Effekt von antihelminthischer Behandlung auf die Impfimmunogenität in einer für Geohelminthen sehr anfälligen Population

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MSc. Sina Christine Brückner

aus Hanau

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„Wer die Natur betrachtet, wird vom Geheimnis des Lebens gefangen genommen.“

(Albert Schweitzer)

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Diese Dissertation basiert auf den nachfolgend angeführten und bereits publizierten bzw. zur Publikation eingereichten Ergebnissen:

Liste der Veröffentlichungen

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Weitere Veröffentlichungen, die nicht in diese Dissertation eingeflossen sind:

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Abkürzungen

Abb.	Abbildung
AIDS	erworbenes Immundefektsyndrom (acquired immunodeficiency syndrome)
ASC	Antikörper-sezernierende Zellen
AT	antihelminthische Behandlung
BCG	Bacillus-Calmette Guérin
bzw.	beziehungsweise
CD	Differenzierungsantigene (Cluster of Differentiation)
CTL	zytotoxische T-Lymphozyten
dl	Deziliter
ELISA	Enzyme-linked Immunosorbent Assay
ELISpot	Enzyme-linked Immunospot Assay
g	Gramm
GMT	Geometrisch gemittelter Titer
HI Test	Hämagglutinin Inhibitionstest
HIV	Humanes Immundefizienzvirus
IFN	Interferon
Ig	Immunglobulin
IL	Interleukin
im	intramuskulär
L1	Larvenstadium 1
mg	Milligramm

MIF	Methionin-Jod-Formaldehyd
OPV	oraler Poliovirus
PBMC	mononukleare Zellen des peripheren Blutes (peripheral blood mononuclear cells)
RKI	Robert-Koch Institut
SBA	Serumbakterizidtest (Serum Bactericidal Assay)
sc	subkutan
spp.	spezies pluralis
STH	über den Boden übertragene Würmer (Soil-transmitted Helminth)
Tab.	Tabelle
TB	Tuberkulose
Th1	T-Helfer Typ 1
Th2	T-Helfer Typ 2
TNF	Tumornekrosefaktor
Treg	regulatorische T-Zellen
vs.	versus
WHO	Weltgesundheitsorganisation
z.B.	zum Beispiel

Zusammenfassung

Wurminfektionen sind vor allem im Sub-Sahara-Raum weit verbreitet und können zu Beeinträchtigungen der kognitiven und körperlichen Fähigkeiten beitragen sowie das Immunsystem beeinflussen, was besonders in Bezug auf Impfungen zu berücksichtigen ist. Kürzlich konnte gezeigt werden, dass nach einer oral verabreichten Impfung gegen Cholera in Personen, die mit *A. lumbricoides* infiziert waren eine verminderte Immunantwort hervorgerufen wurde. Einige wenige andere Studien, durchgeführt in Mäusen und Menschen, gaben Hinweise darauf, dass Infektionen mit Helminthen Impfantworten beeinflussen.

Aus diesem Grund wurde eine randomisierte Placebo-kontrollierte Studie in Gabun, Zentralafrika, durchgeführt, mit dem Ziel einen möglichen Zusammenhang zwischen Immunogenität von Impfungen und Wurminfektionen zu untersuchen. Es wurden drei unterschiedliche Impfungen mit jeweils verschiedener Applikation gewählt, um einen möglichen Unterschied zwischen den unterschiedlichen Anwendungsarten der Impfungen detektieren zu können. Pro Impfgruppe wurden 98, 104 bzw. 105 Schulkinder im Alter von 6-10 Jahren in die Studie eingeschlossen, von denen eine Hälfte eine antihelminthische Behandlung und die andere Hälfte ein Placebo vier Wochen vor der Impfung verabreicht bekam. Anschließend wurden die Probanden entweder mit einer saisonalen Influenza Impfung (intramuskulär), einer Meningokokken Impfung (subkutan) oder einer oralen Cholera Impfung immunisiert.

Es wurden sowohl Antikörpertiter als auch Antikörper-sezernierende Zellen, die Memory-B-Zellen repräsentieren auf das jeweilige Impfantigen untersucht. Es konnten keine statistisch signifikanten Unterschiede der Immunantwort auf die Impfungen festgestellt werden. Weder die Antikörpertiter noch die Antikörper-sezernierenden Zellen lieferten einen signifikanten Unterschied zwischen der antihelminthisch behandelten Gruppe und der Placebo Gruppe. Dennoch ließ sich ein Trend für eine höhere Immunogenität in der antihelminthisch behandelten Gruppe gegenüber der Kontrollgruppe bei der Influenza Impfung feststellen.

Des Weiteren wurden auch die Immunglobulinisotypen untersucht. Dabei wurden signifikant höhere Gesamt-IgA Antikörpertiter in der antihelminthisch behandelten Gruppe gegenüber der Kontrollgruppe für die Influenza Impfung an Tag 28 gefunden. Der Basistiter (vor der Impfung) der beiden Gruppen war nicht unterschiedlich.

Im Generellen ließ sich feststellen, dass sich bei allen Impflingen unabhängig von der Gruppe drei Monate nach der Impfung eine signifikante Immunogenität gegenüber der Basislinie auf die untersuchten Impfantigene zeigte. Der Einfluss einer einmaligen Entwurmungstherapie vier Wochen vor der Impfung in unserem Kollektive aber keinen Einfluss auf die Immunogenität hatte.

Abstract

Helminth infections are distributed worldwide, especially in the sub-Saharan area, and can lead to cognitive and physical impairment as well as to immunological changes. This should be taken into account when it comes to vaccination. Recently it was shown that orally administered vaccines against Cholera mediate a reduced immune response, when individuals were infected with *A. lumbricoides*. Very few other studies in mice and humans gave evidence that vaccine responses are influenced by helminth infection.

Due to this a randomized placebo-controlled trial was conducted in Gabon, Central Africa, to investigate the relationship between immunogenicity and helminth infections. Three differently administered vaccines were chosen to detect a possible difference in terms of vaccine application. For each vaccine cohort 98, 104 and 105 primary school children were enrolled, half of them received an antihelminthic treatment and the other half a placebo. Four weeks later the participants received either a seasonal influenza vaccine (intramuscularly), a meningococcal vaccine (subcutaneous) or an oral cholera vaccine.

Antibody titers and antibody-secreting cells, which represent the memory B cells, were investigated for each vaccine antigen. Neither the antibody titer nor the antibody-secreting cells were significantly different in the pre-treated compared to the placebo group, but there was a trend towards a better immunogenicity in the antihelminthic treated group compared to the control group in the influenza vaccinated arm.

Additionally immunoglobulin isotypes were analyzed. Here significantly elevated total IgA antibody titers were found in the antihelminthic treated group compared to the placebo group at day 28 in the participants vaccinated with the influenza vaccine. The baseline titer (before vaccination) was not different in both groups. In the context of this study no prediction can be made, if the elevation is due to the vaccination or due to the antihelminthic treatment.

Taken together we found that all vaccinees, independent of the group, showed a significant immunogenicity towards the vaccine antigens three months after vaccination compared to baseline. The influence of a single-dose antihelminthic

treatment four weeks before vaccination had no influence towards the immunogenicity in our study.

1. Einleitung

1.1 Allgemeine Informationen

Infektionen mit Geohelminthen oder mit über den Boden übertragenen Würmern (soil-transmitted helminths: STH), wie *Ascaris (A.) lumbricoides, Trichuris (T.) trichiura* und Hakenwürmern (*Necator (N.) americanus* und *Ancyclostoma (A.) duodenale*), sowie mit anderen Arten, wie *Schistosoma* spp., stellen ein großes öffentliches Gesundheitsproblem dar, worunter weltweit mehr als eine Milliarde Menschen vor allem im Sub-Sahara-Raum[1] leiden[2–8].

Der Term “Helminthen” wird verwendet, um wurm-ähnliche Parasiten aus den Stämmen Platyhelminths (Saug- und Bandwürmer), Nematoden (Rundwürmer) und Acanthocephalans (Dornkopfwürmer) zusammenzufassen[9,10]. Die am häufigsten vorkommenden Helminthenarten sind *A. lumbricoides* mit etwa 1,4 Milliarden infizierten Menschen, *T. trichiura* mit 1,3 Milliarden Infizierten und Hakenwürmer mit etwa 1 Milliarde Infizierten, gefolgt von Trematoden wie *Schistosoma* spp., mit rund 200 Millionen infizierten Personen weltweit[2,3,5,11,12].

Im Laufe der Evolution haben Geohelminthen mehrere Übertragungswege entwickelt, um den menschlichen Wirt zu infizieren: oral (z.B. *Ascaris, Trichuris*), durch das Eindringen über die Haut (z.B. *Schistosoma* spp. und Hakenwürmer) oder durch Vektorenübertragung (z.B. Filarien)[13,14].

Darüber hinaus können Helminthen in drei verschiedenen Entwicklungsstadien im Wirt vorkommen: als Eier, Larven und adulte Würmer[2,13]. Sie können außerdem unterschiedliche Organe befallen wie Lunge, Leber, Darm, Kolon, Dünndarm und die Lymphgefäße[9,13,14].

1.2 Lebenszyklen der Geohelminthen

1.2.1 Ascariasis

Adulte Askariden, auch Rundwürmer genannt, scheiden bis zu 200.000 Eier pro Tag aus. Die Eier werden über den Kot ausgeschieden. Bei geeigneten Umweltbedingungen wie ausreichender Feuchtigkeit und Temperaturen zwischen 9°C und 35°C entwickeln sich in den Eiern zwei Larvenstadien (L1 und L2) (Abb. 1, 2-3). Die L2 Larveneier werden dann über die Nahrung aufgenommen (Abb.1, 4) und

gelangen in den Dünndarm, wo diese schlüpfen. Sie bohren sich durch die Darmwand und wandern mit dem Blutstrom in die Leber, in der sie sich zu L3-Larven weiterentwickeln. Über die untere Hohlvene gelangen diese zum Herzen und von dort über die Lungenarterien weiter in die Lungenbläschen, worüber sie in den Luftraum der Alveolen dringen (Abb.1, 6). Hier entstehen nach 10 bis 14 Tagen L4-Larven, die wiederum über die Bronchien und die Luftröhre den Kehlkopf erreichen und dort abgehustet und geschluckt werden (Abb.1, 7). Dadurch gelangen die Larven wieder in den Dünndarm, wo sie sich zu adulten Tieren entwickeln und ohne Behandlung bis zu 1 ½ Jahren überleben können[15,16].

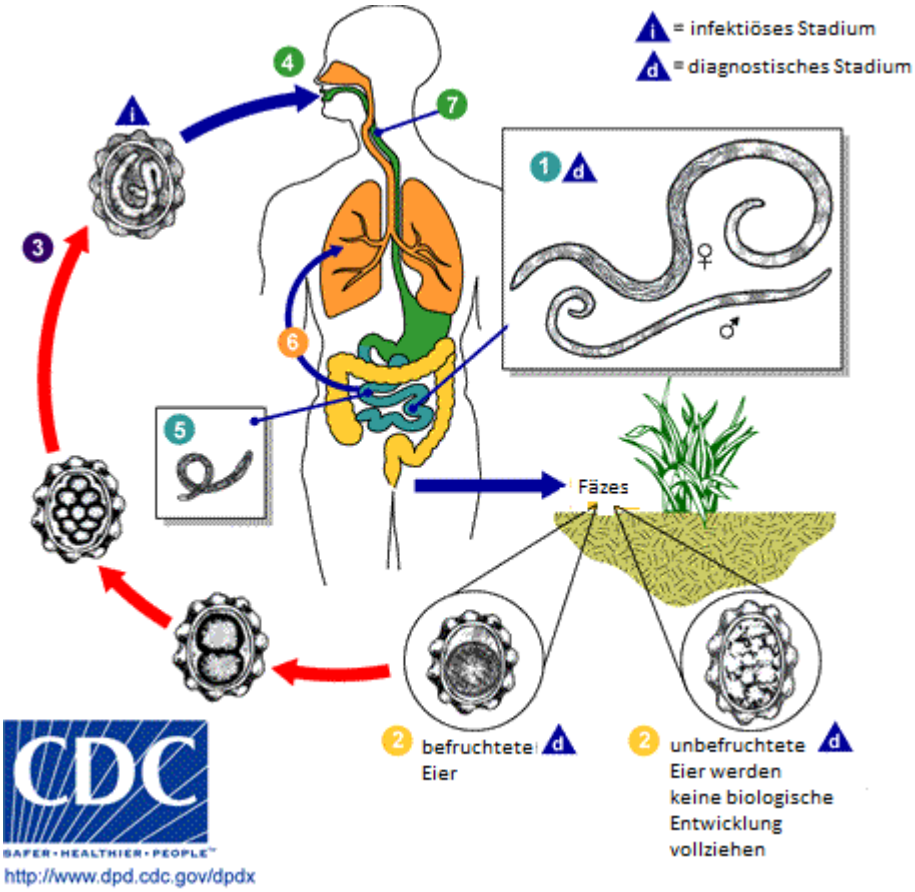

Abbildung 1: Lebenszyklus von *Ascaris lumbricoides*

http://www.cdc.gov/parasites/ascariasis/biology.html, adaptiert nach Sina Brückner, zuletzt auf dieser Webseite gewesen 05.01.2016

1.2.2 Trichuriasis

Auch von den Trichurien (Peitschenwürmern) werden 3.000-20.000 Eier pro Tag über den Kot ausgeschieden (Abb. 2, 1), wo sie drei bis vier Monate überdauern, bevor sie erneut infektiös sind. Wie bei den Askariden werden die Eier über die

Nahrung aufgenommen (Abb. 2, 4) und gelangen in den Darm. Dort schlüpfen die Larven und setzen sich im Dünndarm fest, um zum adulten Wurm heranzuwachsen, der anschließend ins Zäkum wandert (Abb. 2, 6). Der Lebenszeitraum dieser Würmer beträgt unbehandelt etwa ein Jahr[15,17].

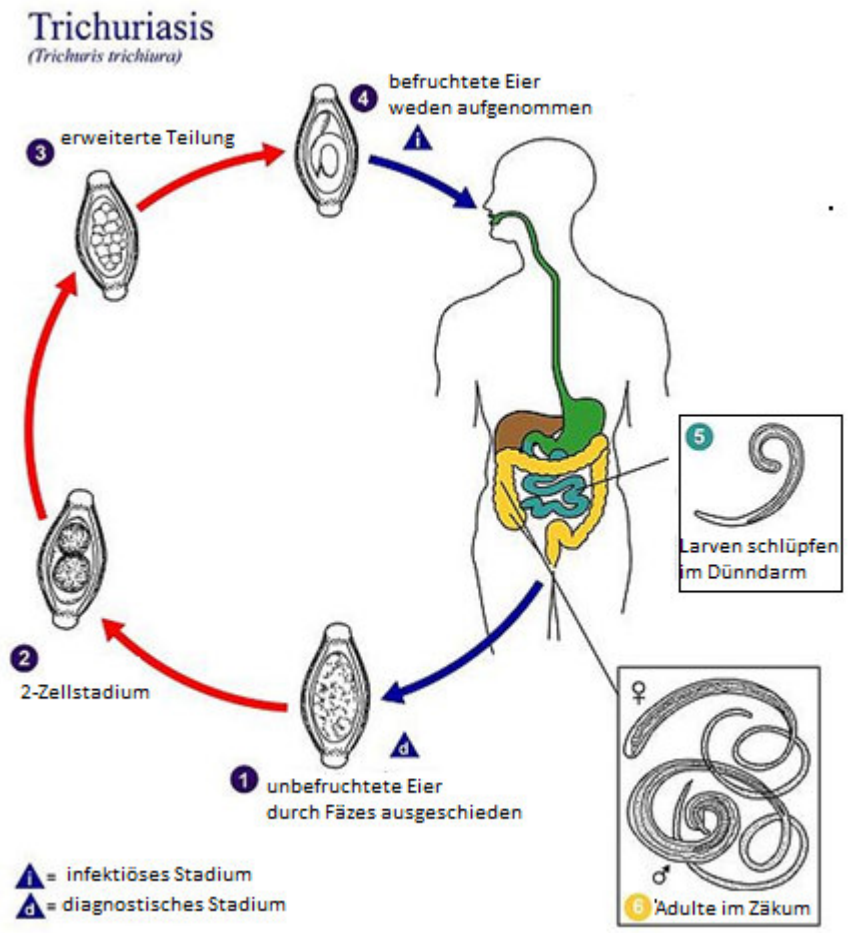

Abbildung 2: Lebenszyklus von *Trichuris trichiura*

http://www.cdc.gov/parasites/whipworm/biology.html, adaptiert nach Sina Brückner, zuletzt auf dieser Webseite gewesen 05.01.2016

1.2.3 Hakenwurm

Ebenso wie bei Ascariasis und Trichuriasis gelangen die Eier des Hakenwurms über die Ausscheidungen in die Umwelt, in der sich die Larvenstadien L1-L3 entwickeln. Während die Stadien L1 und L2 von Bakterien im Kot leben, wandert das Stadium L3 aktiv in die oberste Erdschicht ein und wartet dort auf einen geeigneten Wirt (Abb. 3, 2-3). Bei Hautkontakt bohrt sich die Larve in die Haut und wird zu L4 (Abb. 3, 4), von der Penetrationsstelle gelangt die Larve mit dem Blut in die Lunge, wo sie sich zu L5

weiterentwickelt. Dieses Larvenstadium gelangt über die Bronchien in den Rachen, dort werden sie ausgehustet und geschluckt. Zurück im Darm entwickelt sich der adulte Wurm, der unbehandelt bis zu zwei Jahren Blut an den Darmzotten saugen kann (Abb. 3, 5)[15,18].

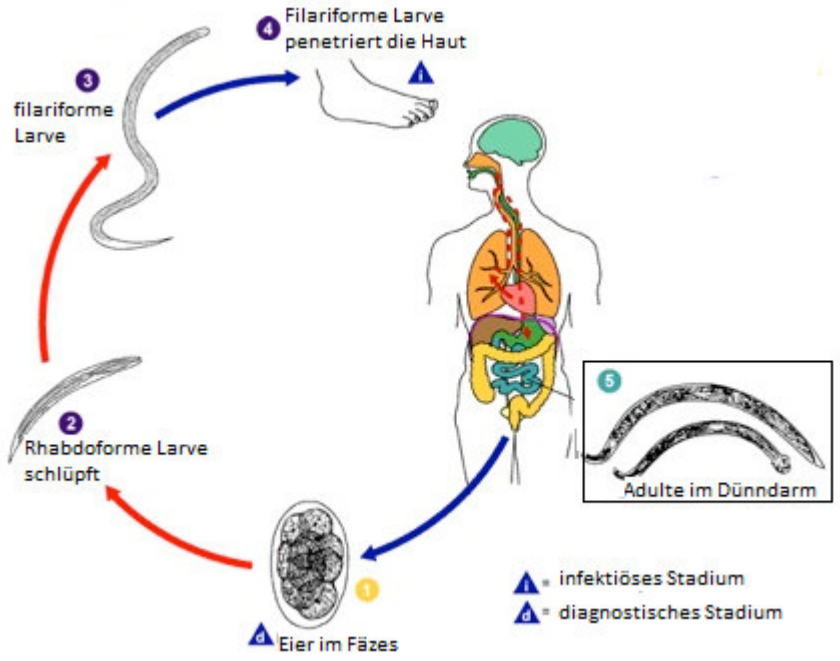

Abbildung 3: Lebenszyklus des Hakenwurms

http://www.cdc.gov/parasites/hookworm/biology.html, adaptiert nach Sina Brückner, zuletzt auf dieser Webseite gewesen 05.01.2016

1.3 Konsequenzen für den humanen Wirt

In vielen Fällen verlaufen Infektionen mit Geohelminthen asymptomatisch[14,19–21], können allerdings auch klinisch relevant werden. Die auftretenden Symptome hängen mit der Schwere der Wurminfektion zusammen[2,21] und werden in akute und chronische Infektionen unterteilt. Eine akute Infektion ist hinsichtlich des Krankheitsanfangs und der Symptome auf einen bestimmten Zeitraum beschränkt. Bleibt die Infektion allerdings unbehandelt, kann sie chronisch werden. Gerade im Sub-Sahara-Raum sind chronische Krankheitsbilder häufig und weit verbreitet. Diese sind nicht fatal, dass heißt sie führen nicht zum Tod, aber zur Morbidität[13,22,23]. Am häufigsten betroffen sind arme Populationen und in diesen besonders Vorschulkinder, Schulkinder und schwangere Frauen[5,6,24,25].

Auf Grund dessen, dass die Folgen einer Geohelminthiasis meistens chronischer Natur und selten wirklich fatal sind, wurden diese Infektionen lange Zeit vernachlässigt. Die Weltgesundheitsorganisation (WHO) zählt Geohelminthen-Infektionen mittlerweile zu einer der am meisten vernachlässigten tropischen Erkrankungen mit ernstzunehmenden Gesundheits-, Ernährungs- und sozialen Beeinträchtigungen für Betroffene[12,26]. Chronische Wurmerkrankungen können zu verringertem Wachstum, körperlicher und geistiger Unterentwicklung, verminderter physischer Fitness, verschlechterter Gedächtnis- und kognitiver Leistung[6,8,12,27–33] und dadurch bedingt zu einer verminderten schulischen Leistung führen. In schwangeren Frauen können Fehlgeburten, Anämie und geringeres Geburtsgewicht des Neugeborenen eine Folge sein[34,35].

In mehreren Studien konnte gezeigt werden, dass vor allem Schulkinder von diesen Infektionen betroffen sind. Es ist bekannt, dass in endemischen Gebieten die Wurmlast mit dem Alter bis zu einem Maximum akkumuliert, um anschließend wieder abzunehmen. Dieses Maximum variiert stark zwischen den einzelnen Wurmarten. Für den Hakenwurm und Filarien liegt es im Erwachsenenalter[2,5,36–39], wohingegen Infektionen mit Askariden und Trichurien ihr Maximum bereits im Alter zwischen 5 und 15 Jahren erreichen[30,40]. Bei Infektionen mit Schistosoma liegt es zwischen 10 und 15 Jahren[41].

Behandelt wird die Infektion mit Geohelminthen für gewöhnlich mit einem der beiden Benzimidazolen (Albendazol oder Mebendazol) über einen Zeitraum von ein bis drei Tagen. Eine Einmaldosis wird nicht empfohlen, da die Effektivität sehr variiert und sie nur gegen Askariden wirksam ist. Für die anderen beiden Spezies ist mindestens eine zweifache Dosis notwendig[42,43].

Auch Polyparasitismus ist weit verbreitet und Ko-Infektionen werden mit höherer Wurmlast verbunden als Einzelinfektionen[2,44]. Des Weiteren deutet das lange Überleben innerhalb des Wirts darauf hin, dass die Helminthen raffinierte Mechanismen entwickelt haben, um dem zytotoxischen Effekt des Immunsystems zu entkommen[10,45]. Einer dieser Mechanismen ist der Wechsel von einer T-Helfer Typ 1 (Th1) zu einer T-Helfer Typ 2 (Th2) Immunantwort[3,19,46].

1.4 Immunomodulation

Infektionen mit Würmern können eine starke Modulation des Wirtsimmunsystems hin zu einer Th2 Immunantwort induzieren, um im menschlichen Wirt überleben zu können. Wurmparasiten haben Strategien entwickelt, um sowohl die angeborenen als auch die erworbenen T- und B-Zell-Antworten durch die Aktivierung von regulatorischen T-Zellen (Treg) und/oder antiinflammatorischen Zytokinen wie Interleukin-10 (IL-10) zu regulieren[13,47]. Treg-Zellen vermindern die Immunantwort und produzieren IL-10. Dieses Zytokin ist bekannt dafür, dass es das Wachstum und die Differenzierung von B-, T- und natürlichen Killerzellen reguliert[48,49]. Diese Mechanismen regulieren das Immunsystem herunter, was sowohl für den Parasiten als auch für den menschlichen Wirt von Nutzen sein kann. Es wird angenommen, dass dieses Phänomen abhängig von der Zeit und der Wurmlast ist[13]. Darüber hinaus werden mehr Immunoglobulin G4 (IgG4), IgG1 und IgE sowie übermäßige Mengen an Eosinophilen und Mastzellen in der Peripherie von infizierten Menschen gefunden[50–52]. Diese Modifikationen des Immunsystems können zu einer höheren Anfälligkeit für Infektionskrankheiten mit Viren oder Bakterien führen[11,14].

T-Lymphozyten können sich entweder in $CD4^+$ oder $CD8^+$ T-Zellen differenzieren. $CD8^+$ T-Zellen sind auch als zytotoxische T-Lymphozyten (CTL) bekannt und zerstören viral infizierte Zellen und Tumorzellen, in dem sie die Apoptose in diesen Zellen einleiten[33,47]. Die $CD4^+$ T-Zellen wiederum werden in Treg- und Th-Zellen klassifiziert, welche in zusätzliche Untergruppen eingeteilt werden können, abhängig davon, welche Zytokine sie produzieren (Th1/ Th2/ Th17)[2,53]. Th1-Zellen produzieren überwiegend IL-2, Interferon-y (IFN-y) und den Tumornekrosefaktor β (TNF-β). Sie sind gegen Viren und intrazelluläre Bakterien aktiv und vermitteln eine pro-inflammatorische Zytokin-Antwort[47]. Im Gegensatz dazu produzieren Th2-Zellen, die gegen extrazelluläre Pathogene gerichtet sind, IL-4, IL-5, IL-9, IL-10, IL-13, IL-21, IL-25 und IL-33[54–62]. Darüber hinaus unterstützen sie B-Zellen in der Produktion von Antikörpern. Th17-Zellen sekretieren überwiegend IL-17, IL-17F und IL-22[63]. In einem Model - vorgeschlagen von Maizels und Yazdanbakhsh im Jahr 2003 - sind Th1-, Th2- und Treg-Zellen ausbalanciert[47]. Während einer chronischen Wurminfektion wird dieses ausbalancierte System polarisiert und Th2-Zellen vertreten die dominante Position. Dies geht einher mit einer erhöhten Konzentration von IgE und IgG4 und einer vermehrten Mastzellaktivierung und

Schleimsekretion[47,50,53,64]. Man nimmt an, dass diese Mechanismen zu einer wurmspezifischen und generellen Immunsuppression führen[1,4,46,47] und dadurch die Reduktion der Parasiten initiiert werden kann[3,14,19,33,52,65].

Während chronischen Wurminfektionen verhindern Th2- und Treg-Antworten starke Immunantworten gegen den parasitären Wurm, sie erlauben Langzeitüberleben und eingeschränkte Pathogenität[11,13,49]. Darüber hinaus beschützen diese Zellen den Wirt nicht *per se*, aber sie schützen vor potentiellem pathogenen Schaden, der von einer unbegrenzten Th1-Zell-vermittelten Entzündung hervorgerufen werden kann[47]. Ferner besitzen Th2-Antworten während Wurminfektionen sowohl pro- als auch anti-inflammatorische Funktionen[47]. Eine besondere Bedeutung wird IL-5, einem Th2 Zytokin, welches auch von Mastzellen produziert wird, zuteil, da Th2-Zellen die Fähigkeit besitzen IL-5-Antworten auf Antigene zu minimieren, was zu einem verringerten einbeziehen von eosinophilen Granulozyten in die Typ-2-vermittelte Entzündung führt[19,47,66,67]. Normalerweise stimuliert IL-5 im Zusammenspiel mit anderen Zytokinen die B-Zellentwicklung und erhöht so die Sekretion von Antikörpern[68]. Außerdem ist dieses Zytokin ein Schlüsselmediator für die Aktivierung von Eosinophilen, deren antiparasitärer Effekt durch eine antikörperabhängige, zellvermittelte zytotoxische Reaktion vermittelt wird[69], da Th2-Zellen nicht in der Lage sind Parasiten alleine zu töten und dementsprechend die Zytokin-vermittelte Aktivierung von angeborenen Effektorzellen benötigen[19].

1.5 Immunogenität/ Impfungen

Impfungen werden als die beste Möglichkeit angesehen, um Infektionskrankheiten zu verhindern bzw. einzuschränken. Dies ist umso wichtiger in Ländern, in denen infektiöse Erreger hoch endemisch sind. Aus den wenigen zu diesem Thema durchgeführten Studien geht hervor, dass Wurminfektionen die Wirkung von Impfungen negativ beeinflussen können[13,24,70].

In endemischen Gebieten sind Kinder die Zielgruppe für Impfungen. Oft sind sie bereits ab einem Alter von 9 Monaten, wenn sie mit Masern und Gelbfieber immunisiert werden sollen, schwerwiegend mit Würmern infiziert[8,71]. Darüber hinaus gibt es Hinweise, dass Wurminfektionen während der Schwangerschaft das Immunsystem eines Neugeborenen beeinflussen könnten und aus diesem Grund eine Auswirkung auf die Impfimmunogenität bei Neugeborenen/Kleinkindern haben

könnten[8,72–75]. Prävention und Behandlung scheinen äußerst wichtig zu sein, um Wurminfektionen zu verringern. Deshalb arbeiten die WHO und andere Institutionen, wie die „Bill und Melinda Gates Fondation“, an der Umsetzung und der Verbesserung von Entwurmungsprogrammen für Kinder und anderen Personen, die einem erhöhten Risiko einer Wurminfektion ausgesetzt sind[76]. Es gibt beispielweise bestehende Empfehlungen zur zweimaligen Entwurmung pro Jahr in hoch endemischen Gebieten[77], was eine große Auswirkung auf verschiedene Gesundheitsparameter, wie generelle Morbidität, Anämie, Wachstums- und Lernfähigkeiten, als auch auf die Wirkung von Impfungen hat[5,71,78–80].

Studien der letzten Jahre zeigten, dass Wurminfektionen die Impfimmunogenität verändern können. Dies wurde sowohl in Mausmodellen, als auch in einzelnen Humanstudien für Impfungen wie Bacillus Calmette-Guérin (BCG), Tetanus, Cholera (oral verabreicht) und Influenza nachgewiesen[24,72,81–84].

Während der klinischen Entwicklung von Impfungen werden die jeweiligen Kandidaten-Impfstoffe häufig zuerst in Industrieländern getestet, um die Sicherheit und Immunogenität des Impfstoffes zu untersuchen. Allerdings ist häufig die Immunogenität wie auch die Wirksamkeit dieser Impfungen in Entwicklungsländern, wo sie am dringendsten benötigt werden, oftmals vermindert[84–86]. Dies trifft zum Beispiel auf die BCG Impfung zu, die gegen Tuberkulose (TB) wirkt und in Entwicklungsländern direkt nach der Geburt verabreicht wird. Die Wirksamkeit der BCG Impfung reicht von 0-80%[7,72,87,88]. Aber auch andere Impfungen vor allem solche, die oral verabreicht werden, wie die oral verabreichte Polioimpfung (OPV) oder die Rotavirus-Impfung fallen diesem Phänomen zum Opfer[11,89–96]. Faktoren für die geringe Wirksamkeit könnten schlechte Gesundheitspflege, hohe maternale Antikörper, schlechte Spurenelementaufnahme oder andere ernährungsbedingte Umstände und Ko-Infektionen sein. Der Einfluss dieser Faktoren variiert eventuell zwischen den verschiedenen Impfungen[11,96,97].

2. Ziel der Arbeit

Nachdem aus wenigen bisher am Menschen durchgeführten Studien hervor geht, dass Würmer die Immunogenität vor allem von oralen Impfungen beeinträchtigen können, stellt sich die Frage, ob dies generell auch bei anders verabreichten Impfungen der Fall ist und ob die Verabreichung einer Einzeldosis eines Antihelminthikums ausreicht, um die Impfantworten auf die Impfungen zu beeinflussen. Deshalb sollte in der vorliegenden doppelblinden randomisierten Studie der Einfluss einer einmaligen antihelminthischen Behandlung (AT) auf die Impfimmunogenität von drei unterschiedlich verabreichten und in Gabun kommerziell erhältlichen Impfungen untersucht werden. Die Impfungen wurden gemäß der unterschiedlichen Applikationsform gewählt. Des Weiteren sollten sie nicht zum erweiterten Programm zur Immunisierung (EPI) in Gabun gehören, um somit eine Grundimmunisierung der Probanden unwahrscheinlich zu machen.

2.1 Studien Design

Im Rahmen einer doppelblinden randomisierten Placebo-kontrollierten Studie wurden Schulkinder im Alter von 6 bis 10 Jahren, die in Lambaréné und Umgebung wohnen eingeschlossen und randomisiert, um entweder eine einmalige antihelminthische Behandlung (Albendazol 400mg) oder ein Placebo zu erhalten[98] (Tag -28). Vier Wochen nach der Behandlung erfolgte die Impfung; entweder eine saisonale Influenza Impfung (VAXIGRIP®) intramuskulär (i.m.), eine Meningokokken Impfung (Polysaccharid Meningokokken A + C®) subkutan (s.c.) oder eine orale Cholera Impfung (Dukoral®) (Tag 0) (Abb. 4). Die beiden erstgenannten Impfungen wurden einmalig und die letztgenannte zweimalig mit einem Abstand von sieben Tagen verabreicht. Einen Monat nach der Impfung gab es einen Folgetermin (Tag 28), um Blut zur Untersuchung von Antikörpern zu erhalten; die Studie endete am Tag 84 also drei Monate nach der Impfung[98]. Die einzelnen Kohorten (Zugehörigkeit nach der jeweiligen Impfung) wurden zeitlich versetzt durchgeführt.

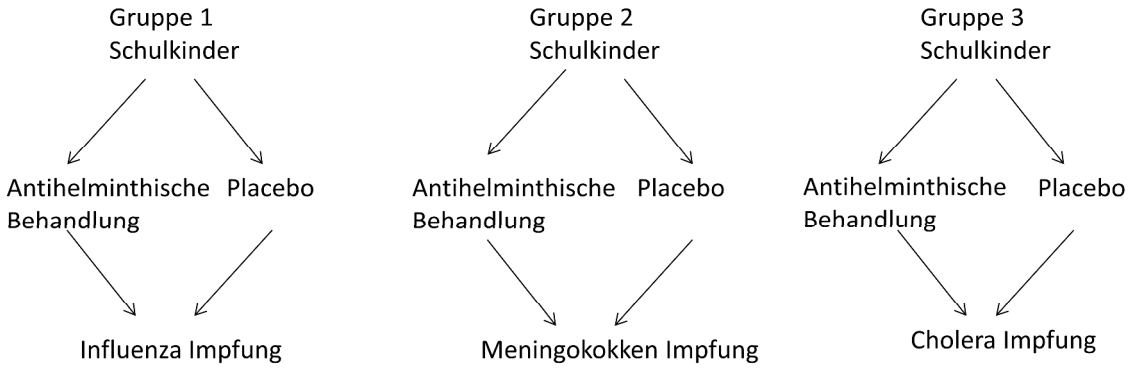

Abbildung 4: Studien Design (HelmVacc)

Die Schulkinder, die in die Studie eingeschlossen wurden, bekamen am gleichen Tag entweder eine antihelminthische Behandlung oder ein Placebo verabreicht (Tag -28). Vier Wochen später wurden diese, je nach Gruppenzugehörigkeit, mit der entsprechenden Impfung immunisiert.

Einschlusskriterien waren eine unterschriebene Einverständniserklärung, keine bekannte Erkrankung und keine Symptome, die auf eine akute oder chronische Erkrankung hinweisen, keine Symptome einer akuten Helmintheninfektion sowie die Absicht, bis zum Ende der Studie in dieser Umgebung wohnhaft zu bleiben. Zu den Ausschlusskriterien zählten: bestehende Teilnahme an einer anderen Studie, bekannte Kontraindikationen gegenüber der antihelminthischen Behandlung oder einer der verabreichten Impfungen, eine bereits bestehende Immunisierung gegen eines der Impfantigene durch vorangegangene Impfung oder eine bekannte vorausgegangene Meningokokken oder Cholera Erkrankung (außer bei Influenza, da die Impfstämme jedes Jahr unterschiedlich sind), bekannte oder verdächtigte immunsuppressive Krankheiten (z.B. HIV). Bei einer fieberhaften Erkrankung am Tag der Impfung, sollte diese verschoben werden, bis die Erkrankung ausgeheilt war. Im Fall einer akuten oder chronischen Erkrankung wie Malaria, AIDS oder Tuberkulose, einem Hämoglobin Level <7g/dl oder Anzeichen für Hämaturie und/oder Proteinurie (mit Hilfe des Combur 9 Tests überprüft) wurde das Kind nicht eingeschlossen und an das Albert-Schweitzer-Krankenhaus verwiesen. Kinder, die mit *Schistosoma (S.) haematobium* infiziert waren oder an einer anderen symptomatischen Infektion litten, wurden aus der Studie ausgeschlossen und gleich behandelt. Alle positiven auf Parasiten getesteten Probanden (auch solche ohne Symptome) bekamen am Ende der Studie eine angemessene Behandlung.

2.2 Immunologische Untersuchungen

Blut wurde an den Tagen 0, 28 und 84 entnommen. Der primäre immunologische Endpunkt der Studie war die Messung des funktionalen Antikörperlevels auf die jeweiligen Impfantigene. Im Falle von Influenza und Meningokokken wurden pathogen spezifische Antikörper gemessen. Influenza-spezifische Antikörper wurden mittels Hämagglutinin-Inhibitionstest (HI) zum Nachweis virusspezifischer Antikörper am Deutschen Referenzzentrum für Influenza (Robert-Koch-Institut, Berlin)[99] gemessen, bei Meningokokken wurde ein Serumbakterizidtest (SBA) am Nationalen Referenzzentrum für Meningokokken (Institut für Hygiene und Mikrobiologie, Würzburg) durchgeführt und bei Cholera wurde dies mit Hilfe eines adaptierten Cholera-spezifischen IgG Enzyme-linked immunosorbent Assays (ELISA) getestet[100]. Als sekundärer immunologischer Endpunkt wurde die Entwicklung von Memory-B-Zellen auf das jeweilige Impfantigen untersucht. Dies geschah mit Enzyme-linked Immunospot (ELISpot) Assays. Des Weiteren wurden auch andere Antikörperklassen wie IgA, IgE, IgM und die IgG-Unterklassen (IgG1, IgG2, IgG3 und IgG4) mittels Bioplex untersucht.

3. Ergebnisse

3.1 Effekt einer antihelminthischen Behandlung auf die Impfimmunogenität einer saisonalen Influenza Impfung in Grundschulkindern in Gabun: eine randominisierte Placebo-kontrollierte Studie

Effect of antihelminthic treatment on vaccine immunogenicity to a seasonal influenza vaccine in primary school children in Gabon: A randomized placebo-controlled trial

Sina Brückner, Selidji T Agnandji, Stefan Berberich, Emmanuel Bache, José F Fernandez, Brunhilde Schweiger, et al.

Im ersten Teil der Studie wurden 98 Grundschulkinder getestet; 50 von diesen erhielten eine antihelminthische Behandlung (AT Gruppe) und 48 ein Placebo (Kontrollgruppe). Am Ende der Studie konnte für 44 Probanden aus der AT Gruppe und 38 aus der Kontrollgruppe der HI-Test durchgeführt werden (Paper 1, Abb. 1). Dabei wurde festgestellt, dass die beiden in der Impfung enthaltenen Influenza A Stämme in der AT Gruppe im Vergleich zu dem ebenfalls in der Impfung enthaltenen Influenza B Stamm zur Kontrollgruppe einen tendenziell, jedoch nicht signifikant höheren Titer an Tag 28 aufwiesen, der sich in beiden Gruppen bis zum Tag 84 etwas verringerte, aber immer noch über dem Basistiter lag (Paper 1, Abb. 2). Darüber hinaus war das Gesamt-IgA am Tag 28 in der AT Gruppe gegenüber der Placebo Gruppe signifikant erhöht (Paper 1, Abb. 4), was sich jedoch nicht auf Influenza-spezifisches IgA übertragen ließ (Paper 1, Abb. 5).

Alle Probanden wiesen drei Monate nach der Impfung (Tag 84) signifikant vermehrte Antikörper-sezernierende Zellen (ASCs) auf (p-Wert <0.001), welche Impfspezifische Memory-B-Zellen repräsentieren (Paper 1, Abb. 6). Betrachtet man dieses Ergebnis innerhalb der beiden Gruppen, ist anzumerken, dass in der AT Gruppe vermehrt ASCs im Vergleich zu der Kontrollgruppe nachzuweisen waren; dieser Unterschied war allerdings nicht statistisch signifikant.

Daraus lässt sich jedoch ein Trend bezüglich einer gesteigerten Immunogenität in der AT Gruppe erkennen.

3.2 Keine Beeinflussung der Impfimmunogenität auf eine Meningokokken und Cholera Impfung nach einer einmaligen antihelminthischen Behandlung in Gabunesischen Schulkindern

A single-dose antihelminthic treatment does not influence immunogenicity of a meningococcal and a cholera vaccine in Gabonese school children

Sina Brückner, Selidji T Agnandji, Johannes Elias, Stefan Berberich, Emmanuel Bache, et al.

Um zu untersuchen, ob eine antihelminthische Behandlung im Vorfeld einer Impfung einen Einfluss auf die Immunogenität zweier Impfungen (Meningokokken Impfung (s.c.) und Cholera Impfung (oral)) besitzt, wurden in diesem Teil der Studie 209 Grundschulkinder aus Lambaréné, Gabun und Umgebung in die vorliegende Studie eingeschlossen. Von diesen 209 Schulkindern bekamen 96 eine Meningokokken und 89 eine Cholera Impfung verabreicht. Es wurden, wie schon in dem vorangegangenen Teil, die bakteriziden Antikörper bzw. impfspezifischen Antikörpertiter sowie die Memory-B-Zellen bestimmt. Im Fall der Meningokokken Impfung waren die Titer in beiden Gruppen über die Zeit bis zu Tag 28 ansteigend und anschließend bis Tag 84 abfallend (Paper 2, Abb. 3A). Dies war bei der, in der Impfung enthaltenen, Serogruppe C etwas stärker in der AT Gruppe ausgeprägt als in der Kontrollgruppe an Tag 84. Dieser Unterschied war nicht statistisch signifikant. In beiden Serogruppen verbleiben die Impftiter an Tag 84 über den Grundtitern. Das gleiche Phänomen lässt sich auch für die Cholera Impfung erkennen. Es besteht jedoch kein Unterschied zwischen der Behandelten und der Placebo Gruppe (Paper 2, Abb. 3B).

Der Anstieg von den Antikörper-sezernierenden Zellen (ASCs) war in beiden Impfungen signifikant. Betrachtet man beide Gruppen im Detail lässt sich feststellen, dass sich in der AT Gruppe bei der Meningokokken Impfung tendenziell mehr ASCs entwickelt haben als in der Kontrollgruppe (Paper 2, Abb. 4A). Dies konnte für die Cholera Impfung nicht gezeigt werden (Paper 2, Abb. 4B).

Alle Ergebnisse sind statistisch nicht signifikant und deshalb lässt sich sagen, dass bezüglich der gemesseneren Parameter kein Unterschied in der Immunität zwischen den vorbehandelten und den Kontrollprobanden zu verzeichnen ist.

4. Diskussion

Wenige kürzlich durchgeführte Studien deuten darauf hin, dass Helminthen Infektionen den immunologischen Schutz einer Impfung beeinflussen können[70,98,101]. Cooper et al. konnte 2000 in einer in Ecuador durchgeführten Studie zeigen, dass Kinder, die mit *A. lumbricoides* infiziert waren, eine verminderte Antikörperreaktion auf die orale Cholera Impfung CVD 103-HgR hatten[101]. In einer Phase Ib Studie in Gabun wurden Kinder mit dem Malaria-Impfstoff-Kandidaten GMZ2 immunisiert. Dabei wurde festgestellt, dass Kinder, die mit *T. trichiura* infiziert waren, eine signifikant reduzierte Antikörperantwort aufwiesen, verglichen mit solchen, die diese Infektion nicht hatten[70]. Des Weiteren gibt es Hinweise, dass die fötale Immunantwort beeinflusst wird, wenn die Mutter mit Geohelminthen infiziert ist. Dieser Effekt überdauert die Kindheit und könnte somit ein Grund für eine Beeinflussung der durch die Impfungen hervorgerufenen schützenden Immunität sein[11]. Besonders mukosale Impfstoffe wie die orale Polioimpfung (OPV) oder Impfungen gegen Rotavirus sind von diesem Phänomen betroffen. Diese Impfungen weisen vor allem in Personen aus ärmeren Regionen eine geringere Immunogenität auf als in wohlhabenden[11,89–91]. Gründe dafür könnten zahlreiche, in einem Haushalt lebende Personen, schlechte Hygiene, hohe maternale Antikörpertiter, Ko-Infektionen, Mangel- oder Fehlernährung sein[96,97,102]. Die Wichtigkeit dieser Faktoren kann von Impfung zu Impfung variieren[11]. Der Effekt der geringeren Immunogenität bestimmter Impfungen konnte auch in Tiermodellen nachgewiesen werden[79].

In Gegenden, in denen Infektionskrankheiten endemisch sind, ist eine effektive Immunantwort auf Impfantigene unerlässlich. Die Antikörperantwort auf diese Impfantigene ist meist ein verlässlicher Parameter für den Impfschutz von Impfungen [98,103–105]. Aus diesem Grund wurden in den vorliegenden Untersuchungen die Antikörpertiter der jeweiligen Impfungen bestimmt, um eine Aussage darüber treffen zu können, ob ein Unterschied bezüglich der Immunogenität der jeweiligen Impfung zwischen der antihelminthisch behandelten und der Placebo Gruppe besteht. Der Basistiter (Tag 0) war, wie erwartet, in zwei der drei getesteten Impfungen gering bis nicht detektierbar. Die geringen Impftiter, die vor der Impfung in der Influenza- und der Meningokokken-Kohorte beobachtet werden konnten, lassen sich auf wahrscheinlich bereits bestehenden Kontakt mit diesen Antigenen zurückführen. Es

ist bekannt, dass Influenza auch in den Tropen zirkuliert[106]. Das positive Ansprechen der Antikörper könnte auch auf eine Kreuzreaktion mit zirkulierenden Virusstämmen oder mit anderen kreuzreaktiven Pathogenen zurückzuführen sein. Die positiven Antikörpertiter bei der Meningokokken-Kohorte könnten darauf hinweisen, dass *Neisseria meningitis* auch in Gabun zirkuliert. Dies ist nicht allzu abwegig, da dieses zentralafrikanische Land von den Meningitisgürtelländern umgeben ist[107] und die Meningitis bis nach Gabun gestreut haben könnte. Um dies nachzuweisen bzw. die Prävalenz in Gabun zu eruieren, wären jedoch weitere Studien notwendig. Die Basistiter in der Cholera-Kohorte waren höher als erwartet. Es ist allerdings unwahrscheinlich, dass dies mit einer vorangegangenen Infektion in Zusammenhang steht. Eher könnte dies auf die gewählte Methode zurückzuführen sein, z.B. Kreuzreaktion der Plasmaantikörper mit den Impfungsantikörpern oder auch, dass sich das Antigen nicht entsprechend blocken ließ. Dies spricht dafür, da auch Kontrollseren von Personen aus Deutschland eine hohe optische Dichte aufwiesen. Eine vorausgegangene Cholerainfektion sowie Infektionen mit enterotoxischen *Escherichia coli* (ETEC) ist auch hier eher unwahrscheinlich. Deshalb haben wir für die vorliegende Studie den Basistiter vom Titer an Tag 28 bzw. Tag 84 nach der Impfung subtrahiert, um jeweils den Anstieg zu berechnen. Als positiv wurden diejenigen Probanden angesehen, die einen 4-fachen Titeranstieg gegenüber dem Basistiter aufwiesen[100].

In dieser Studie gab es einen Anstieg bei den Antikörpertitern bis zum Tag 28 und einen anschließenden Abfall bis zum Tag 84, der aber in allen drei Kohorten über dem Basistiter verbleibt. Vor allem die in der Influenza Impfung enthaltenen Influenza A Stämme (A(H1N1)pdm09 und A(H3N2)) waren in der antihelminthisch behandelten Gruppe im Vergleich zur Kontrollgruppe an Tag 28 tendenziell erhöht. Allerdings war dieser Unterschied zwischen der antihelminthisch behandelten Gruppe und der Kontrolle nicht statistisch signifikant.

Im Falle der Influenza-Kohorte wurde zusätzlich noch der Serum IgA-Titer bestimmt, da IgA in den Schleimhäuten vorkommt und deshalb für die Immunität gegen Influenza eine Rolle spielen kann[108–110]. Hier war ein signifikanter Unterschied zwischen der antihelminthisch behandelten Gruppe und der Kontrollgruppe vier Wochen nach der Impfung zu verzeichnen (Paper 1, Abb. 4)[98]. Die Titer an Tag 0 zeigten keine Gruppenunterschiede. Dennoch kann im Rahmen der durchgeführten

Studie keine Aussage darüber getroffen werden, ob dies mit dem höheren Serum IgA-Spiegel oder mit der Impfung zusammenhängt oder ein verspäteter Effekt der Entwurmung ist. Auch hier sind weiterführende Studien sinnvoll, da IgA wichtige Funktionen bei der Bekämpfung von gastrointestinalen und durch die Luft übertragenen Pathogenen innehat.

Alle drei Kohorten verzeichneten von Tag 0 zu Tag 84 einen signifikanten Anstieg der ASCs, die repräsentativ für die Memory-B-Zellen stehen. Betrachtet man nun die beiden Gruppen im Detail kann kein Unterschied zwischen der antihelminthisch behandelten und der Placebo Gruppe an Tag 84 festgestellt werden.[98,111]

In einer Studie, die 2004 in Gabun durchgeführt wurde und der Berechnung der Studienteilnehmeranzahl dieser Studie zugrunde liegt, wurde eine Prävalenz von Helminthen in der Bevölkerung von 74% in Lambaréné und Umgebung gefunden[112]. Zum Zeitpunkt der Studie ist im untersuchten Kollektiv interessanterweise die Prävalenz mit einer Wurmlast von 21% in der Influenza-Kohorte[98] und von 17,7% an Tag -28 in der Meningokokken-Kohorte wesentlich geringer. In der Cholera-Kohorte wurden zu verschiedene Zeitpunkten unterschiedliche Prävalenzen gemessen (6,7% an Tag 0 zu 22,2% an Tag 84)[111]. Für diesen Rückgang an Wurminfektionen können unterschiedliche Faktoren verantwortlich sein. Zum einen ist die Methode, mit der die Wurminfektionen detektiert wurden eine andere als die, die in den zugrunde liegenden Studien verwendet wurde. In der vorliegenden Studie haben wir uns für die Methionin-Jod-Formaldehyd (MIF-) Technik entschieden, da bei dieser Methode die Stuhlproben konserviert werden konnten im Gegensatz zu der 2004 verwendeten Kato-Katz Methode. Dies gewährleistete, dass alle Forscher, die an dieser Studie beteiligt waren, bis zum Ende verblindet bleiben konnten. Bei der Antragstellung des Projektes wurde von den Reviewern explizit nach einer Methode, die die Verblindung langfristig gewährleistet, verlangt. Da die Sensitivität der Kato-Katz Methode im Vergleich zur MIF-Technik höher ist[113,114] könnte es sein, dass in der Realität dennoch eine leicht höhere Prävalenz, als die in unserer Studie analysierte, existiert.

Zum anderen könnte die bessere Bildung und in diesem Zusammenhang auch Aufklärung über die Risiken und Schädigungen von vor allem chronischen Wurminfektionen eine große Rolle zur Senkung der Prävalenz im Studiengebiet gespielt haben. Auch die unkontrollierte Selbstmedikation, denn die Entwurmungsmittel können frei in Apotheken erworben werden[115,116] oder eine nicht

berichtete, kürzlich vorgenommene Entwurmung, ob nun ärztlich oder selbst verordnet, könnte einen Einfluss auf die geringe Helminthen Prävalenz gehabt haben. All dies zusammengenommen müssen wir feststellen, dass unsere errechnete Studienteilnehmerzahl aufgrund der niedrigen Wurmlast geringer war als ursprünglich berechnet.

Es könnte somit sein, dass eine höhere Probandenzahl evtl. zu einem deutlicheren Ergebnis geführt hätte. Da aber fast alle Probanden einen als schützend geltenden Antikörpertiter auf die getesteten Impfungen entwickelten[100,117,118], stellt sich die Frage, ob eine einmalige antihelminthische Behandlung überhaupt einen Einfluss auf die Immunogenität der getesteten Impfungen hat.

In vielen Hochendemiegebieten sind Massenentwurmungen eine gängige Praxis zur Kontrolle der Helmintheninfektionen[5,42,43,119]. Diese werden häufig in Form einer Einfachdosis in Schulen durchgeführt, um möglichst viele Menschen gleichzeitig zu erreichen. Es könnte jedoch bereits in mehreren kürzlich durchgeführten Studien gezeigt werden, dass eine einmalige Entwurmung nicht effektiv zu sein scheint[42,43], sondern langfristig gesehen einen eher additiven Effekt hat. Dies bezieht sich vor allem auf die Wurmarten *T. trichiura* und den Hakenwurm[43,120]. Um eine Reduktion der Wurmlast insbesondere in diesen zwei Spezies zu erzielen, sollte mindestens eine zweifach Dosis, wenn nicht sogar eine dreifach Dosis in Betracht gezogen werden[43].

Da keine bzw. nur sehr geringe Unterschiede zwischen den beiden Gruppen zu sehen waren, kann keine Empfehlung bezüglich der Effektivität einer Einzeldosis eines Antihelminthikums ausgesprochen werden.

Zusammenfassen lässt sich sagen, dass es in dieser Studie keinen signifikanten Unterschied zwischen der antihelminthisch behandelten und der Kontrollgruppe in Bezug auf die Impf-spezifischen Antikörpertiter und die ASCs in allen drei Kohorten gab. In der Influenza-Kohorte konnte an Tag 28 ein höherer Gesamt IgA-Titer in der antihelminthisch behandelten Gruppe im Vergleich zur Kontrollgruppe festgestellt werden, was sich allerdings nicht auf das Impf-spezifische IgA ausweiten ließ. Ausgehend von diesen Ergebnissen kann nur spekuliert werden, ob es vorteilhaft ist vor einer Impfung zu entwurmen, auch wenn die Antikörpertiter möglicherweise bei Infizierten etwas geringer ausfallen oder ob eine stärkere Behandlung zu einer

besseren Immunantwort nach einer Impfung führen würde. Diese Hypothese bzw. Spekulation müsste in weiteren, detaillierteren Studien verifiziert werden.

Eigenanteil

Hiermit erkläre ich, dass ich der alleinige Autor dieser Dissertation bin, die in der Arbeitsgruppe von Dr. Meral Esen am Institut für Tropenmedizin der Universität Tübingen durchgeführt wurde. Die im Folgenden aufgelisteten experimentellen Arbeiten wurden von mir selbst durchgeführt und sind in die beschriebenen Veröffentlichungen eingeflossen.

Veröffentlichung I:

- Durchführung des Influenza ELISpots
- Total IgA ELISA (BioPlex)
- Impf-spezifischer IgA ELISA

Veröffentlichung II:

- Etablierung des Meningokokken ELISpots
- Durchführung des Meningokokken ELISpots
- Etablierung des Cholera ELISpots
- Durchführung des Cholera ELISpots
- IgG ELISA zur Bestimmung der Cholera Titer (adaptiert nach Chen et al.)

Literaturrecherche, Erstellung und Durchführung des Studiendesigns, Datenanalyse sowie Verfassen der Manuskripte erfolgte weitgehend eigenständig.

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5. Annex

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Effect of Antihelminthic Treatment on Vaccine Immunogenicity to a Seasonal Influenza Vaccine in Primary School Children in Gabon: A Randomized Placebo-Controlled Trial

Sina Brückner[1,2], Selidji T. Agnandji[1,2], Stefan Berberich[2], Emmanuel Bache[2], José F. Fernandes[2], Brunhilde Schweiger[3], Marguerite Massinga Loembe[2], Thomas Engleitner[1¤], Bertrand Lell[1,2], Benjamin Mordmüller[1,2], Ayola A. Adegnika[1,2,4], Maria Yazdanbakhsh[4], Peter G. Kremsner[1,2], Meral Esen[1,2]*

1 Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany, **2** Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospital, Lambaréné, Gabon, **3** Nationales Referenzzentrum für Influenza, Robert-Koch-Institut, Berlin, Germany, **4** Leiden Medical University Center, Department of Parasitology, Leiden, The Netherlands

¤ Current address: Department of Medicine II, Klinikum Rechts der Isar, Technische Universität München, München, Germany
* meral.esen@uni-tuebingen.de

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Abstract

Background

Helminth infections are a major public health problem, especially in the tropics. Infected individuals have an altered immune response with evidence that antibody response to vaccination is impaired. Hence, treatment of helminth infections before vaccination may be a simple intervention to improve vaccine immunogenicity. In the present study we investigated whether a single-dose antihelminthic treatment influences antibody responses to a seasonal influenza vaccine in primary school children living in Gabon, Central Africa.

Methods

In this placebo-controlled double-blind trial conducted in Gabon the effect of a single-dose antihelminthic treatment with 400 mg albendazole versus a placebo one month prior to immunization with a seasonal influenza vaccine was investigated. Antiviral antibody titers against all three vaccine strains were assessed by haemagglutination inhibition (HI) test at baseline (Day 0; vaccination) and four weeks (Day 28) as well as 12 weeks (Day 84) following vaccination. Vaccine-specific memory B-cell response was measured at Day 0 and Day 84 by vaccine-specific Enzyme-linked Immunospot (ELISpot) assay. The trial is registered with the Pan African Clinical Trials Registry (PACTR) (PACTR201303000434188).

Competing Interests: The authors have declared that no competing interests exist.

Results

98 school children aged 6–10 years were randomly allocated to receive either antihelminthic treatment or placebo and were vaccinated one month after the treatment. The prevalence of helminths at baseline was 21%. Vaccine-specific HI titers against at least one of the three vaccine strains increased at Day 28 and Day 84 in all participants. HI titers against both influenza A strains as well as memory B-cell response were modestly higher in the antihelminthic treated group compared to the placebo group but the difference was not statistically significant. Total but not specific IgA was elevated in the antihelminthic treated group compared to the control group at Day 28.

Conclusion

In our setting antihelminthic treatment had no significant effect on influenza vaccine immunogenicity. A trend towards better antiviral and vaccine immunogenicity in the antihelminthic treated group encourages studies to be conducted with alternative treatment schedules or in populations with a higher helminth burden.

Author Summary

Helminth infections are a major health problem in the tropics and most affected are children. The parasites are able to influence the immune system from a T-helper 1 type response to a T-helper 2 type response. There is evidence that in infected individuals the immune response following vaccination is impaired. Thus pre-treatment with a single-dose of an antihelminthic treatment before vaccination could be a simple and cost-effective intervention to improve vaccine efficacy. In the present study we investigated whether a single-dose antihelminthic treatment with albendazole influences the vaccine outcome to a seasonal influenza vaccine in primary school children living in Gabon, Central Africa. We observed a trend towards a higher anti-viral antibody titer after vaccination in the pre-treated group compared to the placebo control group, albeit not statistical significant. Furthermore we detected a higher concentration of total IgA but not of vaccine-specific IgA. In conclusion, our findings show subtle effects of antihelminthic pre-treatment but are not conclusive enough to recommend a single-dose of albendazole before vaccination to improve vaccine immunogenicity but encourage to conduct further studies in endemic areas with other treatment regiments.

Introduction

Infection with geohelminths, mainly *Ascaris (A.) lumbricoides*, *Trichuris (T.) trichiura* and hookworm, is a major public health problem affecting 20% of the world's population, mainly for those living in Sub-Saharan Africa (SSA). As access to public health programs is widely lacking, geohelminthiasis is considered by the World Health Organization (WHO) as one of the most neglected tropical diseases with serious health, nutritional and social outcomes for the affected individuals[1–3]. Vulnerable groups are children[2] and pregnant women[3]. Chronic infection with geohelminths has an impact on health as well as on cognitive skills[4–8] and it is known that infection with helminths leads to immune response alterations. Usually, T-helper type 2 (Th2) immune responses[9–12] are predominant and a general suppression of innate

and adaptive T- and B-cell responses via the activation of regulatory T-cells (Treg) and/or induction of anti-inflammatory cytokines[10,13,14] may lead to general hyporesponsiveness of the immune system[14,15].

Vaccination is one of the most effective tools to prevent infectious diseases. Nonetheless seroconversion and therefore efficacy are variable in vaccinated individuals depending on age, environment and genetic host factors[16–18]. In addition, acute and chronic infections have an influence on vaccine outcome[19,20]. The interaction of geohelminth infection and vaccination is not well investigated although immunization programs for infants are well implemented in areas where geohelminths are highly endemic. Until now it has been shown that *A. lumbricoides* has an impact on the immune response induced by an oral cholera vaccine[21] and that intestinal parasites influence the outcome of a Bacillus-Calmette-Guérin (BCG) vaccination [22]. There is evidence that the presence of geohelminths, especially *T. trichiura* negatively influences immune responses against GMZ2, a malaria vaccine candidate[23].

Past studies from Gabon showed that Gabonese school children are heavily infected with intestinal parasites (infection rates of *A. lumbricoides* 46%, or *T. trichiura* 71%) and 74% of examined children were at least positive for one of the investigated helminths[24–26]. Regular antihelminthic treatment in high-risk groups like school children is considered as an effective tool for controlling the burden of geohelminth infection but is not widely implemented in Gabon. The WHO promotes helminth control by periodic deworming once or twice a year, depending on prevalence, as a cost-effective intervention[27,28]. However regular deworming is not yet implemented in all endemic countries[29].

Antihelminthic treatment would be a cost-effective and easy tool to reduce worm burden [30] and may simultaneously increase vaccine immunogenicity.

Therefore in the present study we investigated the effect on vaccine immunogenicity of pretreatment with a single-dose of albendazole four weeks prior to a scheduled seasonal influenza vaccination in Gabonese primary school children.

Materials and Methods

Trial design and setting

For this double-blinded randomized trial healthy primary school children from Lambaréné and surroundings were randomized to receive either antihelminthic treatment (albendazole 400 mg) (Micro Lab ltd, India) or placebo (Laboratories Sterop, Belgium) four weeks (Day -28) prior to vaccination with either a seasonal influenza vaccination (VAXIGRIP, Sanofi Pasteur, season 2011/2012) intra muscularly (i.m.) (n = 98), Polysaccharide Meningococcal A+C vaccine (Sanofi Pasteur) sub cutaneously (s.c.) (n = 104) or an oral cholera vaccine (Dukoral, Sanofi Pasteur) (n = 106) administered at Day 0. Vaccinations were given in three subsequent time slots. The first cohort of primary school children was vaccinated with the influenza vaccine, the second with meningococcal vaccine and the third cohort received two times the cholera vaccine.

Inclusion criteria were age from 6 to 10 years (primary school children), a signed informed consent form (ICF), good general health upon clinical examination and no acute symptoms of geohelminths infection. Furthermore the participants and their legal representative was asked if he/she will be resident in the area until the end of the study.

Exclusion criteria were the participation in another clinical trial, known contraindication to antihelminthic treatment or to the administration of one of the chosen vaccines including i.m. or s.c. administration, known immunization against the vaccine antigens, known infection with pathogens of one of the vaccine antigens in the past except for influenza (because the influenza vaccine strain composition is different each year), any confirmed or suspected

immunosuppressive or immunodeficient condition resulting from disease (e.g., malignancy, HIV infection) or immunosuppressive/cytotoxic therapy as well as acute disease at the beginning of the study and before vaccination. If a child was febrile at the scheduled time of vaccination, injections were postponed until the infection was cured. If a child had a known acute or chronic disease like malaria, AIDS or tuberculosis as well as a haemoglobin level < 7 g/dl or signs of haematuria and/or proteinuria tested by urine sticks (Combur 9 test) the child was not included and referred to the Albert Schweitzer Hospital (ASH) for treatment. If menarche was reported a pregnancy test was be performed at Day -28 and Day 0 prior to antihelmintic treatment and prior to vaccination.

Here, we report results of the first part of the study, where the children were vaccinated with the seasonal influenza vaccine.

Children, who were infected with *Schistosoma* (*S.*) *haematobium*, as well as children with any other symptomatic infection were excluded from the study and treated accordingly. All parasite positive participants (including those without symptoms) received appropriate treatment after study termination.

Immunological investigations

Blood was taken on the day of vaccine injection (Day 0), Day 28 (four weeks after vaccination) and Day 84 (12 weeks after vaccination). The primary immunological endpoint of the study was functional antibody level measured as haemagglutination inhibition (HI) testing. To assess memory B-cell response (secondary immunological endpoint) against the vaccine antigens, an Enzyme-linked Immunospot (ELISpot) assay was performed.

Assessment of anti-influenza antibody titers

Pre- and post-vaccination samples were analyzed by a validated microtiter haemagglutination inhibition (HI) test at the German National Reference Center for Influenza (NRZ Influenza, Robert Koch Institute (RKI), Berlin) as previously described[31]. Prior to testing, each serum was treated with receptor-degrading enzyme to inactivate non-specific inhibitors at a final serum dilution of 1:10. Sera were then diluted serially two fold into microtiter plates. Each virus strain was adjusted to 4 HA units/25 μl which was verified by back titration and 25 μl of this virus suspension was added to each of the 96 wells. After incubation at room temperature (RT) for 30 min freshly prepared 0.5% turkey red blood cells (RBCs) were added, the plates were mixed, followed by a further incubation at RT for 30 min. Human sera serving as positive controls and negative controls were included on each plate. HI titers were reported as the reciprocal of the last serum dilution that contained non-agglutinated RBCs.

Assessment of total immunoglobuline (Ig) isotypes and subclasses

Pre- and post-vaccination samples (Day 0, Day 28 and Day 84) were analyzed by using a Multiplex assay (Biorad, Germany) for detection of multiple antibodies, like IgG subclasses (IgG1; IgG2, IgG3 and IgG4), total IgE, total IgM and total IgA at baseline, Day 28 and Day 84 post vaccination. The assay was conducted according to the manufacturer's specifications.

Assessment of vaccine-specific IgA by enzyme-linked immunosorbant assay (ELISA)

To assess vaccine-specific IgA concentrations plates (Nunc, Germany) were coated with 0.5 μg/ml of the vaccine antigen, incubated over night at 4°C and blocked with blocking buffer (0.3% milkpowder (Roth, Germany), 0.1% Tween-20 (Sigma, Germany) and PBS (Life

technologies, USA) for 1 h at RT. Samples were plated in serial dilutions for 2 h at RT. As secondary antibody a polyclonal rabbit anti-human IgA/HRP (Dako, Germany) was used. For visualization TMB one (Kem En Tec, Denmark) and H_2SO_4 (Merck, Germany) was used. OD was measured using a Photometer (Phomo, Anthos, Germany) at wave length 450 nm and 620 nm as reference.

Vaccine-specific memory B-cell ELISpot

As previously described, peripheral blood mononuclear cells (PBMCs) were frozen on Day 0 and Day 84 and later used for memory B-cell ELISpot[32,33]. In brief, PBMC were separated from heparinized full blood by gradient centrifugation (Ficoll-Plaque PLUS, GE Healthcare, Sweden), counted and frozen in 90% fetal calf serum (FCS Gold PAA, Germany) and 10% DMSO (Sigma, Germany) at -150°C. Before ELISpot, cells were thawed, counted and seeded at a density of $1^{*}10^{6}$ cells per ml in RPMI 1640 (Sigma, Germany), complemented with sodium pyruvate, non-essential amino acids, L-glutamine, penicillin, streptomycin (all supplemented from Life Technologies, USA) and 10% heat inactivated FCS (FCS Gold, PAA, Germany). Maturation of circulating memory B-cells into antibody-secreting cells (ASC) was performed by *in vitro* stimulation with 2.5 μg/ml CpG-2006 (TIB-MOLBIOL, Germany) and 10 ng/ml recombinant human IL-15 (R&D systems, USA) in 24 wells cell culture plates (Corning Costar) for 6 days at 37°C, 5% CO_2 as described[34]. Following maturation, $2^{*}10^{5}$ cells were serially diluted on Ag-specific 96 well plates (Millipore, Germany). The vaccine (VAXIGRIP) was coated over night at a concentration of 5μg/ml in PBS. Plates were washed and blocked for 1 hour (h) with complete medium. Meanwhile cells were washed and counted. After seeding cells were incubated at 37°C, 5% CO_2 for 2 h allowing them to secrete antibodies. Secreted antigen (Ag)-specific antibodies were detected using biotin labeled anti-human IgG antibody (Sigma, Germany, 1:500 in PBS, 3% BSA) and ExtrAvidin peroxidase (SIGMA, Germany, 1:600 in PBS, 5% BSA). AEC substrate (SIGMA, Germany) was added for 10 min at RT. After staining the plates were dried over night. Spots were counted by the CTL ImmunoSpot (CTL, USA).

Assessment of parasite burden

Stool samples were collected at Day -28, Day 0 and Day 84 and analyzed by using the qualitative Merthiolate-Iodine-Formaldehyde (MIF)-technique[35]. In brief, 10 ml of Merthiolate-Formaldehyde-Solution (5% Formaldehyd, 1% Glycerine (Merck, Germany)) and Lugol's Solution (10% potassium iodine, 5% iodine (Merck, Germany)) were added to each walnut size stool sample (approx. 5 g) filtered through a metal wire, centrifuged for 5 min 1500 rounds per minute (rpm) and analyzed by microscopy at the end of the study.

Urine was examined for the presence of *S. haematobium* by urine filtration method[36] using 10 ml of well mixed urine passed through a filter (12 μm pore size, Millipore, Germany). The filter was transferred to a glass slide, stained with methylene blue and analyzed by microscopy.

At Day 0 and Day 84 thick blood smears were performed to assess malaria parasites retrospectively. If a child presented with fever or any other symptom suggestive of malaria, a rapid test (Paracheck Pf) was performed and the volunteer was treated with appropriate treatment.

Sample size calculation

We assumed that a 30% difference of immune responses between antihelminthic treatment and placebo groups is clinically relevant. In a pilot trial with the malaria vaccine candidate GMZ2 we observed a difference in antibody response greater than 30% with a standard deviation of 0.5[23]. A sample size of 45 individuals (n = 45) per group is required to detect such a

difference with a power of 80% at a significance level of 0.05. To allow for 15% of loss to follow-up a total of 52 (n = 52) schoolchildren per group were required. Sample size calculation was done using R v2.9.0[37].

Randomization, data entry and statistical analysis

The randomization and data management was performed on the "Koordobas" database system (Institute for Clinical Epidemiology and applied Biometry, University of Tübingen) The medical personal treating participants were not involved in the outcome evaluation. Group differences at follow-up visits (Day 28, Day 84) were determined with a rank based ANCOVA (Analysis of Covariance) corrected for baseline titer and gender. Differences in ELISpot counts were assessed by a Wilcoxon test. Geometric mean titers were calculated according to $10^{mean(log_{10}(1+\mathrm{HI-titer}))}$. A two sided alpha of 0.05 was used as significance level.

Ethical approval

The study was approved by the Comité d'Ethique Régional Indépendant de Lambaréné and the Comité National d´Ethique pour la Recherche du Gabon. The trial is registered with PACTR (PACTR201303000434188) and the study was conducted in accordance to the Declaration of Helsinki and followed the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. The child's parents or their legally accepted representatives provided written informed consent before study participation.

Results

From December 2011 until September 2012 out of 113 screened primary school children from Lambaréné and surroundings 98 were randomized to receive either antihelminthic treatment (n = 50) or placebo (n = 48). 92 participants were vaccinated with a seasonal influenza vaccine and 82 terminated the study with visit 4 at Day 84 post vaccination (Fig 1). Baseline characteristics were similar between the two groups except that the fraction of females was higher in the antihelminthic treated group (57% versus 34% in the placebo group) (Table 1). A possible effect of gender was taken into account in exploratory and sensitivity analyses.

Anti-influenza antibody titers

Antibodies against the three influenza vaccine strains A/California/7/09 (A(H1N1)pdm09), A/Perth/16/09 (A(H3N2)) and B/Brisbane/60/08 were determined by HI testing. HI titers increased against at least one vaccine strain after vaccination at Day 28 and Day 84 in all participants (Fig 2). The increase of HI values for A(H1N1)pdm09 and A(H3N2) tended to be higher in the antihelminthic treated group compared to the control group but the difference was not statistically significant (Figs 2 and 3).

Thirty-four participants already had detectable antibodies at baseline for the A(H1N1) pdm09 strain. The baseline titers ranged between 15 and 640 with a median of 120 in the antihelminthic treated group and 80 in the placebo group and the GMT was 28 in the antihelminthic treated group and 15 in the control group; 19 participants in the antihelminthic treated group and 17 participants in the placebo group were without a detectable baseline HI titer. At Day 28 the HI titers increased up to 5000 (median: 640 in both groups and a GMT of 134 in the antihelminthic treated group and 84 in the control group,) and decreased until Day 84 (median: 320 in both groups and GMT of 138 in the antihelminthic treated group and 94 in the control group) (Tables 2 and 3).

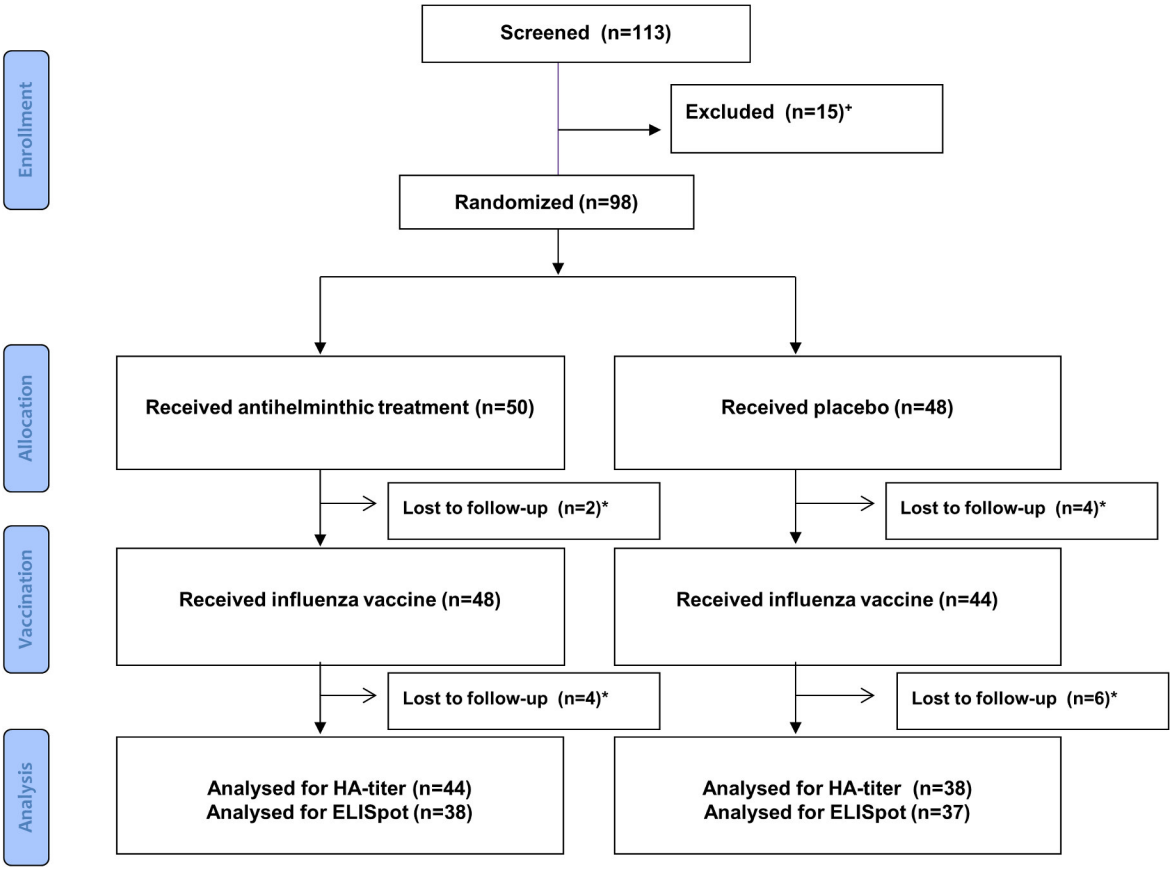

Fig 1. Study profile. +Patients were excluded, because of infection with *S. haematobium*. *Participants not terminating the study are summarized as lost to follow up (n = 16).

doi:10.1371/journal.pntd.0003768.g001

Antibody titers against the strain A(H3N2) ranged from 15 to 160 (median of 120 in the antihelminthic treated group and 80 in the control group), whereas the GMT was 73 for the antihelminthic treated and 72 for the placebo group at Day 0. For this strain also an increase of antibodies at Day 28 with a median of 640 in both groups and a GMT of 554 in the antihelminthic treated group and 516 in the placebo group as well as decreasing values at Day 84 with an median of 320 in both groups and a GMT of 344 in the antihelminthic treated group and 258 in the control group were observed (Tables 2 and 3).

Antibodies against the influenza B vaccine strain had a median of 320 at Day 28 in both groups and declined until Day 84 with a median of 160 in both groups. GMT at Day 28 was 142 in the antihelminthic treated group and 164 in the placebo group and at Day 84 120 in the antihelminthic treated group and 97 in the control group. At baseline the median was 0 in both groups and the GMT was 12 for the antihelminthic treated group and 10 for the placebo group (Tables 2 and 3).

Assessment of total Ig isotypes and subclasses

Ig isotypes and subclasses (IgG1-4, IgA, IgE and IgM) pre- and post-vaccination were assessed by a multiplex system. Total IgA was elevated in the antihelminthic treated group compared to the control group at Day 28 (p-value 0.006) (Fig 4).

Table 1. Baseline characteristics and helminth infection at day -28.

	Antihelminthic treatment	Placebo
Gender		
Male	22 (22.4%)	32 (32.7%)
Female	28 (29.6%)	16 (16.3%)
Age		
6 years	15 (15.3%)	16 (16.3%)
7 years	9 (9.2%)	8 (8.2%)
8 years	14 (14.3%)	12 (12.2%)
9 years	10 (10.2%)	12 (12.2%)
10 years	2 (2%)	0
Mean age	7.43	
Helminth		
Single infection		
A. lumbricoides	2 (2%)	2 (2%)
F. hepatica	0	2 (2%)
T. trichiura	4 (4%)	3 (3%)
Multiple infection		
T. trichiura/ A. lumbricoides	2 (2%)	4 (4%)
T. trichiura/ A. lumbricoides/ A. duodenale	0	1 (1%)
Negative	41 (41.84%)	33 (33.3%)
NA	1 (1%)	3 (3%)

doi:10.1371/journal.pntd.0003768.t001

The total concentration of the subclasses IgG1 and IgG3 were slightly elevated in the antihelminthic treated group (for IgG1 p-value was 0.042 and for IgG3 p-value was 0.03 in the model-based analysis, but was not significant using the Wilcoxon-test (p-value 0.347 and 0.160)).

To evaluate whether the elevation of total IgA indicates a higher amount of vaccine-specific IgA we performed a vaccine-specific ELISA. Here, no difference of vaccine-specific IgA was detected between the antihelminthic treated and control group (Fig 5).

Assessment of ASC

B-cell ELISpot assay to measure vaccine-specific memory B-cells was performed with samples from 75 individuals.

Antigen-specific memory B-cells were detectable in all vaccinated subjects at Day 84 (Fig 6). Median number of vaccine-specific ASC per 100,000 PBMC was 13 at Day 84 (range: 1 to 144) in all participants.

In 10 participants of the antihelminthic treated group and 12 participants of the placebo group a low number of ASCs (range: 0 to 47) was already detected at Day 0 (Fig 6). The number of detectable ASCs in the antihelminthic treated group at Day 84 ranged from 0 to 240 (median 19) and from 0 to 140 (median 3) in the placebo group (Fig 7). Nonetheless this difference was not statistically significant.

Assessment of parasite burden

The percentage of helminth burden in our setting was 21%. From these 21%, infection with *A. lumbricoides* and *T. trichiura* was 6% for each species. The burden did not differ between the visits (Tables 1 and 4).

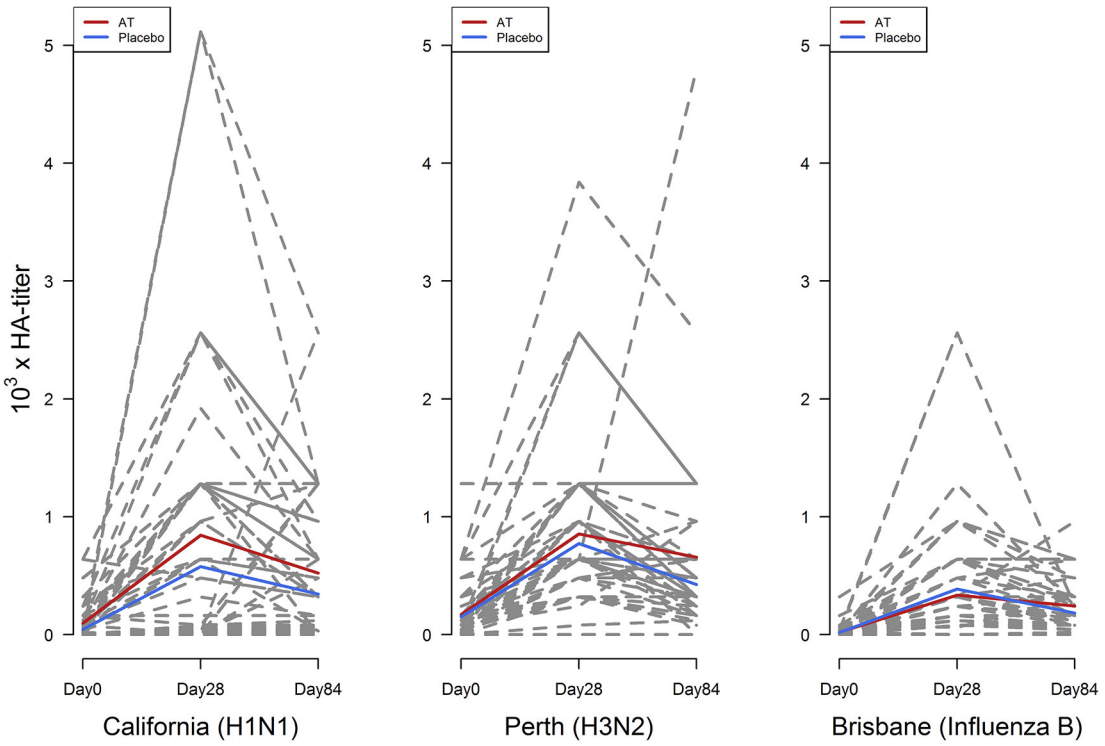

Fig 2. Antibody titers against the three vaccine strains at baseline (day 0), day 28 and day 84. Red lines indicate the mean of all volunteers of the antihelminthic treated group (AT) and blue lines indicate the mean of all participants of the placebo group. Dashed lines indicate antibody titers of each participant.

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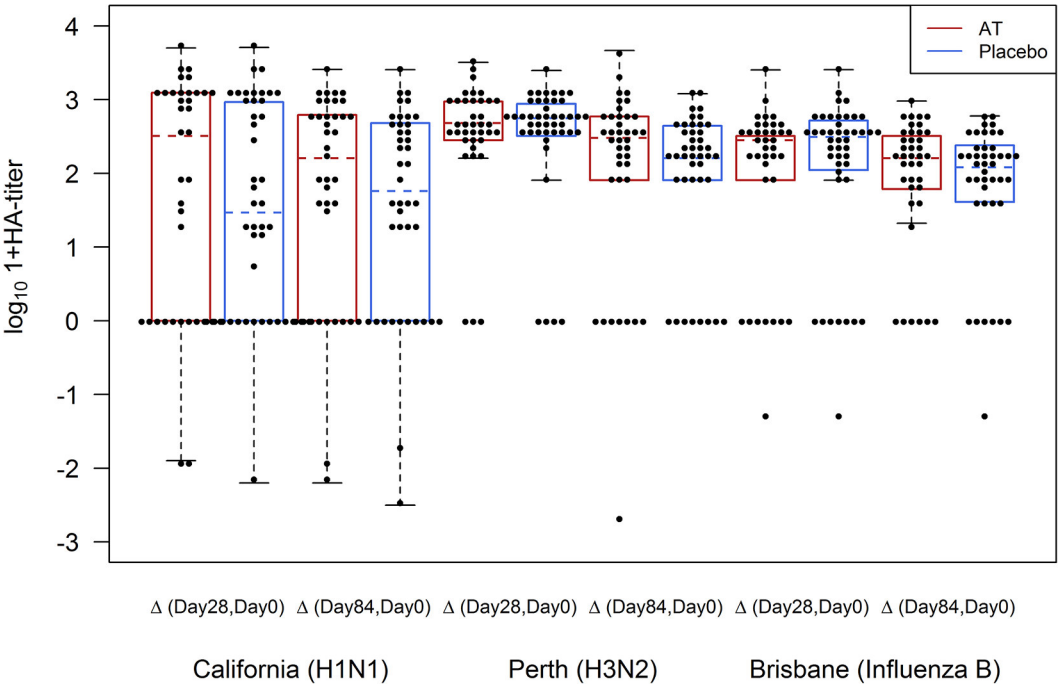

Fig 3. Differences of HI titers between the respective visits (day 28, day 84) and day 0 (baseline). Red and blue colors represent the pre-treated (AT) and control group.

doi:10.1371/journal.pntd.0003768.g003

Table 2. Median, 25% and 75% quartile of vaccine strain specific HI titers.

	Day 0	Day 28	Day 84
A(H1N1)pdm09			
Antihelminthic treatment	120 (0,160)	640 (0,1280)	320 (35,960)
Placebo	80 (0,60)	640 (0,960)	320 (20,480)
A(H3N2)			
Antihelminthic treatment	120 (20,240)	640 (320,960)	320 (280,640)
Placebo	80 (40,160)	640 (480,1280)	320 (240,600)
Influenza B			
Antihelminthic treatment	0 (0,40)	320 (120,320)	160 (80,320)
Placebo	0 (0,20)	320 (120,520)	160 (80,800)

doi:10.1371/journal.pntd.0003768.t002

At Day 0 and Day 84 9 and 10 volunteers were positive in the thick blood smear, respectively.

Discussion

Recent studies suggest that infection with helminths influences the immunological outcome of vaccination. A study recently conducted in rural Gabon showed that children infected with helminths had an impaired antibody response against an influenza vaccine compared to those free of infection[14]. During a phase Ib trial investigating immunogenicity of the malaria vaccine candidate GMZ2 in Gabonese children those infected with *T. trichiura* exhibited a lower antibody response against the vaccine antigens compared to those who were not infected with the parasite[23]. Since these and other studies in animal models and humans [21,38,39] show that helminths and other intestinal infections negatively influence vaccine immunogenicity we conducted a study to test the hypothesis that antihelminthic treatment prior to vaccination will increase immune responses to vaccine antigens.

Because antibody response towards vaccine antigens is a surrogate marker for protection in areas where infectious diseases are highly endemic an effective immune response to vaccinated antigens is very important[40–42].

In the present study anti-viral antibody response was analyzed by HI test for each of the three influenza strains administered with a seasonal vaccine. The influenza vaccine was selected because a single-dose is sufficient to raise antibody responses towards a protective titer and the vaccine is not part of the Expanded Program on Immunization (EPI) in Gabon. Therefore, no or only low baseline titers would presumably be present in the study population. As expected

Table 3. GMT of vaccine strain specific antibodies.

	Day 0	Day 28	Day 84
A(H1N1)pdm09			
Antihelminthic treatment	28	134	138
Placebo	15	84	94
A(H3N2)			
Antihelminthic treatment	73	554	344
Placebo	72	516	258
Influenza B			
Antihelminthic treatment	12	142	120
Placebo	10	164	97

doi:10.1371/journal.pntd.0003768.t003

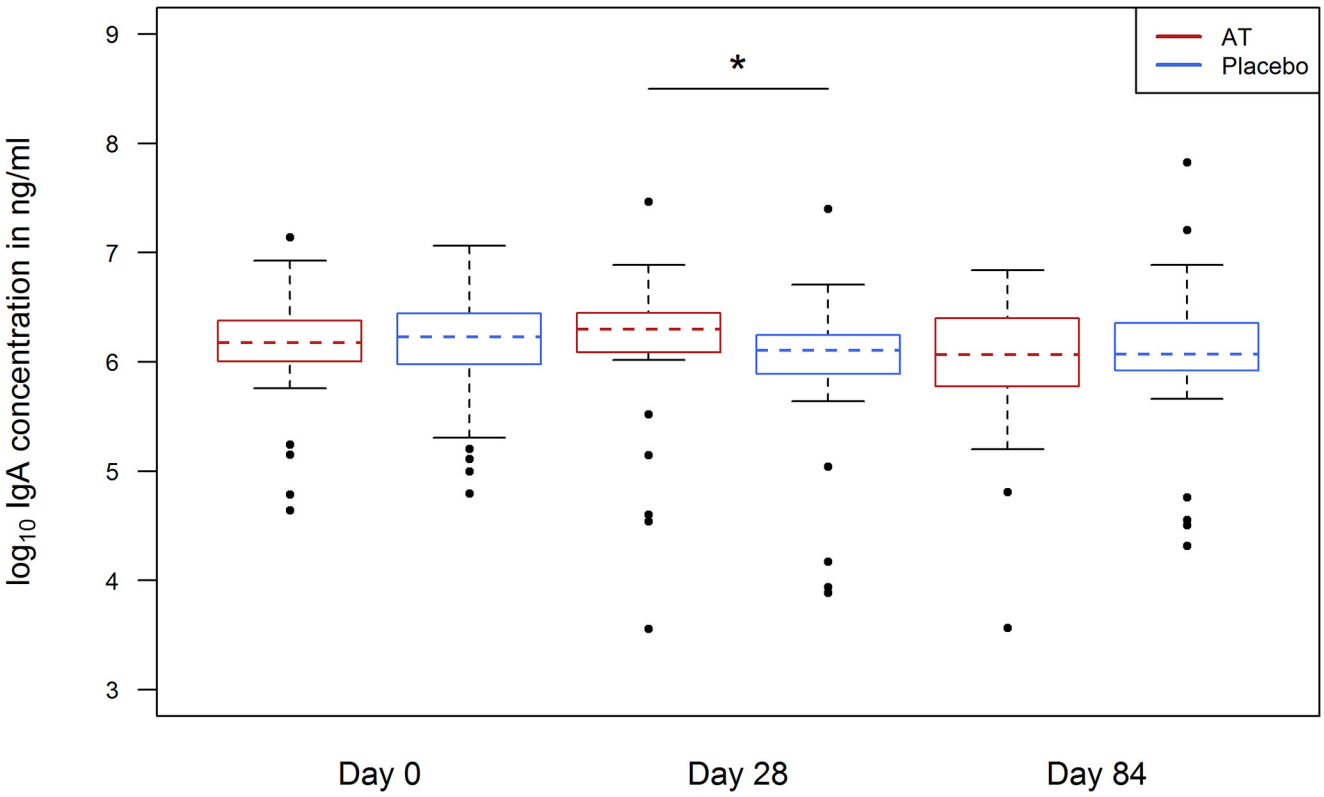

Fig 4. Total IgA at day 0, day 28 and day 84 in antihelminthic treated (AT) (red blots) and placebo group (blue blots).

doi:10.1371/journal.pntd.0003768.g004

there was an increase of HI titers against each vaccine strain after vaccination at Day 28 and Day 84 in all participants. This was observed mainly for the antibody concentration against the influenza A strains A(H1N1)pdm09 and A(H3N2). The low but frequently seen baseline HI titers against the influenza A (H1N1) and A(H3N2) in some participants suggest that these children were already in contact with circulating virus strains or that they have cross-reactive antibodies from recent circulating influenza strains or from other cross-reactive pathogens. In the present study we assessed that total IgA concentrations were higher in individuals of the antihelminthic treated group compared to the control group four weeks following vaccination. Because IgA is crucial for the control of influenza[43–45] and vaccine-specific IgA can be detected in mice following influenza vaccination[46] we also measured vaccine-specific IgA but saw no difference between the groups. Since total IgA was not different at baseline we do not know if the difference of total IgA at Day 28 is an effect of vaccination or a late effect of the antihelminthic treatment. This effect should be further investigated in more detail in particular because IgA has an important function during the defense of airborne and gastrointestinal infections. However in our setting we have not examined the role of this finding and we have not determined secretory IgA.

Besides we could show that the number of vaccine-specific ASCs representing memory B-cells was elevated in the antihelminthic treated group compared to the control group but this difference did also not reach statistical significance. We assume that the already existing ASCs at baseline in samples of some participants are due to previous influenza infections. Since the antibody titers as well as the number of ASCs were not significantly elevated in the antihelminthic treated group the question arises if a better or more effective antihelminthic treatment

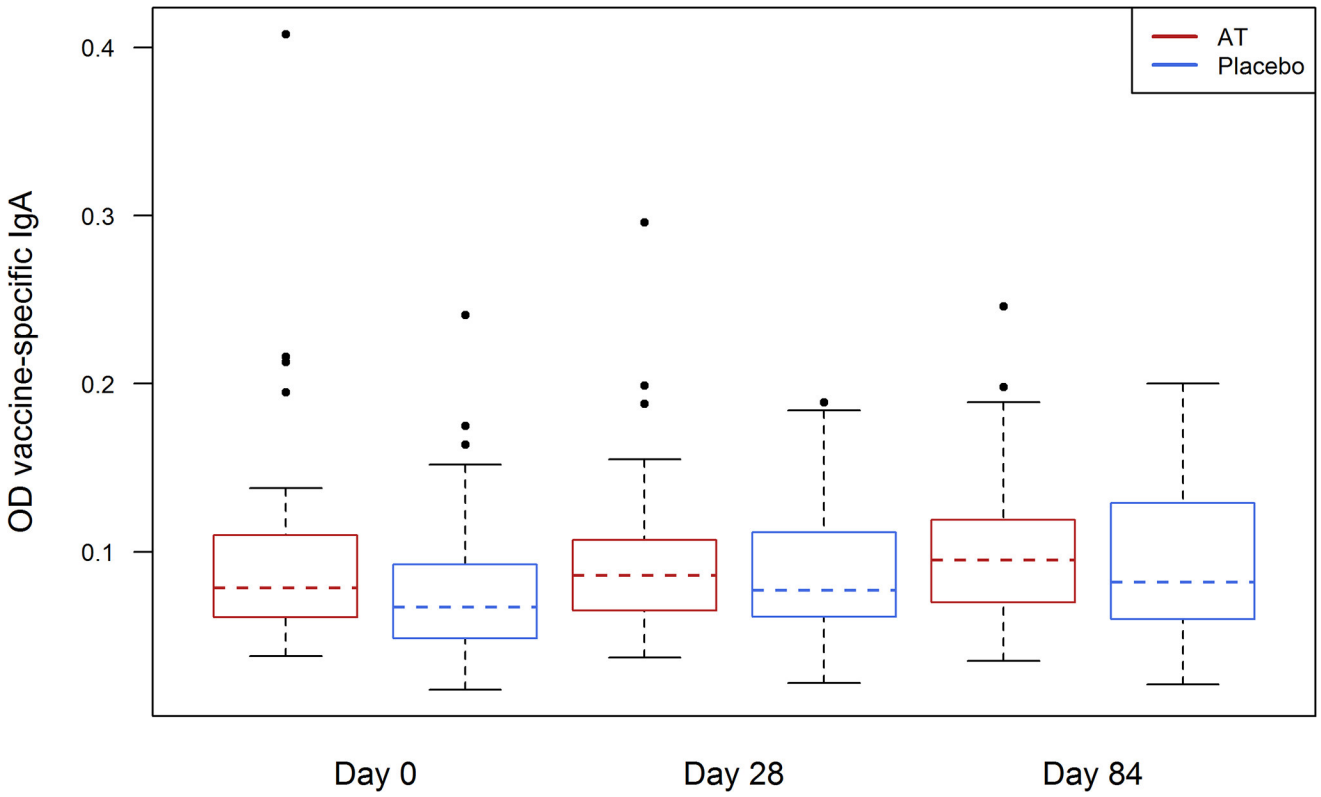

Fig 5. Vaccine specific IgA at day 0, day 28 and day 84 in antihelminthic treated (AT) (red) and placebo group (blue).

doi:10.1371/journal.pntd.0003768.g005

would have had clearer effects on the vaccine immunogenicity. The fact that the overall helminth burden did not differ between the visits implies that single-dose antihelminthic treatment is not sufficient to cure or to prevent relevant helminth infection or that reinfection occurs rapidly. This becomes more evident since Adegnika et al. very recently showed that short antihelminthic treatment regimens are efficacious to cure *A. lumbricoides* but not *T. trichiura* infection. To eliminate *T. trichiura* infection at least a double treatment seems to be necessary[30]. Therefore in our setting the treatment regimen was not adequate to eliminate helminths sufficiently and to reconstitute the immune system. Cooper et al. investigated the effect of a double-dose (2x200mg) albendazole treatment prior to an oral cholera vaccination leading to a highly significant increase in anti-vibriocidal antibodies[21,47]. In contrast to the present study, the investigators only included individuals carrying *A. lumbricoides*[21,30]. In our setting we retrospectively analyzed the helminth burden of the volunteers at the end of the study and noticed that the number of infections amongst primary school children was not as high as originally suggested from recent studies, which reduces the power of our analyses. In a study performed 2004 the overall prevalence of helminth infection was 74%[24], whereas in our study population the worm burden was only 21%. This could be due to different reasons. First, in the study conducted by van den Biggelaar et al. the Kato Katz method to detect the egg load in fresh stool samples was used[24] whereas we used the MIF technique to detect the worm burden in preserved stool samples at the end of the study to ensure all investigators were blind to the infection status of the participants. The Kato Katz method has a higher sensitivity [48] compared to the MIF-technique[49] and this may explain partially the unexpected low prevalence of helminth infection although other studies using the same methodology gave

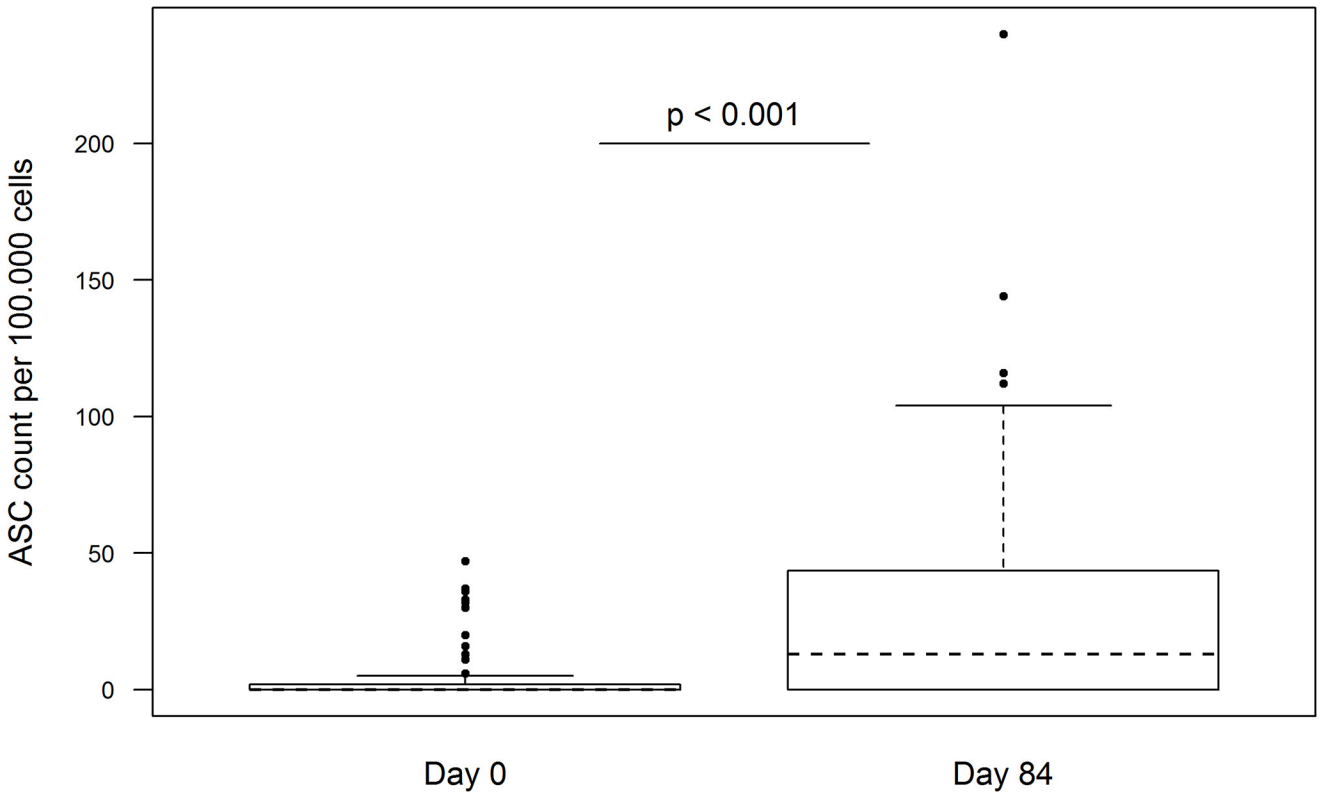

Fig 6. Vaccine-specific IgG ASCs determined by B-cell ELISpot at day 0 and day 84.

doi:10.1371/journal.pntd.0003768.g006

higher prevalence rates. Besides the possibility that it is a chance finding the reduction of worm burden could be due to better public health facilities and better education of the population as well as to mass drug administration (MDA) even when it is administered on an irregular basis or by uncontrolled self-mediation. Gabon is a highly endemic country for STH according to the WHO, therefore MDA is recommended but in the region where the study was performed it is not regularly administered[29]. Private use is difficult to assess since individuals do not need prescription to buy antihelminthic treatment over-the-counter[50,51] and often the individuals do not report the use of antihelminthic treatment. Furthermore it could be that a member of a household was recently treated and therefore also infection of other family members decreased [52]. Taken all this into account our in our study population the helminth burden was lower as expected for reasons which were not elicited in this study and our sample size was not powered for such a low helminth prevalence.

In conclusion, we showed that in our setting there was non-significant difference in virus-specific HI titers and ASCs against the vaccine antigens between the antihelminthic treated and the placebo group. Furthermore at Day 28 post vaccination total IgA was higher in the antihelminthic treated group compared to the control group. But there was no difference comparing vaccine-specific IgA titers. We can only speculate if a single dose antihelminthic treatment is sufficient to increase vaccine immunogenicity in a setting with higher helminth prevalence or if an appropriate more powerful treatment could contribute to a better immune response to vaccination. This has to be investigated in more detail and with different antihelminthic regiments.

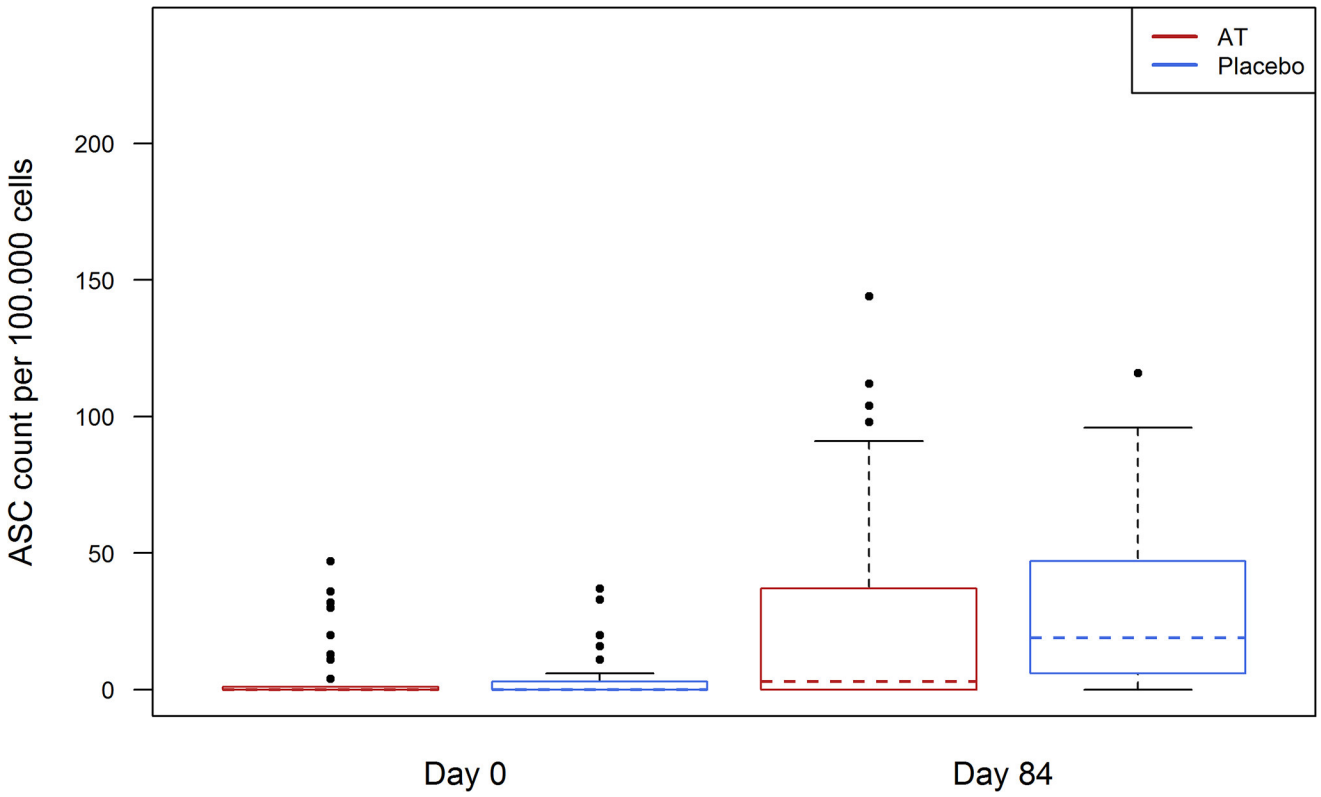

Fig 7. Vaccine-specific IgG ASCs at day 0 and day 84, in antihelminthic treated (AT) and placebo group. Red and blue represent antihelminthic treated (AT) and control group.

doi:10.1371/journal.pntd.0003768.g007

Table 4. Distribution of the worm burden in the two groups at day 0 and day 84.

	Day 0		Day 84	
	Antihelminthic treatment	**Placebo**	**Antihelminthic treatment**	**Placebo**
Single infection				
A. lumbricoides	2 (2.2%)	2 (2.2%)	5 (6.1%)	4 (4.9%)
T. trichiura	2 (2.2%)	3 (3.3%)	3 (3.6%)	1 (1.2%)
S. haematobium	0	0	2 (2.4%)	1 (1.2%)
Taenia	0	0	0	1 (1.2%)
Tapeworm	1 (1.1)	0	0	0
Multiple infection				
T. trichiura/ A. lumbricoides	1 (1.1%)	2 (2.2%)	0	3 (3.7%)
S. haematobium/ T. trichiura	0	0	0	1 (1.2%)
S. haematobium/ A. lumbricoides	0	0	1 (1.2%)	0
T. trichiura/ A. duodenale	1 (1.1%)	0	0	0
T. trichiura/A. lumbricoides/ A. deodenale	0	1 (1.1%)	1 (1.2%)	0
Neg	39 (42.4%)	32 (34.7%)	25 (30.5%)	24 (29.3%)
NA	2 (2.2%)	4 (4.4%)	7 (8.5%)	3 (3.7%)

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Supporting Information

S1 Checklist. Consort checklist.
(PDF)

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Author Contributions

Conceived and designed the experiments: ME STA PGK MY. Performed the experiments: SBr ME STA SBe EB JFF BS TE BM MML. Analyzed the data: SBr ME TE BM. Contributed reagents/materials/analysis tools: ME STA BL BM AAA BS. Wrote the paper: SBr ME BM STA BS TE BL AAA MY PGK.

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A single-dose antihelminthic treatment does not influence immunogenicity of a meningococcal and a cholera vaccine in Gabonese school children

Sina Brückner [a,b], Selidji Todagbe Agnandji [a,b], Johannes Elias [c], Stefan Berberich [b], Emmanuel Bache [b], José Fernandes [b], Marguerite Massinga Loembe [a,b,d], Johanna Hass [a], Bertrand Lell [a,b], Benjamin Mordmüller [a,b], Ayola Akim Adegnika [a,b], Peter Kremsner [a,b], Meral Esen [a,b,*]

[a] Institute of Tropical Medicine, University of Tübingen, Wilhelmstraße 27, D-72074 Tübingen, Germany
[b] Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospital, BP 118 Lambaréné, Gabon
[c] Institute for Hygiene and Microbiology/NRZM, Josef-Schneider-Straße 2, 97080 Würzburg, Germany
[d] Departement de Bacterio-Virologie, Faculté de Medicine, Université des Sciences de la Santé, BP4009 Libreville, Gabon

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ABSTRACT

Background: We recently described the effect of a single-dose antihelminthic treatment on vaccine immunogenicity to a seasonal influenza vaccine. Here we report the effect of antihelminthics on the immunogenicity of a meningococcal vaccine and a cholera vaccine in primary school children living in Lambaréné, Gabon. Since infection with helminths remains a major public health problem and the influence on cognitive and physical development as well as the immunomodulatory effects are well established, we investigated if a single-dose antihelminthic treatment prior to immunization positively influences antibody titers and vaccine-specific memory B-cells.

Methods: In this placebo-controlled, double-blind trial the effect of a single-dose antihelminthic treatment prior to immunization with a meningococcal as well as with a cholera vaccine was investigated. Anti-meningococcal antibodies were assessed by serum bactericidal assay, cholera vaccine-specific antibody titers by Enzyme-linked Immunosorbent Assay (ELISA) at baseline (Day 0; vaccination), four weeks (Day 28) and 12 weeks (Day 84) following vaccination. Meningococcal and cholera vaccine-specific memory B-cells were measured at Day 0 and 84 by vaccine-specific Enzyme-linked Immunospot (ELISpot) assay. The helminth burden of the participants was assessed four weeks before vaccination (Day −28) and at Day 84 by the Merthiolate-Iodine-Formaldehyde technique.

Results: Out of 280 screened school children, 96 received a meningococcal vaccine and 89 a cholera vaccine following allocation to either the single-dose antihelminthic treatment group or the placebo group. Bactericidal antibody titers increased following immunization with the meningococcal vaccine at Day 28 and Day 84 in 68 participants for serogroup A, and in 80 participants for serogroup C. The cholera vaccine titers increased in all participants with a peak at Day 28. The number of memory B-cells increased following vaccination compared to baseline. There was no statistically significant difference in antibody and B-cell response between children receiving albendazole compared to those receiving placebo.

Conclusion: A single-dose treatment with albendazole prior to immunization had no effect on meningococcal or cholera vaccine immunogenicity in our study population.

1. Introduction

Infection with geohelminths, mainly *Ascaris (A.) lumbricoides, Trichuris (T.) trichiura* and hookworm, is a major public health problem affecting 20% of the world's population, especially in Sub-Saharan Africa (SSA). It is one of the most neglected tropical diseases with serious health, nutritional and social outcomes for affected individuals [1–3]. According to the World Health Organization (WHO) in 2014 approximately 2 billion of the world's population were infected with helminths [2], mainly children [3] and pregnant women [4]. Van den Biggelaar reported in 2004 that 46% of children, aged between 5–13 were infected with *A. lumbricoides* and 71% were infected with *T. trichiura* [5]. Three years later van Riet reported that 15% (living in a semi-urban area)

* Corresponding author at: Institute of Tropical Medicine, University of Tübingen, Wilhelmstraße 27, D-72074 Tübingen, Germany.
E-mail address: meral.esen@uni-tuebingen.de (M. Esen).

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and 55% (living in a rural area) of 7–12 year-olds were infected with *A. lumbricoides* and 12% (semi-urban area) and 64% (rural area) had a *T. trichiura* infection [6]. Chronic infection with geohelminths has an impact on health as well as on cognitive skills [7–11] and it has been shown that infection with helminths leads to altered immune responses [12].

Vaccination is one of the most effective tools to prevent infectious diseases. Nonetheless, seroconversion and therefore efficacy is variable in vaccinated individuals depending on age, environment and genetic host factors [13–15]. In addition, acute and chronic infections have an influence on vaccine outcome [16,17]. We and others have recently shown that helminth infections impair immune response to vaccination [5,6,18–22]. The WHO promotes helminth control by periodic deworming once or twice a year, depending on prevalence, as a cost-effective intervention [2,22]. Regular antihelminthic treatment through mass drug administration programs in high-risk groups like school children is considered effective for controlling the helminth infection burden, but it is not regularly applied in Gabon and other endemic countries [23]. Therefore we aimed to investigate the effect of a single-dose antihelminthic treatment on vaccine immunogenicity. Recently, we reported results of the first part of the present study, where primary school children were vaccinated with a seasonal influenza vaccine [18]. Here we report the second part of the study investigating the vaccine immunogenicity of a meningococcal and an oral cholera vaccine following a single-dose of antihelminthic treatment. The vaccines were chosen to assess whether different routes of administration (subcutaneous vs. oral) have different effects on vaccine immunogenicity in helminth infected children. Furthermore these vaccines are not part of the Expanded Program on Immunization (EPI) and we expected that no basic or low level antibody titer would be detectable if the individuals did not report recent infection with these pathogens.

2. Materials and methods

2.1. Trial design and setting

The study design is reported in detail elsewhere [18]. In brief, participants received one dose of antihelminthic treatment (albendazole 400 mg) (Micro Lab ltd, India) or placebo (Laboratories Sterop, Belgium) four weeks (Day −28) prior to vaccination with either a seasonal influenza vaccine (VAXIGRIP®, Sanofi Pasteur, season 2011/2012) intra muscularly ((i.m.) (n = 98) (part I)), meningococcal vaccine containing polysaccharides of *Neisseria (N.) meningitidis* group A and C (Sanofi Pasteur) subcutaneously ((n = 96) (part Ib)) or an oral cholera vaccine containing inactivated *bacteria* and the recombinant cholera toxin B subunit ((Dukoral®, Sanofi Pasteur) (n = 89) (part II)) administered at Day 0. All vaccines are licensed and commercially available in Gabon. Here we focus on part Ib and II (vaccination with the meningococcal vaccine administered once at Day 0 and the oral cholera vaccine that was given twice at Day 0 and Day 7). The study took place in Lambaréné, Gabon.

Inclusion criteria were ages 6 to 10 years (primary school children), a signed informed consent form (ICF) by one of the parents or a legal guardian, no signs of chronic or acute disease upon clinical examination and no symptoms of geohelminth infection, which was assessed by the Merthiolate-Iodine-Formaldehyde (MIF)-technique. Furthermore, the participants and their legal representative were asked to reside in the study area until the end of the study.

Exclusion criteria were the participation in another clinical trial, known contraindication to antihelminthic treatment or to ingredients in one of the chosen vaccines, known immunization with one of the study vaccines; known recent meningococcal or cholera infections and any confirmed or suspected immunosuppressive or immunodeficient condition resulting from disease (e.g. malignancy, HIV infection) or immunosuppressive/cytotoxic therapy. If a child was ill or febrile at the scheduled time of the vaccination injections were postponed until convalescence.

Children who were infected with *Schistosoma (S.) haematobium* assessed by urine filtration, were excluded from the study and were treated accordingly. All parasite positive participants (including those without symptoms) received appropriate treatment after study termination.

2.2. Immunological investigations

Throughout the study period a total of 27 ml blood was collected at baseline (Day 0), Day 28 (four weeks after vaccination) and Day 84 (12 weeks after vaccination). The primary immunological endpoint of the study was functional antibody level measured by a serum bactericidal assay (SBA) for the meningococcal arm and IgG Enzyme-linked immunosorbent assay (ELISA) for the cholera arm. To assess memory B-cells (secondary immunological endpoint) a B-cell Enzyme-linked immunospot (ELISpot) assay was performed. The SBA was performed at the National Reference Center for Meningococcal Disease in Würzburg and all other investigations were performed at CERMEL and at ITM.

2.3. Assessment of anti-meningococcal antibody titers

SBA activity was assessed as described previously [24], with minor modifications. Briefly, serial dilutions of heat-inactivated sera were incubated with defined suspensions of reference strains F8238 and C11 of serogroups A and C, respectively. After 60 min, 10 μl of each well was dropped on tilted plates containing Columbia Agar with 5% sheep blood and allowed to dry. Colonies along trickle tracks were counted after overnight incubation at 35 °C and 5% CO_2 using a photographic counter (ProtoCOL, Synbiosis, Cambridge, UK). For each serum, the SBA titer represented the reciprocal of the highest dilution giving $\geqslant$ 50% killing. Titers above 4 were considered protective [25]. Titers below 4 were assigned a value of 2. A fourfold increase from pre- to post-vaccination titer was regarded as evidence of vaccine immunogenicity.

2.4. Assessment of anti-cholera antibody titers

ELISA to assess cholera-specific IgG concentrations was performed by coating the plates with a final concentration of 1 μg/ml with the *Vibrio (V.) cholerae* strain Inaba 569B (List Biological Laboratories) and incubated for 3 h at 37 °C. After washing plates were blocked over night at 4 °C. 100 μl of serum sample dilution in 10% nonfat dry milk in PBS were incubated for 1 h at 37 °C. Following another washing step, secondary antibody (anti-human IgG γ-chain specific peroxidase conjugate (SIGMA, Germany)) was added. To visualize the bound antibodies, the plate was incubated for 20 min in the dark with a color solution (TMBONE, KemEnTech). The reaction was stopped using 2 M H_2SO_4. The plate was measured at 450 nm (620 nm reference) with an ELISA reader. The OD values were converted using linear regression of a serial dilution of standards into concentrations using the statistical software environment R v2.9.0 [26].

2.5. Vaccine-specific memory B-cell ELISpot

Antibody secreting cells (ASCs) representing memory B-cells were assessed with ELISpot assay as described elsewhere [18] with slight modifications which were the following: Plates were coated directly with 5 μg/ml of the meningococcal vaccine or 10 μg/ml of

the cholera vaccine. Stimulated cells were allowed to secrete antibodies, 16 h for meningococcal vaccine-specific ASCs and 6 h for the cholera-specific ASCs.

2.6. Assessment of helminth infection

Stool samples were collected on Day −28 (four weeks before vaccination), Day 0 and Day 84 and analyzed by qualitative MIF-Technique [18,27].

Urine was examined for the presence of *S. haematobium* by urine filtration method [16,28] using 10 ml of well mixed urine passed through a filter (12 μm pore size, Millipore).

2.7. Sample size calculation

As described in the publication of the first part of the study [18] we estimate that a 30% difference of immune responses between antihelminthic treatment and placebo groups is clinically relevant, when a power of 0.8 and significance level of 0.05 is set. To allow for 15% of loss to follow-up a total of 52 ($n = 52$) schoolchildren per group was required. Sample size calculation was done using R v2.9.0 [26].

2.8. Randomization, data entry and statistical analysis

Randomization and data management was done using the "Koordobas" database system (Institute for Clinical Epidemiology and applied Biometry, University of Tübingen). When not otherwise stated, rank-based ANCOVA (analysis of covariance) to correct for baseline titer and gender was used to compare vaccine-specific antibody titers between serogroups and Mann-Whitney-test was used to test differences in ELISpot counts between groups. For descriptive analyses geometric mean titers (GMT) were calculated. A two-sided alpha of 0.05 was set as significance level.

2.9. Ethical considerations

The study was approved by the Comité d'Ethique Régional Indépendant de Lambaréné and the Comité National d'Ethique pour la Recherche du Gabon. The trial was registered at PACTR (PACTR201303000434188) and conducted in accordance to the Declaration of Helsinki and followed the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. Families with children of primary school age from the semi-urban and rural area of Lambaréné and surroundings were actively asked if they were interested in the study, and if they wished to participate. Children were eligible for screening after a legal guardian was informed about the study and signed an informed consent form.

3. Results

From December 2011 until September 2012, 280 primary school children in Lambaréné and surroundings were screened, from whom 209 were randomized to receive either a single-dose 400 mg albendazole ($n = 104$) or placebo ($n = 105$). 71 participants were excluded due to *S. haematobium* infection, which was an exclusion criterion. One group received a polysaccharide meningococcal A + C vaccine ($n = 96$) and the other group received the oral cholera vaccine ($n = 89$). The study was conducted during different time periods. The first group started with the meningococcal vaccine and three months later the group for cholera vaccine was selected. 89 of the meningococcal group and 71 of the cholera group terminated the study at Day 84 post vaccination (Figs. 1 and 2). Baseline characteristics were similar between the antihelminthic treated and the placebo group (Table 1).

3.1. Assessment of anti-meningococcal antibody titers

Meningococcal titers increased after vaccination up to Day 28 and declined until Day 84 in 68 participants for serogroup A and in 80 participants for serogroup C. Three participants did not respond to the vaccine up to Day 84. Of those participants who had an increase of antibodies, 58 had a protective antibody titer above 4 against serogroup A and 72 against serogroup C. A fourfold increase from pre- to post-vaccination titer was reached in 42 volunteers for serogroup A and in 72 for serogroup C. There was no statistically significant difference between the albendazole treated and the placebo group (Serogroup A: Day 28: $w = 923.5$, $p = 1$, Day 84: $w = 815.5$, $p = 0.2012$; Serogroup C: Day 28: $w = 1068$, $p = 0.2978$, Day 84: $w = 1108$, $p = 0.2321$).

Twenty-one participants (14 in the antihelminthic treated group and 7 in the placebo group) had detectable baseline antibody levels with a titer above or equal to 4 for *Neisseria (N.) meningitidis* serogroup A, 4 participants for *N. meningitidis* serogroup C (3 in the antihelminthic treated group and 1 in the placebo group) and one participant (antihelminthic treated group) had detectable antibody titers for both serogroups (32 for serogroup A and 8 for serogroup C). The values at Day 0 ranged between 16 and 2048 for serogroup A, and from 4 to 64 for serogroup C, with a median of 2 for both serogroups and with a geometric mean titer (GMT) in the pre-treated group of 2.53 for serogroup A and 1.52 for serogroup C and in the control group of 1.98 for serogroup A and 1.42 for serogroup C (S1).

At Day 28 the meningococcal antibody titer values - corrected for Day 0 by subtraction - were increased with a median of 6 (Interquartile Range (IQR): 0; 254) in the antihelminthic treated group and 14 (IQR: 0; 254) in the placebo group for serogroup A and 254 in both groups (IQR: 62; 735 for the antihelminthic treated group and IQR: 77.5; 510 for the control group) for serogroup C. There was a decline at Day 84 (median of 0 (IQR: −2; 126) in the antihelminthic treated and 6 (IQR: 0; 126) in the control group for serogroup A and 126 (IQR: 2; 254) in the antihelminthic treated and 62 (IQR: 2; 252) in the placebo group for serogroup C) (Table 2) and (Fig. 3A).

3.2. Assessment of anti-cholera antibody titers

Antibodies against the cholera vaccine were assessed by vaccine-specific IgG ELISA. The cholera titers increased after vaccination at Day 28 and Day 84 in all participants. The differences in the cholera titer values were not statistically significant (Fig. 3B) (Day 28: $w = 450$, $p = 0.47$; Day 84: $w = 529$, $p = 0.93$).

Since baseline titers were high in all participants statistical tests were done on baseline-corrected values. The concentration increased after vaccination to a median of 11 128.26 ng/ml (IQR: 5500.91; 18369.39) in the antihelminthic treated group and to 13673.56 ng/ml (IQR: 8442.11; 17792.74) in the placebo group and declined to 5587.2 ng/ml (IQR: 2323.78; 8540.27) in the antihelminthic treated group and 4035.44 ng/ml (IQR: 2003.99; 6972.95) in the control group at Day 84 (Table 2). As previously proposed [28], a 4-fold increase after vaccination was considered to be a protective titer (18 in the antihelminthic treated group and 17 in the placebo group). Accordingly at Day 28, 35 participants showed a protective titer and 36 participants did not reach this 4-fold titer increase (20 in the antihelminthic treated group and 16 in the placebo group).

3.3. Assessment of antigen specific memory B-cells

B-cell ELISpot assay to measure vaccine-specific memory B-cells was performed with samples from 83 participants, who received

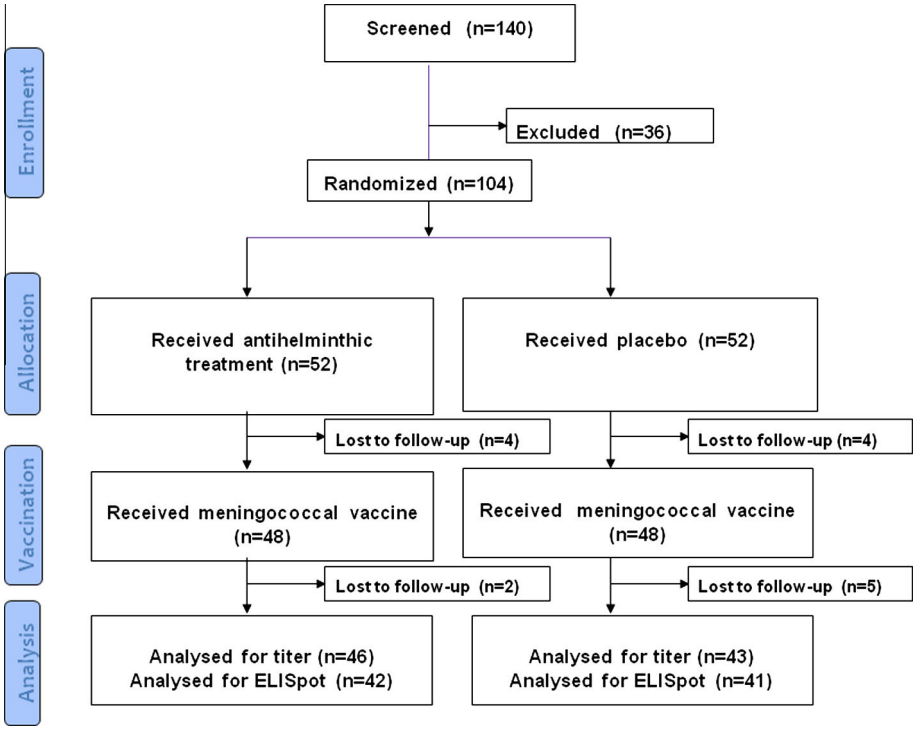

Fig. 1. Study profile for the group immunized with the meningococcal vaccine. [+]Patients were excluded, because of an infection with *S. haematobium*, [*]Participants not terminating the study are summarized as lost to follow-up ($n = 15$).

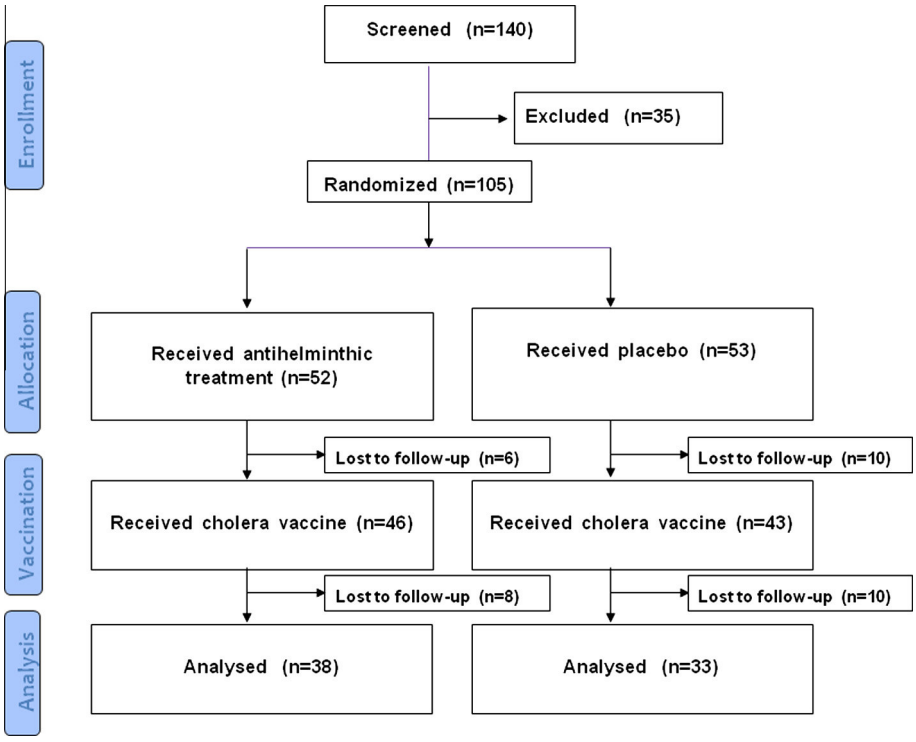

Fig. 2. Study profile for the group immunized with the cholera vaccine. [+]Patients were excluded, because of an infection with *S. haematobium*, [*]Participants not terminating the study are summarized as lost to follow-up ($n = 34$).

the meningococcal vaccine and 71 individuals who received the cholera vaccine.

Antigen-specific memory B-cells were detectable in all vaccinated subjects at Day 84, except for 10 participants vaccinated with the meningococcal vaccine and for 18 participants vaccinated with the cholera vaccine (equally distributed in both groups) (Fig. 4).

Amongst the individuals who were vaccinated with the meningococcal vaccine the median number of vaccine-specific ASC was 1 at Day 0 in the antihelminthic as well as in the placebo

Table 1
Baseline characteristics and helminth infection at Day −28 before vaccination.

	Meningococcal vaccine		Cholera vaccine	
	Antihelminthic treatment	Placebo	Antihelminthic treatment	Placebo
Gender				
Male	30 (31.3%)	30 (31.3%)	23 (25.8%)	20 (22.5%)
Female	18 (17.3%)	18 (17.3%)	23 (25.8%)	23 (25.8%)
Age				
6 years	10 (9.6%)	14 (13.4%)	16 (18.0%)	11 (12.3%)
7 years	4 (4.1%)	5 (5.2%)	11 (12.3%)	7 (7.9%)
8 years	17 (17.7%)	8 (8.3%)	7 (7.9%)	8 (9%)
9 years	14 (14.6%)	13 (13.5%)	8 (9%)	8 (9%)
10 years	3 (3.1%)	8 (8.3%)	4 (4.5%)	9 (10.1%)
Median (IQR)	8 (8; 9)	8 (6; 9)	7 (6; 8.5)	8 (6; 9)
Helminth				
Single infection				
A. lumbricoides	8 (8.3%)	1 (1%)	7 (7.9%)	8 (8.9%)
T. trichiura	2 (2.1%)	4 (4.2%)	3 (3.4%)	2 (2.2%)
More than one pathogen				
T. trichiura/*A. lumbricoides*	0	2 (2.1%)	0	1 (1.1%)
Negative	36 (37.5%)	38 (39.5%)	26 (29.2%)	22 (24.7%)
Not available	2 (2.1%)	3 (3.1%)	10 (11.2%)	10 (11.2%)

Table 2
Antibodies against *N. meningitidis* serogroup A and C and against cholera.

	Antibodies against *N. meningitidis*					
	Serogroup A			Serogroup C		
	Antihelminthic treatment (IQR)	Placebo (IQR)	*p*-value	Antihelminthic treatment (IQR)	Placebo (IQR)	*p*-value
ΔDay28	6 (0; 254)	14 (0; 254)	0.69	254 (62; 735)	254 (77.5; 510)	0.3
ΔDay84	0 (−2; 126)	6 (0; 126)	0.08	126 (2; 254)	62 (2; 252)	0.07
	Cholera-specific IgG titers					
	Antihelminthic treatment (IQR)			Placebo (IQR)		*p*-value
ΔDay28	11 128.26 (5500.91; 18369.39)			13 673.56 (8442.11; 17992.74)		0.47
ΔDay84	5 587.2 (2323.78; 8540.27)			4 035.44 (2003.99; 6972.95)		0.8

group; 18 individuals in the antihelminthic treated group and 16 individuals in the placebo had already a low number of ASCs at baseline (range: 1–20). At Day 84 the antihelminthic treated group had a median ASC number of 9 (IQR: 0; 16, range: 0–46) compared to the control group with 6 (IQR: 0; 16, range: 0–47). In each group 5 participants had no detectable ASCs at all (Fig. 4A).

In the group immunized with the cholera vaccine there were almost no detectable memory B-cells at baseline (mean 0.74 ± 1.56 in the pre-treated group and 0.71 ± 1.57 in the control group), except for 6 participants who had a low number (range: 1–5) of ASCs. All except 18 volunteers (9 in each group) had detectable ASCs at Day 84, and ASCs were slightly but not significantly elevated in the antihelminthic treated group (mean 4.52 ± 7.95; range: 1–32) compared to the placebo group (mean 3.50 ± 4.94; range: 1–17) (Fig. 4B).

3.4. Assessment of helminth burden in pre-school children

The percentage of helminth burden in our setting was 17.7% in meningococcal vaccinated individuals at Day −28, 16.7% at Day 0 and 24.7% at Day 84; the percentage of the assessed samples from the cholera vaccinated individuals was 23.5% at Day −28, 6.7% at Day 0 and 22.2% at Day 84 (Table 1 and S2/S3).

4. Discussion

Recent studies suggest that infection with helminths influences the immunological outcome of vaccination [18,19,21]. In a study conducted in Ecuador Cooper et al. showed that children infected with *A. lumbricoides* had a reduced antibody response towards the oral cholera vaccine CVD 103-HgR [21]. During a Phase 1 trial in Gabon to assess immunogenicity of a malaria vaccine candidate we found that children infected with *T. trichiura* exhibited a lower antibody response compared to those who were not infected with the parasite [19]. These and other studies in animal models and humans show that helminths could negatively influence vaccine immunogenicity [29,30]. Therefore we were interested to assess whether an antihelminthic treatment prior to vaccination has a positive effect on immune responses to vaccine antigens [18].

In the present study bactericidal anti-meningococcal antibody responses were analyzed for *N. meningitidis* serogroup A and C and anti-cholera antibodies following vaccination were assessed. The selected vaccines are not part of the EPI in Gabon. Therefore we assumed that no or only low baseline titers would be present in the study population. As expected there was an increase of anti-meningococcal -, as well as of anti-cholera titers at Day 28 and Day 84 in almost all participants although not all reached titers that are considered protective or vaccine induced. The antibody titer (Day 84) against the meningococcal serogroup C was slightly higher in the albendazole treated group compared to the control group, but the difference was not statistically significant.

Surprisingly 21 participants had an antibody titer >4 (that is considered as a protective meningococcal antibody titer) for serogroup A and 4 participants for serogroup C before vaccination indicating that *N. meningitidis* might circulate in the area. In a report of demographics for 2015 in Gabon, meningococcal meningitis is stated as one of the most important infectious diseases in

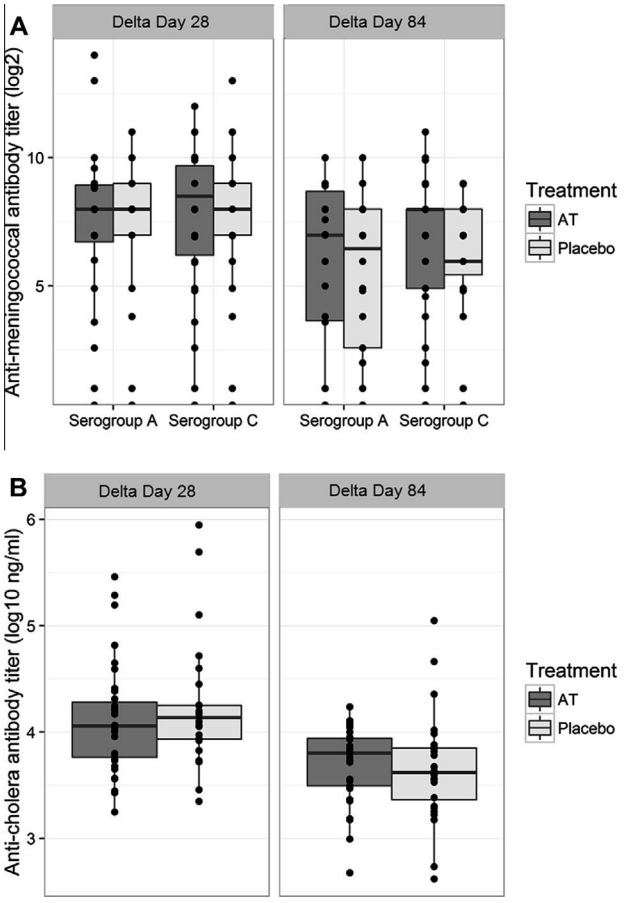

Fig. 3. A: Antibody titer against *N. meningitidis* serogroup A and C. Each boxplot represents the differences of meningococcal titer between the post vaccination visits (Day 28, Day 84) and Day 0 (baseline). B: Cholera vaccine-specific antibody titer. Each boxplot represents the differences of the cholera vaccine-specific IgG titer between the post-vaccination visits (Day 28, Day 84) and Day 0 (baseline).

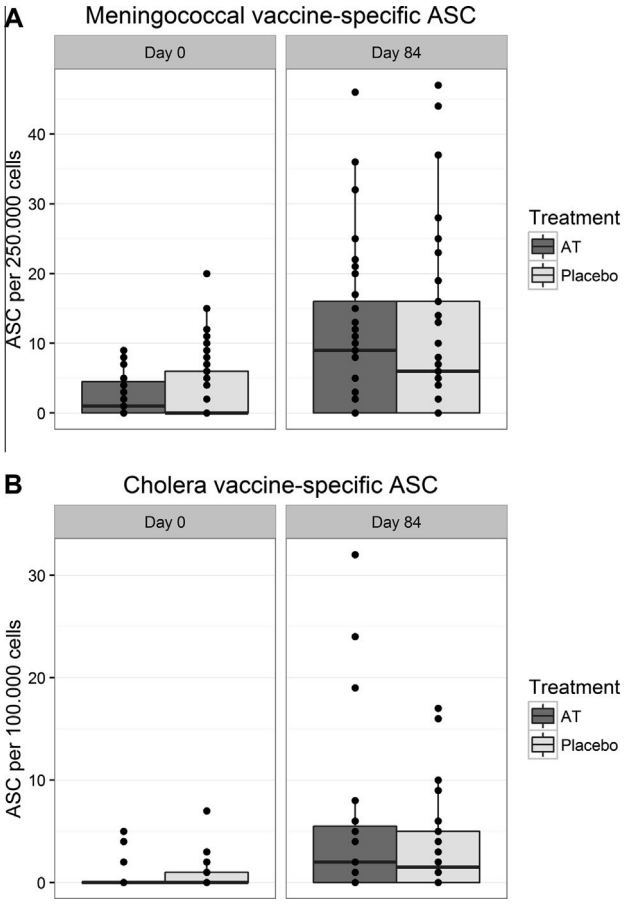

Fig. 4. A: Meningococcal vaccine-specific IgG ELISpot at Day 0 and Day 84. At baseline the majority of the children had no or a low number of meningococcal vaccine-specific IgG ASC representing memory B cells. The number of ASC increases from Day 0 to Day 84 (p = <0.001). There is no statistically significant difference between the antihelminthic treated and placebo group at Day 84. B: Cholera vaccine-specific IgG ELISpot at Day 0 and Day 84. At baseline the majority of the children had no cholera vaccine-specific IgG ASC. The number of ASC increases from Day 0 to Day 84 (p = <0.001) with no difference between the antihelminthic treated and placebo group.

Gabon [31]. The explanation for this could be that Gabon borders countries that belong to the "meningitis belt" [31]. To verify this further studies have to be conducted.

Chen et al. defined a 4-fold antibody increase following cholera vaccination as protective [32]. In the present study, 35 participants had an increase at Day 28 that was equal or higher than 4-fold and 36 participants had an increase that was below this protective titer. This was equally distributed in the antihelminthic treated group and the placebo group and the results correspond with other studies that show that oral cholera vaccines have diminished immunogenicity or efficacy in pre-school- and school-age children [13].

The number of vaccine-specific ASCs representing memory B-cells was slightly higher in the antihelminthic treated group compared to the control group, but this difference did not reach statistical significance neither in the meningococcal nor cholera vaccinated individuals.

In the present study only 16.7% at Day 0 in the group who received the meningococcal vaccine were infected with geohelminths and in the group vaccinated with the cholera vaccine the percentage was even lower. This was unexpected and therefore the power was probably too low to obtain clear and robust results. The sample size calculation was based on a higher prevalence of helminth infection. Our data may not be representative for the whole population of Gabon, but only for the population living in the region where the study was conducted and helminth burden may be higher in other, more remote parts of Gabon. We noted an increase of parasite burden from 6.7% at Day 0 to 22.2% at Day 84 in the group immunized with the cholera vaccine. We think that a more powerful treatment is needed to clear the infection. In addition, the treatment has to be repeated periodically as recommended by the WHO [2,33]. Furthermore it is not clear if the children had a treatment prior to the study without communicating this to the study physician. In 2000 Cooper et al. detected a positive effect on antibody response to an oral cholera vaccine by administering two 200 mg doses of albendazole prior to the vaccine [19,34]. In contrast to the present study the investigators only included volunteers who were infected with *A. lumbricoides* [21].

In conclusion, we showed that the prevalence of geohelminth burden in school children living in the area around Lambaréné was very low compared to earlier studies [21,35] and in this setting a single-dose antihelminthic treatment was not superior to the placebo raising anti-meningococcal and cholera antibody titers as well as ASCs against the vaccine-specific antigens. Most of the children obtained a protective antibody titer following vaccination with the meningococcal vaccine whereas the cholera vaccine did not raise protective antibodies in all participants. Interestingly in some participants high baseline anti-meningococcal titers were present suggesting that *N. meningitidis* might be present in the area, which has to be investigated in the future.

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Conflict of interest

All other authors declare that they have no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.07.040.

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