date of Birth	26.12.1989
Place of Birth	Rotenburg a.d. Fulda
Nationality	German

Professional Experience

Since 01.02.2018	Klinikum Fulda gAG, Med. Klinik I, Fulda, Assistenzärztin für Innere Medizin

Education

06/2016 – 09/2018	doctoral study programme at the Julius Maximilian University of Würzburg
10/2009 – 06/2016	study of Human Medicine at the Julius Maximilian University of Würzburg
since 01/2013	Clinical Research on the „Prevalence of Strongyloides infection and other intestinal parasites in paediatric patients in a referral hospital in Northern Tanzania”
	Tropical Medicine Department at the Missio Clinic in Würzburg
02/2013 – 05/2013	Field Research at Bungando Hospital, Mwanza, Tanzania
2006 – 2009	High School Modellschule Obersberg, Bad Hersfeld
	Graduation: Abitur
2000 – 2006	Secondary School Konrad-Duden Schule, Bad Hersfeld
1996 – 2000	Primary School Linggschule, Bad Hersfeld

VI. Publications

15.03.2014	Short presentation at the DTG Meeting (German Society of Tropical Medicine and Internations Health), Duesseldorf, Germany: Prevalence of intestinal protozoa in paediatric patients in a referral hospital in Northern Tanzania
11.10.2017	Poster presentation at the ECTMIH (European Congress on Tropical Medicine and International Health), Antwerpen, Belgium: Prevalence and associated risk factors of intestinal parasites among children in a referral hospital in Northern Tanzania

Attachment

Questionnaire

Strongyloides/ Intestinal Parasites Prevalence Study – Pediatric Ward

Patient questionnaire:

1. Study Nr:_____ **2. File Nr:_____**

3. Namba ya simu ya wazazi:_____

Mobile number

4. Mwanamke: ☐ **Mwanaume:** ☐

Female *Male*

5. Umri:_____ **6. Uzito: _____** **7.Urefu:_____**

Age *Weight* *Height*

8. Kazi ya wazazi:

Profession of parents/caretakers:

Mvuvi: ☐ Mkulima: ☐ Mama: ☐ Nyingine: ☐

Fishermen *Farmer* *Housewife* *Other*

9. Ni jimbo/ wilaya gani unaishi?

In which Ward/ District do you live?

10. Makazi: Mjini: ☐ Vijijini: ☐ Kitongoji: ☐

Living condition *Urban* *Rural* *Suburb*

11.Maji unayotumia:

Access to water

Bomba: ☐ Kisima: ☐ Chemchemi: ☐ Mto/Ziwa: ☐

Tap *Well* *Spring* *River/Lake*

12. Mna choo: Kichaka: ☐ Choo cha shimo: ☐ Choo cha: ☐

Toilet *Bush* *Latrine* *Flush toilet*

13. Unavaa viatu: Mara chache: ☐ Sivai: ☐ Mara zote: ☐

Wearing shoes *Often* *Never* *Always*

14. Kulazwa kwa ajili: _____

Admission because of:

15.Yakini dalili:

Actual symptoms:

Harisha:☐ Maumivo ya tumbo:☐ Homa:☐

Diarrhea *abdominal pain* *Fever:*

Kutapika:☐ Rehe:☐ Kikohozi: ☐ Upele:☐

Vomiting: *Flatulence:* *Cought:* *Skin lesions/rash:*

16. Kubanwa kifua: Ndio: ☐ Hapana: ☐ Sijui: ☐

Diagnosed Allergic Asthma *Yes* *No* *Don´t know*

17. Umepata dawa za minyoo ndani ya miezi 12?

Deworming/Anthelmintic therapy within the last 12 months?

Ndio: ☐ Hapana: ☐ Sijui: ☐

Yes ⇩ *No* *Don´t know*

Albendazol: ☐ Dozi: Praziquantel: ☐ Dozi: Metronidazole: ☐

Dozi: Nyingine: _____ Dozi: Sijui: ☐

Other *Don´t know*

18. HIV-mtihani kufanyika? Ndio: ☐ Hapana: ☐ Sijui: ☐

HIV-Text done? ⇩ *Yes* *No* *Don't know*

positive: ☐ last CD4-Count:_____ lowest CD4-Count:_____

Negative: ☐ Siju: ☐

19. Treatment with current Corticosteroids: Yes: ☐ No: ☐

(Prednisolon, Dexamethason) ⇩ Dose:

20. Treatment with other immunosuppressing drugs: Yes: ☐ No: ☐

⇩ Dose:

Consent Form, English

Prevalence of *Strongyloides* infection and other intestinal parasites in paediatric patients in a referral hospital in Northern Tanzania – StrongPaed-Study

Consent Form

Aim of the study:

This study is focussing on an insidious parasitic worm infection which is common in tropical climates like Tanzania. In the state of severe immunosuppression this infection may cause serious illness. Testing for this parasite is not a routine procedure. Therefore most infections remain undetected. In addition this study is looking for other parasites like Giardia and Amoeba which may cause long lasting diarrhoea or abdominal discomfort.

The study intends to obtain data on the prevalence of Strongyloides infection as a basis for a preventive deworming therapy and on the prevalence of parasitic intestinal protozoa.

Your voluntary participation is highly appreciated!

What will be done?

You will be asked to answer a few question related to the risk of acquiring these infections. As the worm infection is transmitted via contaminated soil you will be asked questions concerning living conditions, water supply and sanitation. You will be assigned a participant (study) number. You will be asked to donate a blood sample and a stool sample.

What will be done with the samples?

The blood will be analysed for antibodies to Strongyloides and possibly other worm infections. The stool samples will be analysed by different methods for the presence of Strongyloides infection. In addition a sophisticated rapid test will be done looking for parasitic protozoa (Giardia, Amoeba, Cryptosporidia) which are commonly associated with diarrheoa.

What will not be done?

Your personnel data (name, age, hospital file number, mobile phone number) will only be used to report the results of the investigations to you for eventual treatment. You can refuse recording of personnel data but then a report of results will not be possible.

What is the benefit for the participant?

The result of the most sensitive tests for Strongyloides, the culture tests, will be ready one week after delivery of a stool sample. These tests are focussing specifically at Strongyloides – infection and will not give information about other worm infections like ascariasis or schistosomiasis. The results of the test for Giardia, Amoeba and Cryptosporidia will be available within 1 day. Results will be reported to the ward as soon as possible.

What are the risks for the participant?

Blood sampling will be done with sterile safety equipment in the most convenient way for the participant. Blood sampling is a routine procedure in medicine with minor risks only, e.g. local bleeding, swelling or bruises at the site of vein puncture. The risk of an infection by blood sampling is very low. Delivering a stool samples does not have any risks for the participant.

Will the participant be paid?

No. There will be no remuneration for participating in the study.

Will I be offered treatment in case an infection is detected?

If you / your child are staying in the hospital appropriate therapy will be prescribed.

Who can I contact in case of additional questions or to retrieve my results?

Study Nurse: Sr. Lydia Makenge, CTC clinic, Bugando, mobile no. 071 320 3521

Principal Investigator: Prof. Samuel Kalluvya, Bugando, mobile no. 071 333 0766

I have read the information above. All my questions have been answered and I agree to participate in the study.

Date: Place: Signature: Signature of Witness

Consent Form, Swahili

Hali ya maambukizi ya strongyloides na aina nyingine ya minyoo kwa watoto wenye maambukizi ya virusi vya ukimwi kusini mwa Tanzania

Fomu ya Idhini

Utafiti huu unalenga

Utafiti huu unalenga zaidi maambukizi nyemelezi ya vimelea minyoo ambavyo ni kawaida katika hali ya hewa ya Tanzania. Katika upungufu wa kinga haya maambukizi ni hatarishi. Upimaji wa haya magonjwa sio ya utaratibu kwahiyo maambukizi huwa yasiyotambuliwa. Pia utafiti huu unaangalia aina nyinginezo za minyoo kama Giardia na Amoeba ambazo zinaweza kusababisha kuharisha kwa muda mrefu au maumivu ya tumbo.

Utafiti huu utaangalia hali ya maambukizi ya strongyloides ikiwa na lengo la kuzuia maambukizi kwa utumiaji dawa na hali ya minyoo ya utumbo.

Hiari yako katika somo hili ni wa umuhimu!

Nini kitakachofanywa?

Utaulizwa maswali machache kuhusu njia hatarishi zakupata magonjwa haya. Maambukizi hupatikana kupitia udongo wenye vimelea hivyo maswali yatahusisha hali ya maisha, upatikanaji wa maji na usafi. Utapatiwa namba ya somo na kuombwa kutoa sampuli ya damu na choo kikubwa

Nini kitakachofanyika na sampuli hizo?

Damu itafanyiwa utafiti kuona uwepowa chembe chembe za kinga dhidi ya minyoo hao na wengineo, na choo kikubwa pia kitafanyiwa utafiti kwa njia tofauti kuona uwepo wa vimelea hivyo. Na zaidi itabidi ufanyiwe vipimo kwa ajili ya magonchwa ambao ni protozoa.

Nini hakitafanywa?

Mambo yako binafsi (jina, umri, namba ya file, namba ya simu) havitatumika katika ripoti isipokuwa pale katika majibu ya vipimo ili uweze pata tiba. Unaweza kukataa kutoa namba yako ya binafsi ila utashindwa kupata majibu.

Ni faida gani utakayopata?

Kupata majibu ya vipimo baada ya wiki moja. Vipimo vinaangalia vimelea vya strongyloides na sio vinginevyo. Unaweza kupata majibu kutoka kwa nesi kupitia simu. Majibu ya vipimo hivi yatapatikana ndani ya siku moja, na yataripotiwa wodini mapema iwezekanavyo.

Nini ni hatarishi kwako?

Vipimo vya damu vitachukuliwa kwa usafi kwa ushirikiano wa mgonjwa. Unaweza kutoka damu kidogo, kuvimba kwenya sehemu utakayochomwa sindano. Hatari ya kupata maambukizi mengineyo ni kidogo sana. Utoaji wa choo kikubwa hauna hatari yoyote.

Je utalipwa?

Hapana, hapatakuwa na malipo ya aina yoyote.

Je mwanangu atapatiwa tiba kama akigundulika kuwa na vimelea hivyo vya magonjwa?

Ndiyo, mwanao atapatiwa tiba sahihi. Pale atakapogunduliwa kuwa na vimelea hivi, pia tiba hii ni bure.

Nani wakumuulizia iwapo una maswali au kupata majibu?

Nesi, Sr. Lydia Makenge wa CTC Bugando, namba ya simu 0713203521

Mtafiti mkuu, Propesa Samuel Kalluvya, Bugando, namba ya simu 0713330766

Nimesoma taarifa zilizo hapo juu, na nimeridhika na majibu yaliyopo kwa maswali yangu, hivyo ninakubali mwanangu kushiriki zoezi hili.

Tarehe: Sehemu:

Sahihi:

Sahihi ya shahidi: