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DISSERTATION

Untersuchungen zur Klinik, Therapie und Prävention der Tungiasis (Sandflohkrankheit) in Madagaskar, Kenia und Uganda

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1. Zusammenfassung

Hintergrund: Die Tungiasis (Sandflohkrankheit) ist eine in Subsahara-Afrika, Südamerika und der Karibik endemische Tropenkrankheit. Sie gehört zur Gruppe der Neglected Tropical Diseases (NTD) und wird verursacht durch die Penetration des weiblichen Sandflohs *Tunga penetrans* in die Epidermis. Mit der Außenwelt bleibt der Parasit durch eine winzige Öffnung in seinem letzten Abdominalsegment verbunden, über die Eier und Exkremente ausgeschieden werden, Atemluft ausgetauscht und der weibliche Sandfloh befruchtet wird. In der Epidermis eingebettete Sandflöhe verursachen eine starke Entzündungsreaktion, die durch eine - nahezu konstant vorhandene - bakterielle Superinfektion verstärkt wird. Aus Mangel an Alternativen versuchen die Betroffenen eingebettete Sandflöhe mit ungeeigneten, nicht-sterilen Instrumenten zu entfernen.

Methoden: In einer randomisierten, kontrollierten Studie in Madagaskar wurde der präventive Effekt eines Repellents auf Kokosnussölbasis mit dem präventiven Effekt von Schuhen verglichen. In einer proof-of-principle Studie in Kenia wurde an penetrierten Sandflöhen der therapeutische Effekt der topischen Applikation eines niedrig-viskösen Dimeticons mit der Therapieempfehlung des kenianischen Gesundheitsministeriums (Baden des Fußes in KMnO4 und Applikation von Vaseline) verglichen. In Uganda wurde in einer Studie zur Therapieoptimierung geprüft, ob eine gezielte Applikation des Dimeticons auf den Abdominalkonus des penetrierten Parasiten bei geringerem Applikationsvolumen eine schnellere Wirkung erzielen kann. Zudem wurde in Madagaskar ein einzelner, penetrierter Sandfloh systematisch über acht Wochen beobachtet, um Erkenntnisse über die Entwicklung im Wirt zu gewinnen.

Ergebnisse: Die Präventionsstudie bestätigte die effektive Wirkung des Repellents anhand eines signifikanten Rückgangs von Befallsrate, Befallsintensität und klinischer Pathologie im Vergleich zur Kontrollgruppe. Die Bereitstellung von Schuhen zeigte nur eine marginale und verzögerte Wirkung. In der proof-of-principle Studie erwies sich Dimeticon als wirksame Therapie: 78% der behandelten Parasiten waren nach sieben Tagen abgestorben. Die Studie zur Therapieoptimierung zeigte, dass eine gezielte Applikation auf den Abdominalkonus des eingebetteten Sandflohs eine schnellere Wirkung bei geringerem Volumen erzielt. In der Fallstudie wurden zwei Erkenntnisse gewonnen: ein mit großer Wahrscheinlichkeit unbefruchtetes Weibchen lebt deutlich länger als erwartet und scheidet keine Eier aus.

Konklusion: Tungiasis lässt sich durch regelmäßige Applikation eines Repellents auf Kokosnussöl-basis effektiv vorbeugen. Die gezielte Applikation von Dimeticon ist eine hocheffektive Therapie. Ein eingebetteter, gleichwohl nicht befruchteter Sandfloh entwickelt sich nicht regelrecht. Das

unterstützt die Hypothese, dass die Kopulation in situ nach Penetration des Sandflohweibchens in die Epidermis stattfindet.

Abstract

Background: Tungiasis (sand flea disease) is a tropical disease, endemic in sub-Saharan Africa, South America und the Caribbean. It belongs to the list of Neglected Tropical Diseases (NTD) and is caused by the penetration of the female sand flea *Tunga penetrans* into the epidermis. The parasite remains in contact with the environment through a tiny opening in its last abdominal segment, which is needed for expelling eggs and excrement, breathing and fertilization of the female sand flea. The embedded sand fleas provoke a strong inflammatory reaction, often increased by a bacterial superinfection. Due to a lack of alternatives, those affected frequently try to get rid of the imbedded sand fleas by using inappropriate, nonsterile instruments.

Methods: A randomized controlled trial in Madagascar was performed to compare the preventive effect of a repellent, based on coconut oil with the preventive effect of closed shoes. In a proof-of-principle study in Kenya, the therapeutic effect of the topical application of low-viscous dimeticone was compared with the therapy recommendation of the Kenyan Ministry of Health (KMnO4 and vaseline). A subsequent study in Uganda examined whether a targeted application of a lowered application volume of dimeticone to the abdominal rear cone of the penetrated parasite could increase the treatment effect. Additionally, a single, penetrated sand flea was investigated systematically for eight weeks to gain an increased understanding of its on-host development.

Results: The prevention study verified the effective impact of the repellent on the basis of a significant reduction of the attack rate, intensity of infestation and clinical pathology in comparison to the control group. The provision of shoes showed only a marginal and delayed impact. In the proof-of-principle study, dimeticone proved to be an effective therapy: 78% of the treated parasites were dead after seven days. The study for optimization the therapy showed that a targeted application to the abdominal rear cone of the imbedded sand flea achieved faster results with lower application volumes. In the case study, two findings were obtained: a likely unfertilised female sand flea lives much longer than expected and does not excrete eggs.

Conclusions: Tungiasis can be effectively prevented by regular application of a repellent based on coconut oil. The targeted application of dimeticone is a highly effective therapy. An embedded but non-fertilized sand flea does not develop properly. This supports the hypothesis that the copulation takes place in situ after penetration of the female sand flea into the epidermis.

2. Einleitung und Zielsetzung

Die Tungiasis (Sandflohkrankheit) ist eine Ektoparasitose. Die Tropenkrankheit gehört zur Gruppe der Neglected Tropical Diseases (NTD) und ist in weiten Teilen Südamerikas, der Karibik sowie Afrika südlich der Sahara endemisch [1, 2, 3]. Sie wird durch das Penetrieren des weiblichen Sandflohs *Tunga penetrans* in die Epidermis verursacht. Der eingebettete Parasit vergrößert sein Volumen innerhalb von ca. zehn Tagen auf ein 2000-faches [4]. Über eine etwa 250 µm große Öffnung im letzten Abdominalsegment des Sandflohs werden Fäzes in fester und flüssiger Form, sowie Eier ausgeschieden, die sich außerhalb des Wirtes weiter entwickeln [4]. Auch die Atmung erfolgt über den Abdominalkonus. *T. penetrans* durchläuft mehrere spezifische on-host Stadien, die nach der Fortaleza Klassifikation eingeteilt sind [4]:

- Stadium I: Sandfloh in statu penetrandi.
- Stadium II (Tag 1 - 3 nach Penetration): Beginn der Hypertrophie der Abdominalsegmente auf einen Diameter von ca. 8 mm. Zentral in der hellen, im Hautniveau liegenden Läsion ist der Abdominalkonus als dunkler Punkt zu erkennen. Fäzes wird ausgeschieden.
- Stadium III (Tag 4 - Woche 3 nach Penetration): Der penetrierte Sandfloh ist auf eine Größe von ca. 10 mm Durchmesser angewachsen. Von außen betrachtet erscheint die Läsion uhrglasförmig mit einer zentralen kleinen Öffnung. Eier werden expulsiert.
- Stadium IV (Woche 4 - 6 nach Penetration): Der Sandfloh stirbt in situ und stellt sich als dunkle, verkrustete Läsion dar; die chitinösen Reste werden über körpereigene Reparaturmechanismen aus der Epidermis eliminiert.
- Stadium V (Monate bis Jahre nach Penetration): Es verbleibt eine charakteristische kreisrunde Impression im stratum corneum.

99% der Sandflöhe penetrieren an den Füßen [5, 6]. Häufig liegen die penetrierten Sandflöhe in Clustern eng nebeneinander [2].

Die Tungiasis geht mit einer ausgeprägten akuten und chronischen Pathologie einher. Dazu zählen lokale Entzündungsreaktion mit Erythem, Überwärmung, Schmerzen und Ödem bis hin zu Deformation und Verlust von Zehennägeln und Zehen [7, 8, 9, 10]. Die Befallsintensität (Gesamtzahl aller Läsionen) und das Ausmaß der klinischen Pathologie korrelieren signifikant [2, 5]. Eine Superinfektion mit Aerobiern und Anaerobiern ist konstant; Infektionen mit *Clostridium tetani* haben bei ungeimpften Personen bereits mehrfach zum Tod des Patienten geführt [8, 11, 12, 13]. Aus Mangel an geeigneten Therapiemaßnahmen versuchen die Betroffenen oder die Familienangehörigen die penetrierten Sandflöhe mit toxischen Substanzen wie Petroleum oder Benzin abzutöten oder sie mit

spitzen Instrumenten wie Sicherheitsnadeln, Nähnadeln, Messern, Hölzchen, Dornen etc. zu extrahieren [2, 6, 10]. Durch die Manipulation steigt das Risiko einer bakteriellen Superinfektion. Durch die Wiederverwendung desselben Instruments bei anderen Patienten besteht zudem ein Risiko der Übertragung von HBV, HCV oder HIV [6].

Bei der Tungiasis handelt es sich um eine mit Armut assoziierte Krankheit [1, 2, 14, 15]. Auf Bevölkerungsebene betrifft sie vor allem Kinder zwischen fünf und 14 Jahren sowie ältere Menschen [2, 5, 9]. In einigen Endemiegebieten beträgt die Prävalenz in der allgemeinen Bevölkerung bis zu 50% und bei Kindern bis zu 80% [5, 16, 17]. Kinder mit Tungiasis leiden oftmals unter Stigmatisierung bis hin zur sozialen Exklusion. Ihre Lebensqualität ist deutlich eingeschränkt [8].

Zahlreiche Aspekte der Tungiasis und von *T. penetrans* sind bisher nicht oder nur unzureichend erforscht. Dazu gehören Möglichkeiten der Prävention, der Therapie sowie die Fortpflanzungsbiologie des Parasiten.

Studien in Nordbrasilien haben gezeigt, dass ein Repellent auf Kokosnussölbasis (Zanzarin®) effektiv gegen Sandflöhe schützt [18, 19]. Die Wirksamkeit im Vergleich zu anderen Präventionsmaßnahmen ist bislang jedoch unbekannt.

Therapieversuche mit Anthelminthika wie Metrifonat, Thiabendazol oder Ivermectin zeigten nur unbefriedigende Ergebnisse [20, 21]. Ein völlig neuer Therapieansatz ist die topische Anwendung von Dimeticonen mit hoher Kriechfähigkeit und sehr niedriger Viskosität. Therapeutische Angriffsfläche ist dabei der Abdominalkonus von *T. penetrans.*

Die Kenntnisse zur Fortpflanzungsbiologie von *T. penetrans* stammen aus der Zeit von vor 1960 [22]. Es ist nach wie vor ungeklärt, ob die Kopulation außerhalb des Wirtes, also vor der Penetration des weiblichen Flohs in die Epidermis, oder in situ nach der Penetration stattfindet.

Die Zielsetzungen meiner Dissertation sind:

1. Die protektive Wirkung der topischen Applikation eines Repellents auf Kokosnussölbasis in einer bevölkerungsbasierten randomisierten, kontrollierten Studie mit der protektiven Wirkung von geschlossenen Schuhen zu vergleichen.
2. Die therapeutische Wirksamkeit der topischen Anwendung eines zwei-Komponenten-Dimeticons zu überprüfen und die Therapie zu optimieren.
3. Neue Erkenntnisse über die Fortpflanzungsbiologie von *T. penetrans* anhand eines akzidentiellen Selbstversuchs zu gewinnen.

Weitere im Rahmen dieser Dissertation gewonnene und publizierte Daten sind aus der Publikationsliste ersichtlich.

3. Methodik

3.1 Studiengebiete und Studienpopulationen

Die vier Studien wurden jeweils in der Trockenzeit - also der Periode mit dem höchsten Infektionsdruck - durchgeführt. Die Präventionsstudie wurde im Distrikt Moramanga, in Zentralmadagaskar in den Dörfern Tanambe II (507 Einwohner) und Tanambaovao (486 Einwohner) realisiert: Die Bewohner leben vorwiegend von Subsistenzlandwirtschaft und bauen Reis, Maniok und Mais auf angrenzenden Feldern an. Die Häuser der Dorfbewohner sind aus Holz gebaut; einige stehen auf Stelzen (Abb. 1A). Die Straßen sind nicht geteert, sondern sind in der Regel unbefestigt und sandig (Abb. 1B). Die meisten Dorfbewohner tragen keine Schuhe. Die Prävalenz der Tungiasis in der allgemeinen Bevölkerung betrug 43% in Tanambaovao und 69% in Tanambe II. Insgesamt wurden 219 Individuen aus 62 Haushalten aus den beiden Dörfern in die Studie aufgenommen.

Abb. 1A und B: Häuser und unbefestigte Wege in Tanambe II.

Die proof-of-principle Studie zur Überprüfung der Effektivität von Dimeticon wurde im Gatundu North District in Zentralkenia durchgeführt. Die Menschen leben von Subsistenzlandwirtschaft. In früheren Studien wurde eine Prävalenz der Tungiasis von 15 - 40 % in der allgemeinen Bevölkerung dokumentiert [23]. Die Studie wurde in zwei benachbarten Grundschulen durchgeführt. Die meisten Schulkinder trugen keine Schuhe. 47 Kinder zwischen 5 und 16 Jahren nahmen an der Studie teil.

Die Studie zur Optimierung der Dimeticonapplikation wurde im Bugiri District, 150 km nordöstlich von Kampala in Uganda durchgeführt. Die Menschen hier leben ebenfalls von Subsistenzland-wirtschaft. Die Prävalenz der Tungiasis betrug 72% in der Kinderpopulation. Die Studie wurde in acht Grundschulen realisiert. Die meisten Schulkinder hatten keine geschlossenen

Schuhe und trugen Sandalen oder Flipflops. 60 Kinder im Alter von fünf bis 12 Jahren wurden in der Studie eingeschlossen.

3.2. Studiendesign und Untersuchungsmethoden

3.2.1. Prävention der Tungiasis durch Einsatz eines Repellents

Die Präventionsstudie wurde über 12 Wochen durchgeführt und beinhaltete eine Basisuntersuchung zu Beginn der Studie und fünf Folgeuntersuchungen. Einschlusskriterien für die Studienteilnahme waren ein Alter ≥ fünf Jahre, Präsenz im Dorf für die gesamte Studiendauer, sowie mindestens eine Person im Haushalt, die mindestens sieben Sandflohläsionen an beiden Füßen hatte.

Die Studienteilnehmer wurden in drei Gruppen randomisiert: zwei Gruppen erhielten eine Prävention, die dritte diente als unbehandelte Kontrollgruppe. Aus praktischen Gründen wurde die Randomisierung nach Haushalten durchgeführt. Jeweils 21 Haushalte gehörten zu den Gruppen I und III, zu Gruppe II gehörten 20 Haushalte.

In Gruppe I (Schuh-Gruppe, N=77) bekamen alle Studienteilnehmer zu Beginn der Studie geschlossene, der Fußgröße angepasste Sportschuhe. Sie wurden ermutigt die Schuhe regelmäßig zu tragen und ermahnt diese nicht mit anderen Leuten zu teilen oder zu verkaufen. Drei Dorfgesundheitshelfer beobachteten und dokumentierten in regelmäßigen Abständen, ob die Teilnehmer der Schuhgruppe die Schuhe trugen. Anhand ihrer Beobachtungen konnten die Studienteilnehmer am Ende der Studie in folgende Kategorien eingeteilt werden: „seltene Schuhträger“ (sie trugen die Schuhe in weniger als 30% der beobachteten Fälle), „unregelmäßige Schuhträger“ (sie trugen die Schuhe in 30 - 60% der Fälle) und „regelmäßige Schuhträger“ (sie trugen die Schuhe in mehr als 60% der Fälle).

In Gruppe II (Repellent-Gruppe, N=72) wurden die Studienteilnehmer zweimal täglich mit dem Repellent Zanzarin® (Engelhard Arzeimittel GmbH & Co. KG, Niederdorfelden) behandelt. Das Repellent basiert auf Kokonussöl und enthält zusätzlich die antientzündlichen Wirkstoffe Jojobaöl und *Aloe vera.* Jeweils morgens und abends applizierten geschulte Dorfgesundheitshelfer durchschnittlich 1,5 ml von Zanzarin® sorgfältig auf die zuvor gewaschenen und abgetrockneten Füße von den Zehen bis zu den Knöcheln. Die Applikation des Repellents wurde jedes Mal durch die Unterschrift oder den Fingerabdruck des Studienteilnehmers auf einem Dokumentationsbogen bestätigt.

Bei Gruppe III (N=70) handelte es sich um die Kontrollgruppe, die weder Schuhe noch eine Behandlung mit dem Repellent erhielt. Ob in den teilnehmenden Haushalten bereits Schuhe

existierten und die Studienteilnehmer diese trugen, wurde weder in der Kontroll-, noch in der Repellent-Gruppe erfasst.

Alle Studienteilnehmer wurden alle zwei Wochen klinisch und parasitologisch untersucht. Die drei Zielgrößen Befallsintensität, Befallsrate und Ausmaß der klinischen Pathologie wurden für beide Füße zusammen erfasst. Um die Befallsintensität zu ermitteln, wurde die Gesamtzahl der penetrierten Sandflöhe (einschließlich vitaler, toter und manipulierter Läsionen) dokumentiert. Die Befallsrate entspricht der Anzahl der Sandflöhe, die die Haut des Patienten seit der letzten Untersuchung neu penetriert hatten. Dazu wurde bei jeder Untersuchung die topographische Lokalisation jeder Sandflohläsion und ihr jeweiliges Stadium auf einem Fuß-Schema eingezeichnet und mit einer Digitalkamera, ausgestattet mit einem Makroobjektiv, dokumentiert (EOS 450 D, Canon, Tokyo, Japan). Die Tungiasis-assoziierte Pathologie wurde semi-quantitativ mit dem Index für akute Tungiasis (*severity score for acute tungiasis* = SSAT, 0 – 35 Punkte) und dem Index für chronische Tungiasis (*severity score for chronic tungiasis* = SSCT, 0 – 30 Punkte) bestimmt [7].

3.2.2. Therapie der Tungiasis mit Dimeticon - eine proof-of-principle Studie

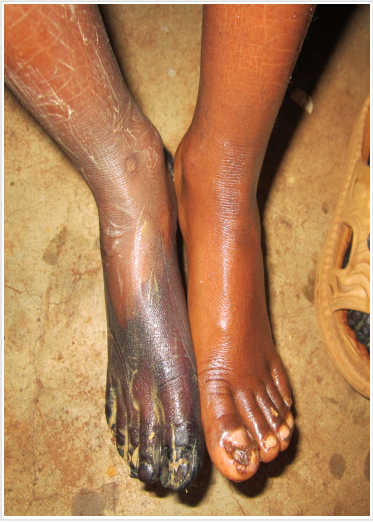
Einschlusskriterien für Studienteilnehmer waren ein Alter ≥ fünf Jahre und das Vorhandensein von mindestens einer Sandflohläsion pro Fuß im Fortaleza-Stadium II oder III. Es wurden pro Fuß maximal drei Läsionen in die Studie aufgenommen, die ausreichend entfernt voneinander liegen mussten, um jedes Entwicklungsstadium gut beurteilen und die lokale Entzündungsreaktion quantifizieren zu können. Läsionen, die bereits manipuliert worden waren oder in Clustern eng nebeneinander lagen, wurden nicht ausgewertet.

Die Füße der Teilnehmer wurden in zwei Therapiegruppen randomisiert. In Gruppe 1 wurde der linke Fuß drei Mal innerhalb von zehn Minuten gleichmäßig mit dem zwei-Komponenten-Dimeticon NYDA® (Pohl-Boskamp GmbH & Co. KG, Hohenlockstedt) gleichmäßig von den Zehen bis zum Knöchel eingerieben. Dabei handelt es sich um eine Mischung aus einem niedrig viskösen mit einem höher viskösen Dimeticon mit niedriger Oberflächenspannung und exzellenten Kriecheigenschaften, die in alle vitalen Öffnungen des Ektoparasiten, unter anderem in die Atemwege eindringen kann [24]. Dimeticone gelten als Standardtherapie gegen *Pediculus humanus capitis* [25, 26].

Gleichzeitig wurde in Gruppe 2 der rechte Fuß bis zum Knöchel für zehn Minuten in einer 0,05% KMnO4-Lösung gebadet. Nach dem Fußbad trocknete der Fuß an der warmen Luft und wurde daraufhin mit Vaseline eingefettet, um die durch KMnO4 verursachte Austrocknung der Haut zu

kompensieren (Abb. 2). Dabei handelt es sich um die von den kenianischen Gesundheitsbehörden empfohlene Behandlung der Tungiasis.

Abb. 2: Rechter und linker Fuß nach Behandlung.



Da KMnO4 die Haut für einige Tage violett färbt, war weder für den Patienten noch für den Untersucher eine Verblindung möglich. In die Behandlungsgruppe mit NYDA® wurden 88, in die Kontrollgruppe mit KMnO4 82 Läsionen aufgenommen. Vor der Behandlung wurden die Füße der Studienteilnehmer gewaschen und getrocknet.

Die einmalig behandelten Sandflohläsionen wurden anschließend über einen Zeitraum von sieben Tagen auf ihre Viabilität und morphologische Entwicklung untersucht. Es wurden folgende Vitalitätszeichen identifiziert: Ausscheiden von Fäzes in flüssiger und fester Form, Expulsion von Eiern und Pulsationen des Flohkörpers. Vitalitätszeichen wurden mit einem digitalen Video-Mikroskop (eScope iTEZ, Hongkong, China) für maximal 15 Minuten detektiert. Normale und abnormale Entwicklungen wurden anhand der Fortaleza-Klassifikation beurteilt [4]. Die Periode einer Woche deckt den Zeitraum der normalen Entwicklung eines eingebetteten Sandflohs von Stadium II bis Stadium III ab [4].

Um eine Reduktion der Tungiasis-assoziierten Entzündung pro Fuß-Einheit messen zu können, wurde ein periläsionaler semiquantitativer Entzündungsindex entwickelt. Er beinhaltet die Symptome Erythem, Schwellung, Überwärmung, Suppuration, Ulzeration, Abszess, sowie Juckreiz und Schmerz und reicht von 0 bis maximal 27 Punkten.

Die erste Zielgröße war der prozentuale Anteil abgestorbener Sandflöhe im Verhältnis zu allen in die Auswertung einbezogenen Parasiten am Ende der Nachbehandlungszeit. Ein eingebetteter Sandfloh wurde als tot definiert, wenn während zwei aufeinander folgender Verlaufskontrollen jegliche Vitalitätszeichen fehlten. Die zweite Zielgröße war der prozentuale Anteil der Sandflöhe mit einer gestörten morphologischen Entwicklung. Die Entwicklung des penetrierten Parasiten wurde als gestört definiert, wenn er innerhalb von zwei aufeinander folgender Verlaufskontrollen das nachfolgende Entwicklungsstadium nicht erreichte und/oder sich morphologische Abnormalitäten, wie eine Verfärbung oder Austrocknung des Abdominalkonus zeigten.

3.2.3. Optimierung der Therapie der Tungiasis mit Dimeticon

Die Ein- und Ausschlusskriterien waren identisch zu der unter 3.2.2. beschriebenen proof-of-principle Studie. Ein erweitertes Einschlusskriterium war die Lokalisation der Läsion an einer für das digitale Video-Mikroskop leicht zugänglichen Stelle, um die Vitalitätszeichen leichter bestimmen zu können. Die Füße jeden Studienteilnehmers wurden in zwei Therapiegruppen randomisiert.

Bei dem einen Fuß wurde NYDA® auf die gesamte Haut appliziert. Dazu wurde wie in der proof-of-principle Studie das Dimeticon von den Zehen bis zum Knöchel drei Mal innerhalb von zehn Minuten gleichmäßig aufgetragen (insgesamt ca. 2 – 5 ml Dimeticon pro Fuß je nach Fußgröße). An dem anderen Fuß wurde eine gezielte Applikation vorgenommen. Dazu wurde NYDA® drei Mal innerhalb von zehn Minuten tropfenweise mithilfe einer 5 ml Spritze direkt auf den Abdominalkonus der penetrierten Sandflöhe aufgetragen (insgesamt ca. 450 µl Dimeticon pro Läsion). In der Gruppe der Ganzfuß-Therapie waren 139 Läsionen, in der Gruppe der gezielten Therapie 156 Läsionen eingeschlossen.

Vitalitätszeichen, morphologische Entwicklung und das Ausmaß der Tungiasis-assoziierten Entzündung wurden wie unter 3.2.2. beschrieben für sieben Tage dokumentiert. Über visuelle Skalen wurde zudem das Ausmaß von Schmerz, Juckreiz, Schmerz- und Juckreiz-assoziierte Schlafstörungen und Mobilitätseinschränkungen ermittelt. Die subjektive Beurteilung des Patienten konnte jeweils zwischen 0 (keine Beschwerden) und 4 (sehr starke Beschwerden) variieren und bezog sich auf beide Füße.

3.2.4. Fallbeobachtung: Unterbrechung der Entwicklung von *T. penetrans* im Wirt

Im Rahmen der Projektarbeiten im ländlichen Madagaskar penetrierte ein Sandfloh akzidentiell und unbemerkt in die Sohle meines rechten Fußes. Ich entdeckte den Sandfloh drei Tage nach Penetration im Stadium II. Zu diesem Zeitpunkt bestanden weder Juckreiz noch Schmerzen, sondern lediglich ein lokales Erythem. Ich beobachtete und dokumentierte die Entwicklung des Parasiten und die klinische Pathologie über einen Zeitraum von 60 Tagen. Um zu verhindern, dass weitere weibliche Sandflöhe meine Füße penetrierten bzw. ein männlicher Sandfloh das penetrierte Weibchen begattete, trug ich fortan konstant Socken und geschlossene Schuhe. Es wurde sonst nichts unternommen, um die natürliche Entwicklung des eingebetteten Parasiten zu beeinflussen.

Alle fünf Tage wurde die Läsion mit einem digitalen Video-Mikroskop (eScope iTEZ, Hongkong, China) auf die unter 3.2.2. beschriebenen vier Vitalitätszeichen untersucht und mit einer Digital-

kamera mit einem Makroobjektiv (EOS 450 D, Canon, Tokyo, Japan) fotografiert. Folgende Symptome wurden dokumentiert: Spontanschmerz, Druckschmerz, Juckreiz, sowie Schmerz- und Juckreiz-assoziierte Schlafstörungen und Mobilitätseinschränkungen; Präsenz von Erythem, Ödem und Überwärmung.

3.3. Datenanalyse

Die Stichprobenberechnung für die Präventionsstudie beruhte auf der Annahme, dass beide Präventionsmaßnahmen eine ähnlich schützende Wirkung haben würden. Um einen Unterschied in der Zielgröße Befallsintensität von 50% zwischen einer Interventions- und der Kontrollgruppe zu detektieren, bedurfte es mindestens 75 Teilnehmer pro Studiengruppe (confidence level = 95%; power of the test = 86%). In der proof-of-principle Studie benötigte man 45 Sandflohläsionen pro Therapiegruppe, um einen Unterschied in der Zielgröße Viabilität von 35% zwischen den Gruppen festzustellen (confidence level = 95%; power of the test = 90%). In der Optimierungsstudie wurden 140 Läsionen pro Therapiegruppe gebraucht, um einen Unterschied von 15% zwischen den Gruppen aufzeigen zu können (confidence level = 95%; power of the test = 90%).

Die Daten wurden in Epi Info (Version 3.4.3 CDC Atlanta, USA) gespeichert. Da die Zielgrößen nicht normverteilt waren, wurden in der Präventionsstudie der Median und die Interquartilsabstände (IQR) als Indikatoren für zentrale Tendenz und Dispersion der Daten genutzt. Aufgrund der Randomisierung nach Haushalten wurde angenommen, dass die Ergebniswerte der einzelnen Teilnehmer nicht unabhängig waren. Zur Datenauswertung wurde deshalb die Methode der Generalized Estimating Equations (GEE) verwendet. Es wurde eine Intention-to-treat-Analyse und eine per-Protokoll-Analyse durchgeführt.

In den Therapiestudien wurde der Fisher´s Exakt Test zum Vergleich von Proportionen verwendet. Für die Analyse des Entzündungsindex und der anhand der visuellen Skalen gemessenen Variablen wurde der Kruskal-Wallis Test angewendet.

3.4. Ethische Aspekte

Die Ziele und Maßnahmen der jeweiligen Studie wurden jedem Teilnehmer bzw. den gesetzlichen Vertretern mündlich in Englisch (in Kenia und Uganda), Französisch (in Madagaskar) bzw. über einen Dorfgesundheitshelfer in einer der lokalen Sprache erklärt. Für die bevölkerungsbasierte Studie in Madagaskar geschah dies in Form einer öffentlichen Dorfversammlung. Das Recht auf jederzeitiges Ausscheiden aus der Studie wurde betont. Jeder Teilnehmer bzw. sein gesetzlicher

Vertreter gab eine schriftliche Einverständniserklärung ab. Am Ende der Studien wurden alle verbliebenen Sandflöhe entweder chirurgisch extrahiert (in Madagaskar und Kenia) oder gezielt mit Dimeticon (in Uganda) behandelt. Alle Studienteilnehmer erhielten zudem ein Paar geschlossene Schuhe. Die Studien wurden unter controlled-trials.com registriert und durch die Ethikkommission des Gesundheitsministeriums von Madagaskar und Kenia bzw. der Makarere Universität, Kampala, Uganda geprüft und genehmigt.

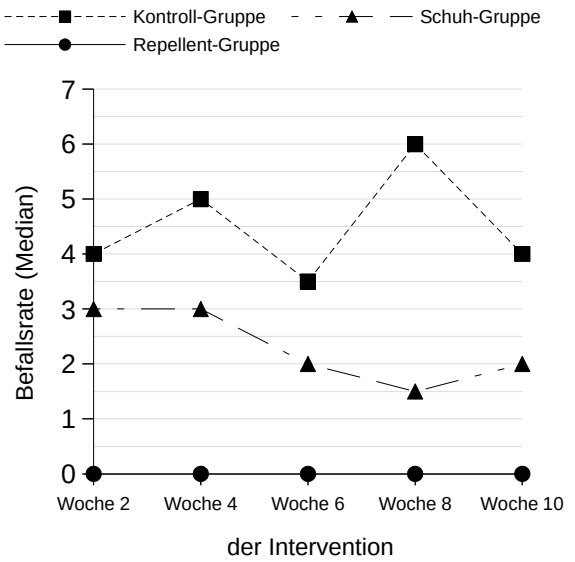
4. Ergebnisse

4.1. Prävention der Tungiasis durch Einsatz eines Repellents

Die Befallsrate sank in der Repellent-Gruppe bereits in Woche 2 auf Null und blieb während der gesamten Intervention Null. In der Schuh-Gruppe sank die Befallsrate maximal um einen Median von 1.5 in Woche 8. Außer am Ende der Beobachtungszeit (Woche 10) gab es keinen signifikanten Unterschied zwischen Schuh- und Kontroll-Gruppe (Grafik 1).

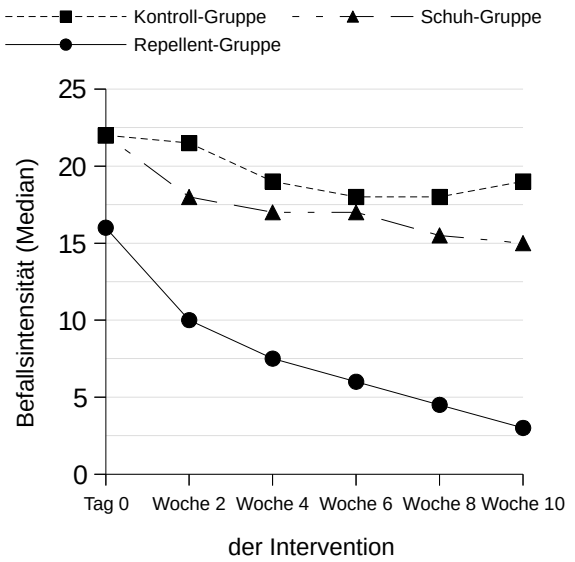
In der Repellent-Gruppe konnte ein rascher und kontinuierlicher Abfall der Befallsintensität beobachtet werden, während diese in der Schuh- und Kontrollgruppe lediglich marginal abnahm.

Grafik 1: Befallsrate im Beobachtungszeitraum.



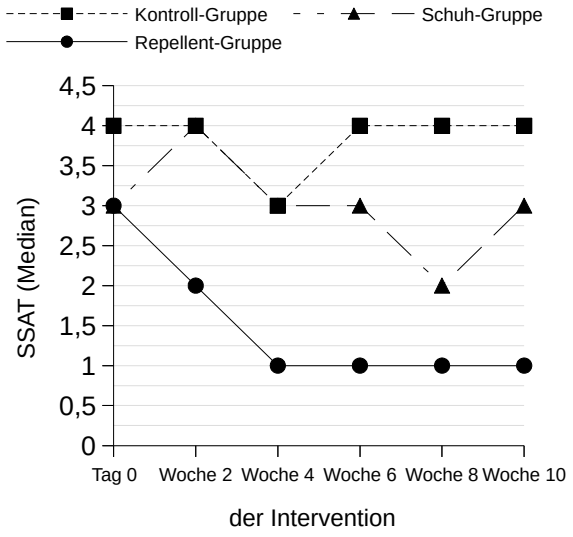
p <0.001 ab Woche 2 Repellent-Gruppe versus Kontroll-Gruppe; p = 0.049 in Woche 10 Schuh-Gruppe versus Kontroll-Gruppe

Grafik 2: Befallsintensität im Beobachtungszeitraum.



p = 0.02 in Woche 4, p = 0.004 in Woche 6, p <0.002 in Woche 8 und p <0.001 in Woche 10 Repellent-Gruppe versus Kontroll-Gruppe

Grafik 3: SSAT im Beobachtungszeitraum.



p <0.001 ab Woche 2 Repellent-Gruppe versus Kontroll-Gruppe

Auch der SSAT sank rasch in der Repellent-Gruppe und erreichte bereits in Woche 4 einen Median von 1. In der Schuh-Gruppe zeigte sich eine abfallende Tendenz. Der Unterschied zur Kontroll-Gruppe war jedoch zu keinem Zeitpunkt signifikant. In der Kontroll-Gruppe änderte sich der SSAT nicht.

Der SSCT änderte sich bei keiner Interventionsgruppe. In der Repellent-Gruppe zeigte sich allerdings eine abnehmende, jedoch nicht signifikante Tendenz: Der Median von 2 zur Basisuntersuchung fiel auf einen Median von 0.5 in Woche 10.

Intention-to-treat-Analyse und per-Protokoll-Analyse

Um die Effektivität der Applikation des Repellents mit der Verfügbarkeit von geschlossenen Schuhen zu vergleichen, wurde eine Intention-to-treat-Analyse am Ende der Beobachtungszeit (Woche 10) durchgeführt. Bezüglich der Befallsrate sowie der Befallsintensität war der Unterschied zwischen den Gruppen signifikant ($p = 0.001$ und $p = 0.03$). Auch der SSAT unterschied sich signifikant zwischen beiden Gruppen ($p < 0.001$). Den SSCT betreffend gab es in beiden Gruppen eine ähnlich abnehmende Tendenz, hier gab es keinen signifikanten Unterschied ($p = 0.17$).

Außerdem wurde eine per-Protokoll-Analyse für die Schuhgruppe durchgeführt. 14 Teilnehmer der Schuh-Gruppe trugen die Schuhe nie bzw. selten, 24 Teilnehmer unregelmäßig und 25 Personen regelmäßig; 14 Teilnehmer konnten nicht klassifiziert werden. Die durchschnittliche Compliance betrug 51.6% (Spannweite 0% - 98%). Aus Tabelle 1 wird ein reziprokes Verhältnis zwischen der Compliance und der Befallsintensität ersichtlich.

Die Befallsintensität sank signifikant während der Studienperiode in der Gruppe der regelmäßigen Schuhträger ($p = 0.03$ für Basisuntersuchung versus Woche 10). In der Gruppe der unregelmäßigen Schuhträger war ein Abfall der Befallsintensität ersichtlich, aber nicht signifikant ($p = 0.72$ für Basisuntersuchung versus Woche 10). Im Gegensatz dazu blieb die Befallsintensität der seltenen Schuhträger während der gesamten Studienphase unverändert. Ähnlich verhielt es sich mit der

Befallsrate: bei den regelmäßigen Schuhträgern nahm sie ab, während sie in der Gruppe der seltenen und der unregelmäßigen Schuhträger unverändert blieb.

Tabelle 1: Befallsrate und Befallsintensität in der Schuhgruppe je nach Compliance.

	Tragen der Schuhe					
	Nie/selten (n=14)	Unregelmäßig (n=24)	Regelmäßig (n=25)	Nie/selten (n=14)	Unregelmäßig (n=24)	Regelmäßig (n=25)
Zeitpunkt		**Befallsrate** [a]			**Befallsintensität** [a]	
Basis-Untersuchung	n. b.	n. b.	n. b.	24.5 (16-55)	18.5 (9.5-28)	19 (11.5-29)
Woche 2	2 (0-6)	2 (1-8)	2 (1-5.5)	23 (15-50)	13 (7.5-26)	16.5 (8-27)
Woche 4	5 (1-8)	1 (1-7)	3 (1-10)	23 (16-62)	12.5 (5-32)	11 (5-30)
Woche 6	4.5 (0-7)	3 (0-4)	1 (0-3)	30.5 (20-67)	12 (7-26)	12 (4-32)
Woche 8	5 (0-7)	2 (0-8.5)	0 (0-2)	32 (15-77)	10.5 (5.5-31)	9 (3-27)
Woche 10	2 (1-7)	3 (1-7)	0.5 (0-3)	21 (15-61)	11.5 (5.8-28.5)	9 (4-20)

[a] siehe Definition im Methodenteil, n.b. = nicht bestimmt

4.2. Therapie der Tungiasis mit Dimeticon - eine proof-of-principle Studie

Bereits drei Tage nach Applikation des Dimeticons hatten 50% der Parasiten alle Vitalitätszeichen verloren, im Gegensatz zu nur 14% in der KMnO4-Gruppe ($p < 0.001$) (Tabelle 2). Am Ende der Beobachtungszeit (Tag 7) betrug die Wirksamkeit der Dimeticon-Behandlung 78%, in der KMnO4-Gruppe war der Anteil an abgestorbenen Parasiten nur halb so hoch ($p < 0.001$).

Tabelle 2: Vitalität/ Morphologische Entwicklung nach Behandlung mit NYDA® versus KMnO4.

	nicht-vitale [a] /alle Läsionen [b] (%)/ 95% KI (%)			abnormale Entwicklung [a] /alle Läsionen [b] (%)/ 95% KI (%)		
	NYDA®	**KMnO4**	p-Wert [c]	**NYDA®**	**KMnO4**	p-Wert [c]
Basis-Untersuchung	0/ 89 (0%)	0/82 (0%)		n.b.	n.b.	
Tag 3	27/54 (50%) (36-64%)	7/50 (14%) (6-27%)	<0.001	41/54 (76%) (62-86%)	22/50 (44%) (30-59%)	<0.001
Tag 5	39/72 (54%) (42-66%)	15/58 (26%) (16-39%)	<0.001	65/72 (90%) (80-96%)	31/58 (53%) (40-66%)	<0.001

Tag 7	67/86 (78%) (67-86%)	28/71 (39%) (28-52%)	<0.001	79/86 (92%) (83-96%)	45/71 (63%) (51-74%)	<0.001

[a] siehe Definition im Methodenteil,[b] Die Gesamtzahl der untersuchten Sandflöhe variierte von Untersuchung zu Untersuchung, da nicht immer alle Patienten wie vorgesehen untersucht werden konnten,[c] Dimeticon versus KMnO4-Behandlung, n.b. = nicht bestimmt

Auch in der morphologischen Entwicklung zeigten sich deutliche Unterschiede: In der Dimeticon-Gruppe zeigten bereits nach drei Tagen 76% der penetrierten Sandflöhe eine abnormale Entwicklung, im Vergleich zu 44% in der KMnO4-Gruppe (p <0.001). Bis Tag 7 waren in der Dimeticon-Gruppe 92% der Parasiten in ihrer Entwicklung gestört, in der KMnO4-Gruppe lediglich 63 % (p <0.001).

In der Dimeticon-Gruppe fiel der Entzündungsindex von einem Median von 6 während der Basis-Untersuchung auf einen Median von 4.75 Punkten an Tag 7 (p <0.001). Im Gegensatz dazu stieg der Index in der KMnO4-Gruppe von einem Median von 4.5 auf einen Median von 5 Punkten (p = 0.009).

4.3. Optimierung der Therapie der Tungiasis mit Dimeticon

In beiden Gruppen sank bereits zwei Tage nach Behandlung mit Dimeticon die Anzahl an vitalen Sandflöhen signifikant (je p <0.001 zur Basisuntersuchung) (Tabelle 3). Nach einer Woche hatten in der Gruppe der Ganzfuß-Therapie 95% und in der Gruppe der gezielten Therapie 98% der behandelten Sandflöhe alle Vitalitätszeichen verloren. Die Differenz der Wirksamkeit von 3% zwischen beiden Therapiegruppen war nicht signifikant. In beiden Gruppen konnte bei den bis zum Ende der Beobachtungszeit vitalen Sandflöhen zu keinem Zeitpunkt ein Ablegen von Eiern beobachtet werden.

Tabelle 3: Vitalität der eingebetteten Sandflöhe nach Ganzfuß-Therapie versus gezielter Therapie.

	nicht-vitale [a] /alle Läsionen [b] (%)/ 95% KI (%)		
	Applikation auf den ganzen Fuß	**Gezielte Applikation**	p-Wert [c]
Basis-Untersuchung	0/139 (0%)	0/156 (0%)	
Tag 3	105/137 (77%) (68-83%)	132/142 (93%) (87-96%)	<0.001
Tag 5	105/120 (87%) (80-93%)	122/127 (96%) (91-99%)	0.018
Tag 7	128/134 (95%) (90-98%)	137/140 (98%) (93-99%)	0.326

[a] siehe Definition im Methodenteil, [b] Die Gesamtzahl der untersuchten Sandflöhe variierte von Untersuchung zu Untersuchung, da nicht immer alle Patienten wie vorgesehen untersucht werden konnten, [c] Ganzfuß-Therapie versus gezielte Therapie

Der Entzündungsindex sowie das Ausmaß von Juckreiz, Schmerz und einhergehende Schlaf- und Mobilitätseinschränkungen zeigten einen signifikanten Rückgang zwischen Basisuntersuchung und Tag 7 (je $p < 0.001$): Am Ende der Studie waren die anhand der visuellen Skalen gemessenen Symptome Schmerz und Juckreiz von einem anfänglichen Median von 3 Punkten sowie die Symptome Schlafstörungen und Schwierigkeiten zu Laufen von einem anfänglichen Median von 2 Punkten jeweils auf einen Median von Null zurückgegangen.

4.4. Fallbeobachtung: Unterbrechung der Entwicklung von *T. penetrans* im Wirt

Als ich den Sandfloh drei Tage nach Penetration unter dem rechten Fuß entdeckte, hatte er eine Größe von ca. 8 mm. Es bestand ein ca. 12 mm durchmessendes, schmerzloses Erythem (Abb. 3A). Innerhalb von fünf Tagen entwickelte sich der Sandfloh wie erwartet zu seiner vollen Größe von 10 mm und imponierte durch die charakteristische uhrglasförmige Konvexität (Abb. 3B-C). Das morphologische Erscheinungsbild blieb über die nächsten vier Wochen unverändert.

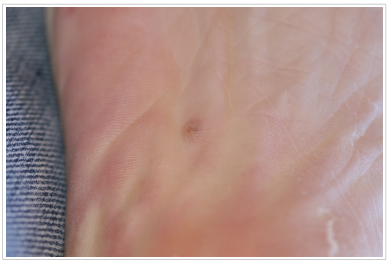
Zwei Wochen nach Penetration trat nachts zu Schlafstörungen führender Juckreiz auf. Nach vier weiteren Wochen entwickelte sich Druckschmerz und die Symptome einer lokalen Entzündung nahmen zu. Zu diesem Zeitpunkt hatte sich die Läsion dunkelbraun verfärbt und war eingesunken (Abb. 3D).

Nach zwei weiteren Wochen (in Woche 8) verstärkte sich der Juckreiz und trat auch während des Tages auf. Zudem hatte sich ein ausgeprägtes Ödem entwickelt, das zu Schmerzen beim Auftreten und zu Schwierigkeiten beim Gehen führte (Abb. 3E). Dies resultierte in der Entscheidung den Parasiten chirurgisch entfernen zu lassen (Abb. 3F).

Bis zum Ende der Beobachtungsperiode zeigte der weibliche Sandfloh Vitalitätszeichen in Form von Pulsationen und Ausscheidung von Fäzes. Allerdings wurde in der ganzen Periode kein einziges Ei expulsiert.

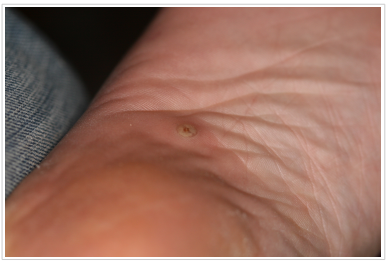
Abb. 3 A-F: Fotostrecke des einzelnen penetrierten Sandflohs.

A)



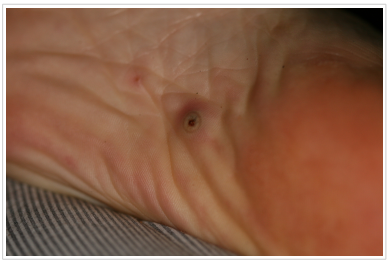
Tag 4

B)



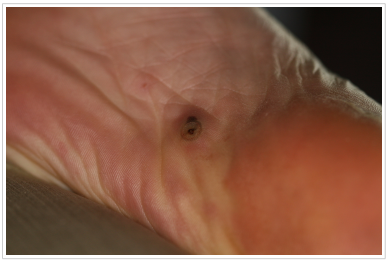
Tag 18

C)



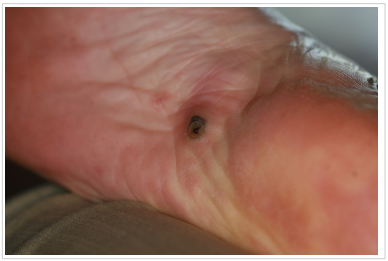
Tag 34

D)



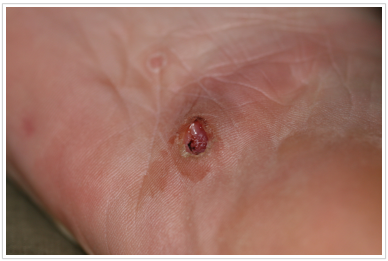
Tag 46

E)



Tag 60

F)



Tag 61, nach Extraktion

5. Diskussion

5.1. Prävention der Tungiasis durch Einsatz eines Repellents

In der Trockenzeit steigt die Inzidenz der Tungiasis rasch an und die Befallsintensität akkumuliert. Betroffene haben dann Dutzende (manchmal mehr als Hundert) penetrierter Sandflöhe mit einer entsprechend schweren klinischen Pathologie [2, 27]. Gelingt es die Re-Infektionen zu verhindern oder zumindest die Befallsintensität gering zu halten, entwickelt sich keine bzw. nur eine minimale klinische Pathologie [2, 7].

Die Ergebnisse vorausgegangener Studien in Brasilien, die einen schützenden Effekt der regelmäßigen Applikation eines Repellents auf Kokosnussölbasis (Zanzarin®) gezeigt hatten, konnten in der Studie in Madagaskar bestätigt werden: Die Wirksamkeit der zweimal-täglichen Applikation des Repellents zeigte sich in einem sofortigen Rückgang der Befallsrate auf Null, sowie einer kontinuierlich abnehmenden Befallsintensität. Die zu Studienbeginn bereits vorhandenen Sandflöhe starben bis zum Studienende in situ ab und die Residuen wurden durch Reparaturmechanismen aus der Haut eliminiert. So kam es zu einem generellen Rückgang der Entzündung und die Haut konnte heilen. Dies erklärt die nahezu vollständige Resolution der akuten mit Tungiasis-assoziierten klinischen Pathologie (SSAT) bis zum Ende der Beobachtungszeit.

Das Tragen geschlossener Schuhe als mechanische Barriere gegen *T. penetrans* wird in tropenmedizinischen Lehrbüchern als Präventionsmaßnahme empfohlen [28]. Im Falle von Individuen, die sich nur vorübergehend in einem Endemiegebiet aufhalten, ist ein schützender Effekt von geschlossenem Schuhwerk wahrscheinlich (siehe Fallbeobachtung). Die Präventionsstudie in Madagaskar zeigte jedoch, dass bei Bewohnern von Endemiegebieten die Zurverfügungstellung von Schuhen im Vergleich zur Applikation des Repellents lediglich eine marginale und verzögerte präventive Wirkung hatte (siehe Intention-to-treat-Analyse). Auffällig dabei war die niedrige Compliance: nur 39% der Teilnehmer der Schuhgruppe trugen die Schuhe regelmäßig. Es wurden

verschiedene Gründe dafür angeben und beobachtet. Zum einen waren die Dorfbewohner festes Schuhwerk nicht gewöhnt und beklagten sich über Unbequemlichkeit, vermehrtes Schwitzen und schlechten Geruch der Füße, sowie über Unpraktikabilität beispielsweise beim Bewirtschaften ihrer Felder. Zudem stellt festes Schuhwerk in den ärmlichen Endemiegebieten ein wertvolles Gut dar, das nur zu besonderen Anlässen wie Festen oder Kirchgängen getragen wird. Zum anderen gaben Individuen mit vielen Sandflohläsionen Schmerzen beim Laufen in geschlossenen Schuhen an. Tatsächlich hatten die Studienteilnehmer mit der schlechtesten Compliance die höchste Befallsintensität.

Auch vom Gesichtspunkt der Nachhaltigkeit sind Schuhe nicht sehr sinnvoll. Durch Tragen auf rauen Wegen und Straßen (Abb. 1B) würden schon nach einigen Monaten Löcher und Risse in den Schuhen entstehen. Hingegen ist ein Repellent auf Kokosnussölbasis als Präventionsmaßnahme insofern nachhaltig, als dass Kokosnussöl in nahezu allen Endemiegebieten kostengünstig hergestellt werden kann.

5.2. Therapie der Tungiasis mit Dimeticon

In Anbetracht der hohen Prävalenz von Tungiasis und den gravierenden Gesundheitsproblemen, die mit Tungiasis und den traditionellen Behandlungsmethoden einhergehen, ist die Bereitstellung einer sicheren Therapie dringend erforderlich.

Als Ziel für einen neuen Therapieansatz zogen wir die winzige ca. 250 µm durchmessende Öffnung im letzten Abdominalsegment des eingebetteten Sandflohs in Betracht. In Analogie zu der Wirkung des Dimeticons bei *Pediculus humanus capitis*, postulieren wir, dass NYDA® in die Atemwege des Parasiten eindringt und die Atemluft verdrängt. Die Todesursache wäre dann eine akute Asphyxie. Da das Wirkprinzip rein physikalisch ist, ist eine Resistenzentwicklung des Parasiten quasi ausgeschlossen [25, 29].

In der proof-of-prinicple Studie zeigten sieben Tage nach der Behandlung mit NYDA® 78% der Sandflohläsionen keine Vitalitätszeichen mehr. Zudem war der Entzündungsindex signifikant gesunken, was den schnellen Tod der eingebetteten Parasiten widerspiegelt. Vorausgegangene Studien konnten zeigen, dass die Tungiasis-assoziierte Entzündung sistiert bzw. zurückgeht, sobald der Parasit abgestorben ist [30].

Nachdem sich Dimeticon als wirksames Therapeutikum erwiesen hatte, konnten wir in der Optimierungsstudie zeigen, dass eine gezielte Applikation des Dimeticons auf den Abdominalkonus penetrierte Sandflöhe schneller abtötete: bereits zwei Tage nach der Behandlung waren nur noch 7%

der Parasiten vital. Es ist anzunehmen, dass durch eine gezielte Applikation mehr Dimeticon pro Zeiteinheit in den Abdominalkonus eindringen kann, als wenn die gesamte Haut mit Dimeticon benetzt wird. Ein schnellerer Tod von penetrierten Sandflöhen hat den Vorteil, dass die mit Tungiasis-assoziierte Entzündungsreaktion schneller zurückgeht. Der rasche Rückgang der Entzündungsreaktion nach Ableben der penetrierten Sandflöhe konnte in dieser Studie bestätigt werden.

Die Effektivität der Ganzfuß-Therapie war in der Optimierungsstudie höher als in der proof-of principle Studie (95% versus 78%). Je dicker und uneben die Hornhaut, desto länger benötigt Dimeticon um die Epidermis zu erreichen, in die der Parasit eingebettet ist und desto mehr Dimeticon wird dafür benötigt. Während die kenianischen Kinder keinerlei Schuhwerk besaßen, trugen die Kinder in Uganda zum Teil Sandalen oder Flipflops, wodurch ihre Füße vermutlich wenig hyperkeratotisch waren. Außerdem waren in der proof-of-principle Studie mitunter für das Dimeticon schwer zugängliche Läsionen eingeschlossen (unterhalb von Zehennägeln und Hornhautkrusten liegend), wohingegen für die Optimierungsstudie nur Läsionen ausgewählt wurden, die leicht für das digitale Video-Mikroskop zugänglich waren und auf die das Dimeticon gezielt appliziert werden konnte.

Errechnet aus der durchschnittlichen Menge an benötigtem Dimeticon pro Therapiegruppe erwies sich die gezielte Applikation von Dimeticon nicht nur als schneller wirksam, sondern auch als sparsamer, solange die Anzahl der vitalen Sandflöhe nicht sieben pro Fuß überschreitet. Bei schweren Fällen von Tungiasis ist die Ganzfuß-Behandlung kostengünstiger und praktischer in der Durchführung.

5.3. Unterbrechung der Entwicklung von *T. penetrans* im Wirt

Das einzelne Sandflohweibchen entwickelte sich in zweierlei Hinsicht untypisch. Erstens hatte es eine Lebensdauer von über acht Wochen, anstatt von üblichen vier bis sechs Wochen. Zweitens expulsierte es während der gesamten Beobachtungsperiode kein einziges Ei, obwohl nach morphologischen Aspekten die Verdauungsorgane sowie der Reproduktionstrakt hypertrophierten (Abb. 3B-C). Beide Auffälligkeiten sprechen für die Hypothese, dass die Kopulation nach der Penetration des Weibchens in die Epidermis stattfindet und dass eine Befruchtung notwendig ist, damit sich ein Sandflohweibchen normal im Wirt entwickelt.

Ein weiterer Befall von Sandflöhen wurde durch permanentes Tragen von Socken und geschlossenen Schuhen unterbunden. Dadurch konnten weder weitere Sandflohweibchen penetrieren noch

männliche Sandflöhe die Haut nach Weibchen explorieren und diese begatten. In Folge dessen blieben die bereits herangereiften Eier des penetrierten Sandflohs vermutlich unbefruchtet. Die außergewöhnlich lange Vitalität des eingebetteten Sandflohs lässt sich durch einen physiologischen Mechanismus der Art "Warten auf die Kopulation" erklären. Nachdem diese ausblieb, entwickelten sich die Eier schließlich zurück - die uhrglasförmige Konvexität verschwand.

Zwei weitere Argumente sprechen für die Hypothese, dass die Kopulation von *T. penetrans* in situ stattfindet: Erstens wird so sichergestellt, dass sich das Weibchen bereits am idealen Ort für die Entwicklung der Eier befindet und kein Sperma verschwendet wird. Zweitens liegen häufig Dutzende weibliche Sandflöhe dicht beieinander in Clustern, sodass männliche Sandflöhe, die die Haut nach penetrierten Weibchen sondieren, innerhalb kurzer Zeit mit mehreren weiblichen Sandflöhen kopulieren können.

5.4. Schlussfolgerungen

1. Die tägliche topische Applikation eines Repellents auf Kokosnussölbasis (Zanzarin®) ist eine wirksame Methode der Prävention der Tungiasis im Endemiegebiet. Die Zurverfügungstellung von geschlossenen Schuhen hatte durch eine niedrige Compliance der Studienteilnehmer einen vergleichsweise geringen präventiven Effekt.
2. Die topische Applikation eines zwei-Komponenten-Dimeticons (NYDA®) hat sich in zwei verschiedenen Studien als wirksame Therapie gegen Tungiasis erwiesen. Eine gezielte Applikation auf den Abdominalkonus des penetrierten Sandflohs wirkt schneller und spart Dimeticon, insofern der Patient nicht mehr als sieben vitale Parasiten pro Fuß hat. Die Behandlung ist unschädlich und kann vom Patienten selbst durchgeführt werden.
3. Die ungewöhnliche Entwicklung eines einzelnen Sandflohweibchens nach akzidenteller Infektion zeigt, dass bei *T. penetrans* die Kopulation nach der Penetration des Weibchens in die Epidermis des Wirts stattfindet.

Die effektiven Präventions- und Therapiemaßnahmen gegen Tungiasis ersetzen die mit vielen Risiken behafteten traditionellen Methoden von Extraktion bzw. Manipulation mit ungeeigneten Instrumenten. Außerdem können sie - langfristig und auf Bevölkerungsebene durchgeführt - die Transmission von *T. penetrans* zumindest teilweise unterbrechen.

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Eidesstattliche Versicherung

„Ich, Marlene Thielecke, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Untersuchungen zur Klinik, Therapie und Prävention der Tungiasis (Sandflohkrankheit) in Madagaskar, Kenia und Uganda“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Betreuer, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Anteilserklärung

Marlene Thielecke hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Thielecke M, Raharimanga V, Rogier C, Stauss-Grabo M, Richard V, Feldmeier H. Prevention of tungiasis and tungiasis-associated morbidity using the plant-based repellent Zanzarin: A randomized, controlled field study in rural Madagascar. PloS Neglected Tropical Diseases. 2013; 7(9): e2462. doi:10.1371/journal.pntd.0002426
60 Prozent
Beitrag im Einzelnen: Beteiligung an der Planung der Studie, Durchführung der Studie in Madagaskar, Erhebung der klinischen Daten, teilweise Datenanalyse, Erstellung von Grafiken und Tabellen, Verfassen des Textes

Publikation 2: Thielecke M, Nordin P, Ngomi N, Feldmeier H. Treatment of tungiasis with dimeticone: A proof-of-principle study in rural Kenya. PloS Neglected Tropical Diseases. 2014; 8(7): e3058. doi:10.1371/journal.pntd.0003058
75 Prozent
Beitrag im Einzelnen: Beteiligung an der Planung der Studie, Durchführung der Studie in Kenia, Erhebung der klinischen Daten, Fotodokumentation und Videomikroskopie, Dateneingabe und -analyse, Erstellung von Grafiken und Tabellen, Verfassen des Textes

Publikation 3: Nordin P, Thielecke M, Ngomi N, Mukone Mudanga G, Krantz I, Feldmeier H. Treatment of tungiasis with a two-component dimeticone: a comparison between moistening the whole foot and directly targeting the embedded sand fleas. Tropical Medicine and Health. 2017; 45(6): doi:10.1186/s41182-017-0046-9
30 Prozent
Beitrag im Einzelnen: Beteiligung an der Planung der Studie, Dateneingabe, Korrektur des Textes

Publikation 4: Thielecke M, Feldmeier H. The fate of the embedded virgin sand flea *Tunga penetrans:* Hypothesis, self-experimentation and photographic sequence. Travel Medicine and Infectious Disease. 2013; 11(6): 440-443.
90 Prozent

Beitrag im Einzelnen: Mitbeteiligung an der Planung der Studie, Durchführung der Studie, Erhebung klinischer Daten, Fotodokumentation und Videomikroskopie, Erstellung von Tabellen, Verfassen des Textes

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Komplette Publikationsliste

Wissenschaftliche Artikel

1) Thielecke M, Raharimanga V, Rogier C, Stauss-Grabo M, Richard V, Feldmeier H (2013) Prevention of tungiasis and tungiasis-associated morbidity using the plant-based repellent Zanzarin: A randomized, controlled field study in rural Madagascar. PloS Neglected Tropical Diseases 7(9): e2462. doi:10.1371/journal.pntd.0002426

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2) Thielecke M, Raharimanga V, Stauss-Grabo M, Rogier C, Richard V, Feldmeier H (2013) Regression of severe tungiasis-associated morbidity after prevention of re-infestation: A case series from rural Madagascar. American Journal of Tropical Medicine and Hygiene 89(5): 932-936.

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3) Thielecke M, Feldmeier H (2013) The fate of the embedded virgin sand flea *Tunga penetrans:* Hypothesis, self-experimentation and photographic sequence. Travel Medicine and Infectious Disease, 11(6): 440-443.

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PLOS | NEGLECTED TROPICAL DISEASES

Prevention of Tungiasis and Tungiasis-Associated Morbidity Using the Plant-Based Repellent Zanzarin: A Randomized, Controlled Field Study in Rural Madagascar

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Abstract

Background: Tungiasis, a parasitic skin disease caused by the female sand flea *Tunga penetrans*, is a prevalent condition in impoverished communities in the tropics. In this setting, the ectoparasitosis is associated with important morbidity. It causes disfigurement and mutilation of the feet. Feasible and effective treatment is not available. So far prevention is the only means to control tungiasis-associated morbidity.

Methodology: In two villages in Central Madagascar, we assessed the efficacy of the availability of closed shoes and the twice-daily application of a plant-based repellent active against sand fleas (Zanzarin) in comparison to a control group without intervention. The study population was randomized into three groups: shoe group, repellent group and control group and monitored for ten weeks. The intensity of infestation, the attack rate and the severity of tungiasis-associated morbidity were assessed every two weeks.

Findings: In the repellent group, the median attack rate became zero already after two weeks. The intensity of the infestation decreased constantly during the observation period and tungiasis-associated morbidity was lowered to an insignificant level. In the shoe group, only a marginal decrease in the intensity of infestation and in the attack rate was observed. At week 10, the intensity of infestation, the attack rate and the severity score for acute tungiasis remained significantly higher in the shoe group than in the repellent group. Per protocol analysis showed that the protective effect of shoes was closely related to the regularity with which shoes were worn.

Conclusions: Although shoes were requested by the villagers and wearing shoes was encouraged by the investigators at the beginning of the study, the availability of shoes only marginally influenced the attack rate of female sand fleas. The twice-daily application of a plant-based repellent active against sand fleas reduced the attack to zero and lowered tungiasis-associated morbidity to an insignificant level.

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Introduction

Tungiasis is a neglected tropical disease, which is widespread in South America, the Caribbean and sub-Saharan Africa [1,2]. It is a zoonosis with various domestic and sylvatic animals acting as reservoirs [3]. Tungiasis is associated with poverty and mainly affects marginalized populations living in urban squatter settlements, in villages in the rural hinterland or in traditional fishing communities along coastal areas [4–9]. Crude prevalences up to 50 percent are not uncommon in endemic areas [6–12]. Tungiasis is acquired when walking barefoot on soil, in which off-host stages of *T. penetrans* have propagated. 99% of all penetrated sand fleas are located at the feet [10,13] Tungiasis may be acquired peri-domiciliary and inside houses [14].

In resource-poor settings tungiasis is associated with important and debilitating morbidity, such as intense inflammation of toes

Author Summary

Tungiasis (sand flea disease) is a parasitic skin disease present in many resource-poor communities in South America, the Caribbean and sub-Saharan Africa. In this setting tungiasis is associated with important morbidity. Hitherto, the only effective treatment is the surgical extraction of embedded sand fleas. In the endemic areas this is done using inappropriate sharp instruments and causes more harm than good. The prevention of the infestation is the only option to control morbidity. In this study we show that the twice daily application of a herbal repellent based on coconut-oil (Zanzarin), is highly effective in preventing sand flea disease in a heavily affected community in Madagascar. The attack rate became zero immediately after starting the application of the repellent. The degree of tungiasis associated morbidity approached zero within 10 weeks. In contrast, the availability of closed solid shoes had only a marginal protective effect; although shoes were requested by the villagers and wearing shoes was encouraged by the investigators at the beginning of the study. In a control group from the same village the attack rate, the intensity of infestation and of tungiasis-associated morbidity remained unchanged. Our study in rural Madagascar shows that effective und sustainable morbidity control is possible using a repellent derived from coconut oil.

and heels, formation of painful fissures and ulcers, and deformation and loss of nails [7,15]. Predilection sites are the toes, the heel and the sole. A common finding are walking difficulties [15,16]. Suppuration, lymphangitis and gangrene reflect the almost constant bacterial superinfection of sand flea lesions [17–19]. Tetanus is a life threatening sequel of tungiasis [20,21]. In areas where tungiasis is common and people are not or only insufficiently vaccinated against tetanus, embedded sand fleas are considered as the port of entry for *Clostridium tetani* [22].

Epidemiological studies suggest that individuals with a high parasite burden are most prone to develop severe disease sequelae [16,23]. In endemic areas, these are children five to 14 years of age and the elderly [7,16]. Tungiasis does not induce a protective immunity.

Hitherto, there is no effective chemotherapy available and embedded parasites need to be extracted surgically [2,24]. This requires a skilled hand and good eyesight. In resource-poor communities, surgical removal is inconsistently performed and usually causes more harm than good, because inappropriate instruments such as pins, needles or thorns are used [16]. Since it is virtually impossible to eliminate tungiasis as long as the precarious living conditions characteristic for the endemic areas exist, and control of animal reservoirs is currently not feasible, prevention of infestation so far remains the only option.

We have previously shown that the regular application of Zanzarin, a repellent based on coconut oil, reduced the attack rate of invading sand fleas by 92%, and almost completely resolved tungiasis-associated morbidity in resource-poor settings in Northeast Brazil [25,26]. In this study we compared the protective effect of a twice daily application of the repellent with the availability of closed shoes in a rural area in Madagascar, where the crude prevalence of tungiasis varied between 30 to 69% [6]. The decision of comparing the protective efficacy of the repellent to the protective efficacy of shoes was the result of extensive discussions with the villagers who voted for shoes as the second type of intervention when the study was planned. The decision of comparing the protective efficacy of shoes was based on extensive discussions with the villagers, who voted to receive shoes as the second type of intervention.

Materials and Methods

Ethics statements

The study was approved by the Ethical Committee of the Ministry of Health (MINSANP/CE ref.-nr. 051) and was registered at Controlled-trials.com (ISRCTN 11415557). The study was conducted according to the principles expressed in the Declaration of Helsinki. Informed written consent was obtained from all participants in Malagasy and in the case of minors from the parents or legal guardians. At the end of the study, all participants received a pair of closed solid shoes. In each participant, any remaining viable sand fleas were removed under sterile conditions. The flow chart of the study is depicted in Figure 1.

Study area

The study was conducted in the villages Tanambe II (507 inhabitants) and Tanambaovao (486 inhabitants) located in Andasibe community, Moramanga district, central Madagascar. The villages are traditional communities, where people live from subsistence farming. Similar to other communities in the hinterland of Madagascar, tungiasis is common. Local people consider sand fleas disease to be inevitable and remember that it always existed.

In the villages roads are not paved. The houses are made from wood and many are constructed on stilts (Figure 2A, 2B). The houses possess a front court used for cooking, washing and drying clothes. Chicken, pigs, dogs and cats are kept as domestic animals and live inside the compound. Water for cooking is derived from public pumps, water for cleaning the body and washing clothes is taken from a river close to the villages. Rice, cassava and pineapples are the main crops. Children usually do not possess shoes. Adolescents and adults have shoes, but do not wear them regularly. Most sand flea lesions are manipulated. Extraction of embedded sand fleas is attempted as soon as the lesions become painful. This is done using non-sterile instruments, such as fixing pins or needles. Neither the instrument nor the skin is disinfected. Often, the extraction is performed by elder members of the household and not by the patient himself (M. Thielecke unpublished observation 2011).

In Madagascar, the occurrence of tungiasis follows a characteristic seasonal pattern: the incidence starts to rise with the beginning of the hot and dry season (May), peaks around September and starts to decline with the beginning of the rainy season (November).

Study design

A randomized controlled field study with three arms was carried out between May 31, 2011 (baseline investigation) and August 24, 2011 (final follow up). This period was chosen to coincide with the beginning of the dry season and the high transmission of *T. penetrans*. The study was conceived as a comparison of individuals who were provided with and encouraged to wear closed shoes and those who got a twice-daily application of the repellent to untreated individuals.

Individuals with tungiasis were identified with the assistance of community health workers. Inclusion criteria were: age $\geq$5 years and at least one person in the household with $\geq$7 lesions at both feet. The latter prerequisite was based on the rationale that, in this case, the average attack rate in household members should be high. Exclusion criteria were the presence of $\geq$70 lesions (total

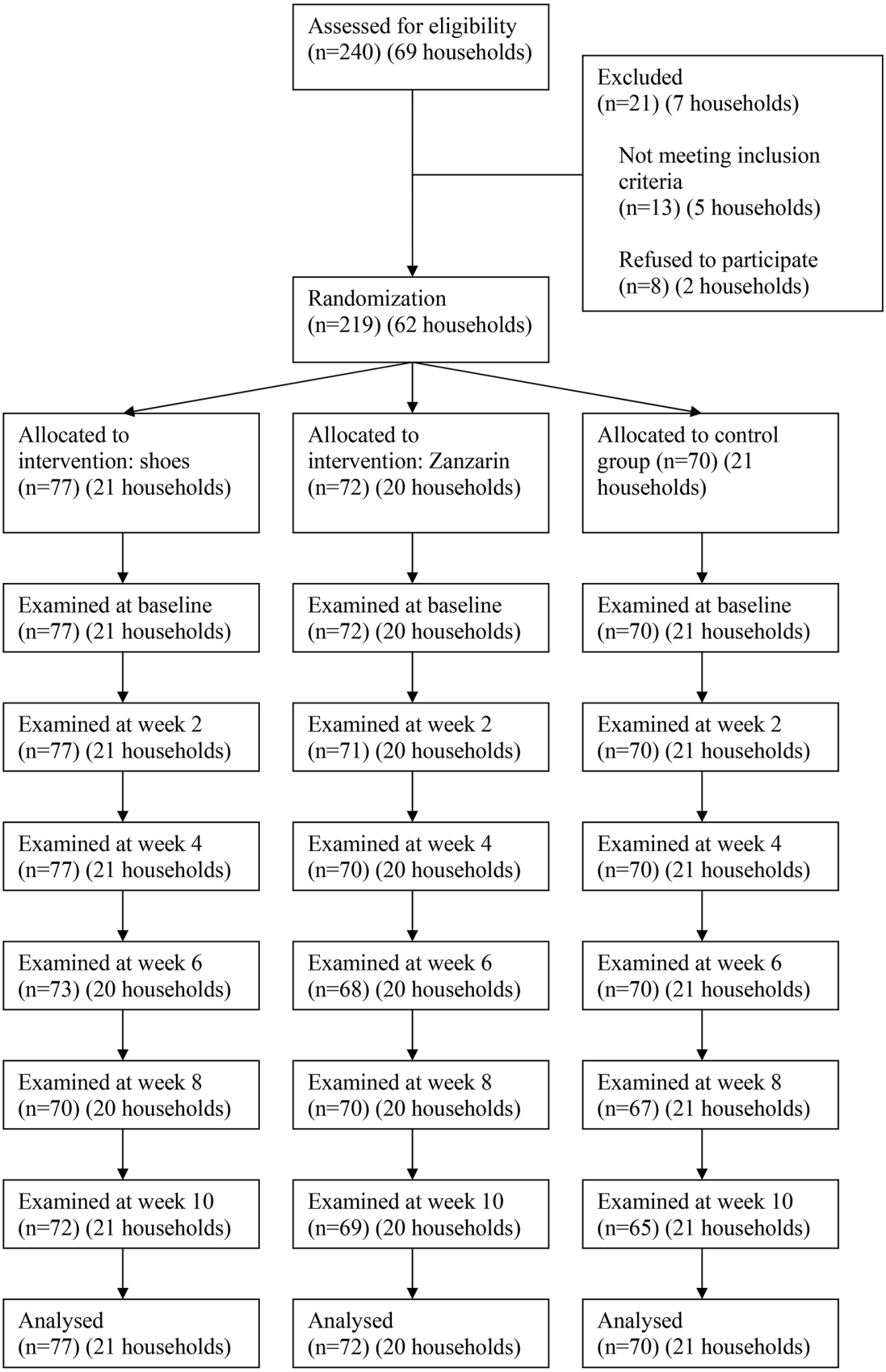

Figure 1. Flow diagram of the study.
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A

B

Figure 2. View of Tanambe II village. Houses are constructed from wood and are built on stilts (A). Roads and paths are not paved (B).
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Table 1. Demographic and clinical characteristic of the study population.

Characteristic	Control group (n = 70)	Shoe group (n = 77)	Repellent group (n = 72)
Age in years, median (range)	25.5 (5–73)	26.7 (5–93)	26.8 (5–80)
Male-female ratio (%)	44/56	39/61	40/60
Number of household members, median (range)	3 (1–6)	3 (1–7)	4 (1–6)
Number of household members with tungiasis, median (range)	3 (1–6)	3 (1–7)	3.5 (1–6)
Intensity of infestation in household members[a], median (range)	10.5 (2.3–33.3)	9.8 (2.3–40.3)	10.2 (1.7–27.3)
Intensity of infestation in participants[b], median (range)	22 (1–99)	22 (1–179)	16 (1–91)
Manipulated lesions[c], median (range)	83.2 (0–100)	88.8 (0–100)	85.4 (0–100)
SSAT[d], median (range)	4 (0–17)	3 (0–19)	3 (0–20)
SSCT[e], median (range)	1 (0–10)	2 (0–8.5)	2 (0–11.5)

[a]total number of viable, dead and manipulated lesions; only individuals with tungiasis.
[b]total number of viable, dead and manipulated lesions.
[c]the patient or a caretaker had attempted to take out the embedded sand flea; in percent of total lesions.
[d]severity score for acute tungiasis (see material and methods).
[e]severity score for chronic tungiasis (see material and methods).
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number of viable or manipulated sand flea lesions), or the presence of severe acute morbidity necessitating immediate surgical or antibiotic treatment. Eligible individuals were only enrolled if they were permanent residents in the villages, intended to stay in the community for the next four months and provided an informed written consent. In total, 240 participants were recruited and 219 were randomized.

The major outcome measures were the intensity of infestation, i.e. the number of sand flea lesions present on both feet at the time of examination, the number of newly penetrated sand fleas since the last examination (considered as a proxy of the attack rate [25,27]), and the severity of acute and chronic pathology as measured by the severity score for acute tungiasis (SSAT) and the severity score for chronic tungiasis (SSCT) [28]. Outcome measures were assessed every two weeks by the same investigator (M. T. and V. R.). No participant was missing on more than one consecutive follow-up examination.

To avoid that members of one and the same household would participate in different treatment arms, we randomized by household. Randomization into three groups was performed using a computer-generated random number table. Participants of group I received a pair of closed solid shoes. To the feet of the participants of group II the repellent was applied twice daily. Group III did not receive any intervention. 21 households belonged to the shoe group, 20 to the repellent group and 21 households to the control group. Households with study-participants were homogenously distributed across the village. The median number of household members was 3 (interquartile range [IQR] 2–5) in the shoe, 4 (IQR 2–5) in the repellent and 3 (IQR 2–5) in the control group.

Shoe group. A pair of shoes was given to all members of this cohort by the research team. Before the donation of shoes, key informants of the village explained to the participants why shoes should be worn and encouraged them to wear the donated shoes regularly. They were advised not to share the shoes with other members of the household or to sell them. Shoes were closed, solid and fitted to the size of the feet. To avoid envy and mental strain all shoes were from the same type. To avoid envy and mental strain all shoes were from the same type.

Health workers who knew each participant observed the compliance of members of the shoe group by casually walking through the villages and taking notes whether a member of the shoe group wore the donated shoes or not. Compliance was assessed daily between week 2 and week 10. Individuals seen with shoes at $<30\%$ of the inspections were considered to wear protective footgear only seldom. Those who were seen with shoes at 30–60% of the occasions were considered to wear footgear irregularly. Wearing shoes at $>60\%$ of the inspections was classified as regular users.

Repellent group. Zanzarin (Engelhard Arzneimittel GmbH & Co. KG, Niederdorfelden, Germany) contains coconut oil (*Cocos nucifera*), jojoba oil (*Simmondsia chinesis*) and *Aloe vera*. The lotion is sold as a biocide with repellent activities against ticks and biting insects. In the morning (5:30–7:30 a.m.) and in the evening (5:00–7:00 p.m.), the repellent was applied by trained community health workers on the skin of both feet, up to the ankle including the interdigital areas, after washing the feet with water in a bowl. The average volume applied was 3 ml for 2 feet per person and day. The minimal volume was 2 ml (for children) and the maximal volume was 5 ml (for large adult feet). The procedure of applying the repellent was identical in children and adults. The application of the repellent was checked regularly by members of the team and had to be confirmed by the participant on a documentation sheet either by signing or by fingerprint. This ensured that the repellent was applied exactly as defined in the study protocol, not spilled or given away for money. The participants were asked not to wash their feet for at least two hours after the application of the repellent but they were allowed to take a shower whenever they wanted. Whether shoes already existing in a household were worn or not by the participants of the repellent group was not assessed.

Control group. The control group had no access to the repellent and did not receive shoes. Whether shoes already existing in the households were worn or not by the participants of the control group was not assessed.

Definitions and documentation of lesions

The intensity of infestation, the attack rate and the degree of tungiasis-associated morbidity were assessed as described previously [25,28]. Staging of lesions was performed according to the Fortaleza Classification [29]. The following findings were considered diagnostic for tungiasis:

- Flea in *statu penetrandi* (stage I)

Table 2. Parasitological and clinical characteristics of study groups at baseline and during intervention.

Point of time/group	Outcome measure							
	Intensity of infestation[a]	P-value[b]	Attack rate[c]	P-value[b]	SSAT[d)]	P-value[b]	SSCT[e]	P-value[b]
Baseline								
Control group (n = 70)	22 (13–33)		n.a.		4 (3–7)		1 (0–2)	
Shoe group (n = 77)	22 (10–35)	0.58	n.a.		3 (2–7)	0.63	2 (0–3.5)	0.18
Repellent group (n = 72)	16 (5–31.5)	0.36	n.a.		3 (1–7)	0.50	2 (0–3)	0.15
Week 2								
Control group (n = 70)	21.5 (12–35)		4 (1–8)		4 (2–7)		1 (0–2)	
Shoe group (n = 77)	18 (9–34)	0.31	3 (1–6)	0.21	4 (2–6)	0.72	1 (0–3)	0.36
Repellent group (n = 71)	10 (3–27)	0.06	0 (0–1)	**< 0.001**	2 (1–4)	**< 0.001**	1 (0–2.5)	0.35
Week 4								
Control group (n = 70)	19 (11–32)		5 (1–9)		3 (2–7)		1 (0–2)	
Shoe group (n = 77)	17 (8–35)	0.34	3 (1–9)	0.62	3 (2–6)	0.21	1 (0–3.5)	0.52
Repellent group (70)	7.5 (2–21)	**0.02**	0 (0–1)	**< 0.001**	1 (1–3)	**< 0.001**	1 (0–2.5)	0.79
Week 6								
Control group (n = 70)	18 (12–36)		3.5 (1–9)		4 (2–7)		1 (0–2.5)	
Shoe group (n = 73)	17 (7–36)	0.67	2 (0–7)	0.31	3 (2–6)	0.05	1 (0.2.5)	0.32
Repellent group (n = 68)	6 (1.5–18)	**0.004**	0 (0–0)	**< 0.001**	1 (0–2)	**< 0.001**	1 (0–2.3)	0.64
Week 8								
Control group (n = 67)	18 (11–40)		6 (1–11)		4 (2–9)		1 (0–2.5)	
Shoe group (n = 70)	15.5 (5–41)	0.83	1.5 (0–6)	0.27	2 (1–5)	0.14	1 (0–3)	0.60
Repellent group (n = 70)	4.5 (1–17)	**< 0.002**	0 (0–1)	**< 0.001**	1 (0–2)	**< 0.001**	0.8 (0–1.5)	0.37
Week 10								
Control group (n = 65)	19 (10–35)		4 (2–8)		4 (2–6)		1 (0–2.5)	
Shoe group (n = 72)	15 (6–36)	0.85	2 (0–4.5)	**0.049**	3 (1–4)	0.11	1 (0–3)	0.43
Repellent group (n = 69)	3 (1–13)	**<0.001**	0 (0–0)	**<0.001**	1 (0–1)	**<0.001**	0.5 (0–2)	0.36

Data indicate the median and the interquartile range (IQR).
n. a. = not applicable.
[a]total number of viable, dead and manipulated lesions.
[b]shoe group versus control group, and repellent group versus control group, respectively.
[c]number of newly penetrated sand fleas since last examination.
[d]severity score for acute tungiasis.
[e]severity score for chronic tungiasis.
doi:10.1371/journal.pntd.0002426.t002

- A dark and itching spot in the epidermis with a diameter of 1 to 2 mm, with or without local pain and itching (early lesion, stage II)
- Lesions presenting as a white halo with a diameter of 3 to 10 mm with a central black dot (mature egg producing flea, stage III)
- A brownish–black circular crust with or without surrounding necrosis of the epidermis (dead parasite, stage IV).

At each examination the number of viable (stage I to III) and dead (stage IV) fleas, the total number of embedded fleas (= intensity of infestation), and the number of parasites having penetrated since the previous examination (= attack rate) were documented. Manipulated lesions (such as partially or totally removed parasites leaving a characteristic crater-like sore in the skin), and suppurative lesions caused by the use of non-sterile perforating instruments were noted as well. At each examination data of the two feet were combined.

The exact topographic localization of each lesion, its stage, and appearance were documented on a visual record sheet and lesions were photographed, using a digital camera equipped with a macro objective (EOS 450 D, Canon, Tokyo, Japan).

Clinical pathology was assessed in a semi-quantitative manner using the severity score for acute tungiasis (SSAT), and the severity score for chronic tungiasis (SSCT) [28]. The SSAT score comprises the following signs and symptoms: erythema, edema, pain upon pressure or spontaneously, itching, sleep disturbance due to itching or pain, difficulty walking as indicated by an altered gait; abscess, and suppuration as indicators of superinfection; fissures, perilesional desquamation and ulcers as characteristic chronic skin defects. The score can take a value from 0–35 points. The SSCT ranges from 0 to 30 points and comprises the presence of nail deformation, nail loss, deformation of toes, hypertrophic nail rim, and hyperkeratosis; all of those characteristics are indicators of repeated episodes of tungiasis experienced in the past [28].

Statistical considerations

Since the distributions of the outcome measures were skewed, the median and the interquartile ranges were used to indicate the

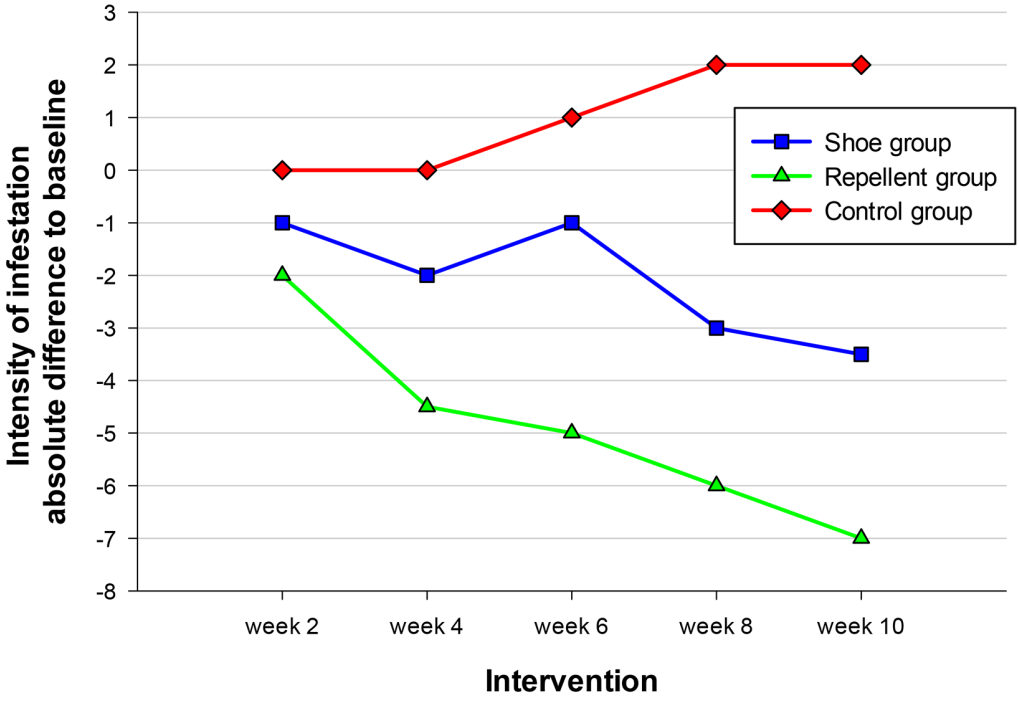

Figure 3. Total number of sand flea lesions (viable, dead and manipulated lesions during the intervention phase. Data indicate medians.
doi:10.1371/journal.pntd.0002426.g003

central tendency and dispersion of data, respectively. Since the randomization was based on households, outcome measures in participants were considered to be correlated (i. e. not to be independent). To compare measurements between groups the method of generalized estimating equations (GEE) was used [30]. GEEs are appropriate to analyze longitudinal and other correlated data, especially when they are in the form of counts [30]. In all GEE models the baseline measure of the dependent variable was

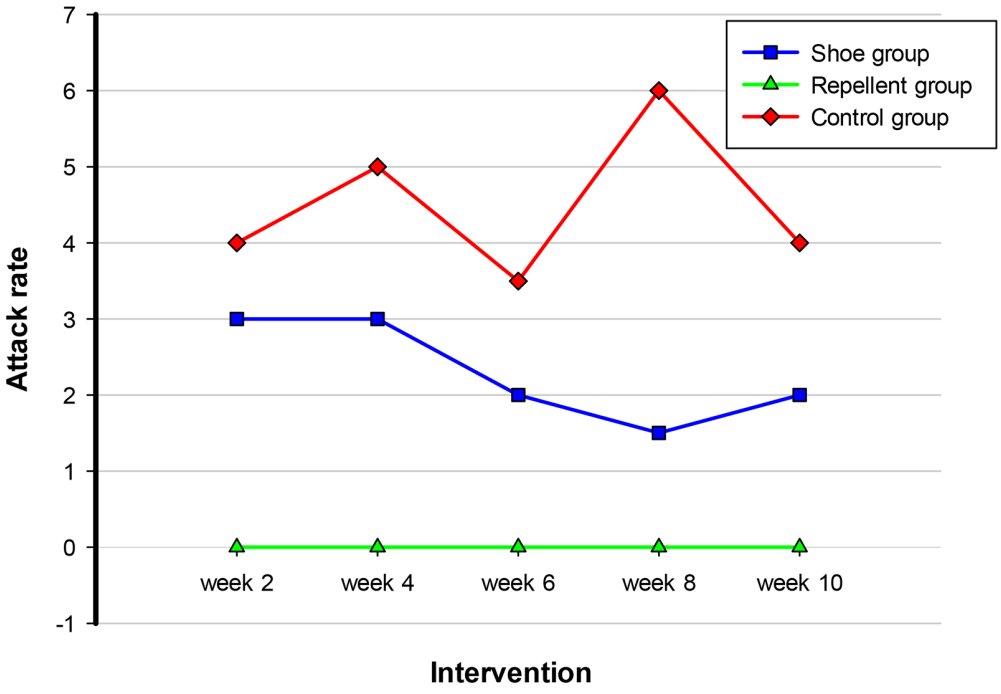

Figure 4. Number of newly penetrated sand fleas during the intervention phase. Data indicate medians.
doi:10.1371/journal.pntd.0002426.g004

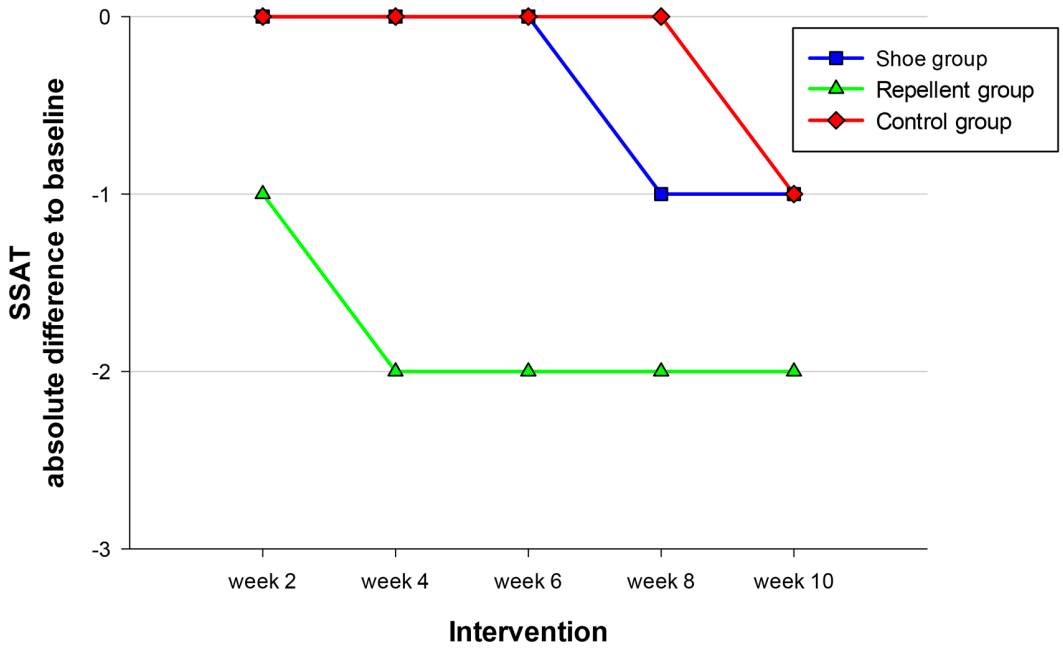

Figure 5. Dynamics of the Severity Score for Acute Tungiasis (SSAT) during the intervention phase. Data indicate medians.
doi:10.1371/journal.pntd.0002426.g005

used as a covariate. Intention-to treat and per protocol data analyses were performed.

Assuming that the application of the repellent and the availability of shoes would have a similar protective effect, 75 participants were needed in each of the three groups to detect a difference of 50% in the intensity of infection between an intervention group and the control group ($\alpha = 5\%$; power of the test = 86%). A dropout rate of 25% until the end of the study was included in the calculation.

Results

Baseline characteristics

The demographic and clinical characteristics of the study population at baseline are shown in Table 1. There were more female participants in each group than males. This was due to the fact that many of the male inhabitants were not eligible for the study as they worked in town during the week or were absent >8 hours, so that they could not be examined. The median intensity of infestation was identical in the shoe and the control group (22 lesions), but slightly lower in the repellent group (16 lesions).

Major outcome measures

Table 2 depicts the time course of the four major outcome measures. Onward from week 2, the attack rate became zero and the intensity of infestation and the SSAT decreased significantly in the repellent group, whereas no significant change occurred in the control group. In the shoe group, there was a tendency of decrease in the intensity of infestation and of the SSAT over time.

In order to avoid bias in the comparisons of the follow-up data between the groups, the absolute difference to baseline was calculated for the intensity of infestation, the SSAT and the SSCT. Figure 3 shows the time course of the intensity of infestation. In the repellent group the decrease was already significant at week 4 ($p = 0.02$ compared to the control group). In contrast, at no point of time the difference between the shoe cohort and the control cohort was significant.

The time course of the attack rate in the three cohorts is depicted in Figure 4. The median attack rate in the repellent group became zero already in week 2 and remained so until the end of the study. In contrast, in the shoe cohort the median attack rate started to decrease slightly in week 6. Only in week 10, it was significantly lower as compared to the control cohort ($p = 0.049$). In the control group, the attack rate varied over time.

The time course of SSAT is shown in Figure 5. At baseline, this indicator of acute tungiasis-associated morbidity was rather low in the three cohorts: median 4 (IQR 3–7), 3 (IQR 2–7) and 3 (IQR 1–7) in the control, the shoe and the repellent cohort, respectively (Table 2). Whereas in the repellent cohort the absolute difference to the baseline value became already significant in week 2 ($p<0.001$ compared to the control group), in the shoe cohort the absolute difference of the SSAT to the baseline value remained insignificant during all follow ups.

In all cohorts, the SSCT for chronic tungiasis was very low: median 1 (IQR 0–2), 2 (IQR 0–3.5) and 2 (IQR 0–3) in the control, the shoe and the repellent cohort, respectively (Table 2). In the repellent and the shoe cohort, a slight reduction became obvious with time. However, these differences were not significant.

To compare the efficacy of the application of the repellent with the protective effect of the availability of closed shoes, we compared the four major outcome measures at week 10. Whereas in the repellent group the intensity of infestation had decreased to a median of 3 (IQR 1–13), in the shoe group this outcome measure remained high: median 15 (IQR 6–36; $p = 0.001$). The attack rate was zero in the repellent group (IQR 0-0), and 2 in the shoe group (IQR 0–4.5; $p = 0.03$). The SSAT showed a similar tendency: median 1 (IQR 0–1) versus median 3 (IQR 1–4; $p<0.0001$). No significant difference between the repellent and the shoe group existed with regard to the SSCT ($p = 0.17$).

Table 3. The intensity of infestation and the attack rate in the shoe group stratified according to compliance.

	Wearing shoes					
	never/rarely (n = 14)	irregularly (n = 24)	regularly (n = 25)	never/rarely (n = 14)	irregularly (n = 24)	regularly (n = 25)
Point of time	Intensity of infestation[a]			Attack rate[b]		
Baseline (n = 77)	24.5 (16–55)	18.5 (9.5–28)	19 (11.5–29)	n. a.	n. a.	n. a.
Week 2 (n = 77)	23 (15–50)	13 (7.5–26)	16.5 (8–27)	2 (0–6)	2 (1–8)	2 (1–5.5)
Week 4 (n = 77)	23 (16–62)	12.5 (5–32)	11 (5–30)	5 (1–8)	1 (1–7)	3 (1–10)
Week 6 (n = 73)	30.5 (19.5–67)	12 (7–26)	12 (4–32)	4.5 (0–7)	3 (0–4)	1 (0–3)
Week 8 (n = 70)	32 (15–77)	10.5 (5.5–31)	9 (3–27)	5 (0–7)	2 (0–8.5)	0 (0–2)
Week 10 (n = 72)	21 (15–61)	11.5 (5.75–28.5)	9 (4–20)	2 (1–7)	3 (1–7)	0.5 (0–3)

n. a. = not applicable.
[a]total number of lesions.
[b]newly penetrated sand fleas since the previous examination.
Data indicate the median and the interquartile range (IQR).
doi:10.1371/journal.pntd.0002426.t003

Per protocol analysis

Of the 5.042 applications of Zanzarin foreseen in the repellent group, 4.832 (96%) had been applied as foreseen in the protocol. A per protocol analysis was, therefore, not considered to be meaningful in this group. Wearing shoes in the shoe group was assessed during 62 days. Fourteen participants wore shoes never/seldomly, 24 irregularly and 25 regularly; 14 were not classified, since the number of observations was too low to permit a conclusion. The average compliance was 51.6% (range 0%–98%). Stratification of members of the shoe group into individuals who never/seldomly, irregularly, or regularly wore the donated shoes is shown in Table 3. The intensity of infestation decreased continuously in the subgroup of participants wearing the shoes regularly: median 19 (IQR 11.5–29) at baseline versus median 9

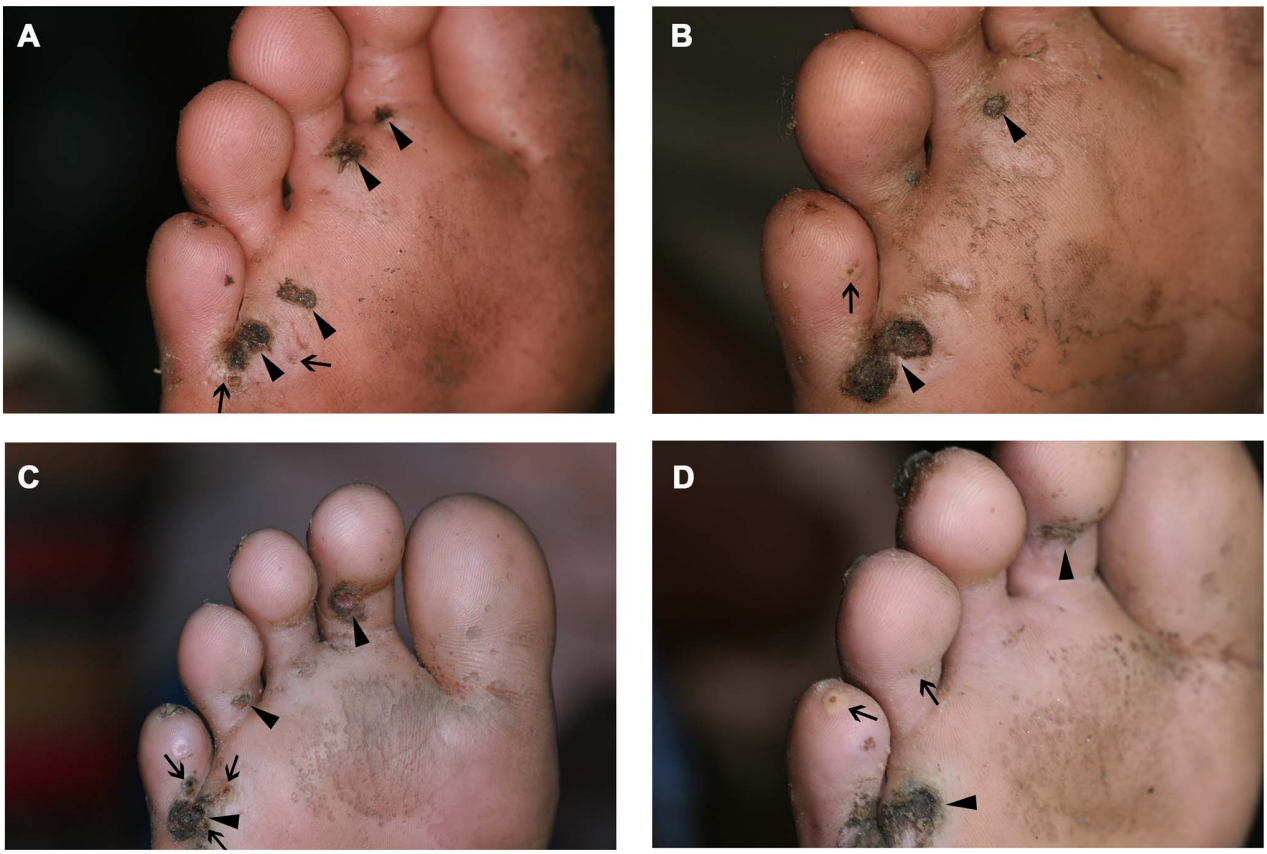

Figure 6. [→ triangle indicates newly penetrated sand fleas; ▲ indicates older lesions) Picture series of an individual of the control cohort with typical clinical pathology at the sole; (A) baseline examination, (B) week 2, (C) week 6 and (D) week 10 of follow up.
doi:10.1371/journal.pntd.0002426.g006

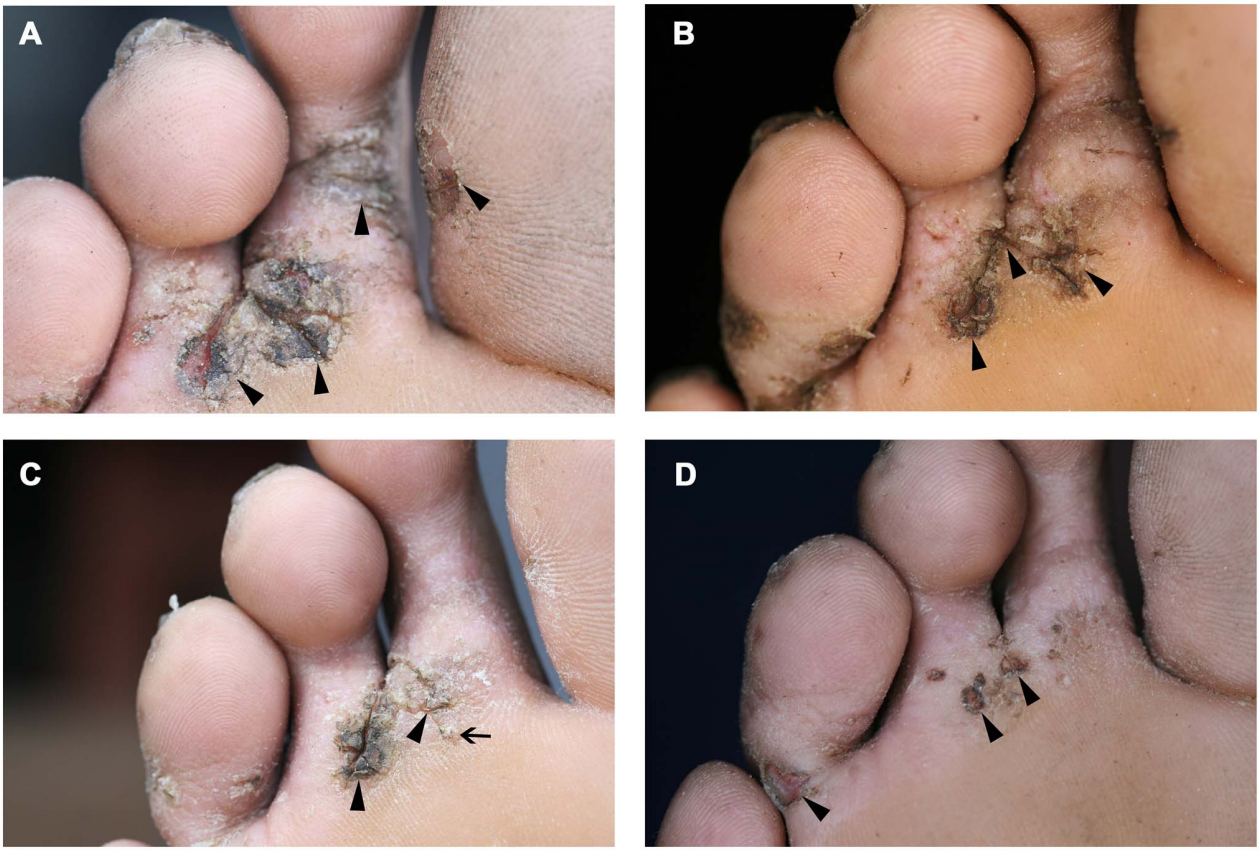

Figure 7. Picture series of an individual of the shoe cohort with typical clinical pathology at the sole; (A) baseline examination, (B) week 2, (C) week 6 and (D) week 10 of follow up.
doi:10.1371/journal.pntd.0002426.g007

(IQR 4–20) at week 10 ($p = 0.03$). In the subgroup of people wearing shoes irregularly the decrease was less obvious: median at baseline 18.5 (IQR 9.5–28) versus median 11.5 (IQR 5.75–28.5) at week 10 ($p = 0.72$). In contrast, individuals who never or rarely wore their shoes remained infested to the same degree during the entire observation period. Similarly, the attack rate decreased in participants of the two former groups, but not in individuals who never/rarely wore shoes (Table 3).

At baseline, there was inverse relationship between the intensity of infestation and wearing of shoes later: median = 24.5 lesions (IQR 16–52) in participants who later wore their shoes seldomly, median = 18.5 (IQR 10–28) in participants who later wore their shoes irregularly and median = 16 (13–29) in participants who later wore their shoes regularly.

Clinical pathology

Figure 6, 7 and 8 show a photo series of typical clinical pathology presentations at the soles of individuals from the control, shoe and repellent cohort. The type and degree of clinical pathology in the individual of the control cohort remained essentially the same during the observation period: several lesions disappeared and new lesions developed, and the soles remained heavily inflamed (Figure 6 A, B, C, D). In the individual of the shoe cohort, a slight amelioration was apparent onwards from week 6. However, at week 10, signs of inflammation still persisted (Figure 7 A, B, C, D). In contrast, in the individual of the repellent group, a reduction of tungiasis-related inflammation became apparent already at week 3. At week 10, the sole of this individual only showed residues of sand flea lesions without signs of inflammation (Figure 8 A, B, C, D).

Discussion

Tungiasis is a neglected tropical disease for which no drug treatment is available [2]. During the transmission season constant reinfestation is the rule and individuals frequently harbor dozens, sometimes hundreds of embedded parasites [16]. Previous studies showed that the intensity of infestation and the degree of tungiasis-associated morbidity are correlated [16,28]. Hence, even a partial reduction of the intensity of infestation would lower the risk for severe morbidity in an affected individual.

In the endemic areas people try to extract embedded sand fleas with kitchen utensils, sewing needles, safety pins, sharpened nails or even thorns, which usually causes more harm than good: either this induces a bacterial superinfection of the lesion or the parasite ruptures in situ, thereby, intensifying the existing inflammation [15]. Since the extraction of a burrowed sand flea with inappropriate instruments invariably causes a (micro) haemorrhage, traditional treatment may expose individuals with tungiasis at risk for transmission of viruses such as HCB and HCV in areas where the prevalence of these infections are high and non-sterile instruments are used in consecutive patients [15].

Since no drug treatment is at hand, the prevention of the infestation is the only means to control tungiasis-associated morbidity. Here we compare the efficacy of making closed shoes

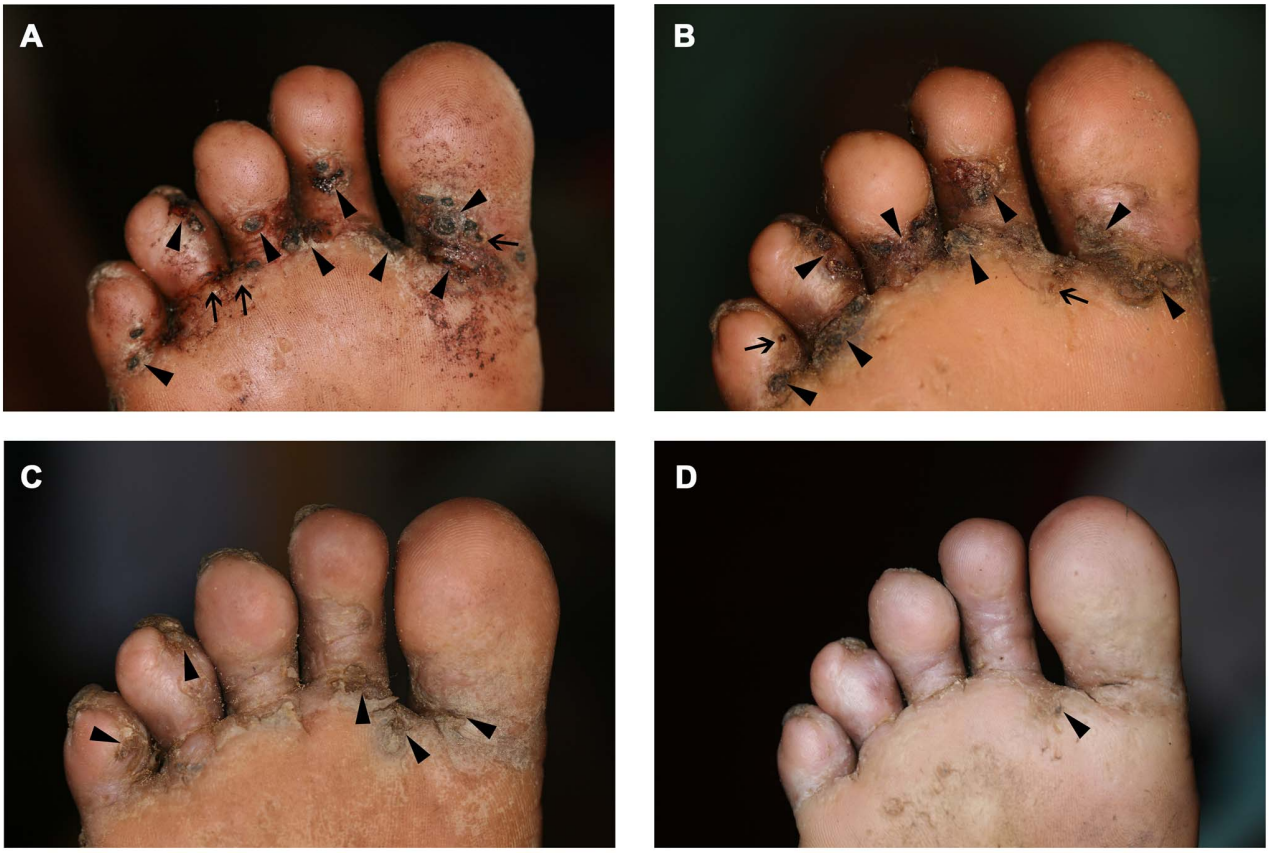

Figure 8. Picture series of an individual of the repellent cohort with typical clinical pathology at the sole; (A) baseline examination, (B) week 2, (C) week 6 and (D) week 10 of follow up.
doi:10.1371/journal.pntd.0002426.g008

available by donation with the application of a repellent based on coconut oil to the feet. In previous studies in Northeast Brazil, we have demonstrated that the regular application of this repellent to the feet reversed tungiasis-associated clinical pathology to an insignificant level within four weeks [25]. Even if the repellent is only applied intermittently, e. g. daily every second week, its protective effect is remarkable [26]. In this study in rural Madagascar, the efficacy of the repellent to rapidly reduce the intensity of infestation and to resolve acute tungiasis-associated clinical pathology was also very high. This reflects the fact that the attack decreased to zero already after two weeks and remained so until the end of the study.

In tungiasis, acute clinical pathology is essentially inflammation-related, and can be measured by the SSAT [23]. Two explanations for the rapid resolution of inflammation and respectively the decrease of the SSAT in participants of the repellent group seem plausible. First, since intensity of inflammation correlates to the accumulation of penetrated sand fleas per unit of time [23], after a couple of weeks of the application of the repellent the number of remaining sand flea lesions had fallen below a threshold at which no significant acute clinical pathology develops. Second, jojoba oil and *Aloe vera*, in the concentrations present in the lotion, may have an anti-inflammatory effect and, thus, also may have contributed to the rapid decrease of inflammation-related pathology.

Within two weeks after the application of the repellent, the median attack rate was reduced to zero, and after 10 weeks even the interquartile range of this outcome measure was zero to zero. In previous studies in areas with different transmission dynamics, the twice daily application reduced the median attack rate by 92% [25,26]. The higher efficacy observed in this study might be explained by the moderate attack rate in the two Malagasy villages in contrast to an extremely high attack rate in the Brazilian populations.

Textbooks on tropical medicine suggest that wearing footgear protects against invading sand fleas [18]. This assumption is supported by anecdotes from colonial times, indicating that in East and Central Africa local soldiers - which possessed no shoes at all - frequently developed a high intensity of infestation and severe morbidity, whereas European officers and sergeants equipped with solid footgear rarely were affected [31]. However, personal experience of two of the investigators confirms that wearing closed shoes - even with socks - does not completely protect against invading sand fleas (H. Feldmeier, unpublished observation 2004; M. Thielecke, unpublished observation 2011).

In this study, the availability of shoes had only a marginal protective effect which manifested with a delay of several weeks. At week 10, the attack rate, the intensity of infestation and the SSAT still were significantly different between the shoe group and the repellent group. Surprisingly, the compliance in the shoe group was rather low, and only 39% of group members wore the donated shoes regularly. This may have several reasons: First, in the villages wearing footwear is not a custom and people may find wearing solid closed shoes uncomfortable in comparison with flip-flops or walking barefoot. In fact, some people complained about perspiration and unpleasant odor when wearing the shoes (V.

Raharimanga and M. Thielecke, unpublished observation 2011). Second, as a part of their daily routine, participants regularly came into contact with water or wet soil (e. g. when tilling their rice fields). Such work makes wearing shoes rather impracticable. Third, solid shoes are considered to be very valuable commodities in the villages, and are preferred to be used for special activities, such as going to church. Fourth, people care for their clothes very properly. Accordingly, when the donated shoes got dirty (which was almost inevitable when leaving the house), they washed and dried them. This means that shoes - even when a person was willing to wear protective footwear - were simply not available all the time. Fifth, some individuals did not wear the donated shoes in order not to be recognized as participants to avoid jealousy of other inhabitants of the village (V. Raharimanga and M. Thielecke, unpublished observation 2011). Sixth, individuals with many sand flea lesions felt pain when walking in solid shoes. This might explain why participants, never or only rarely wearing the donated shoes, were those with the highest intensity of infestation. Finally, when shoes are worn daily in rural Madagascar - where rough roads prevail and where paths are either dusty or muddy – they get cracks and are worn out after a couple of months. Frequently, they are so holey, that they should have lost any protective effect (M. Thielecke, unpublished observation 2011).

The weakness of the study is obvious. Although the wearing of shoes was encouraged at the beginning of the study, compliance was not enforced. This means, the decision whether or not to wear solid footwear was reserved for each participant itself. In contrast, the application of the repellent was performed by community health workers and strictly executed as foreseen by the protocol. Hence, no personal initiative was required from the participants of the repellent group. We deliberately decided to apply the repellent in an active way, to get in an idea to which extent tungiasis-associated morbidity can be controlled if the repellent is applied in an optimal way.

Supporting Information

Supporting Information S1 Study protocol in French.
(DOC)

Supporting Information S2 CONSORT checklist.
(DOC)

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Author Contributions

Conceived and designed the experiments: HF CR MSG. Performed the experiments: MT VRa. Analyzed the data: MT HF CR. Contributed reagents/materials/analysis tools: VRi CR MSG. Wrote the paper: MT HF. Critical revision of the manuscript for important intellectual content: HF CR MSG MT VRa VRi.

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PLOS | NEGLECTED TROPICAL DISEASES

Treatment of Tungiasis with Dimeticone: A Proof-of-Principle Study in Rural Kenya

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Abstract

Tungiasis (sand flea disease) is a neglected tropical disease, prevalent in resource-poor communities in South America and sub-Saharan Africa. It is caused by an inflammatory response against penetrated female sand fleas (*Tunga penetrans*) embedded in the skin of the host. Although associated with debilitating acute and chronic morbidity, there is no proven effective drug treatment. By consequence patients attempt to remove embedded sand fleas with non-sterile sharp instruments, such as safety pins, a procedure that represents a health threat by itself. In this proof-of-principle study we compared the topical application of a mixture of two dimeticones of low viscosity (NYDA) to the topical application of a 0.05% solution of KMnO4 in 47 school children in an endemic area in rural Kenya. The efficacy of the treatment was assessed during a follow up period of seven days using viability signs of the embedded parasites, alterations in the natural development of lesion morphology and the degree of local inflammation as outcome measures. Seven days after treatment, in the dimeticone group 78% (95% CI 67–86%) of the parasites had lost all signs of viability as compared to 39% (95% CI 28–52%) in the KMnO4 group ($p<0.001$). In the dimeticone group 90% (95% CI 80–95%) of the penetrated sand fleas showed an abnormal development already after 5 days, compared to 53% (95% CI 40–66%; $p<0.001$) in the KMnO4 group. Seven days after treatment, signs of local skin inflammation had significantly decreased in the dimeticone group ($p<0.001$). This study identified the topical application of dimeticones of low viscosity (NYDA) as an effective means to kill embedded sand fleas. In view of the efficacy and safety of the topical treatment with dimeticone, the mechanical extraction of embedded sand fleas using hazardous instruments is no longer warranted.

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Introduction

Tungiasis (sand flea disease) is a neglected tropical disease frequent in South America, The Caribbean and in sub-Saharan Africa. [1,2,3]. It is prevalent in resource-poor rural and urban communities, where animal reservoirs are present and people live in poverty [2,4,5,6,7,8]. In the last decade, tungiasis has re-emerged in East Africa in epidemic dimensions [9]. In 2010, Ahadi Kenya Trust, a non-governmental organization, reported several hundred thousand cases of tungiasis in Kenya alone, of which the majority were children [4,10,11].

Sand flea disease is the result of an intense inflammatory response against penetrated sand fleas embedded in the skin of the host. The mechanisms underlying the inflammation are complex and only partially understood [11,12,13]. Immediately after a successful penetration the female sand flea starts to hypertrophy reaching the size of a pea after 10 days [14]. Through its abdominal rear cone the parasite remains in contact with the environment [14]. The tiny opening in the skin (250 to 500 µm) is needed for copulation with male sand fleas, breathing, defecation and expelling eggs [14]. After expulsion of all eggs the female sand flea dies in situ and is discarded from the epidermis by tissue repair mechanisms [14].

Although by its nature a self-limiting infection, tungiasis is actually a debilitating disease in endemic areas [15]. Sequels are common and are related to repeated and severe infection. They include acute and chronic inflammation of toes, deformation and loss of toe nails, fissures and lymphoedema [11].

Bacterial super-infection is almost invariably present [13]. It increases the inflammation and leads to intense pain [16]. If embedded sand fleas are removed by using inappropriate sharp instruments, severe mutilation of the feet may develop including deep ulcers, gangrene and loss of toes [15]. Septicaemia has also been described [17] and tetanus is a known deadly sequel in non-vaccinated individuals [18].

Hitherto, the only effective treatment is the surgical extraction of embedded sand fleas under sterile conditions in medical facilities. However, in the endemic areas patients do not have

Author Summary

Tungiasis (sand flea disease), a parasitic skin disease, causes important morbidity, and eventually leads to mutilation of the feet. Hitherto, the only effective treatment is the surgical extraction of embedded sand fleas. In the endemic areas this is done using inappropriate sharp instruments and causes more harm than good. We identified the three last abdominal segments of *Tunga penetrans* which protrude through the skin and through which the parasite breathes, defecates, and expels eggs - as an Achilles heel of embedded sand fleas. In a proof-of-principle study we investigated whether this Achilles heel is vulnerable to dimeticone with a low viscosity and a high creeping property. We randomized the left and the right feet to either receive a topical application of KMnO4 (the standard treatment in Kenya) or of dimeticone. The major outcome measure was the absence of viability signs of the treated sand fleas. The study shows that the topical application of a mixture of two dimeticones (NYDA) effectively kills embedded sand fleas within seven days. Since dimeticones are considered to be wholly non-toxic and are not expensive the new treatment could become a means to control tungiasis-associated morbidity on the population level.

access to appropriately equipped health centers and therefore use any kind of sharp instruments (safety pins, sewing needles, hair pins, sharpened pieces of wood, etc.) to remove embedded sand fleas. Attempts to remove the embedded parasites by using a sharp instrument, invariably causes a (micro) hemorrhage [9]. As the same instrument is frequently used to remove embedded sand fleas from different persons, this procedure increases the risk of the transmission of blood-borne pathogens, such as hepatitis B and C virus [19].

In an act of desperation, patients may apply toxic substances to the skin with the intention of killing the embedded parasites. In Brazil and Madagascar, for instance, kerosene, used petrol, and insecticides are used [9,20]. In rural Uganda, a crop pesticide used in tomato cultivation is applied (H. Feldmeier, unpublished observation 2013).

In the absence of safe and effective treatment options, Ahadi Kenya Trust recommends to bath the feet in a 0.05% solution of potassium permanganate (KMnO4) for 10 minutes [10]. However, the efficacy of this approach is not known. In Brazil several antihelminthic compounds, including ivermectin, have been tested, but none proved to be a really effective [21].

Dimeticones are silicone oils of low viscosity with a low surface tension and excellent creeping properties. They are highly effective against head lice [22]. The substance creeps into the tracheae of head lice and leads to lethal asphyxia within one minute [23]. The mode of action is purely physical. Dimeticones are biochemically inert and are not absorbed when applied to the skin or swallowed [24]. They are neither carcinogenic nor teratogenic and are considered wholly non-toxic [24].

Previous observation in rats infested with *T. penetrans* showed that if a drop of a solution of two dimeticones of low viscosity (NYDA) was applied on top of the protruding rear cone of an embedded sand flea, the parasite rapidly lost signs of viability (H. Feldmeier, unpublished observation 2011). Based on this observation we decided to investigate the efficacy of the dimeticone for the treatment of tungiasis in a proof-of-principle study in rural Kenya. The results show that wetting the skin of the feet with dimeticones with low viscosity effectively kills embedded sand fleas and reduces tungiasis-associated inflammation within seven days.

Materials and Methods

Study area and study population

The study was performed in Gatundu North District, central Kenya, approximately one hour north of Nairobi. Tungiasis is endemic in this region. People live in small hamlets in houses made of wood or bricks. Families earn their living from subsistence farming. Most households possess animals, dogs, chicken and pigs. The animals live on the compound or are brought back to it in the night. Living conditions are generally very poor.

The study participants were school children aged five to sixteen years enrolled at the public Kiamwangi Primary School and Ikuma Primary School, which are situated five km to each other. The classrooms consist of simple houses without a solid floor. Both schools have a limited access to water, so that the schoolyards and rooms cannot be cleaned regularly. Most pupils wore worn-out sandals or walked barefoot. The study was carried out between January 10 and February 17, 2012. This period coincides with the high transmission period of *T. penetrans*.

Study design

To allow comparison between the new approach (the application of the dimeticone) and the local reference procedure (bathing feet in a 0.05% solution of KMnO4), one foot was bathed in the KMnO4 solution for 10 minutes and to the other foot the dimeticone was applied three times during this period (see below). Since bathing a foot in a 0.05% KMnO4 solution changes the color of the skin into dark purple, neither the patient nor the examiner were blinded with regard to the treatment applied.

Individuals, aged $\geq$5 years, with at least one lesion in stage IIa – IIIa of the Fortaleza classification on each foot were eligible [14]. In IIa the sand flea is already completely embedded in the skin of the host and has started to hypertrophy [14]. Lesions in stage IIIa correspond to a fully developed parasite with a characteristic watchglass-like appearance. In this stage the female sand flea starts to expel eggs [14]. In stage IIIb egg expulsion stops, thereafter the sand flea dies and the lesion changes into stage IV: the lesion becomes crusted, viability signs become rare and eventually completely disappear [14]. Hence, sand fleas in stage IIa – IIIa are most suitable to assess viability and alterations in the normal development of the parasites.

The inclusion criterion for an eligible lesion was the presence of at least 2 out of 4 viability signs at the baseline examination: expulsion of eggs, excretion of a faecal thread, excretion of faecal liquid or pulsations/contractions of the parasite. Viability signs were determined using a handheld digital video microscope (eScope iTEZ, Hongkong, China) (see supplementary electronic material 1).

When several eligible lesions were present on one foot only those (at most three) were selected for evaluation that allowed a clear discernment of the developmental stage of the embedded parasite and a quantification of the inflammatory response around the lesion. Hence, lesions occurring in cluster and lesions which the patient had attempted to manipulate were excluded. Other exclusion criteria were: Presence of gross inflammation, abscess or ascending lymphangitis or lymphedema on either foot. Children with such complications of tungiasis were referred to the nearest health facility for treatment.

For practical reasons we decided to treat always the same foot with dimeticone and KMnO4, respectively. At the beginning of the study a coin was tossed for randomizing the two treatments.

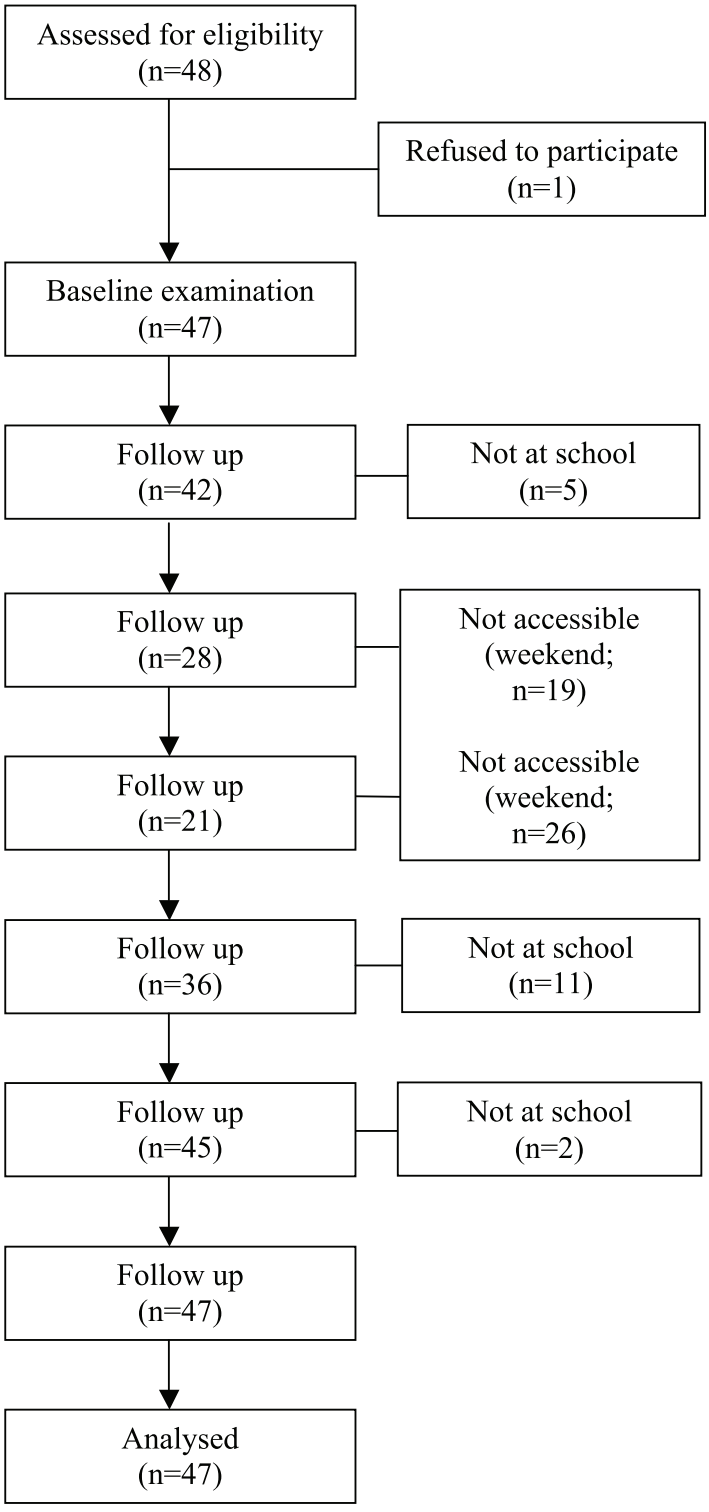

Figure 1. Flow diagram.
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This resulted in application of the dimeticone to the left foot and of KMnO4 to the right foot. Children were informed not to manipulate the lesions during the next seven days.

Before each examination the feet of the participants were washed properly with water and soap and dried with a clean towel. Then, the left foot was wetted with NYDA up to the ankle three times within

Table 1. Demographic and clinical data of study participants at baseline.

	Treatment applied	
Variable	**NYDA (left foot)**	**KMnO4 (right foot)**
Median number of lesions on respective foot (range)[a]	25 (8–112)	25 (6–107)
Median of viable lesions (range)[b]	3 (1–29)	2 (1–25)
Median of non-viable lesions (range)[c]	3 (0–30)	2 (0–36)
Median of manipulated lesions (range)[d]	18 (6–53)	18 (4–54)
Number of viable lesions included in the study[e]:	88	82
stage IIa	52	51
stage IIb	35	31
stage IIIa	1	0

[a]total number of viable, non-viable and manipulated sand flea lesions.
[b]sand flea lesions in stage I to IIIb, according to the Fortaleza Classification.
[c]lesions in stage IV and V, according to the Fortaleza Classification.
[d]lesions manipulated with a sharp instrument by the patient himself or a caregiver.
[e]maximum of 3 lesions per foot (see material and methods).
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10 minutes. In the interval, the foot was kept in an upright position to allow surplus dimeticone to evaporate. Simultaneously, the right foot was put into a bucket containing a 0.05% KMnO4 solution, and remained there for 10 minutes. After sun drying the right foot, vaseline was applied to compensate the desiccation of the skin caused by KMnO4. The immersion of the foot in 0.05% KMnO4 for 10 minutes and the subsequent oiling with vaseline is the standard procedure applied by Ahadi Kenya Trust. After treatment the children were allowed to continue their daily activities.

The lesions were monitored daily for viability signs and the abnormal development of the embedded parasite for a total of seven days. One week reflects the period of normal development of a sand flea from stage IIa to stage IIIa [14]. Thereafter, it looses its characteristic watchglass-like appearance, but does not increase in size anymore [14]. Hence, abnormalities in development are difficult to be detected.

In order to detect a change of tungiasis-associated inflammation an inflammation score was developed. In addition to the classic signs of local inflammation (erythema, oedema and warmness) the score included the presence of suppuration, ulcers and fissures as well as itching and pain. The inflammation score ranged from 0 to 27 points [25].

In total, 48 participants were recruited and 47 were randomized. The flow diagram is shown in Figure 1.

Table 2. Efficacy of treatment based on viability of embedded sand fleas.

			Treatment			
		NYDA viable/total lesions (%)	**Efficacy (%)[a]**	**KMn04 viable/total lesions (%)**	**Efficacy (%)[a]**	**p-value[b]**
Baseline	All lesions[c]	89/89 (100%)	0%	82/82 (100%)	0%	
	- early stages (IIa)[d]	52/52 (100%)	0%	52/52 (100%)	0%	
	- later stages (IIb–IIIa)[d]	37/37 (100%)	0%	30/30 (100%)	0%	
Day 3	All lesions[c]	27/54 (50%)	50%	43/50 (86%)	14%	<0.001
	- early stages (IIa)[d]	12/28 (43%)	67%	29/33 (88%)	12%	<0.001
	- later stages (IIb–IIIa)[d]	15/26 (58%)	42%	14/17 (82%)	18%	0.10
Day 5	All lesions[c]	33/72 (46%)	54%	43/58 (74%)	26%	0.001
	- early stages (IIa)[d]	18/43 (42%)	58%	26/37 (70%)	30%	0.01
	- later stages (IIb–IIIa)[d]	15/29 (52%)	46%	17/21 (81%)	19%	0.04
Day 7	All lesions[c]	19/86 (22%)	78%	43/71 (61%)	39%	<0.001
	- early stages (IIa)[d]	6/49 (12%)	88%	27/45 (60%)	40%	<0.001
	- later stages (IIb–IIIa)[d]	13/37 (35%)	65%	16/26 (62%)	38%	0.04

[a]proportion of parasites which lost all viability signs.
[b]dimeticone versus KMnO4 treatment.
[c]The total number of lesions examined varied at follow up examinations, because some participants could not be examined at the days foreseen, especially at the weekends (see flow diagram).
[d]according to the Fortaleza classification.
doi:10.1371/journal.pntd.0003058.t002

Table 3. Efficacy of treatment based on the morphological development of sand flea lesions.

		Treatment applied		
		NYDA	KMnO4	p-value[b]
		Abnormal development/total lesions (%)[a]		
Day 3	All lesions[c]	41/54 (76%)	22/50 (44%)	<0.001
	- early stages (IIa)[d]	19/28 (68%)	12/33 (36%)	0.021
	- later stages (IIb–IIIa)[d]	22/26 (85%)	10/17 (59%)	0.080
Day 5	All lesions[c]	65/72 (90%)	31/58 (53%)	<0.001
	- early stages (IIa)[d]	40/43 (93%)	20/37 (54%)	<0.001
	- later stages (IIb–IIIa)[d]	25/29 (86%)	11/21 (52%)	0.012
Day 7	All lesions[c]	79/86 (92%)	45/71 (63%)	<0.001
	- early stages (IIa)[d]	45/49 (92%)	27/45 (60%)	<0.001
	- later stages (IIb–IIIa)[d]	34/37 (92%)	18/26 (69%)	0.040

[a]see definition in materials and methods.
[b]dimeticone versus KMnO4 treatment.
[c]The total number of lesions examined varied at follow up examinations, because some participants could not be examined at the days foreseen, especially at the weekends (see flow diagram).
[d]according to the Fortaleza classification.
doi:10.1371/journal.pntd.0003058.t003

Outcome measures

Two major outcome measures were defined. First, the proportion of viable embedded sand fleas which lost viability signs after seven days of follow-up. An embedded sand flea was considered to be dead when none of the four viability signs (expulsion of egg, excretion of faecal thread, excretion of faecal liquid, pulsations/contractions) was detected during 15 minutes of observation with the digital handhold video microscope on two consecutive follow-up examinations. Videos were recorded and reviewed in the evening of the examination day (see supplementary electronic material 1). Second, the proportion of embedded sand fleas in which the normal development was interrupted. We defined a development as abnormal, when the lesion did not change its size within two consecutive follow ups and/or

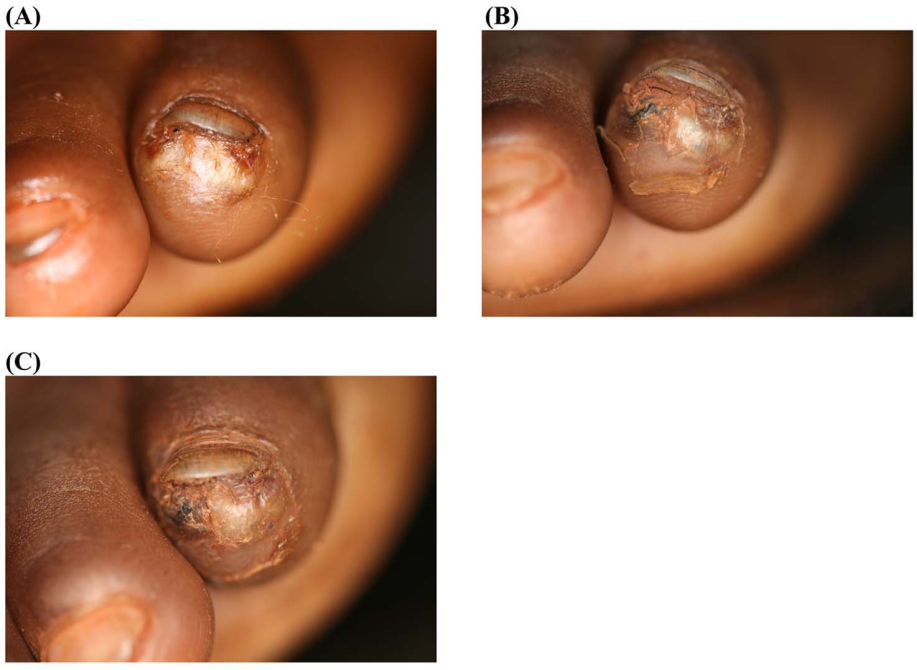

Figure 2. Photo series of two lesions located next to the nail rim of the fifth toe; treatment with dimeticone. (A) Baseline: Two sand flea lesions in stage IIIa are located next to each other with the characteristic watchglass-like elevation. The abdominal cone is the circular brownish protrusion in the center of the lesions. (B) Day 3: The abdominal cones have changed in a brownish-black crust, the watchglass-like elevations have vanished and the lesions have dried out. Desquamation of the stratum corneum around the lesions has started. No signs of viability were detected. (C) Day 7: The appearance of the lesions has not changed; desquamation has slightly increased.
doi:10.1371/journal.pntd.0003058.g002

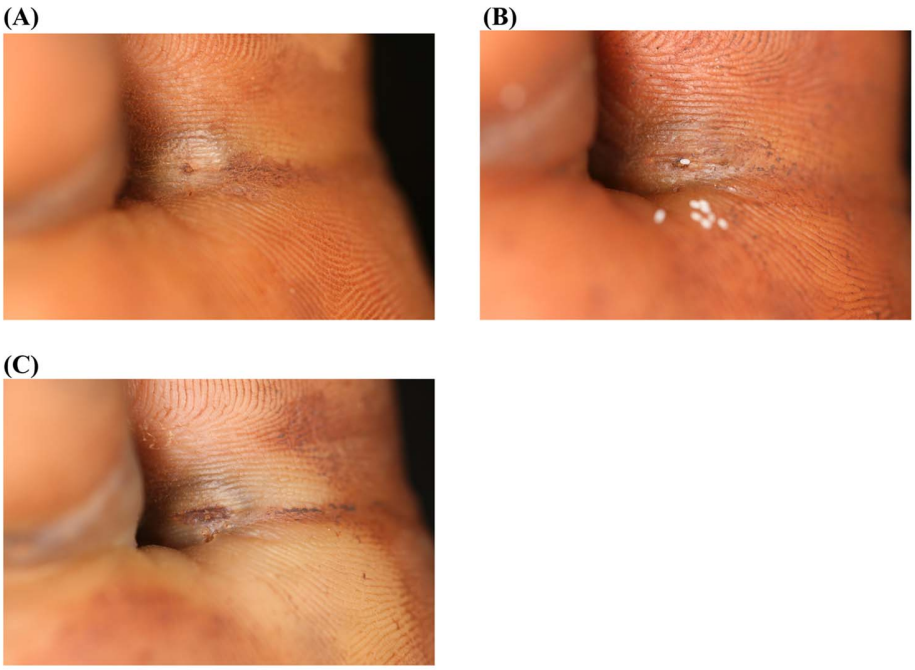

Figure 3. Photo series of a lesion located at the base of the first toe; treatment with KMnO4. (A) Baseline: A lesion in stage IIIa with a diameter of 10 mm at the base of the first toe. The abdominal cone is the circular brownish protrusion in the center of the elevation. The dermal papillae next to the lesion contain faecal material expelled by the parasite. (B) Day 3: The sand flea has expulsed several eggs (white oval dots). One of the eggs is in progress of being expelled. The appearance of the lesion has not changed. (C) Day 7: The lesion has retained its size and remains elevated. Recently excreted faecal material has spread into the dermal papillae next to the lesion, another indicator that the parasite remained viable.
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morphological abnormalities developed, e.g. discoloring or desiccation of the abdominal rear cone [14].

A secondary outcome measure was the intensity of local inflammation, as assessed semi-quantitatively by the inflammation score. The observation units for all outcome measures were single sand flea lesions.

Statistical analysis

The sample size calculation was based on the following assumptions: with a level of confidence set at 95% together with a power of 90% assuming equal number of lesions in treatment and control group, 45 lesions in each group were needed to determine a difference of 35% in the major outcome measure between the two treatments assuming a 40% effect of the standard treatment.

Fisher's exact test was used to compare proportions. General estimation equations were used to analyze the evolution of the inflammation score during the observation period.

Ethical considerations

The study was approved by the Ethics Committee of the Ministry of Health, Nairobi (MMS/ADM/3/8/Vol 111), and was registered at Controlled-trials.com (ISRCTN: 91405042). The study was performed in accordance with the ethical standards of the Ethics Committee of the Ministry of Health, and with the Declaration of Helsinki as amended 2013 by the World Medical Association. Informed written consent was obtained from the guardians of the participants in English before starting the study. For ethical reasons no controls were included. During the study, food was provided free of charge to the participants. At the end of the study, any remaining viable sand fleas were removed under sterile conditions and the wounds were dressed following standard procedures. All patients received a new pair of closed solid shoes.

Results

Baseline characteristics

The baseline characteristics of the feet of the 47 participants are summarized in Table 1. None of the variables differed significantly between the two feet. In the NYDA group, 88 lesions were included in the study, in the KMnO4 group 82.

Major outcome measures

Table 2 shows the efficacy of treatment based on the disappearance of viability signs. Already three days after application of dimeticone 50% of the parasites lost all viability signs (efficacy = 50%), whereas the efficacy in the KMnO4 group was 14% ($p<0.001$). At day 7 the efficacy was 78% (95% CI 67–86%) after treatment with dimeticone and 39% (95% CI 28–52%) after treatment with KMnO4 ($p<0.001$); a difference of 39% (95% CI 23–54%). In the dimeticone group, lesions in an early stage of development lost viability signs more often than lesions in later stages (efficacy = 88% (95% CI 75–95%) versus 65% (95% CI 47–79%) at day 7 ($p = 0.01$)). In the KMnO4 group, there was no difference between lesions in early and later stages of development.

The effect of treatment on the morphological development of the lesions is shown in Table 3. Already after 5 days in the dimeticone group 90% (95% CI 80–95%) of sand flea lesions showed an abnormal development as compared to 53% (95% CI 40–66%) ($p<0.001$) in the KMnO4 group.

Figure 2A–C and 3A–C show the macroscopic development of lesions after the treatment with dimeticone or KMnO4, respectively. Figure 4A–D and 5A–D depict the microscopic development of

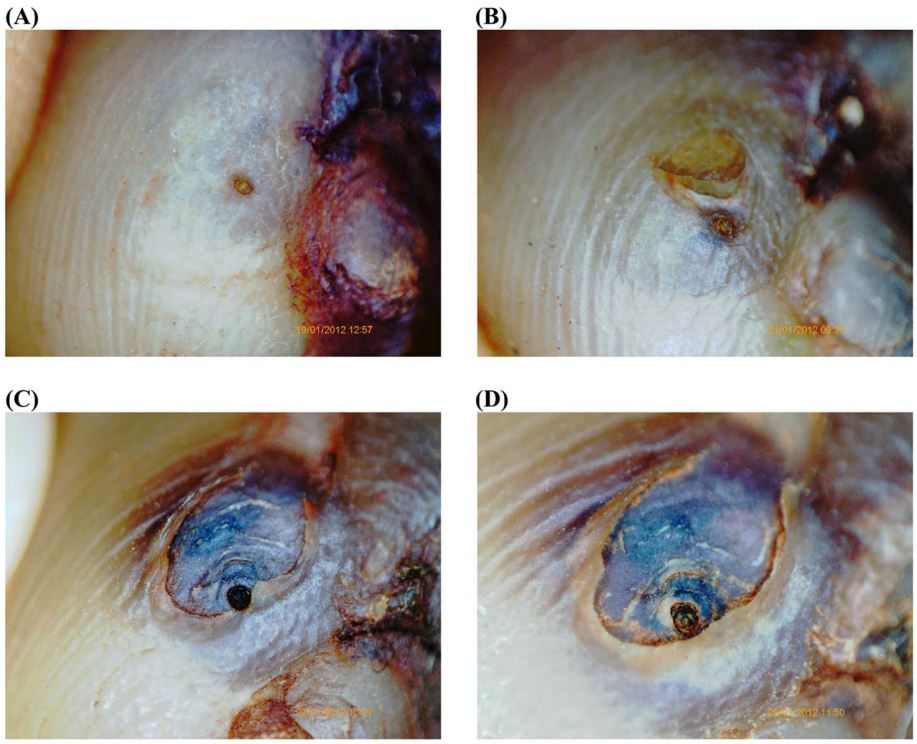

Figure 4. Photo series of a lesion documented by the digital handhold video microscope at 200 fold magnification; treatment with dimeticone. (A) Baseline: Lesion in stage IIb. The abdominal cone is the circular brownish protrusion in the center. The cone is surrounded by a slightly elevated circle. The dark area on the right is part of the toe nail. (B) Day 3: The abdominal cone has changed in a brownish crust. The stratum corneum covering the embedded parasite has started to desquamate. No viability signs detectable. (C) Day 5: The rear cone has changed into a black crust. The desquamation has significantly enlarged. The uncovered intersegmental skin of the abdomen of the parasite has turned into dark-purple. (D) Day 7: The appearance of the lesion has remained similar; desiccation and desquamation have continued.
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lesions after treatment as seen through the digital handhold video microscope.

Inflammation score

In the dimeticone group the inflammation score decreased from a median of 6.0 at baseline to a median of 4.75 at day 7. In contrast, in the KMnO4 group, the inflammation score increased (median 4.5 versus 5.0). Both differences were significant ($p < 0.0001$ and $p = 0.009$, respectively).

Ancillary findings

During the study period three sand fleas were extracted by the participants or their caregiver in the NYDA group and 11 in the KMnO4 group.

Discussion

Tungiasis, a wide spread neglected tropical disease, is prevalent in resource-poor rural and urban communities, where animal reservoirs are present and people live in poverty [2,4,5,6,7,8]. Elimination of sand flea disease is not possible as long as the precarious living conditions, which are characteristic of the endemic areas, prevail and animal reservoirs exist.

Taking into consideration the high prevalence of tungiasis, the absence of appropriate infrastructure in the endemic areas and the health hazards associated with the traditional treatment, there is an urgent need for a safe and effective drug treatment. Recently, dimeticones have emerged as highly effective chemicals against ectoparasites such as head lice [26]. Since dimeticones have a purely physical mode of action and are considered to be non-toxic, they have become the standard treatment of pediculosis capitis in Europe [22].

We considered the last abdominal segments of an embedded sand flea, which protrude through the skin by forming a miniature cone and through which the parasite breathes, defecates and excretes eggs, as an Achilles heel, which can be targeted by dimeticone. Since the opening leading to internal organs measures less than 1 mm, we decided to use a combination of two dimeticones of very low viscosity with a low surface tension and excellent creeping properties (NYDA) [23].

We defined a set of viability signs of embedded sand fleas detectable through a handhold digital video microscope. We used the presence of viability signs as the major outcome measure and compared the efficacy of a 0.05% solution of KMnO4 – the standard treatment used in mass campaigns in Kenya – to wetting the foot with dimeticone three times during a period of 10 minutes. The observation period was limited to seven days, since a certain number of embedded sand fleas will die even without any intervention during this period [14].

After 7 days, 78% of the lesions did not show any sign of viability in the dimeticone group, whereas the proportion was 39% in the KMnO4 group. True efficacy of a 0.05% solution of KMnO4 alone may be lower since KMnO4 is a disinfectant and has no insecticidal properties. It is unlikely that KMnO4 diluted in water will creep into vital organs of embedded sand fleas through the parasite's abdominal cone. Presumably, the observed effect in

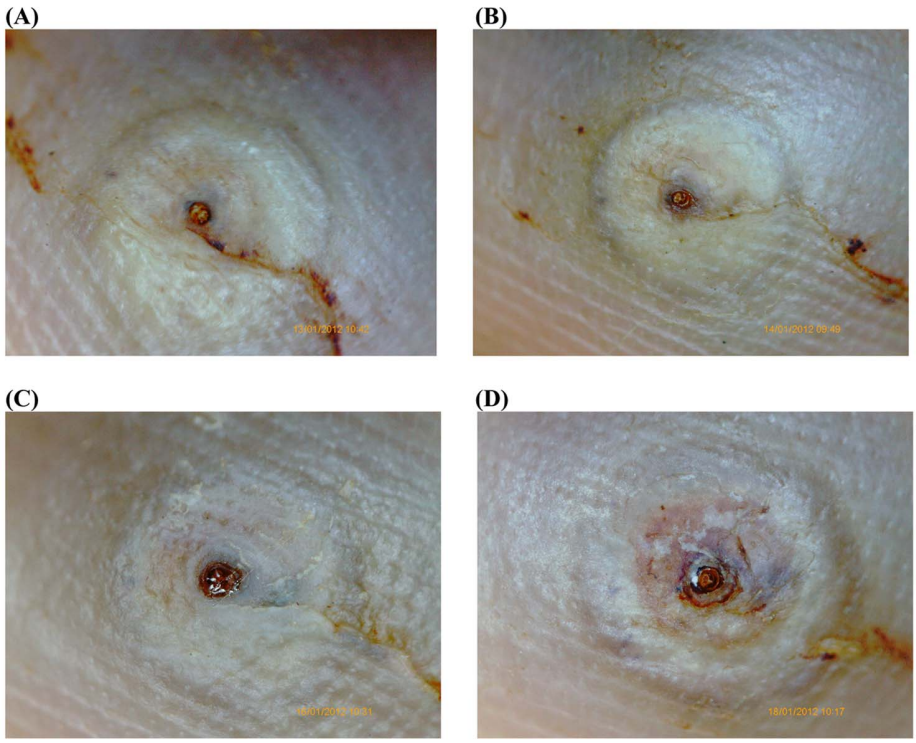

Figure 5. Photo series of a lesion documented by the digital handhold video microscope at 200 fold magnification; treatment with KMnO4. (A) Baseline: Lesion in stage IIIa. The abdominal cone is the circular brownish protrusion surrounded by the characteristic watchglass-like elevation. The curved line is faecal material of the parasite that has spread into dermal papillae. (B) Day 3: The embedded parasite has grown slightly and the convex elevation is more embossed. The abdominal cone is still brownish and shining. (C) Day 5: The appearance of the lesion has not changed. Faecal liquid is excreted through the abdominal cone and appears as a clear, light-reflecting "pond" on the top of the cone. (D) Day 7: The abdominal cone is still brownish and shining. The lesion has a convex double-rim appearance. Two viability signs (pulsation of the parasite and excretion of liquid) were present at this moment.
doi:10.1371/journal.pntd.0003058.g005

the KMnO4 treated lesions was due to the vaseline which was applied to the skin for cosmetic reasons (because bathing the feet in KMnO4 makes the skin rough and cracked). Applied on the skin, vaseline rapidly turns into oil, particularly in hot climate countries. Liquid fatty acids of the vaseline may thereby creep into the abdominal rear cone and suffocate the parasite.

Interestingly, the efficacy of dimeticone to kill embedded sand fleas depended on the stage of development: parasites being in an early stage of development were more susceptible than those who had already fully developed (efficacy = 88% versus 66%). This is plausible, since embedded sand fleas increase their size by a factor of approximately 2000 within 6–7 days during the development from stage IIa to stage IIIa [14]. Such a rapid growth requires an intense metabolism, which in turn needs constant supply of oxygen. During the early stages of development supply of oxygen might be at a critical limit. This makes the parasite vulnerable for suffocating compounds such as low-viscosity.

Since it is important to kill sand fleas as soon as they have penetrated in order to prevent the development of clinical pathology [16], the enhanced effect of dimeticone on early developmental stages is an additional advantage. The early death of the embedded parasite will also prevent the expulsion of eggs – which starts about one week after penetration – and, thereby, may have an impact on transmission.

92% of the embedded fleas treated with dimeticone showed an abnormal development. This could indicate that no (or fewer) eggs are produced and released into the environment. Hence, if applied on the population level, treatment with dimeticones could have even an impact on the off-host cycle of the parasite, possibly resulting in lower attack rates over time.

In the dimeticone group, the inflammation score started to decrease after 3 days and became significantly lower after 7 days, whereas in the KMnO4 group the inflammation slightly increased. It is conceivable that the resolution of inflammation reflects the rapid death of the parasites. Previous studies have shown that tungiasis-associated inflammation comes to a halt and tissue repair mechanism begins, when the parasites are dead [25,27].

Another indicator of the efficacy of the dimeticone was that in the course of the study 11 sand fleas were extracted from the feet treated with KMnO4 by the patients themselves, whereas in the NYDA treated feet only 3 sand fleas were removed. Similarly, when the study participants were asked at the end of the study about their satisfaction, only 10 participants preferred KMnO4, but 37 preferred the dimeticone. Children also disliked that KMnO4 colored the skin into deep purple for a few days which led to teasing in school (Figure 6).

This study on the treatment of a neglected parasitic disease is particularly in the sense that an Achilles heel of the parasite was identified first and then a compound was identified that is able to target the vulnerable body part. The abdominal cone which protrudes through the skin and through which the parasite breathes, defecates, excretes liquids and expels eggs was considered to be an ideal target for a dimeticone with a low viscosity and excellent creeping properties.

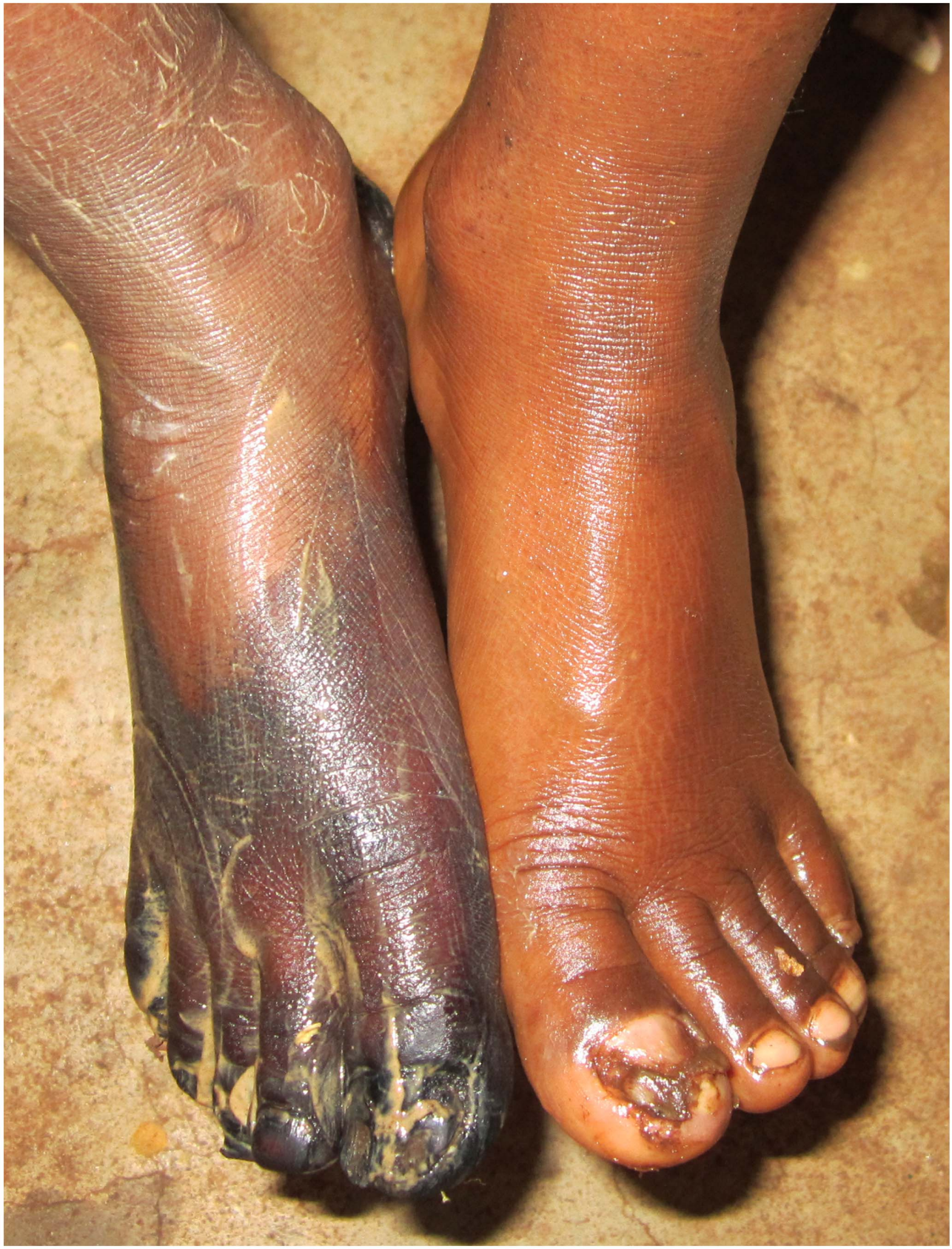

Figure 6. Left and right foot after the application of the dimeticone and KMnO4, respectively. The dark coloring of the right foot is due to KMnO4. The yellow jelly on the right foot is vaseline being in the process of dissolution.
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Although this was a proof-of-principle study with a small number of units of observations, it can be concluded that the topical application of a mixture of two dimeticones (NYDA) comprises a promising approach to treat sand flea disease. The treatment can be performed by the patient himself with minimal input from the health sector. Hence, surgical extraction with all its associated complications is no longer warrantable. After the sand flea has died in situ, the inflammation resolved. Importantly, future resistance of the parasites against dimeticone treatment is highly unlikely to evolve, since the drug acts only physically.

Supporting Information

Video S1 Embedded sand flea produces faecal thread. Video of an embedded sand flea in stage IIb using a handheld digital video microscope. The lightly brownish abdominal rear cone is magnified 200 fold. The cone is contracting and producing a black faecal thread. In the surrounding of the cone pulsations of the intestines are visible.
(MP4)

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Author Contributions

Conceived and designed the experiments: HF PN NN. Performed the experiments: MT. Analyzed the data: MT PN. Contributed reagents/materials/analysis tools: HF. Contributed to the writing of the manuscript: MT HF. Literature research: HF. Data entry: MT. Study supervision: HF. Interpretation of data: HF MT NN. Critical revision of the manuscript for important intellectual content: MT PN NN HF.

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Tropical Medicine and Health

RESEARCH Open Access

Treatment of tungiasis with a two-component dimeticone: a comparison between moistening the whole foot and directly targeting the embedded sand fleas

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Abstract

Background: Tungiasis (sand flea disease) is caused by the penetration of female sand fleas (*Tunga penetrans*, Siphonaptera) into the skin. It belongs to the neglected tropical diseases and is prevalent in South America, the Caribbean and sub-Saharan Africa. Tungiasis predominantly affects marginalized populations and resource-poor communities in both urban and rural areas. In the endemic areas, patients do not have access to an effective and safe treatment. A proof-of-principle study in rural Kenya has shown that the application of a two-component dimeticone (NYDA®) which is a mixture of two low viscosity silicone oils caused almost 80% of the embedded sand fleas to lose their viability within 7 days.

Methods: In this study we compared the efficacy of two distinct modes of application of NYDA®; one targeted application to the area where the parasite protrudes through the skin and one comprehensive application to the whole foot.

Results: Independent of the two modes of application, the dimeticone caused more than 95% of embedded sand fleas to lose all signs of viability within 7 days. The targeted application killed embedded sand fleas more rapidly compared to when the whole foot was covered. The proportion of viable lesions at day two were 7.0 versus 23.4% ($p < 0.01$) and at day five 3.9 versus 12.5% ($p < 0.02$).

Conclusions: Our findings suggest that the dimeticone could provide a safe and effective treatment for tungiasis in areas with difficult access to health care.

Trial registration: ISRCTN ISRCTN74306878

Keywords: Tungiasis, Treatment, Dimeticone, Public health

Background

Tungiasis (sand flea disease) belongs to the family of neglected tropical diseases and is prevalent in South America, the Caribbean and sub-Saharan Africa [1]. It is caused by penetration of female sand fleas (*Tunga penetrans*) into the skin and the ensuing inflammatory response [2]. The inflammation is intensified by an almost unavoidable bacterial super-infection [3]. Just about all of the lesions are found in the feet [4, 5]. The consequences of sand flea disease are debilitating, eventually leading to chronic morbidity with impaired mobility and quality of life [6, 7].

The prevalence of tungiasis varies between settings; prevalences up to 60% have been reported in various populations with up to 80% in children [8–11]. Children and the elderly are more likely to develop severe disease [4, 11, 12]. Tungiasis predominantly affects marginalized populations and people living in resource-poor communities in both urban and rural areas [8, 10, 13–15].

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Various drugs have been examined for their efficacy against embedded sand fleas in humans. Randomized controlled trials using topical or oral administration of antihelminthic drugs such as metrifonate, thiabendazole or ivermectin showed little or no efficacy at all [16–19].

The only treatment option patients in the endemic areas have is to try to kill embedded sand fleas chemically or mechanically. People often apply toxic substances such as kerosene, used engine oil or household insecticides. Alternatively, the lesions are manipulated with sharp instruments such as needles, safety pins, razor blades or thorns, a health risk by itself. Such practices can also cause additional bacterial super-infections or transmit viral pathogens such as HBV, HCV and HIV [7].

The last three abdominal segments of an embedded sand flea form a miniature cone through which the parasite remains in contact with the environment through an opening in the skin of about 250 μm. Through this opening, the female sand flea takes up oxygen, expels eggs, defecates and gets fertilized. The abdominal cone protrudes through the skin and has been identified as a target for drug treatment [20, 21]. As the skin around the abdominal cone is painful, patients usually know exactly how to localize an embedded sand flea.

A proof-of-principle study in rural Kenya has shown that the application of a two-component dimeticone (NYDA®) to the skin of the feet, repeated two times within 5 min, kills almost 80% of the embedded fleas within 7 days [21]. Furthermore, assessments of lesion morphology indicate that normal development was interrupted in those parasites not killed: the female fleas became unable to produce and/or expel eggs. Lesion-associated inflammation significantly decreased within 7 days after application of dimeticone [21].

NYDA® contains two dimeticones or silicone oils with different viscosities and a high creeping property. It is commercialized as a medical device for the treatment of head lice infestation in many European countries [22, 23]. Its mode of action is purely physical [24].

In this study, we compared the efficacy of two distinct modes of application of NYDA®: one targeted application to the area where the abdominal cone of the parasite protrudes through the skin and one general application to the whole foot.

The rationale for the targeted application was twofold: first, to minimize the volume of the dimeticone and, second, to direct the dimeticone to where it should act, namely the vital organs of the parasite located inside the abdominal cone. By consequence, a targeted application of the dimeticone should lead to a more rapid death of an embedded sand flea.

Methods

The study took place from the end of February till the end of March 2014, i.e. during the end of the dry season, when transmission of tungiasis peaks.

Study area

The study was conducted in eight primary schools in Bugiri district, Bulidha sub-county, eastern Uganda. The schools were located in the following villages: Makoma, Isaka Bisolo, Kibuye, Businda, Busakira, Nakawa and Wakawaka.

Study population

Sixty children aged 5 to 12 years selected from eight primary schools were included in the study. The number of children sampled from each school was based on the school and class size as well as on the organizational convenience. The number of children enrolled per school varied from 3 to 21.

Children from classes one to six present at the day of the investigation were eligible for the study provided they had at least three viable sand flea lesions on each foot (stage 2 and/or 3 according to the Fortaleza classification [25]) as evaluated by a rapid assessment method [26].

They should furthermore not show clinical symptoms requiring immediate medical attention such as abscesses, ulcers or intense pain. When multiple embedded sand fleas of stage 2 or 3 were present, only lesions which could be clearly distinguished from one another were included in the study.

Lesions were chosen so that their location made it possible to use the handheld digital microscope, such as at the toe, the sole or the rim of the foot. Each lesion was photographed, and its location and stage was noted in the patient's record. The number of lesions included in the study was limited to three per foot and were followed up at regular intervals for 7 days (Fig. 1).

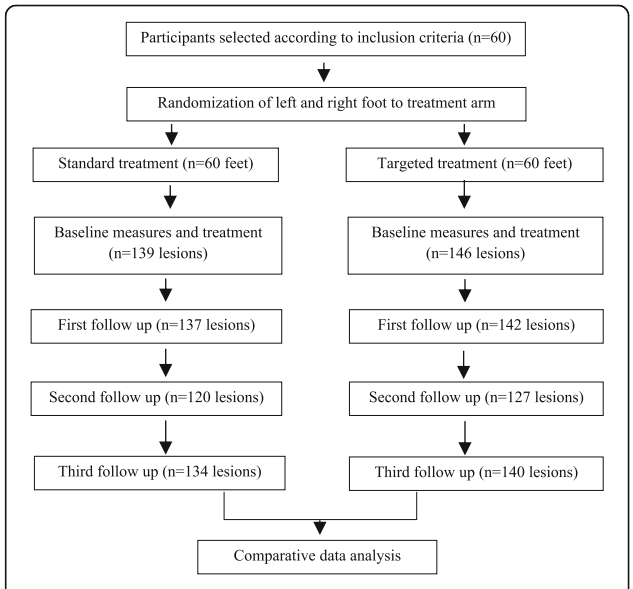

Fig. 1 Sampling of the patients, allocation of feet to treatment arms and number of lesions included

Study design

Two topical regimens of the two-component dimeticone (NYDA®) (Pohl-Boskamp GmbH & Co. KG, Hohenlockstedt, Germany) were randomly allocated to either the left or the right foot of the patient. Before the application of dimeticone, both feet were washed with water and soap and dried with a clean towel.

The regimens were labeled 'whole foot treatment' and 'targeted treatment'. Whole foot treatment meant that dimeticone was applied to the skin of the foot up to the ankle, as previously described [21]. The application was stopped when the skin became shiny, indicating that it was wetted with the dimeticone. This procedure was repeated three times within 10 min and required 2 to 5 ml depending on the size of the foot.

Targeted treatment meant that dimeticone was aspired into a 5-ml syringe to which a flexible tube was mounted. Three drops were applied to the area where the parasite's abdominal cone protruded through the skin. One drop corresponds to approximately 50 μl of dimeticone. This procedure was repeated three times within 10 min to ensure that a maximum amount of dimeticone entered into the abdominal cone of the parasite within a short period of time. This required approximately 450 μl per embedded sand flea. In both groups, the dimeticone was only applied at baseline.

For each patient, demographic data as well as baseline parasitological measurements were conducted as previously described [21, 25, 27]. Staging was performed according to the Fortaleza Classification.

- Stage I: penetrating sand flea
- Stage II: brownish-black dot with a diameter of 1-2 mm
- Stage III: circular yellow-white watch glass-like patch with a diameter of 3–10 mm and with a central black dot
- Stage IV: brownish-black crust with or without surrounding necrosis

Stage I to III are viable sand fleas; in stage IV, the parasite is dying or already dead [25].

Using a digital handheld microscope (dnt DigiMicro Mobile 5-megapixel-handheld-microscope, ITEZ, Hong Kong, China) viability signs (expulsion of eggs, excretion of faeces threads, excretion of liquid, pulsations/contractions) were recorded as present or not.

Outcome measures

All data were collected by the same investigator at baseline and during a 7-day follow-up: day two, day five and day seven.

Two major outcome measures were defined: the viability of the embedded sand flea and the intensity of the local inflammation. The primary outcome measure was the viability of the embedded sand flea according to the Fortaleza Classification [25]. An embedded sand flea was considered to be dead, if none of the four viability signs was detected during 15 min of observation by the digital handheld microscope on two consecutive follow-up examinations [20]. A secondary outcome measure was the intensity of the local inflammation, as assessed semi-quantitatively by an inflammation score [21]. Lesions manipulated by the patient or the caregiver were also documented.

Another set of outcome measures were based on visual scales depicting how the impact of tungiasis was perceived by the patient. The scales consist of a series of simple pictures illustrating itching, pain, itching-related sleep disturbance, pain-related sleep disturbance and mobility impairment as perceived by the patient. The patient was asked to classify the degree of each complaint by pointing to the corresponding picture. Zero meant no complaint at all, 1 = little complaint, 2 = moderate complaint, 3 = severe complaint and 4 = very severe complaint. These outcome measures encompass both feet, since it was considered impossible for the participants to discern the impact of embedded sand fleas separately for each foot.

Statistical methods

The sample size of circa 140 lesions per treatment group is based on a 15% difference in treatment effect, a power of 90% and a significance level of 5%. The assumption was based on our previous findings [21]. Fisher's exact test was used to compare proportions, and the Kruskal-Wallis test was used to analyze the inflammation score and self-reported tungiasis-related characteristics. A relationship was considered statistically significant when a p value was less than 5%. All presented confidence interval (CI) have a confidence level set at 95%.

Results

Baseline

At baseline, the two treatment groups displayed similar characteristics concerning the distribution of lesion types (Table 1).

The difference between the number of lesions found on the two feet for the same individual were never larger than 12 in all cases except one. In this particular case, the foot intended for whole foot treatment had 75 lesions and the foot intended for targeted treatment only nine lesions. The distribution of differences in number of lesions between the paired feet, i.e. feet on the same individual are considered a pair, showed a mean of 0.1 together with a standard deviation of 9.7, and the related median had a value of 0. The average numbers of viable

Table 1 Types of lesions at baseline in the two treatment groups

	Whole foot treatment		Targeted treatment	
Lesion type[a]	Median	(Min–max)	Median	(Min–max)
Viable lesion	4	(1–75)	5	(1–50)
Non-viable lesion	4	(0–30)	4	(0–30)
Manipulated lesion	1	(0–12)	1	(0–15)
All lesions	9	(1–94)	10	(2–70)

[a]According to definition in subjects and methods

lesions were close to seven per foot with a substantial variation in both treatment groups. The two groups also displayed a similar distribution of frequencies across the range of the parasites' developmental stages (Table 2).

During and after treatment

The outcome measured as the loss of viability of embedded sand fleas after the application of the dimeticone is shown in Fig. 2.

At the first follow-up, 2 days after treatment, the number of viable parasites decreased significantly in both groups (whole foot treatment $p < 0.001$; targeted treatment $p < 0.001$). The loss of viability was higher in the targeted treatment group ($p < 0.001$). At the second follow-up, after 5 days, the loss of viability was still higher in the targeted treatment group compared to the whole foot treatment group ($p < 0.02$). After 7 days, the reduction of the number of viable sand fleas was similar in both groups: 95% (CI 92; 99) of the parasites in the whole foot treatment group had lost all signs of viability and 97% (CI 94; 99) in the targeted treatment group (Table 3). Furthermore, in both groups, sand fleas which remained viable did not expel eggs during the 7 days. Whether lesions were in stage 2 or 3 at baseline had no impact on the efficacy of either treatment (Table 3).

The inflammation scores and the visual scale measurements showed significant reductions between baseline and day seven (Table 4).

After completion of the study (day 7), the medians of all visual scales had decreased to 0.

Table 2 Number of viable lesions per developmental stage of lesions in the two treatment groups at baseline

Stage[a]	Whole foot treatment	Targeted treatment
2a	30	39
2b	96	93
3a	12	12
3b	1	2
Total number	139	146

[a]According to the Fortaleza classification [25]

Discussion

Tungiasis is endemic in resource-poor populations in many countries of sub-Saharan Africa (8, 10, 13, 14, 15). It is associated with important morbidity, and children and the elderly carry the highest disease burden (4, 11, 12). Nonetheless, hitherto there is no approved treatment. In Kenya, the Ministry of Health recommends bathing the feet for 10 min in KMnO4, an approach with a rather low efficacy [21]. Besides, KMnO4 stains the skin in deep purple. This makes the treatment visible for everyone and the patient vulnerable to ridiculosity [21]. Hence, there is an urgent need for a safe and effective treatment of tungiasis.

The study showed that with the targeted application of the dimeticone, parasites were killed more rapidly and that 2 days after the topical application, only 7% of the parasites remained viable (Table 3). After 7 days, though, in both treatment groups, >95% of the embedded sand fleas had lost all viability signs. A more rapid death of the parasites is an advantage, because inflammation resolves as soon as an embedded sand flea has died (Thielecke M, unpublished observation 2014, [28]).

It is likely that by a repeated targeted application, more dimeticone crept into the abdominal cone per unit of time compared to when the skin of the whole foot is wetted and that this resulted in a rapid death.

Actually, when looking at the abdominal cone with the digital microscope, one could see how the dimeticone creeps into the opening in the skin and then disappears, as it spreads to the microscopic surfaces located within the abdominal cone. A similar observation has been made when dimeticone is applied to free running insects such as head lice and crickets [23].

Seven days after the application of the dimeticone, 98% of the parasites had lost all their viability signs in the targeted treatment group and 95.5% in the whole foot treatment group. Only a few sand fleas withstand the treatment, but these were not observed to expel eggs during the remainder of the study, indicating that the dimeticone had abrogated the normal development of the female sand flea as observed previously [21]. The implication of this finding is that a scaled-up treatment program could have an effect on the transmission.

Tungiasis-related symptoms decreased rapidly, when the sand fleas lost their viability by the dimeticone. This was visible both as indicated by the inflammation score assessed by the investigator, as well as by the visual scales as expressed by the patients themselves (Table 4). Notably, the median became 0 7 days after treatment in all visual scales. One week after treatment, healing was evident as seen by the decrease in the inflammation score as well as the visual scales confirming a previous finding [21].

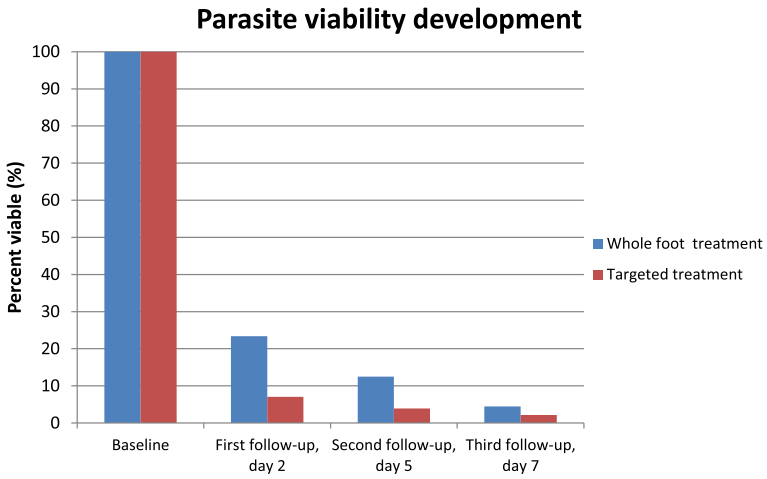

Fig. 2 Decline of parasite viability from baseline with treatment for each group through the three follow-up examinations at day 2, day 5 and day 7

During a previous study, we have observed that the inflammation around the embedded sand fleas increase when they continue to grow and intensify their metabolic activity in stages 2 and 3 [27]. The inflammation recedes, however, when the parasites develop from stage 3 to 4 [25]. In these stages, the signs of viability slowly disappear and eventually the parasites die.

The efficacy of the whole foot treatment here was higher compared to the proof-of-principle study in Kenya where dimeticone applied to the feet up to ankles killed 78% of the embedded sand fleas within 7 days [21]. Apart from the difficulty of achieving a standardized application procedure giving a precise dosage for each foot, there are also biological explanations for this difference. Rapid penetration of the dimeticone into the last abdominal segments of the embedded sand flea may depend on how deep a parasite is located in the skin. The thicker the corneal layer of the skin, the longer the dimeticone will need to reach the stratum of the epidermis in which the parasites are located. In addition, only a part of the dimeticone will be absorbed by a rough and thickened epidermis. The children in Kenya did not use shoes at all, whereas the children in Uganda, at least partially, had sandals or flip-flops. Thus, the higher efficacy of the dimeticone in this study might reflect a thinner and smoother corneal layer of the feet of the Ugandan participants.

Table 3 Viability of embedded sand fleas in the treatment groups at baseline and the subsequent follow-ups[a]

		Whole foot treatment			Targeted Treatment			
	Lesion stage[b]	No. viable	No. non-viable	Viable (%)	No. viable	No. non-viable	Viable (%)	*p* value
Baseline (day 0)	All lesions	139	0	100	146	0	100	n.a.
	stage 2	126	0	100	132	0	100	n.a.
	stage 3	13	0	100	14	0	100	n.a.
First follow-up (day 2)	All lesions	32	105	23.4	10	132	7.0	<0.001
	stage 2	31	92	25.2	8	116	6.5	<0.001
	stage 3	1	13	7.1	2	16	11.1	0.600
Second follow-up (day 5)	All lesions	15	105	12.5	5	122	3.9	0.018
	stage 2	14	95	12.8	5	109	4.4	0.030
	stage 3	1	10	9.1	0	13	0	0.458
Third follow-up (day 7)	All lesions	6	128	4.5	3	137	2.1	0.326
	stage 2	5	115	4.2	3	120	2.4	0.496
	stage 3	1	13	7.1	0	17	0.0	0.452

n.a. not applicable
[a]Not all participants and all lesions could be examined at every occasion, which explains differences in the numbers of examined lesions during follow-ups
[b]According to the Fortaleza classification [25]

Table 4 Secondary outcome measures at baseline and at day seven for both feet combined

	Baseline					Day 7					
Outcome	N	Median	IQR[a]	Min	Max	N	Median	IQR[a]	Min	Max	*p* value[b]
Inflammation score	56	4.3	3.4	1	22	57	0.5	1.5	0	12	<0.001
Visual scales											
Intensity of spontaneous pain	60	3	1	1	4	60	0	1	0	4	<0.001
Intensity of itching	60	3	1	1	4	60	0	1	0	3	<0.001
Itch-related sleep disturbance	60	2	1	1	4	60	0	1	0	4	<0.001
Pain-related sleep disturbance	60	2	1	1	4	60	0	0	0	3	<0.001
Degree of mobility impairment	52	2	1	1	4	59	0	1	0	4	<0.001

[a]Interquartile range
[b]Kruskall-Wallis test

Lesions localized to the tip of the toes, the sole and the rim of the foot were deliberately chosen so that the handheld digital microscope easily could be applied in order to assess viability signs with a higher degree of precision. At those selected sites, it can be suspected that the dimeticone might be targeted more precisely and/or penetrate the parasite more rapidly. No such selections were made in the Kenyan study. There, the included lesions were also located under the nail, under thick crusts of the corneal layer or in necrotic tissue areas into which the dimeticone cannot penetrate easily. A difference in penetration efficiency could thus possibly also explain the differing results.

The number of lesions varied considerably within the group of included children (Table 2). When the number of lesions are more than three, they often occur in clusters [29] making it difficult to distinguish the characteristics of an individual lesion, which also impairs assessments of morphological changes and the lesion-associated inflammation [21]. We, therefore, deliberately limited the number of included lesions to three per foot.

The results of this study are based on highly controlled and monitored procedures. This means one ought to be cautious when considering the true effectiveness of treatment of tungiasis with dimeticone under field conditions. Based on the average required volume of dimeticone for treatment of the whole foot and what is used in the targeted treatment, we find that the targeted application is more parsimonious as long as the number of viable embedded sand fleas does not exceed seven lesions per foot. Hence, in severe cases where individuals suffer from dozens of embedded viable sand fleas, the whole foot treatment should be more cost-effective and also more practicable.

Conclusions

The application of a mixture of a two-component dimeticone (NYDA®) caused more than 95% of embedded sand fleas to lose all defined signs of viability within 7 days. The targeted topical application worked faster compared to when the whole foot was covered. Our findings suggest that the dimeticone could provide a safe and effective treatment for tungiasis in areas with difficult access to health care.

Abbreviations

CI: Confidence interval; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; ISRCTN: International standard randomised controlled trials number; WHO: World Health Organization

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the fact that they in their raw form contain information that theoretically could make it possible to identify an individual. The data can be made available from the corresponding author on reasonable request, but to protect the integrity of the examined individuals and based on the nature of the request, we reserve the right to remove such data used to identify particular individuals.

Authors' contributions

HF, PN and MT conceived and designed the experiments. GM and NN performed the field work. PN, HF and IK analyzed the data. PN, MT, HF and IK wrote the paper. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing interests

HF has received lecture fees from Pohl-Boskamp GmbH and Co KG, Hohenlockstedt, Germany, the producer of NYDA. The company had no role in the design, execution or interpretation of the study. The other authors do not have any conflicts of interest to declare.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was registered in the Current Controlled Trials 2014 (ISRCTN74306878) and approved by the Ethics Committee of Makerere University College of Health Sciences, School of Public Health, Makerere, Uganda, (Higher Degrees Research and Ethics Committee Protocol No. 157). Written consent was signed, either by signature or fingerprint, by the

guardians of the participants after they were informed about the trial in the local language Lusoga. The headmaster of the respective schools acted as a witness. At the end of the study, any remaining viable sand fleas were treated with a targeted application of dimeticone. All participants received a new pair of closed shoes, since wearing such shoes has been shown to reduce the incidence of sand flea disease [25]. Children not eligible for the study because of symptoms, such as intense pain, abscesses or ulcers, which required immediate treatment, were referred to the nearest Community Health Center.

Endnotes
Not applicable.

Publisher's Note

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