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# Treatment of tungiasis using a tea tree oil-based gel formulation: protocol for a randomised controlled proof-of-principle trial

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## ABSTRACT

**Introduction:** Tungiasis (sand flea disease or jigger infestation) is a neglected tropical disease caused by penetration of female sand fleas, *Tunga penetrans*, in the skin. The disease inflicts immense pain and suffering on millions of people, particularly children, in Latin America, the Caribbean and sub-Saharan Africa. Currently, there is no standard treatment for tungiasis, and a simple, safe, and effective tungiasis treatment option is required. Tea tree oil (TTO) has long been used as parasiticidal agent against ectoparasites such as headlice, mites, and fleas with proven safety and efficacy data. However, current data are insufficient to warrant a recommendation for its use in tungiasis. This trial aims to generate these data by comparing the safety and efficacy of a 5% (v/w) TTO proprietary gel formulation with 0.05% (w/v) potassium permanganate ($KMnO_4$) solution for tungiasis treatment.

**Methods and analysis:** This trial is a randomised controlled trial (RCT) in primary schools (n=8) in South-Western Kenya. The study will include school children (n=88) aged 6-15 years with a confirmed diagnosis of tungiasis. The participants will be randomised in a 1:1 ratio to receive a 3-day twice daily treatment of either 5% TTO gel or 0.05% $KMnO_4$ solution. Two viable embedded sandflea lesions per participant will be targeted and the viability of these lesions will be followed throughout the study using a digital handheld microscope. The primary outcome is the proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 (9 days after first treatment). Secondary outcomes include improvement in acute tungiasis morbidities assessed using a validated severity score for tungiasis, safety assessed through adverse events (AEs), and product acceptability assessed by interviewing the participants to rate the treatment in terms of effectiveness, side effects, convenience, suitability, and overall satisfaction.

**Ethics and dissemination:** The trial protocol has been reviewed and approved by the University of Canberra Human Research Ethics Committee (HREC-2019-2114). The findings of the study will be presented at scientific conferences and published in a peer-reviewed journal.

**Trial registration**: ACTRN12619001610123; PACTR202003651095100; and Universal Trial Number-U1111-1243-2294.


**Keywords:** Children, Protocol, Randomised controlled trial, Tea tree oil, Tropical medicine, Skin infection, Tungiasis, NTD

## STRENGTHS AND LIMITATIONS OF THIS STUDY

### Strengths

- Given tungiasis affects the most disadvantaged communities, this work has an ultimate objective of reducing the tungiasis burden in endemic settings and improving the health and wellbeing of affected children and.
- Educational and community support packages (e.g. health education using flip charts) delivered to the participating communities as part of this study, will help facilitate the appropriate control of tungiasis with sustainable benefits to the community.
- If TTO gel effectively treats tungiasis, this would provide compelling evidence for a simple, affordable and effective treatment for tungiasis, which does not require direct supervision by a trained health worker, essentially enabling the communities and/or individuals to manage their own health.

### Limitations

- Compliance to the treatment protocol is likely to be less than ideal in the targeted study settings in Kenya, and we also expect considerable attrition; however, regular follow up is likely to improve patient compliance and limit attrition.
- The identity of trial interventions couldn't not be concealed to the study participants and caregivers.

## INTRODUCTION

Tungiasis (sand flea disease or jigger infestation), is a Neglected Tropical Disease (NTD) caused by penetration of an ectoparasite, female sand flea *Tunga penetrans*.[1-3] It is rampant in resource-limited communities in Latin America, the Caribbean, and sub-Saharan Africa,[2] where children (aged 5-14 years) and the elderly (≥60 years) are most heavily affected, with prevalence data ranging from 50% -85%.[4-6] No clear estimates of global burden of tungiasis exist,[7] but according to the World Health Organisation (WHO), 20 million people are estimated to be at risk of developing tungiasis in South America alone.[8] Based on Kenyan and Ugandan Ministries of Health,[9] [10] about 4 million people suffer from the tungiasis, with another 16 million are at risk. A tungiasis infected person can harbour up to hundreds of parasites, usually on the feet and hands with toes, soles, and heels are the sites most frequently affected.[6] [11] The infection results in intense inflammation and itching, and frequently occurs with secondary bacterial infections, resulting in abscesses, suppuration, cellulitis, lymphangitis, sepsis, tetanus, and post-streptococcal glomerulonephritis. Repeated infection can lead to deformation and loss

of nails, and disfigurement of the feet.[8 12-15] Tungiasis negatively impacts education (in children), quality of life, household economy, and wellbeing for affected individuals.[8 9 16-19]

Currently, there is no standard treatment for tungiasis.[19] Parasiticides such as oral thiabendazole,[20] oral ivermectin,[21] and topical benzyl benzoate[22] and disinfectants like hydrogen peroxide,[9] have been explored for tungiasis treatment, but there is little conclusive evidence available on their safety or effectiveness. Our seminal systematic review on this topic identified (Abrha *et al*, Lancet infectious disease , 2020) eight RCTs[23-30] investigated interventions for tungiasis. These included: coconut oil-based lotion (Zanzarin®) for prevention; and oral – niridazole and ivermectin; topical–ivermectin lotion, metrifonate lotion, thiabendazole lotion, thiabendazole ointment, dimeticones (NYDA®), and a neem seed and coconut oils-based mixture for treatment. Among these, the coconut oil-based lotion for prevention, and dimeticones for treatment of tungiasis displayed the most promise. However, the coconut oil-based lotion is no longer commercially available and dimeticones are expensive and currently not available in tungiasis endemic areas in sub-Saharan Africa including Kenya, thus limiting treatment options to surgical extraction of embedded fleas and bathing feet with 0.05% potassium permanganate ($KMnO_4$) solution.[30 31] In such settings, surgical extraction is frequently performed using unsafe procedures involving sharing of sharp instruments, leading to additional bacterial superinfections, and potential transmission of viral pathogens like HIV, Hepatitis B, and Hepatitis C.[12,32 33] Although bathing feet with 0.05% $KMnO_4$ solution is widely used in Kenya and is recommended by the country's Ministry of Health,[9] recent trials[24 30] have revealed that it was only marginally effective, killing less than 40% of embedded fleas.

Thus, there is a critical need for new, safe, effective, and affordable treatments for tungiasis. This trial aims to address this unmet critical need by trialling a novel 5% tea tee oil (TTO) gel-based skin formulation. Unlike current treatment agents used, TTO possesses a unique combination of potent insecticidal, acaricidal, antibacterial, anti-inflammatory, and wound healing properties. [34 35] It has long been used as a helpful topical treatment agent for a variety of epidermal parasitic skin diseases in Australia and Europe, with good safety and efficacy data.[36] The insecticidal and acaricidal effects of topical formulations of TTO for a range of medical ectoparasites/pests, including house dust mites, *Demodex* mites, ticks, scabies mites, headlice and fleas, have been investigated in several *in vitro*, animal and clinical studies, reporting an efficacy range of 70-100% for these vectors.[37-42] TTO is also effective at low concentrations (*in vitro*) as a bactericide (at 0.002–2%; including against MRSA [methicillin-

resistant *S. aureus*]), and as an anti-inflammatory agent (≤ 0.125%).[35] In sum, an ideal therapeutic candidate for tungiasis should be able to kill the embedded parasite, prevent inflammatory skin reactions, and block bacterial infection. The unique parasiticidal, antibacterial, and anti-inflammatory properties of TTO appear to hold tremendous potential in reducing the burden of tungiasis and its deadly sequalae. The aim of this RCT is to investigate the safety and efficacy of a 5% v/w TTO-proprietary gel formulation in comparison with the locally endorsed, 0.05% w/v $KMnO_4$ solution for tungiasis treatment in children.

## METHODS AND ANALYSIS

This protocol has been written in line with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (Supplemental file 1).[43]

### Study setting and design overview

The study will be conducted at eight selected primary schools (permission letters obtained from the respective directors of the schools) in Kisii and Nyamira counties, South-Western Kenya where tungiasis is endemic.[9] Schools have been selected based on the presence of students with tungiasis and willingness of the principals to collaborate in the study. Schools already have strong collaborative working relationships with our community collaborator, Global School Partners (GSP), a local non-government and not-for-profit charity organisation in Kenya (GSP).[44] This pre-existing network of the GSP with school directors and student parents will be utilised to facilitate the successful completion of this study.

The study is designed as an assessor-blinded Phase II RCT. It will be conducted in the dry season as tungiasis peaks during this period.[14] Eighty-eight participants with tungiasis will be recruited and randomised in a 1:1 ratio to receive either the 5% TTO proprietary gel formulation or 0.05% $KMnO_4$ solution. TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. It contains 5% v/w pure and standard Australian TTO (ISO 4730: 2017 and AS 2782: 2017), approximately 14% poloxamer 407 gel, and other excipients such as formulation stabilisers, penetration enhancers, and preservatives. It will be prepared following the WHO's current Good Manufacturing Practice (Institute of Drug Technology (IDT) Limited, Australia). $KMnO_4$ solution contains 0.05g $KMnO_4$ in a litre of water. The selection of $KMnO_4$ solution as the active comparator in this study reflects its status as a local tungiasis treatment used in mass campaigns in children (and adults) in Kenya,[45] and its being the recommended tungiasis treatment by the Kenyan Ministry of Health.[9] Study participants' feet will be fully assessed as

more than 95% of embedded sand flea lesions are localised to this site (toes, soles, and heels),[6 7 46] with lesions staged according to Fortaleza classification system (Supplemental file 2).[47] The test and control interventions will then be applied twice-daily on days 1, 4, and 7. These treatment days are selected based on the lifecycle of the embedded sand flea. As a sand flea can take up to 1-2 weeks to develop from stage II/III (viable embedded lesions) to stage IV (dying or dead embedded sand flea),[27 47] the use of the 3 treatment doses is designed to ensure that any stage II or III embedded sand flea lesion would be killed by the treatments before they die due to their natural course. After the treatment, viability signs of embedded fleas in each participant will be monitored. The proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 will be determined and compared between test and control groups, as the primary outcome.

**Study personnel**

The trial will be conducted by a recruitment team and a study team in each school. These teams will be composed of staff members of GSP.[44] The recruitment team will consist of school nurses led by a recruitment officer. This team will be responsible for liaising with the school directors and caregivers to facilitate the participants' informed consent and allocation procedures. The school directors will be used as mediators to reach out to caregivers and potential participants. The members of the team will receive information and training about the trial particularly the recruitment procedure.

The study team will comprise clinical advisors and clinical assessors, led by one of the clinical advisors. The clinical advisors are experienced medical doctors working in hospitals located in the study areas. The clinical assessors are school nurses who will be responsible for collecting baseline demographic and disease characteristics, treating participants and performing outcome assessments. They will be trained on the overall trial and outcome assessment (viability assessment and staging of the embedded sand fleas), intervention application, and safety monitoring procedures. The clinical advisors will supervise the clinical assessors, particularly in outcome assessment procedures, and be consulted in any case of diagnostic uncertainty. The reports of clinical assessors will further be evaluated by a panel of infectious disease specialists or offsite clinical assessors by evaluating the photograph records of each participant.

**Sample size calculation**

The sample size calculation is based on the primary outcome measure, assuming the 0.05% $KMnO_4$ solution will have a 40% efficacy[24], and the 5% TTO proprietary gel formulation will

have a 70% efficacy at 10 days. There are no reports of clinical trials exploring TTO proprietary gel formulation for tungiasis treatment. Hence, the estimated efficacy of TTO was estimated based on the existing observational studies on tungiasis, the clinical experience of our team members, findings from similar trials exploring other tungiasis treatments, and findings of studies (*in vitro* and *in vivo*) on TTO against other ectoparasites. To enable the detection of this 30% difference with at least 80% power at a significance level of 5%, a sample size of 40 participants per arm (88 in total accounting for 10% attrition, as seen in similar settings[26]) is required.

**Study participants**

The study population will consist of school children aged 6–15 years from eight schools with a confirmed diagnosis of tungiasis. The age range of 6–15 years was selected because tungiasis is highly prevalent in this group.[48 49]

**Consent and assent**

Before starting the study, face-to-face meetings with caregivers, participants, and school directors will be held to explain the objectives of the research and to facilitate an understanding of how the research aligns with community values. The overall procedure of the study, the nature of the disease, the preventive strategies, details of the treatments, and risks and benefits of participation will also be explained to caregivers and participants using instruction manuals containing coloured photographic images to ensure they fully understand the consequences of participation. A pictorial consent flipchart will be used and any study documents including information booklet will be translated into the locally spoken language to assist and facilitate the consent process After this explanation, the participant's legally responsible caregivers caregiver/parent will be provided a participant information sheet and asked to complete an informed consent with written assent (if aged 12-15 years) or verbal assent (if aged 6-11 years) provided by children (Supplemental file 3 and 4). If a subject and his/her caregiver are unable to read, an impartial witness must be present during the entire informed consent discussion. The signature of the impartial witness will certify the subject's consent. The participant's parent/caregiver subject will receive a signed and dated copy of the consent from.

**Recruitment and enrolment**

Potential participants with tungiasis will be identified in each school and recruited by the recruitment team over three months. Eligibility assessment (presence of viable embedded sand fleas) will be initiated by the clinical assessors under the supervision of the clinical advisor as per the inclusion and exclusion criteria. If a potential participant meets the study criteria, he or she will be invited to a room designated for study procedures, referred to as a study centre, for further examination.

Participants' must have at least one viable embedded sand flea lesion (stage II or Stage III) as inclusion criterium. Viable embedded sand flea lesions located at the tip of toes, soles, and rim of heels will be exclusively selected for this purpose.

Participants' exclusion criteria are 1) participants with cluster lesions (≥3 together) or manipulated lesions. 2) the presence of complicated sand flea lesions requiring antibiotic treatment (these children will be referred to nearby health facilities for appropriate management); 3) evidence that guardian/parent/caregiver intend to change their place of residence during the study period; 4) known history of allergy to any of the study medications (TTO or other essential oils and/or $KMnO_4$); and 5) the use of systemic or topical drugs or medications, particularly antibiotics, which may interfere with the study results.

Eligible participants will be instructed to come back to the study centre located in each school for randomisation, baseline assessment, treatment, and outcome assessments. An outline of the recruitment and enrolment process with study timeline is given in **Figure** 1.

Figure 1: Overview of the study process. *BID- twice daily

**Randomisation and treatment allocation**

Participants will be allocated to either the test (5% TTO gel) or control (0.05% $KMnO_4$ solution) group in a 1:1 ratio using a predetermined, computer-generated randomisation schedule developed by an independent statistician who will not be directly involved in the study. All participants in each school will be allocated in the study with participant from new schools included to the study until the minimum sample is reached. The randomisation schedule will be kept secure (password-protected) by the statistician. The randomisation schedule will be concealed from trial participants, clinical assessors, and data assessors (who will be analysing the data) until the participants have been assigned into the trial.

**Blinding**

Foot bathing with the $KMnO_4$ solution may change the colour of the skin to dark purple. As a result, the trial participants and onsite clinical assessors cannot be blinded to the trial interventions. However, a blind assessment of photographs of tungiasis lesions by an expert panel of clinicians (offsite clinical assessors) during the data analysis phase will prevent any likelihood of investigator bias in the outcome assessments. To keep the offsite clinical assessors and data assessors blind, they will not be involved in the clinical trial procedures or have any contact with trial participants. Given the primary efficacy outcome will be measured three days after the last treatment, we do not consider that the colour of $KMnO_4$ solution on the feet would compromise the blinding of offsite clinical assessors. The onsite team will carefully assess the skin surrounding the targeted lesions and ensure the absence of an any trace of purple staining prior to taking the photographs. In any case that the blinding is broken, the study team will document the date and reason for breaking.

**Study participant treatment**

The randomised participants will receive either the test (5% TTO-proprietary gel formulation) or control (0.05% $KMnO_4$ solution) intervention. They will be required to attend the study centre in each school twice-daily (AM and PM) on days 1, 4 and 7 for the treatments. At each visit the feet of the participants will be washed with water and soap, dried with a clean towel, and toenails clipped as necessary to enable the easier application of the products. The allocated treatments will be applied by the clinical assessors. The test product will be applied by smearing the required amount (up to 8g/day) of the product on the palms and spreading it over the skin surface of the feet up to the ankle including the soles, and interdigital areas (between toes). The treated feet will then be left for about 15 minutes to allow the test products to dry. In contrast, the comparator product will be applied by immersing and bathing the feet up to the ankle in a bucket containing a 0.05% KMnO4 solution (up to 2.5 litres) for 15 minutes. After sun drying the feet, a thin layer of petroleum jelly, fully covering the treated surface, will be applied for the purpose of softening the roughness on the skin caused by the KMnO4 treatment.

After the initial treatment (Day 1 AM), all participants will be given pairs of closed shoes to be worn throughout the study period and to be kept after the study participation. This will help the treatments remain on the feet and protect the feet from contamination with dirt and water. Also, wearing closed shoes may decrease reinfestation. Participants will be advised to avoid using or mixing any other tungiasis treatments with trial medications during the study period.

They will also be advised at each visit to regularly wear the provided pair of shoes throughout the study period. Dates and times of start and end of treatment application, as well as any noncompliance with the trial protocol will be documented in the CRF.

**Outcome assessment**

*Primary outcome*

The primary and secondary efficacy end points are the proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 (9 days after first treatment) and by day 5 (4 days after the first treatment), respectively. Participants will be required to attend the study centre in each school once daily (AM or PM) at baseline, Days 5 and 10 for the outcome assessment. At baseline, viability of the embedded sand flea lesions located in the feet will be assessed using a handheld digital video microscope, assisted with pictorial flipcharts. Sites of all viable (stage II – III) lesions will be recorded on the foot diagram sheets and the entire feet and appearance will be photographed to document the baseline characteristics of the embedded sandflea lesions. Two viable embedded sand flea lesions will be selected as target lesions and will then be observed for their viability at each outcome assessment visit. All the information collected at baseline, such as the number of viable embedded sand flea lesions, non-viable lesions, manipulated lesions, SSAT, itching, pain, and pain-related and itching-related sleep disturbance, will be documented and recorded in each participant's case report form (CRF). The photographs will also be linked to the participant's CRF (Supplemental file 5). At each follow up visit, the entire feet of participants will also be thoroughly examined and the two target lesions per participant, selected during bassline assessment, will be observed for their viability on days 5 and 10. The number of target lesions that become non-viable after the interventions will be recorded for each study participant at each follow-up visit. Photographs will be recorded and reviewed during the analysis phase to confirm observations recorded in the CRF

A panel of blinded offsite clinical assessors will independently evaluate photographs of the targeted embedded sand flea lesions taken at baseline, Days 5 and 10 independently of the onsite clinical assessors and the primary outcome measure will be determined by the blinded photograph assessment of the offsite clinical assessor. Any discrepancy in the assessment results will be adjudicated by a third person. An empirical evaluation of the onsite versus offsite agreement, using the kappa coefficient will be performed to determine reliability of the

assessment. To evaluate the efficacy of the test intervention, the proportions of non-viable lesions in the test group will be compared with the control groups at day 10.

*Secondary outcomes*

The secondary outcomes are severity score for acute morbidities (SSAT), itching, pain, pain-related and itching-related sleep disturbance, safety, and participant acceptability of the trial intervention/s. The SSAT, which includes typical signs of local inflammation (erythema, oedema and warmness) and the presence of suppuration, ulcers and fissures, will be evaluated by the clinical assessors at baseline, days 5 and 10, using a validated scoring system designed for tungiasis morbidity assessment.[50] The entire feet and appearance will be photographed and recorded in the CRF to evaluate this outcome measure. The itch-man scale for pain,[51] and 4 point tungiasis pictorial scales[18] for pain, and pain-related and itching related sleep disturbance will be used to evaluate these outcomes.

Safety will be assessed through adverse events (AEs) and evaluations of the skin irritation during each visit (days 1, 4, 5, 7 and 10). Participants/caregivers (in-person or on the phone) will be asked at each follow-up visit by the study team about the occurrence of local (stinging/burning, irritation and itching) or systemic AEs (nausea and headache). Children will be physically examined for evidence of local swelling, erythema and fever. The severity of the AEs will be categorised as mild (Grade 1), moderate (Grade 2), severe (Grade 3) and life-threatening (Grade 4) according to the common terminology criteria for adverse events (CTCAE) v5.0 guideline ( Supplemental file 2).[52] Acceptability of the treatments will be assessed at the end of the study (day 10) by asking the participants to rate the treatment in terms of effectiveness, side effects, convenience, suitability, and overall satisfaction. Responses to these questions will be recorded in the CRF.

**Adherence and retention**

Continuous motivation and advice will be given by the clinical assessors to the participants at each visit throughout the study to promote study retention. Community home visits will also be organised, if required (e.g. in case of absenteeism from school).

**Monitoring and reporting of adverse events (AEs)**

If AEs occur, the clinical advisors will determine the relationship between the AEs and the trial medication. AEs considered related to the trial medication will be followed up until either resolution, or the event is considered stable. All Grade 1 and 2 AEs reported spontaneously by the subject or observed by the study team will be recorded in the AE form and documented in

each participant's CRF. The following information about each AE will also be recorded where available: description, onset and end date, severity, expectedness, assessment of relatedness to trial medication, what action was taken afterwards, and whether the participant was withdrawn from the trial.

The SAEs will also be reported to the Human Ethics Committees and regulatory bodies as per the reporting schedule stipulated in their guidelines. The following information will be documented in the SAE form: description, classification, start date, status/outcome, relatedness to study intervention, therapy given, and any actions taken to study intervention.

**Statistical analysis**

All data will be reported following the Consolidated Standards of Reporting Trial (CONSORT) guidelines (Supplemental file 6).[53] A detailed analysis plan will be approved by all investigators before any data analysis. The data will be analysed by the study statistician who will be blinded to the treatment allocation. Statistical analyses will be performed for both the intention to treat (ITT) and per-protocol (PP) populations. The ITT population will include all randomised participants treated or not, and any participants who withdraw prematurely or poorly comply with the protocol. The PP population will be all subjects who are enrolled in the study, randomly assigned to the treatment regimen, received three doses (twice daily) and did not deviate from the study protocol in a clinically significant manner. Results will be considered significant if $p \leq 0.05$.

Baseline characteristics collected on each patient will be reported and compared between randomisation group including age, sex, number of viable embedded sand flea lesions, SSAT, as well as scores for pain, itching and sleep disturbance. Categorical (qualitative) variables will be summarised by frequency and percentage. Continuous variables will be summarised as mean and standard deviation in case of normal distribution and as median and interquartile range in case of non-normal distribution. The Shapiro-Wilk test will be used to assess the normality of the distribution of outcome variables for both groups. Independent student's t or Mann-Whitney tests will be used to investigate differences in continuous variables, and chi-squared tests will be used to identify significant variations in proportions across treatment groups.

Based on the change in primary outcome, the efficacy of test and comparator products will be compared at each follow up visit. The difference in proportion of non-viable lesions between the test and control groups will be compared using student's t-test or Mann-Whitney tests depending on the distribution and presented as relative and absolute risk reductions with their

respective 95% confidence intervals and P values. Further, within-group differences will be assessed using paired t-test in case of normally distributed data and a Wilcoxon signed-rank test in case of non-normally distributed data. Secondary outcomes will be compared in the same fashion as the primary outcome.

### Study management

Quality assurance audits of the clinical trial and related documentation will be performed during and after this study in accordance with the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines and recommendations.[54 55] The quality assurance will also consider the Kenyan Good Clinical Practice (GCP) guideline and the Pharmacy and Poisons Board (PPB) requirements. Trial SOPs will be used to ensure that the trial will be conducted, and data are generated, documented (recorded), and reported in compliance with the latest approved protocol, ICH-GCP, Declaration of Helsinki, Kenyan GCP, PPB and National Commission for Science Technology and Innovation (NACOSTI) requirements. The data monitoring committee will involve a medical practitioner, toxicologist and pharmacist..

## ETHICS AND DISSEMINATION

### Ethical approval

The trial protocol has been approved on August 29, 2019 by the University of Canberra Human Research Ethics Committee (HREC20192114) and registered with WHO accredited registries (Supplemental files 7–9). Further, the investigators will secure ethical approvals from one of the National Commission for Science Technology and Innovation (NACOSTI, Kenya) accredited ethics review committees and will seek letters of support from both the Kenyan Ministry of Health and Ministry of Education.

### Confidentiality and access to data

The privacy of participants will be protected by appropriate collection and storage of data. Participants will be identified only by initials and a participant ID number on the CRFs and in any electronic databases. Data collection forms will be stored in locked filing cabinets in a locked office at the participating schools until the end of the study period, which will then be transferred to the University of Canberra and handled as per the university's recommended data storage guideline for clinical trials. All documents will only be accessible by trial staff and authorised personnel. Documents containing participant's identifying information will not be stored electronically and will be anonymised as soon as practical. Participants will be advised

their records may be examined by lawful authorities but will be treated with strict confidentiality and will not be made publicly available.

### Dissemination

Study results and feedback will be disseminated to end-users (participants and community members counties' health bureaus and other relevant government organisations) in formats that are useful and understandable, such as community meetings, presentations, websites, and social media. The findings of this study will also be disseminated through peer-reviewed journals and national and international scientific meetings.

### Patient and public involvement

Patients and/or the public were not involved in the study design, or conduct, or reporting, or dissemination plans of this research. Study results and feedback will be disseminated to patients by local trial team in formats that are useful and understandable, such as community meetings, presentations, websites, and social media

## DISCUSSION

In endemic communities, tungiasis morbidity is caused by the parasite and associated inflammatory skin reactions and secondary bacterial infections. Thus, proposed treatment options should have the potential to address the morbidities caused by the parasite and treat secondary bacterial complications. In this vein, TTO is a strong fit for tungiasis treatment as it possesses a unique combination of parasiticidal, antibacterial, anti-inflammatory, and wound healing properties.[35] There has been a claim that TTO causes skin irritation or allergic contact dermatitis.[56] In a suitable pharmaceutical base at concentrations ≤25%, multiple clinical studies[57-63] have shown that TTO has no or low risk of adverse skin reactions. While potential toxicity in children is yet to be extensively evaluated, a report from a RCT[64] in children (mean age 6.3+5.1 years) with viral molluscum contagiosum demonstrated that 75% (v/v) TTO was well tolerated in the 30-day treatment period. TTO's sensitising potential is largely due to elevated levels of peroxides and other degradation products from oxidised oil.[65] When correctly stored in amber glass bottles with polypropylene caps, TTO has no appreciable degradation for up to 12 months.[35 56] Due to its high volatility, 90% of the applied TTO rapidly evaporates, minimising the potential for components to permeate the dermis and bloodstream. Nevertheless, key active components (terpinen-4-ol, α-terpineol, and 1,8-cineole) have sufficient epidermal penetration to provide antimicrobial, anti-inflammatory and potentially insecticidal and acaricidal effects.[34]

If TTO gel effectively treats tungiasis, this trial will provide compelling evidence for a simple, affordable and effective treatment for disadvantaged populations with a significant health burden. This will lead to a significant change in the treatment of this neglected condition. While the tungiasis-affected children in selected Kenyan villages are intended as the primary beneficiaries of this research, the pattern of tungiasis and associated bacterial complications among children is analogous to that observed in resource-poor and underprivileged endemic communities in many parts of the world, especially in sub-Saharan Africa. Thus, the results from this study have the potential to provide evidence for a global health role of TTO in managing tungiasis and its associated complications in children.

**Authors' contributions:** SA and JT conceived the study. GP, AB, JCK, JH, JM and SC contributed to the study design. SA and JT drafted the manuscript. JKC, JM, WT, SVN, AYC TS, SK, GK, SC, JH, HF, AB, MD, and GMP assisted in developing the protocol and have reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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**Competing interests' statement:** None

**Patient consent for publication**: Not required.

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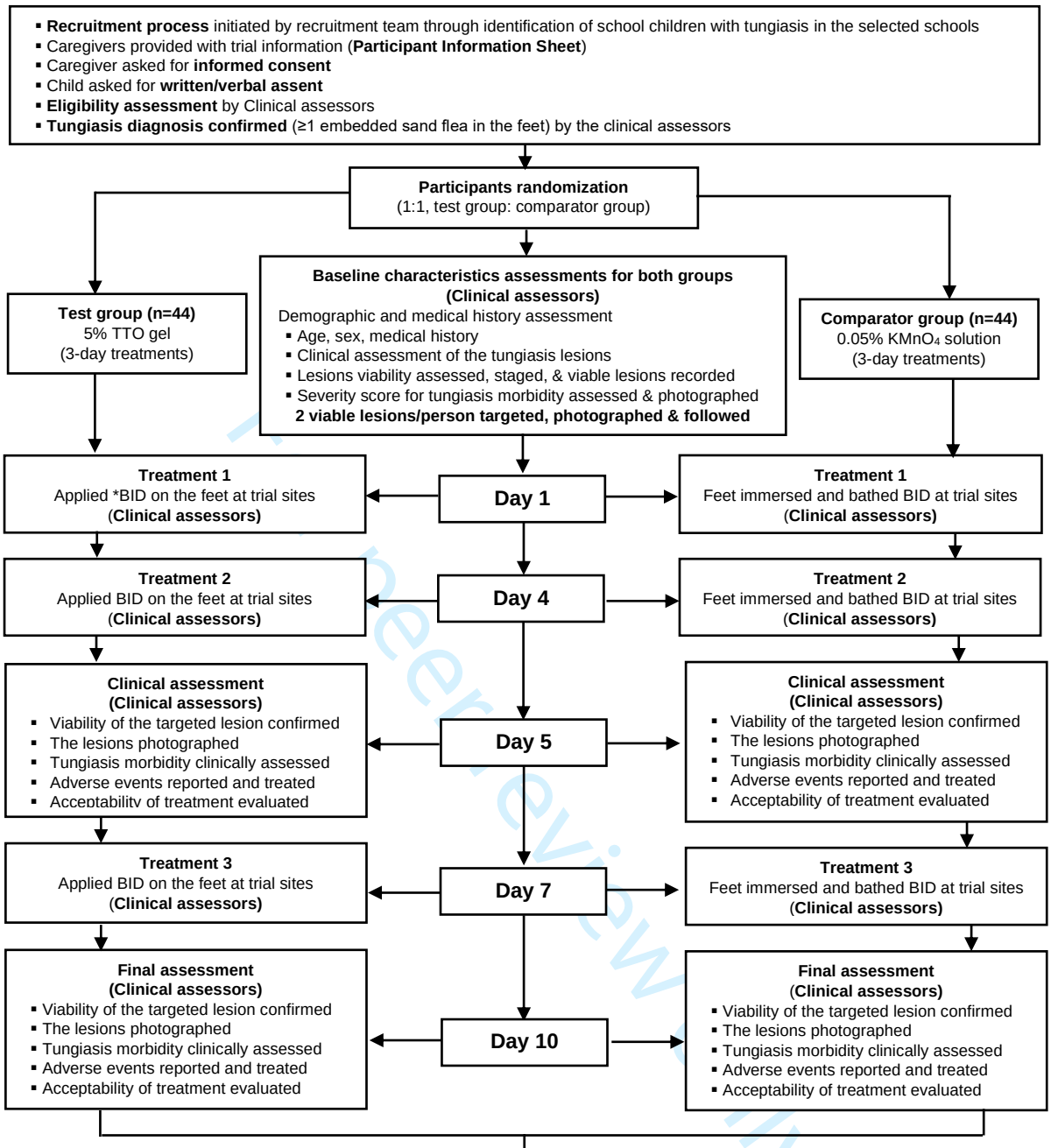


Figure 1: Overview of the study process. *BID- twice daily


SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Page NO |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 2 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | Attached as supplement |
| Protocol version | 3 | Date and version identifier | Attached as supplement |
| Funding | 4 | Sources and types of financial, material, and other support | 15 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1 &15 |
| | 5b | Name and contact information for the trial sponsor | 1 & 15 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | None |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see item 21a for data monitoring committee) | 6 |

| | | | |
|---|---|---|---|
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 3-5 |
| | 6b | Explanation for choice of comparators | 5-6 |
| Objectives | 7 | Specific objectives or hypotheses | 5 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 5 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 5-6 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 8 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 5-6 & 8-9 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 12-13 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 11 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 8 |

| | | | |
|---|---|---|---|
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 7-11, Figure 1 and Supplemental file 2 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 7 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 8 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 8 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 9 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 9 |
| **Methods: Data collection, management, and analysis** | | | |

| | | | |
|---|---|---|---|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10-11 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10-11 & 12-13 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 13-14 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12-13 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 12-13 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12-13 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 13 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 13-14 |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 11-12 |

| | | | |
|---|---|---|---|
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 13 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | Attached as supplement |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 6-7 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 13-14 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 15 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 13-14 |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 11-12 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 14 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 14 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 13-14 |
| **Appendices** | | | |

| | | | |
|---|---|---|---|
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Attached as supplement |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

# Supplemental file 2

## Supplemental file 2.1: Fortaleza classification system

Embedded sand flea lesions are stratified into different developmental stages, as per the Fortaleza classification system[1] .

**Table 1:** Fortaleza classification system

| Stages | Appearance/phases | Symptoms | Time span |
|---|---|---|---|
| Stage I | Penetrating flea (penetration) | Erythema, and itching | 30 min–several hours |
| Stage II | Brownish-black dot (beginning of hypertrophy) | Erythema surrounding a central black dot, unpleasant itching, and pain | 1–2 days after penetration |
| Stage $III_a$ | White (tender) halo with black dot at the centre (hypertrophy) | Eggs expulsion, faecal coil, brownish-watery secretion, pulsation, severe itching, pain, and tenderness | 2– 6 days after penetration |
| Stage $III_b$ | White (non-tender) halo with caldera formation, discoloration, and skin peeling around lesion (hypertrophy) | Eggs (white and shining) expulsion, faecal coil, pulsation, watery secretion, severe pain while walking, and loss of tenderness | 6 days–3 weeks after penetration |
| Stage $IV_a$ | Brownish-black wrinkled lesion (involution) | Rare egg expulsion and pulsation, sporadic faecal expulsion, and watery secretion | 3–4 weeks after penetration |
| Stage $IV_b$ | Brownish-black, necrotised, desiccated lesion (crust) (involution) | No vital signs (pulsation, egg, faeces, and watery secretion), (dead flea) | 4–6 weeks after penetration |
| Stage V | Circular depression in the stratum corneum (residue) | No flea | 6 weeks–several months after penetration |

Stage II and III lesions can be classified as viable embedded sand flea lesions, whereas stage IV is classified as a lesion with either a dying ($IV_a$) or dead ($IV_b$) embedded flea. An embedded sand flea is considered to be viable when any of the viability signs (expulsion of eggs, excretion of faecal threads, excretion of faecal liquid, and/or pulsations/contractions) are observed using diagnostic tools (hand held digital microscope).[1]

## Supplemental file 2.2: Study schedule

**Table 2**: Study schedule of enrolment, interventions, and assessments.

| Study procedures | Time points | | | | | |
|---|---|---|---|---|---|---|
| | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 |
| | Day 0* | Day 1 | Day 4 | Day 5 | Day 7 | Day 10 |
| **Recruitment and enrolment** | | | | | | |
| Training clinical recruitment and study team | X | | | | | |
| Identifying potential participants with tungiasis | X | | | | | |
| Participant information sheet | X | | | | | |
| Informed consent/assent | X | | | | | |
| Subject demographics / medical history | X | | | | | |
| Inclusion/exclusion criteria - review | X | | | | | |
| Concomitant medications - review | X | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Subjects instructions | X | | | | | |
| Subject randomisation | X | | | | | |
| Baseline assessment-lesion viability & staging | X | | | | | |
| Baseline assessment-acute tungiasis morbidity | X | | | | | |
| **Study intervention** | | | | | | |
| Distribution of intervention products | X | X | X | | X | |
| Application of test intervention | | X | X | | X | |
| Application of control intervention | | X | X | | X | |
| **Outcome assessment** | | | | | | |
| Efficacy outcome-viability of embedded sand flea | | | | X | | X |
| Acute morbidity outcome-SSAT, itching & sleep disturbance | | | | X | | X |
| Safety outcome-monitoring AEs | | X | X | X | X | X |
| Product acceptability outcome | | | | X | | X |
| Study compliance confirmation | | X | X | X | X | X |

## Supplemental file 2.3: Adverse events grading

**Table 3**: Grading severity of adverse events.

| Grade | Type | Description |
|---|---|---|
| Grade 1 | Mild | Signs or symptoms which are easily tolerated, does not interfere with the subject's usual function; clinical or diagnostic observations only; intervention not indicated |
| Grade 2 | Moderate | Signs or symptoms causes interference with usual activity or affects clinical status; minimal, local or non-invasive intervention indicated |
| Grade 3 | Severe | Signs or symptoms affect clinical status and likely requires medical intervention and/or close follow-up |
| Grade 4 | Life-threatening | Sign or symptom results in a potential threat to life; urgent intervention indicated<br>This grade will be considered as SAE |

## Supplemental file 2.4: Parasiticidal and repellent effects of tea tree oil (TTO)

**Table 4:** Summary of studies on the insecticidal, acaricidal, and repellent effects of TTO.

| Study setting | Study design | TTO concentration or volume tested | Ectoparasite (insect or arachnid) | Treatment outcome |
|---|---|---|---|---|
| Akkad *et al*. 2016,[2] Egypt | *In vitro* | 5% TTO Head Lice Gel | Louse (*Pediculus humanus capitis*) | 96.7% mortality |
| Alver *et al*. 2017,[3] Turkey | *In vivo* | 10% TTO eye shampoo with 4% gel | Mite (*Demodex folliculorum* & *D. brevis*) | 82.1% improvement in blepharitis |
| Barker & Altman 2010,[4] Australia | RCT | 10% w/v TTO and 1% w/v lavender oil NeutraLice Lotion® (TTO/LO) | Louse (*Pediculus humanus capitis*) | 97.6% cure rate |
| Benelli *et al*. 2013,[5] Italy | *In vitro* | 1.5-3 µL oil/cm2 TTO | Mediterranean fruit fly (*Ceratitis capitate*) | >60% mortality |
| Callander & James 2012,[6] Australia | *In vitro* | 2.5-3% TTO | Blow fly (*Lucilia cuprina*) | 100 % ovicidal and larvicidal (1st instar) & 100% repellent effect for 7hrs |
| De Wolff 2008,[7] USA | *In vitro* | 20% TTO | Fleas (*Siphonaptera*) | 78% mortality(in1hr) and |

| | | | | |
|---|---|---|---|---|
| | | | | 100% mortality (in day) |
| Di Campli *et al*. 2012,[8] Italy | *In vitro* | 1-8 % TTO | Louse (*Pediculus humanus capitis*) | 100 % mortality |
| Ellse *et al*. 2013,[9] UK | *In vitro*<br>*In vivo* | 5% & 10% TTO<br>5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | >80% mortality |
| Ellse *et al*. 2016,[10] UK | *In vivo* | 5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 78% mortality |
| Fitzjarrell 1995,[11] USA | *In vivo* | 2–10% v/v TTO | Fleas (*Siphonaptera*) | 100% mortality |
| Gao *et al*. 2005,[12] USA | *In vitro* and *in vivo* | 50–100% TTO | Mite (*Demodex folliculorum*) | 100% mortality |
| Iori *et al*. 2005,[13] Italy | *In vitro* | 8 -10µl TTO | Tick (*Ixodes ricinus*) | >80% mortality |
| James & Callander 2012,[14] Australia | *In vitro* | 1–20% TTO | Sheep louse (*Bovicola ovis Schrank*) | 100% mortality (adult lice and eggs) |
| James & Callander 2012,[15] Australia | *In vivo* | 1–2% TTO | Sheep louse (*Bovicola ovis Schrank*) | 100% mortality |
| Klauck *et al*. 2014,[16] Brazil. | *In vitro* | 5.0% TTO | Houseflies (*Musca domestica* & *H. irritans*) | 100% mortality |
| Maher 2018,[17] United Arab Emirates | *In vivo* | 5% TTO eyelid scrub | Mite (*Demodex folliculorum*) | 100% improvement in symptoms |
| Nicholls *et al*. 2016,[18] Australia | *Case series (in vivo)* | 5 % TTO | Mites (*Demodex folliculorum* & *D. brevis*) | 91% improvement in symptoms |
| Pazinato *et al*. 2014,[19] Brazil | *In vitro* | 1–10 % TTO & 0.075–0.75 % TTO nanoparticles | Tick (*Rhipicephalus (Booophilus) microplus*) | 100 % reproductive inhibition<br>70 % mortality |
| Sands *et al*. 2016,[20] UK | *In vitro* | 5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 100% mortality |
| Talbert & Wall 2012,[21] UK | *In vitro* | 0.5–10% *TTO* | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 100% mortality |
| Walton *et al*. 2004,[22] Australia | *In vitro* | 5% TTO | Scabies mite (*S scabiei var hominis*) | 100% mortality |
| Walton *et al*. 2000,[23] Australia | *in vitro* | 5% TTO | Scabies mite (*S scabiei var hominis*) | 100% mortality |
| Williamson *et al*. 2007,[24] UK | *In vitro* | 10% TTO | House dust mites **(***Dermatophagoides pteronyssinus* & *D. farinae*); Louse (*Pediculus humanus capitis*) | 100% immobility<br>100% mortality |
| Yim *et al*. 2016,[25] Australia | *In vivo* | 2–5% TTO | Cattle tick (*Rhipicephalus australis*) | 78–100% repellent effect for 2 days |

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UNIVERSITY OF CANBERRA

# PARTICIPANT INFORMATION SHEET AND ASSENT FORM

## Tea tree oil gel for Tungiasis (Jiggers) Treatment

***What the study is about?***

We are testing whether tea tree oil (TTO)-based gel can kill the jiggers in your feet without causing you any pain or discomfort compared to the purple medicine called potassium permanganate, in a10-day treatment period.

***What would I have to do?***

If you agree to be a part of the study, you will be asked to sign this form and to:

1. Allow the study team to wash and carefully examine your feet using a handheld digital microscope
2. Allow the study team to take photographs of your feet
3. Allow the study team to apply the treatment on days 1, 4, and 7
4. Wear a pair of new closed shoes throughout the study period (which we will be provided on day 1)
5. Attend the clinic for treatment and examination on Days 1,4, 5, 7 and 10
6. Avoid applying any other medicine or skin products on the jiggers affected skin area during the study period (1-10 days).
7. Avoid cutting your jiggers affected skin during the study period
8. Follow the study instructions explained to you by the study team

***What are the side effects of taking part?***

TTO has long been used as a medicine by indigenous communities in Australia and internationally and the likelihood for developing sever skin reactions are minimal. However, the trail medication may have some side effects. It may cause skin discomfort with an allergic or irritant reaction. If you suffer from these or any other symptoms you should report them immediately to the study team. If you are concerned in any way, you can speak to study team at the school. As for the purple medicine, it will not hurt you, but it will change the colour of your feet. This colour will go away after a few days. However, the provided closed footwear will adequately mask this skin colouration – and this is likely to prevent other students from giving you a hard time.

***What happens if something goes wrong during the trial?***

The risk of serious side effects is small compared to the risk you face as a result of having jiggers. If you do experience side effects as result trial medications, you will be referred to the nearby health facility for appropriate treatment and medical care.

***What would I benefit from the participation?***

We hope that the TTO gel will help you, but this cannot be guaranteed. The information we get from this study may help us to improve the treatments available for jiggers in the future.

***Will my taking part in this study be kept confidential?***

The information gathered about you by the investigator or obtained during the study will be held by the investigators in strict confidence. All the people who handle your information will adhere to traditional standards of confidentiality and will also comply with all relevant privacy legislation, in Australia and Kenya.

If needed, summary data without your name attached will be made available, to government regulatory bodies in Kenya and Australia.

***Do I have to take part?***

You do not have to be in this study if you do not want to be, even if your parents and teachers said it is okay for you to be in the study. If you decide to stop after we begin, that's okay too. Your parents know about the study too.

# PARTICIPANT INFORMATION SHEET AND ASSENT FORM

UNIVERSITY OF CANBERRA

## Consent approval

1. I have been given clear information, both verbally and in writing, about this study and, having had time to consider it, am able to make an informed decision to participate.
2. I have read and understood the Patient Information Sheet and have retained a copy of it.
3. I have been given the opportunity to ask the investigator questions about the study.
4. I have been told about the possible benefits and risks of taking part and I understand what I am being asked to do.
5. I understand that I may withdraw from the study at any time without affecting any future medical treatment, or the treatment of the condition which is the subject of the trial.
6. I agree to take part in this research and for the data obtained to be published provided that my name or other identifying data is not used.
7. I understand that if I leave the study for any reason, the information and samples collected will still be used unless I specifically ask for them to be removed from the study at the time I leave.
8. I understand that the investigators of the trial will adhere to usual standards of confidentiality in the collection and handling of my personal information.

I, _____, agree to participate in the above study. I have read and understood the Participant Information Sheet and I have been given a copy of it. I have been given the opportunity to ask questions about the study. I understand that I may withdraw from the study at any time without affecting my future medical treatment, or the treatment of the condition which is the subject of the trial.

Participant Name: _____ Signature: _____

Date: _____/_____/_____

Investigator Name: _____ Signature: _____

Date: _____/_____/_____

**Thank you for your interest in the study.**

# PARTICIPANT INFORMATION SHEET AND CONSENT FORM

## Tea tree oil gel for Tungiasis (Jiggers) Treatment

You are being invited to take part in this research study because your child has been identified with jiggers in his/her feet. We are asking for your willingness to allow your child to take part in this study. Please take time to carefully read the following information. Ask us if there is anything that is not clear or if you would like more information. Consider carefully before you make your decision whether or not you wish to take part. You may also wish to discuss the study with a relative, friend or your friendly clinical staff at the school.

***What is the objective of this study?***

This study aims to evaluate whether tea tree oil (TTO 5% v/w) gel can kill the embedded jiggers better than the locally recommended potassium permanganate solution followed by Vaseline® application (within a 10-day study period). The study also aims to determine whether the TTO gel can reduce skin inflammation, pain and itching caused by the jiggers better than the potassium permanganate/ Vaseline® treatment. If the proposed treatment is effective, this study might help us improve the treatment outcomes for jiggers.

***What would I have to do?***

We are asking for your willingness to allow your child to take part in this study. If you agree to proceed, you will be given this information sheet to keep and be asked to sign a consent form.

***If we choose to participate, will our participation be kept confidential?***

The information gathered about you child during the study will not be shared. All of the people who handle your information will maintain confidentiality and will also comply with NHMRC clinical trial guidelines and local privacy laws.

***What will happen to my child if we take part?***

Your child will be randomly allocated to either the TTO gel or potassium permanganate treatment. Treatments will be given twice daily on days 1, 4, and 7. In addition, your child will be given a pair of new closed shoes as part of the study. The clinical investigator will then make careful observations about the jiggers on days 5 and 10. The doctor will also ask your child about how much pain and itching he/she is feeling. In summary, your child will be asked to attend the clinics at the school 6 times during the treatment phase (i.e. AM and PM on days 1, 4, and 7), and 2 follow up visits on days 5 and 10. Each clinic-visit will take about 30 mins.

***What would be expected from us during study period?***

It is **VERY** important that you and your child, **DO NOT** cut out any jiggers from the child's foot during this time.

You should not use any other jigger medicine or any other skin products on the affected skin area during this time (days 1-10). We would like you to maintain the daily diary of events during study participation (1- 10 days).

If your child develops a reaction to the trial medication, you should notify the study clinical team as soon as possible.

***What information would be collected?***

The study will not be collecting any samples from your child. We will only make observations of the jiggers. If your child is found to have any other disease, we will advise you on the best way to manage it. The information we collect from your child will be entered into a computer system along with information from other study participants. The study team based at University of Canberra, will analyse the data and prepare a report with findings from this study and necessary recommendations. These findings will be communicated with other organizations, the Kisii, and Nyamira counties and National Ministry of Health, Kenya.

***What would be the risks of participation for the child?***

Tea tree oil (TTO) has been documented as a topical antiseptic (nationally and internationally) for over 90 years and even longer in the indigenous communities in Australia as a bush medicine. The treatment is unlikely to pose any serious health risk to your child. However, the trail medication may have some side effects. It may cause skin discomfort with an allergic or irritant reaction. If your child suffers from these or any other symptoms you should report them immediately to the study team. If you are concerned in any way, you can speak to study team at the school.

# PARTICIPANT INFORMATION SHEET AND CONSENT FORM

UNIVERSITY OF CANBERRA

***Your Right to Refuse or Withdraw from the study***

The decision to participate in the study is entirely voluntary. Clinical examination and treatment will be conducted in the school. This research study has received support and endorsement from the participating school. And you are free to withdraw at any time and without giving a reason.

***What is the contact for further information?***

If you need any further information or have any concerns, you can speak to the school health officer or study team or Doctor Stanislous Misati (GSP: +254 710 521804).

**Consent approval**

The undersigned _____ (full name) testifies that she/he is the legal guardian of _____ (name of child) and that she/he has read and understood the consent form which was also read aloud and explained by _____ _____.

I understand the objectives, the necessities, the potential risks and benefits regarding the participation of my child in the study, including the time commitment during the treatment, assessment and follow-up period.

I agree that any living sand fleas remained at the end of the study will be treated with the local government/medical recommendations.

I am aware of the fact that all information which could lead to an identification of my child will be kept strictly confidential. I have the right to withdraw my child from the study at any time without giving any justification for the removal. I voluntarily agree for my child to participate in this study based on these conditions.

School _____ Date: _____/_____/_____
Subject Study ID-No: _____
Parent/Caregiver
Name: _____ Signature: _____
Date: _____/_____/_____
Investigator who provided the information:
Name: _____ Signature: _____
Date: _____/_____/_____
Witness:
Name: _____ Signature: _____
Date: _____/_____/_____

**Thank you for your interest in this study.**

Version 2.0 dated 20/08/2019

UNIVERSITY OF CANBERRA

# Case Report Form (CRF)

## Treatment of tungiasis using a 5% v/w tea tree oil (TTO) gel: A randomised, controlled, proof-of-principle trial

## Subject Study ID:





Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

UNIVERSITY OF CANBERRA

## A. Recruitment Form

Please complete this form for every child who is identified as a potential participant in the TTO (5% v/w) gel tungiasis Trial

Investigator: _____ Date: ___/___/_____ [dd/mm/yyyy]

School: _____

| **Question** | **Response** (tick one) |
|---|---|
| 1. *Has the child been identified as having active embedded jiggers?*<br>- *If No, excuse participant*<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 2. *Is the child aged between 6 and 15 years?*<br>- *If No, excuse participant*<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 3. Explain the study protocol to the caregiver (and the child if appropriate) with the aid of the Participant Information Sheet.<br>- *Once done, tick 'Done' and proceed* | ☐ Done |
| 4. Is the caregiver able and willing to provide written informed consent for the child to take part in the study?<br>- *If No, record reason (if given) and excuse participant*<br>_____<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 5. Is the caregiver able and willing to be contacted by telephone (voice call and SMS) after the initial assessment? | ☐ Yes ☐ No |
| 6. Is the child willing to participate in the study?<br>- *If No, excuse participant*<br>- *If Yes, ask child to fill in Written Assent if aged ≥12 years, then proceed* | ☐ Yes ☐ No |
| 7. *Ensure that the child's caregiver has signed informed consent for the child to participate in the study*<br>- *If 'Done', proceed to Eligibility Assessment Form*<br>- *If consent was not given, provide reason below (if given) and excuse participant*<br>_____ | ☐ Done<br>☐ Consent not given |



Investigator signature_____

## B. Eligibility Assessment Form

Please complete this form for every participant who is recruited to the TTO (5% v/w) gel tungiasis trial. This form is used to assess whether the participant meets the criteria to be eligible for enrolment into the study.

Investigator: _____ Date assessed: ___/___/_____ [dd/mm/yyyy]

School: _____

### Study Eligibility Criteria

### Inclusion criteria

Please tick 'Yes' or 'No' for each item.

Both 2 items must be marked 'Yes' for the child to be eligible for enrolment.

| Inclusion criteria | Yes | No |
|---|---|---|
| 1. Is the child aged from 6–15 years with at least 1 viable (stage II and Stage III) lesion according to the Fortaleza classification on the child's feet? *Perform clinical examination of the lesions and confirm their viability based on the four viability signs using the handheld digital microscope. Refer to Figure 1 and Figure 2 on page 10 and 11 of Case Report Form.* | ☐ | ☐ |
| 2. Is the caregiver able and willing to provide written informed consent for the child to take part in the study? | ☐ | ☐ |

### Exclusion Criteria

Please tick 'Yes' or 'No' for each item.

All items must be marked 'No' for the child to be eligible for enrolment

| Exclusion criteria | Yes | No |
|---|---|---|
| 1. Are there any cluster lesions (more than 3 lesions together) and manipulated lesions? | ☐ | ☐ |
| 2. Are there any complicated lesions (severe) requiring antibiotic treatment? | ☐ | ☐ |
| 3. Do the caregivers intend to change their place of residence during the study period? | ☐ | ☐ |
| 4. Has the child used any medication (systemic or topical drugs medication) in the past week? This could include antibiotics, prescription or non-prescription medications, creams, ointments, medicated wash products, etc.<br>If Yes, please tick all that apply and provide name of medication (if known).<br>☐ Oral medication (specify) _____<br>☐ Cream/ointment (specify) _____<br>☐ Anti-itch preparation, e.g. steroid (specify) _____<br>☐ other (specify) _____ | ☐ | ☐ |
| 5. Does the child have a known history of allergy to any of the study medications listed below?<br>If Yes, please tick all that apply.<br>☐ Potassium permanganate<br>☐ Tea tree oil or other essential oils | ☐ | ☐ |



Investigator signature_____

**Eligibility outcome**

The child is eligible for enrolment into the TTO (5% v/w) gel Tungiasis Trial only if they meet all of the inclusion criteria and do not meet any of the exclusion criteria.

For an eligible child, the answer must be 'Yes' to question 1 and 'No' to question 2 below.

| | Yes | No |
|---|---|---|
| 1. Does the child meet all the **Inclusion Criteria** (answered 'Yes' to both 2 questions on page 1)? | ☐ | ☐ |
| 2. Does the child meet any of the **Exclusion criteria** (answered 'Yes' to any of the 5 questions on pages 2)? | ☐ | ☐ |

The participant is

☐ **Not eligible** for the trial

- Please excuse child and caregiver

☐ Eligible for the trial but will not be randomized due to other reasons

- Please specify reason: _____

_____

☐ **Eligible** for the trial and will be randomized

- Proceed to Baseline Assessment form

Form completed by: _____ Date: ___/___/_____ [dd/mm/yyyy]

Signature: _____

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## C. Baseline Assessment

Please complete this assessment form at the participant's first visit (Day 0, Week 0).
Investigator: _____ Date assessed: ___/___/_____ [dd/mm/yyyy]
School: _____

**Participant details**

**Demographics**

| Clinical Assessment 1 - Demographics | Response |
|---|---|
| Age | |
| Sex | ☐ Male ☐ Female |
| Date of Informed Consent from legal caregiver (dd/mm/yyyy) | ___/___/_____ |
| School | |
| Usual place(s) of residence | ☐ Rural ☐ Remote |
| Usual place(s) of residence (name of suburb, town or community) | _____ _____ |

**Physical Examination**

Please record any existing medical conditions (e.g. diabetes), allergies, illnesses (e.g. gastroenteritis). Provide further detail in 'comments' below if necessary.

| Clinical Assessment 2 – Physical examination | | | | | Response |
|---|---|---|---|---|---|
| Height (cm) | | | | | __ __ __ . __ cm |
| Weight (kg) | | | | | __ __ __ . __ kg |
| Date assessed | Study day | BP | Pulse | Temp. | Comment |
| ___/___/_____ | Day 1 | | | | |
| ___/___/_____ | Day 4 | | | | |
| ___/___/_____ | Day 5 | | | | |
| ___/___/_____ | Day 7 | | | | |
| ___/___/_____ | Day 10 | | | | |



Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

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## Medical history

| **Medical condition/illness/allergy** | **Start date** (dd/mm/yyyy) | **End date** (dd/mm/yyyy) | **Condition ongoing** (Y/N) |
|---|---|---|---|
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |

Please record any medications taken by the child in the last 1 week.

| **Medication name** | **Indication** | **Dose** | **Start date** (dd/mm/yyyy) | **End date** (dd/mm/yyyy) | **Ongoing** (Y/N) |
|---|---|---|---|---|---|
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |

Comments:

_____

_____

_____

_____

_____

## History of Jiggers

Please record answers to these questions about jiggers in the child and their community.

| Clinical Assessment 1 - History of jiggers | Response |
|---|---|
| 1. How long ago did the child's jiggers start? (tick one) | ☐ < 1 week<br>☐ 1-3 weeks<br>☐ 3-6 weeks<br>☐ > 6 weeks |
| 2. Has the child previously been diagnosed with jiggers by a health worker or doctor? | ☐ Yes ☐ No |

| Clinical Assessment 1 - History of jiggers | Response |
|---|---|
| 3. How many times in the past has the child had jiggers? (tick one) | ☐ 0<br>☐ 1-2 times<br>☐ 3-5 times<br>☐ >5 times<br>☐ unknown |
| 4. Has the child been treated with any jigger's medication <u>at any time in the past</u>?<br><br>If Yes, please tick all that apply<br>☐ Potassium permanganate (KMNo4) and Vaseline<br>☐ Vaseline<br>☐ Neem extracts<br>☐ Coconut oil<br>☐ Other (specify) _____<br>☐ Skin cream/ointment, name unknown<br>☐ Oral medication, name unknown<br>If Yes, how long ago did the most recent treatment end?<br>☐ < 1 week<br>☐ 1-2 weeks<br>☐ > 2 weeks | ☐ Yes ☐ No |
| 5. Has the child been treated with any antibiotics in the last 1 week?<br><br>If Yes, what is the name and indication of the antibiotic?<br><br>Name: _____ ☐ Unknown<br><br>Indication: _____ ☐ Unknown | ☐ Yes ☐ No |

## D. Study drug administration

Please record the type of intervention and time of application in this form.

| **Treatment applied** | **Amount applied (g)** | **Date of application** dd/mm/yyyy | **Time of application** 24-hr time |
|---|---|---|---|
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |



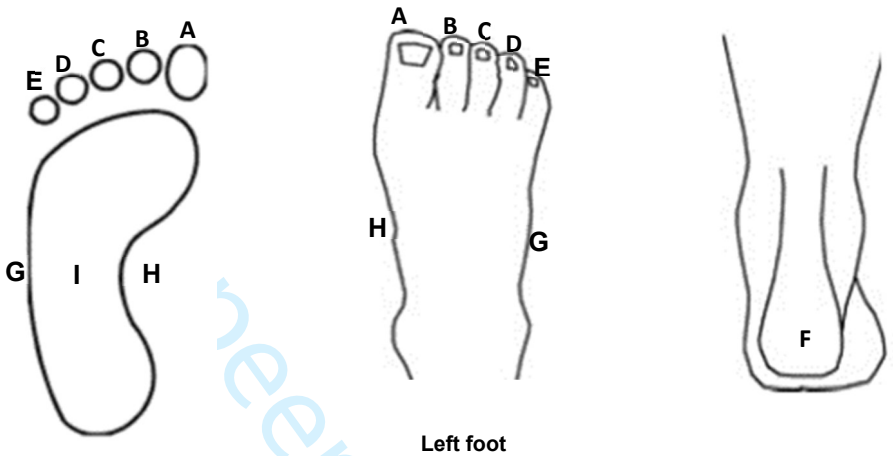
Investigator signature_____

## E. Clinical and symptomatic assessment -1

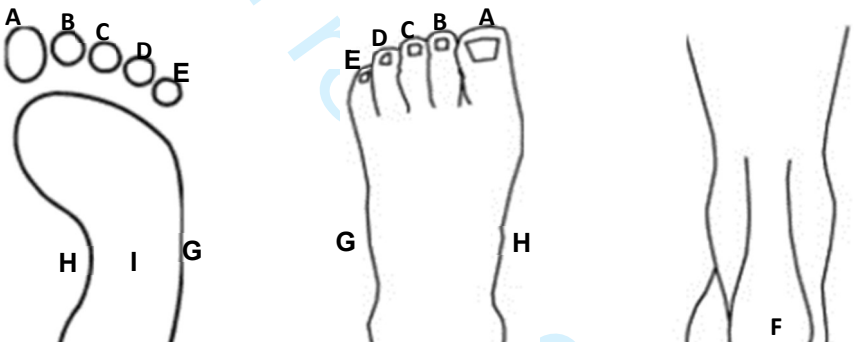
1. Please assess each of the following foot parts for typical jigger lesions (tick if present).

| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side- **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side- **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole- **I** |

**Right foot**




**Left foot**




| | |
|---|---|
| 2. Are any viable lesions present on the child's feet? | ☐ Yes ☐ No |
| 3. Mark all sites of viable lesions on the feet diagrams on pages 9 and 10. | ☐ Done |

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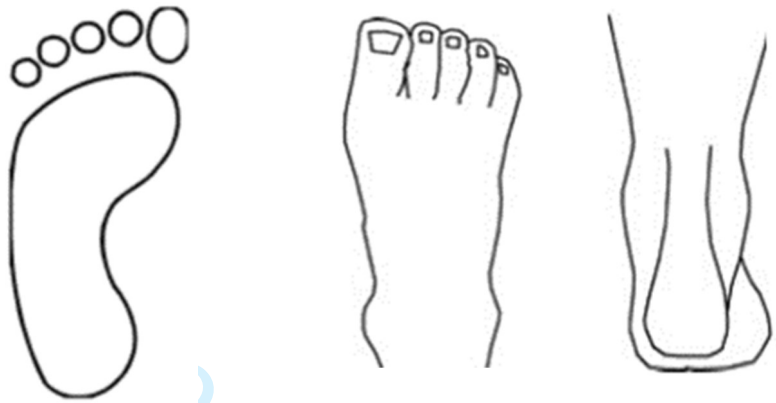
## E. Clinical and symptomatic assessment -1

**Feet diagram – Full**

Mark all sites of active jigger lesions with an X. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **E. Clinical and symptomatic assessment -1** | |
|---|---|
| 4. Select and record 2 target lesion in 2 target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 5. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 6. Photograph each of the 2 viable lesions together with their target sites | ☐ Done |
| 7. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |
| 8. Assess the viability of 2 target lesions. Tick all that apply for each site. | |

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

| | |
|---|---|
| 9. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 10. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***numbers of viable lesions*** (Stages II & III, total) are there on the child's feet? | |
| 12. How many ***numbers of manipulated lesions (total)*** are there on the child's feet? | |
| 13. How many ***numbers of cluster lesions (total)*** are there on the child's feet? | |

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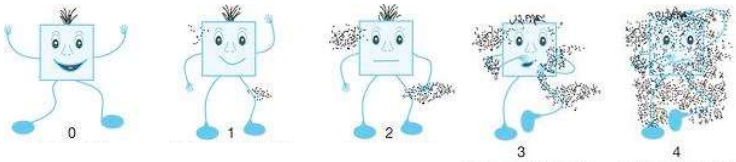
## E. Clinical and symptomatic assessment -1

14. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



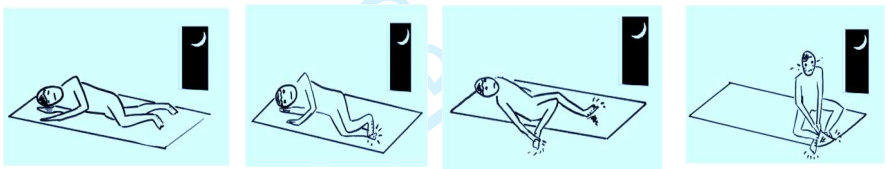

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

16. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

**Acute pathology examination and scoring**

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

| | | Sites on the left foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

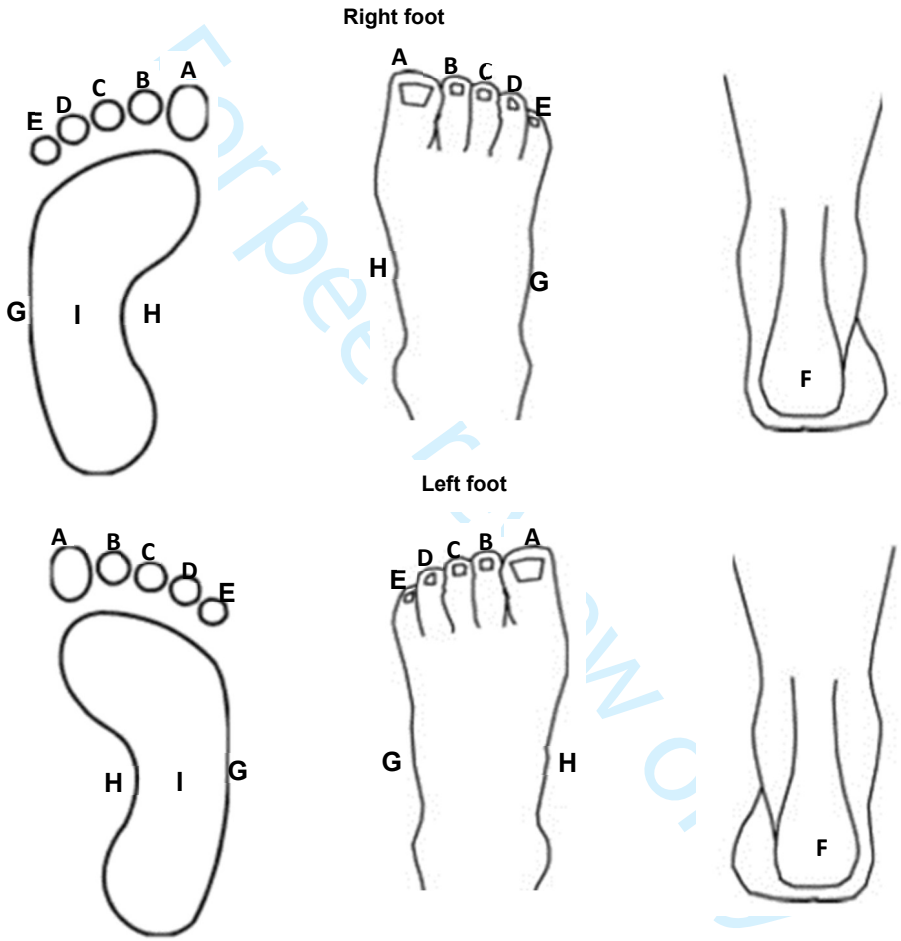


Investigator signature_____

## F. Clinical and symptomatic assessment- 2

1. Please assess each of the following foot parts for new and existing jigger lesions (tick if present).

| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side- **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side- **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole- **I** |




| | |
|---|---|
| 2. Are any new embedded jiggers present on the child's feet? | ☐ Yes ☐ No |
| 3. How many numbers of **newly embedded sand fleas** since the last examination? | |

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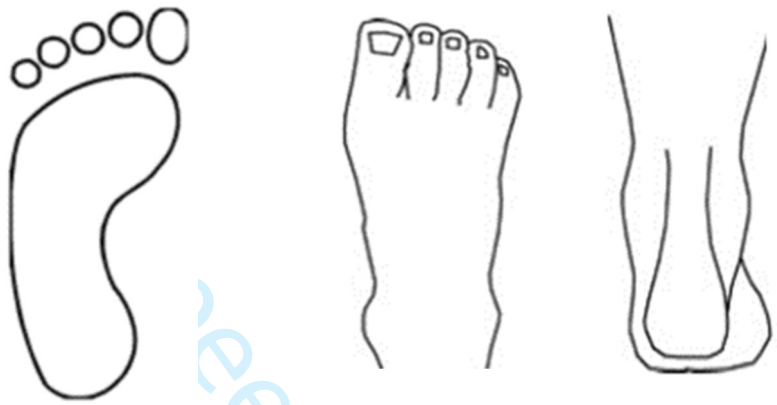
## F. Clinical and symptomatic assessment- 2

| | |
|---|---|
| 4. Mark all sites of new embedded jiggers and existing viable lesions on the feet diagrams on pages 13 and 14. | ☐ Done |

**Feet diagram – Full**

Mark all sites of active jigger lesions with X and newly embedded jiggers with Y. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **F. Clinical and symptomatic assessment- 2** | |
|---|---|
| 5. Follow the selected viable lesions together with their target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 6. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 7. Photograph the 2 target lesions together with their target sites | ☐ Done |
| 8. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |
| 9. Assess the viability of 2 target lesions. Tick all that apply for each site. | |

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

| | |
|---|---|
| 10. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 12. How many ***total numbers of viable lesions*** (sage II & III) are there on the child's feet | |

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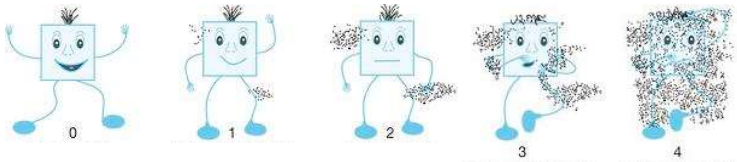
## F. Clinical and symptomatic assessment- 2

13. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



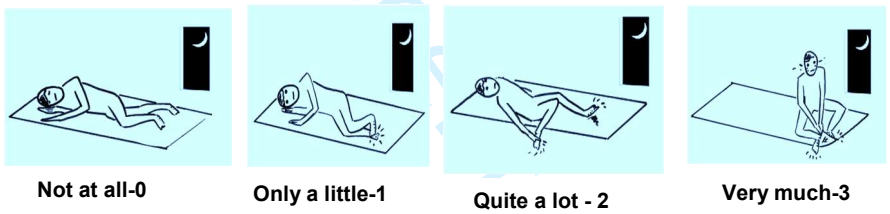

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

14. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

### Acute pathology examination and scoring

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

| | | Sites on the left foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |



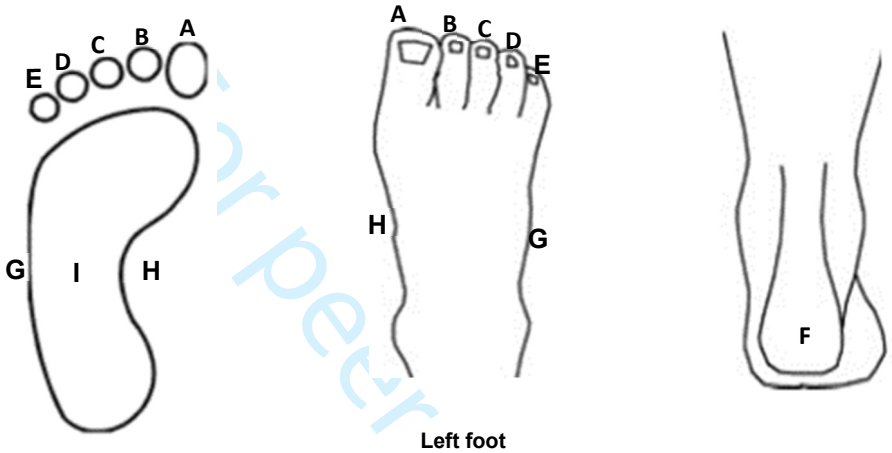
Investigator signature_____

## G. Clinical and symptomatic assessment - 3

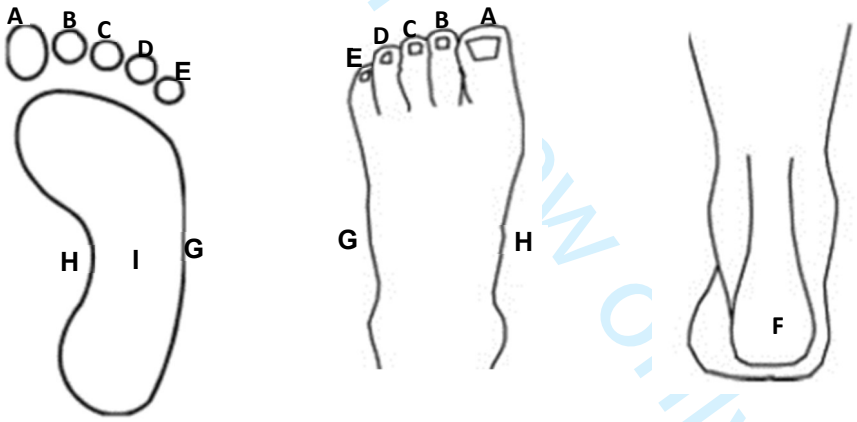
1. Please assess each of the following foot parts for new and existing jigger lesions (tick if present).

| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side - **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side - **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole - **I** |

**Right foot**




**Left foot**




| | |
|---|---|
| 2. Are any new embedded jiggers present on the child's feet? | ☐ Yes ☐ No |
| 3. How many numbers of **newly embedded sand fleas** since the last examination? | |

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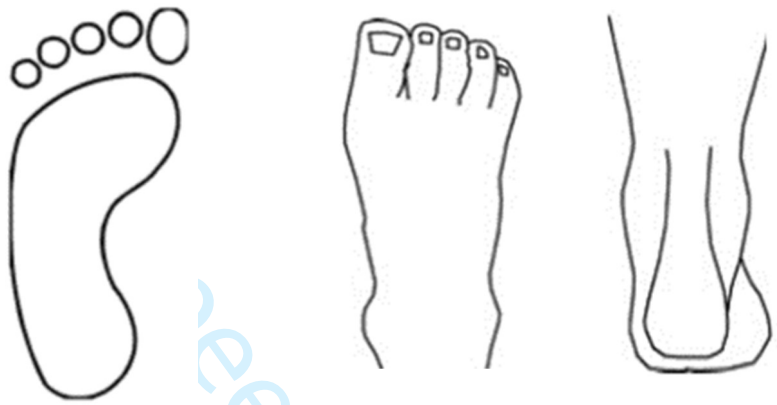
## G. Clinical and symptomatic assessment - 3

| | |
|---|---|
| 4. Mark all sites of new embedded jiggers and existing viable lesions on the feet diagrams on pages 13 and 14. | ☐ Done |

**Feet diagram – Full**

Mark all sites of active jigger lesions with X and newly embedded jiggers with Y. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **G. Clinical and symptomatic assessment - 3** | |
|---|---|
| 5. Follow the selected viable lesions together with their target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 6. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 7. Photograph the 2 target lesions together with their target sites | ☐ Done |
| 8. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |

9. Assess the viability of 2 target lesions. Tick all that apply for each site.

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

| | |
|---|---|
| 10. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 12. How many ***total numbers of viable lesions*** (sage II & III) are there on the child's feet | |

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

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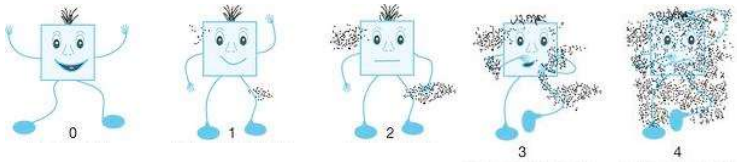
## G. Clinical and symptomatic assessment - 3

13. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



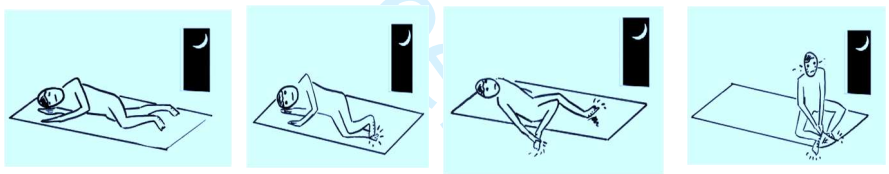

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

14. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

**Acute pathology examination and scoring**

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |
| | | Sites on the left foot | | | | | | | | |
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |



Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

UNIVERSITY OF CANBERRA

## H. Adverse Event Log

| Date of entry | Adverse Event | Grade/ Severity | Serious | Date/time of Onset | Date/time of Resolution | Relation to study drugs | Action taken | Treatment given | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| dd/mm/yyyy | Diagnosis (if known) or Signs/symptoms (list one per line) | 1=mild 2=moderate 3=severe | (Y/N) * | dd/mm/yyyy 24-hr time | dd/mm/yyyy 24-hr time | 1=related 2=not related 3=other Specify | 1=none 2=interrupted 3=patient withdrawn 4=medication discontinued 5=other??? | (Y/N) | 1=resolved 2=resolved w sequelae 3=ongoing 4=death 5=unknown |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |

*For any Serious Adverse Events, participant must be immediately referred to nearby healthcare facility for medic



Investigator signature_____

UNIVERSITY OF CANBERRA

# I. Final Study Outcome

| Question | Response (tick one) |
|---|---|
| Has the subject completed the study? | ☐ Yes ☐ No |
| If yes, indicte the completion date dd/mm/yyyy | ___/___/_____ |
| If NO, specify last follow up date dd/mm/yyyy | ___/___/_____ |
| What are the reasons for not completing the study? | ☐ Significant non-compliance |
| | ☐ Drug-related AE |
| | ☐ Treatment failure |
| | ☐ Consent withdrawn |
| | ☐ Lost to follow-up |
| | ☐ Other (specify) |
| | |
| | |
| | |
| Remarks | |
| | |
| | |
| | |
| ***Investigator's Statement*** ***I have reviewed the data recorded in this CRF and confirm that the data are complete and accurate*** | |
| Investigator (Full name) | |
| Investigator signature | |
| Signature Date /dd/mm/yyyy/: | ___/___/_____ |



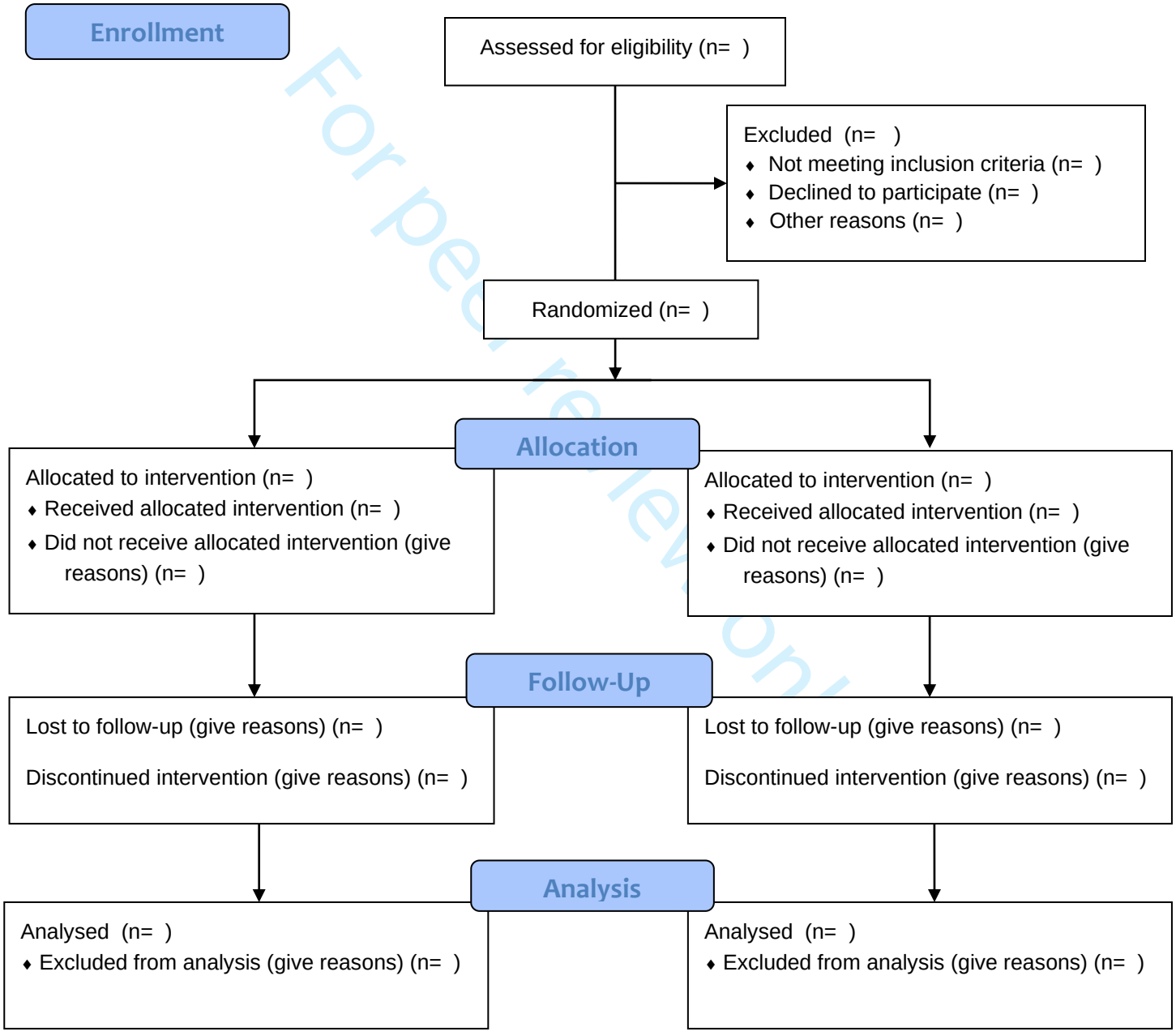


Investigator signature_____

CONSORT

TRANSPARENT REPORTING of TRIALS

# CONSORT 2010 Flow Diagram



# CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | |
| | 2b | Specific objectives or hypotheses | |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | |
| Participants | 4a | Eligibility criteria for participants | |
| | 4b | Settings and locations where the data were collected | |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | |
| Sample size | 7a | How sample size was determined | |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | |
| Randomisation: Sequence | 8a | Method used to generate the random allocation sequence | |

| | | | |
|---|---|---|---|
| | generation | 8b | Type of randomisation; details of any restriction (such as blocking and block size) |
| | Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned |
| | Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions |
| | Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how |
| | | 11b | If relevant, description of the similarity of interventions |
| | Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes |
| | | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses |
| **Results** | | | |
| | Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome |
| | | 13b | For each group, losses and exclusions after randomisation, together with reasons |
| | Recruitment | 14a | Dates defining the periods of recruitment and follow-up |
| | | 14b | Why the trial ended or was stopped |
| | Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group |
| | Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups |
| | Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) |
| | | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended |
| | Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory |

| | | | |
|---|---|---|---|
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.




## Main

*Note: This record shows only 22 elements of the WHO Trial Registration Data Set. To view changes that have been made to the source record, or for additional information about this trial, click on the URL below to go to the source record in the primary register.*

| | |
|---|---|
| **Register:** | ANZCTR |
| **Last refreshed on:** | 10 December 2019 |
| **Main ID:** | ACTRN12619001610123 |
| **Date of registration:** | 21/11/2019 |
| **Prospective Registration:** | Yes |
| **Primary sponsor:** | University of Canberra |
| **Public title:** | Exploring a tea tree oil (TTO)-based skin treatment for tungiasis in children |
| **Scientific title:** | Treatment of tungiasis using a proprietary tea tree oil (TTO)-gel formulation in children: Protocol for a randomised, controlled, proof-of principle trial |
| **Date of first enrolment:** | 03/02/2020 |
| **Target sample size:** | 88 |
| **Recruitment status:** | Not yet recruiting |
| **URL:** | https://anzctr.org.au/ACTRN12619001610123.aspx |
| **Study type:** | Interventional |
| **Study design:** | Purpose: Treatment; Allocation: Randomised controlled trial; Masking: Blinded (masking used);Assignment: Parallel;Type of endpoint: Safety/efficacy; |
| **Phase:** | Phase 2 |

## Countries of recruitment

Kenya

## Contacts

| | | | |
|---|---|---|---|
| **Name:** | A/Prof Jackson Thomas | **Name:** | A/Prof Jackson Thomas |
| **Address:** | Faculty of Health University of Canberra Building 12 Level D Office 36 Kirinari Street Bruce ACT 2601 Australia | **Address:** | Faculty of Health University of Canberra Building 12 Level D Office 36 Kirinari Street Bruce ACT 2601 Australia |
| **Telephone:** | +61 2 62068928 | **Telephone:** | +61 2 62068928 |
| **Email:** | Jackson.Thomas@canberra.edu.au | **Email:** | Jackson.Thomas@canberra.edu.au |
| **Affiliation:** | | **Affiliation:** | |

## Key inclusion & exclusion criteria

Inclusion criteria: 1. Children aged 6-15 years with at least 1 viable (stage II and Stage III) lesions according to the Fortaleza classification and a maximum of 2 viable sand flea lesions will be targeted.
2. Children whose legal guardians are willing to give informed written consents after having been oral and written informed about benefits and potential risks of the trial
Exclusion criteria: 1. Children with cluster lesions and manipulated lesions.
2. Children with complicated lesions requiring antibiotic treatment. They will be referred to the nearby health facilities for appropriate clinical management.
3. Children whose guardian/parents intend to change their place of residence during the study period
4. Children with known history of allergy to any of the study medications (Tea Tree Oil or other essential oils and potassium permanganate)
5. Individuals have/had systemic or topical drugs or medications, including systemic antibiotics, which may interfere with the study results (based on clinical team's assessment).

Age minimum: 6 Years
Age maximum: 15 Years
Gender: Both males and females

## Health Condition(s) or Problem(s) studied

Infection - Other infectious diseases

Public Health - Other public health

Skin - Dermatological conditions

Tungiasis (sand flea disease) ;
Tungiasis (sand flea disease)

**Intervention(s)**

Test group- treatment of tungiasis with a 5% (v/w), proprietary tea tree oil (TTO) gel

The feet of the participants will be washed with water and non-medicated soap, dried with a clean towel, and the participants' toenails will be clipped to enable easier application of the test medication. Then, the test medication will be applied twice daily on days 1, 4 and 7 by trained study personnel (concerned case officers from participating schools). The mode of administration of the test medication is by taking the required amount of the gel on the palms (up to 8g/day) and spreading it over the infested skin areas until it provides a full coverage of the affected area (skin surface of the feet up to the ankle) and the feet will then be left for 15 minutes to allow the medication to dry.

**Primary Outcome(s)**

Proportion of non-viable fleas

Determination of viability of the sand flea lesions will be performed using a handheld digital video microscope, assisted with pictorial flipcharts. Expulsion of eggs, excretion of faecal threads, excretion of faecal liquid, and pulsations/contractions in the abdomen of the embedded flea will be considered as four viability signs and lesions with 2 out of 4 viability signs will be recorded viable. Lesions will be considered dead (non-viable) if their viability signs are not detected during the 10 min follow-up examinations. Differences in the proportion of non-viable lesions between test and control groups will be compared and presented with their respective confidence intervals at 95% and p-values. [Day 10 (9 days after the first treatment).]

**Secondary Outcome(s)**

Acute morbidity evaluation

The severity score for acute morbidities (SSAT; which includes typical signs of local inflammation, the presence of suppuration, ulcers and fissures) will be assessed using a validated scoring system designed for tungiasis morbidity assessment.
In addition to SSAT, a visual analogue scale (VAS) called the 'Itch-man scale'-- a 5-point pictorial Likert scale, validated for paediatric burn survivors, will be adopted to evaluate itching. Finally, a 4 point pictorial scale, validated in paediatric tungiasis patients will be adopted to assess the pain, as well as pain-related and itching related sleep disturbances (QoL assessment).
[Days 0 (baseline), 5 and 10 (post treatment)]

Participant acceptability of the trial intervention/s

Participants/caregivers will be asked to rate the acceptability of the treatment in terms of effectiveness, side effects, convenience, and overall satisfaction on a 0-5 visual analogue scale. [Day 10 (9 days after the first treatment).

]

Proportion of participants with side effects (adverse events)

Safety will be assessed through evaluation of treatment related adverse events and skin irritation.
Participants/caregivers (in person or on the phone) will be asked about the occurrence of any solicited or unsolicited adverse reactions to the treatment during each follow-up visit. The trial team (clinical officer and health officers) will also carefully follow-up the trial participants on a regular basis at the trial site, until the end of trial period. This will be done using a pre-specified list of possible AEs, including local adverse reactions (swelling, stinging/burning, itching, induration, erythema) and systemic adverse reactions (fever, nausea and headache). Caregivers of participants will also be given a diary card to record ongoing solicited adverse events. The severity of the adverse events will be categorized as mild, moderate and severe according to common terminology criteria for adverse events (CTCAE) v5.0 guideline[Days 1 (PM), 4, 5, 7 and 10 (post-treatment)

]

**Secondary ID(s)**

None

**Source(s) of Monetary Support**

University of Canberra

**Secondary Sponsor(s)**

**Ethics review**

Status: Approved
Approval date:
Contact:
University of Canberra Human Ethics Research Committee

Results

**Results available:**
**Date Posted:**
**Date Completed:**
**URL:**

# Pan African Clinical Trials Registry

South African Medical Research Council, South African Cochrane Centre
PO Box 19070, Tygerberg, 7505, South Africa
Telephone: +27 21 938 0506 / +27 21 938 0834 Fax: +27 21 938 0836
Email: pactradmin@mrc.ac.za Website: www.pactr.org

| | | | |
|---|---|---|---|
| **Trial no.:** | PACTR202003651095100 | **Date registered:** | 26/02/2020 |
| **Trial Status:** | Registered in accordance with WHO and ICMJE standards | | |

## TRIAL DESCRIPTION

| | |
|---|---|
| **Public title** | Exploring a tea tree oil (TTO)-based skin treatment for tungiasis in children |
| **Official scientific title** | Treatment of tungiasis using a proprietary tea tree oil (TTO)-gel formulation in children: Protocol for a randomized, controlled, proof-of-principle trial |
| **Brief summary describing the background and objectives of the trial** | Tungiasis is a neglected parasitic skin disease caused by the female sand fleas (Tunga penetrans), which is highly prevalent in central and south America, the Caribbean, and Sub-Saharan Africa. The disease inflicts pain and suffering on millions of people, particularly children, and yet it is neglected by donors, governments, the scientific community, and health care providers. Left untreated, tungiasis can lead to substantial human consequences including impaired sleep, school absenteeism social isolation, difficulty in walking, auto-amputation, childhood disability, and immobility in severe cases. There is no approved drug treatment for tungiasis, and the available treatment options are very limited. There is a clear need for new, safe, effective, affordable and culturally acceptable tungiasis treatment options. Topical treatment is most ideally suited in endemic settings and the treatment should be simple, enabling self-administration, and should be started as soon as symptoms appear so that it can kill the embedded parasite at an early stage, prevent secondary bacterial complications, and substantially reduce the occurrence of acute and chronic morbidities. This trial aims to investigate the safety and efficacy of a proprietary tea tree oil gel (TTO) formulation (5% v/w) in comparison with an active comparator (i.e. 0.05% w/v potassium permanganate solution) for the treatment of tungiasis in children, over a 10-day period. TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. Unlike other tungiasis treatments, the TTO proprietary treatment offers a unique combination of parasiticidal, antibacterial, wound-healing, anti-inflammatory and anti-itch properties. |
| **Type of trial** | RCT |
| **Acronym (If the trial has an acronym then please provide)** | |
| **Disease(s) or condition(s) being studied** | Paediatrics,Skin and Connective Tissue Diseases |
| **Sub-Disease(s) or condition(s) being studied** | |
| **Purpose of the trial** | Treatment: Drugs |
| **Anticipated trial start date** | 01/06/2020 |
| **Actual trial start date** | |
| **Anticipated date of last follow up** | 04/09/2020 |
| **Actual Last follow-up date** | |
| **Anticipated target sample size (number of participants)** | 88 |
| **Actual target sample size (number of participants)** | |
| **Recruitment status** | Not yet recruiting |
| **Publication URL** | |

| Secondary Ids | Issuing authority/Trial register |
|---|---|
| ACTRN12619001610123 | Australian New Zealand Clinical Trial Registry, ANZCTR |
| U111112432294 | World Health Organization, Universal Trial Number |
| HREC20192114 | University of Canberra Human Research Ethics Committee |

## STUDY DESIGN

| Intervention assignment | Allocation to intervention | If randomised, describe how the allocation sequence was generated | Describe how the allocation sequence/code was concealed from the person allocating the participants to the intervention arms | Masking | If masking / blinding was used |
|---|---|---|---|---|---|
| | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Parallel: different groups receive different interventions at same time during study | Randomised | Simple randomization using a randomization table created by a computer software program | Sealed opaque envelopes | Masking/ blinding used | Outcome Assessors |

## INTERVENTIONS

| Intervention type | Intervention name | Dose | Duration | Intervention description | Group size | Nature of control |
|---|---|---|---|---|---|---|
| Experimental Group | Tea tree oil gel | Up to 8g/day | Twice daily for three days (Days 1, 4 and 7) | TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. It contains 5% (v/w) tea tree oil as an active ingredient. It will be applied by taking the required amount of the gel on the palms (up to 8g/day) and spreading it over the infested skin areas until it provides a full coverage of the affected area (skin surface of the feet up to the ankle) and the feet will then be left for 15 minutes to allow the medication to dry. | 44 | |
| Control Group | Potassium permanganate solution | Up to 2.5 liters of 0.05% potassium permanganate solution solution | Twice daily for three days (Day 1, 4 and 7) | Potassium permanganate solution contains 0.05g of potassium permanganate in a liter of water. It will be applied by immersing and bathing the feet of the participants in a bucket containing a required volume of 0.05% potassium permanganate solution for 15 minutes. After air-drying the feet (for about 15 mins), petroleum jelly will be applied to soften the skin, which may get rough and irritated after bathing with potassium permanganate solution. | 44 | Active-Treatment of Control Group |

## ELIGIBILITY CRITERIA

| List inclusion criteria | List exclusion criteria | Age Category | Minimum age | Maximum age | Gender |
|---|---|---|---|---|---|
| Children aged 6–15 years with at least 1 viable stage II and Stage III embedded sand flea lesions according to the Fortaleza classification. A maximum of 2 viable sand flea lesions will be targeted and the lesions must be the sum of the feet and lesions located at the tip of toes, soles, and rim of heels will be selected properly. Children whose legal guardians are willing to give informed written consents after having been oral and written informed about benefits and potential risks of the trial | Children with cluster lesions and manipulated lesions. Children with complicated lesions requiring antibiotic treatment. They will be referred to the nearby health facilities for appropriate clinical management. Children whose guardian/parents intend to change their place of residence during the study period Children with known histories of allergy to any of the study medications (Tea Tree Oil or other essential oils and potassium permanganate) Children have/had systemic or topical drugs or medications, including systemic antibiotics, which may interfere with the study results (based on the clinical team's assessment). | Adolescent: 13 Year-18 Year,Child: 6 Year-12 Year | 6 Year(s) | 15 Year(s) | Both |

## ETHICS APPROVAL

| Has the study received appropriate ethics committee approval | Date the study will be submitted for approval | Date of approval | Name of the ethics committee |
|---|---|---|---|
| Yes | | 28/08/2019 | University of Canberra Human Ethics Research Committee |

**Ethics Committee Address**

| Street address | City | Postal code | Country |
|---|---|---|---|
| Kirinari Street | Canberra | 2617 | Australia |

## OUTCOMES

| Type of outcome | Outcome | Timepoint(s) at which outcome measured |
|---|---|---|
| Primary Outcome | The proportion of non-viable embedded sand fleas | Day 10 or 9 days after the first treatment |
| Secondary Outcome | Acute morbidity evaluation | Days 0 or baseline, 5 and 10 or post treatment |
| Secondary Outcome | The proportion of participants with adverse events | Days 1 at PM, 4, 5, 7 and 10 |
| Secondary Outcome | Participant acceptability of the trial intervention/s | Day 10 or 9 days after the first treatment |

## RECRUITMENT CENTRES

| Name of recruitment centre | Street address | City | Postal code | Country |
|---|---|---|---|---|
| Riamajeshi Bright Start Academy | Sotik Ikonge Road | Nyamira | 0800 | Kenya |

## FUNDING SOURCES

| Name of source | Street address | City | Postal code | Country |
|---|---|---|---|---|

## SPONSORS

| Sponsor level | Name | Street address | City | Postal code | Country | Nature of sponsor |
|---|---|---|---|---|---|---|
| Primary Sponsor | University of Canberra | Kirinari St | Canberra | 2617 | Australia | University |

## COLLABORATORS

| Name | Street address | City | Postal code | Country |
|---|---|---|---|---|
| Global School Partners Kenya Chapter | Sotik Ikonge Road | Nyamira | 0800 | Kenya |
| Global School Partners Australia Chapter | Deakin | Canberra | 2603 | Australia |

## CONTACT PEOPLE

| Role | Name | Email | Phone | Street address |
|---|---|---|---|---|
| **Principal Investigator** | Solomon Abrha Bezabh | Solomon.Bezabh@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | PhD student | |
| **Role** | **Name** | **Email** | **Phone** | **Street address** |
| **Public Enquiries** | Jackson Thomas | Jackson.Thomas@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | Academic staff and researcher | |
| **Role** | **Name** | **Email** | **Phone** | **Street address** |
| **Scientific Enquiries** | Jackson Thomas | Jackson.Thomas@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | Academic staff and researcher | |

## REPORTING

| Share IPD | Description | Additional Document Types | Sharing Time Frame | Key Access Criteria |
|---|---|---|---|---|
| Yes | The ethical approval for this study requires the individual participant data to be kept confidential. However, the deidentified pooled data per intervention will be made | Informed Consent Form,Statistical Analysis Plan,Study Protocol | The findings of the trial will be available through peer-reviewed journals as well as national and international scientific conference meetings once the primary outcome paper is published. | Through open access research publications, with no restriction. |

| | | | | |
|---|---|---|---|---|
| | available through open access research publications. | | The additional document types will also be shared during the publication. | |
| **URL** | **Results Available** | **Results Summary** | **Result Posting Date** | **First Journal Publication Date** |
| | No | | | |
| **Result URL Hyperlinks** | **Baseline Characters** | **Participant Flow** | **Adverse Events** | **Outcome Measures Description** |
| **Link To Protocol** | | | | |

**Changes to trial information**

| Section Name | Field Name | Date | Reason | Old Value | Updated Value |
|---|---|---|---|---|---|
| Reporting | Plan to share IPD | 06/03/2020 | it was indicated 'No' in the previous submission but modified to elaborate on how and where results will be stored and how they will be available for the public good. | No | Yes |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD description | 06/03/2020 | It was not included in the previous submission. | | The ethical approval for this study requires the individual participant data to be kept confidential. However, the deidentified pooled data per intervention will be made available through open access research publications. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD-Sharing time frame | 06/03/2020 | It was not included in the previous submission. | | The findings of the trial will be available through peer-reviewed journals and national and international scientific meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | Key access criteria | 06/03/2020 | It was not included in the previous submission. | | Through open access research publications, with no restriction. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | Study protocol document | 06/03/2020 | It was not included in the previous submission. | | Study Protocol, Statistical Analysis Plan, Informed Consent Form |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD-Sharing time frame | 06/03/2020 | It was not included in the previous submission. | The findings of the trial will be available through peer-reviewed journals and national and international scientific meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. | The findings of the trial will be available through peer-reviewed journals as well as national and international scientific conference meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. |

Supplementary Table S3: Protocol Amendment History – Tea Tree Oil tungiasis Trial

| Amendment No | Protocol version No | Date issued | Author(s) of changes | Details of changes made |
|---|---|---|---|---|
| 1 | Version 1 | 2019 | Solomon Abrha Bezabh | Original version |
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# Treatment of tungiasis using a tea tree oil-based gel formulation: protocol for a randomised controlled proof-of-principle trial

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# Treatment of tungiasis using a tea tree oil-based gel formulation: protocol for a randomised controlled proof-of-principle trial

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## ABSTRACT

**Introduction:** Tungiasis (sand flea disease or jigger infestation) is a neglected tropical disease caused by penetration of female sand fleas, *Tunga penetrans*, in the skin. The disease inflicts immense pain and suffering on millions of people, particularly children, in Latin America, the Caribbean, and sub-Saharan Africa. Currently, there is no standard treatment for tungiasis, and a simple, safe, and effective tungiasis treatment option is required. Tea tree oil (TTO) has long been used as parasiticidal agent against ectoparasites such as headlice, mites, and fleas with proven safety and efficacy data. However, current data are insufficient to warrant a recommendation for its use in tungiasis. This trial aims to generate these data by comparing the safety and efficacy of a 5% (v/w) TTO proprietary gel formulation with 0.05% (w/v) potassium permanganate ($KMnO_4$) solution for tungiasis treatment.

**Methods and analysis:** This trial is a randomised controlled trial (RCT) in primary schools (n=8) in South-Western Kenya. The study will include school children (n=88) aged 6-15 years with a confirmed diagnosis of tungiasis. The participants will be randomised in a 1:1 ratio to receive a 3-day twice daily treatment of either 5% TTO gel or 0.05% $KMnO_4$ solution. Two viable embedded sandflea lesions per participant will be targeted and the viability of these lesions will be followed throughout the study using a digital handheld microscope. The primary outcome is the proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 (9 days after first treatment). Secondary outcomes include improvement in acute tungiasis morbidities assessed using a validated severity score for tungiasis, safety assessed through adverse events (AEs), and product acceptability assessed by interviewing the participants to rate the treatment in terms of effectiveness, side effects, convenience, suitability, and overall satisfaction.

**Ethics and dissemination:** The trial protocol has been reviewed and approved by the University of Canberra Human Research Ethics Committee (HREC-2019-2114). The findings of the study will be presented at scientific conferences and published in a peer-reviewed journal.

**Trial registration**: ACTRN12619001610123; PACTR202003651095100; and Universal Trial Number-U1111-1243-2294.


**Keywords:** Children, Protocol, Randomised controlled trial, Tea tree oil, Tropical medicine, Skin infection, Tungiasis, NTD

## STRENGTHS AND LIMITATIONS OF THIS STUDY

### Strengths

- Given tungiasis affects the most disadvantaged communities, this work has an ultimate objective of reducing the tungiasis burden and improving the health and wellbeing of affected children and communities in Kenya.
- Educational and community support packages (e.g. health education using flip charts) delivered to the participating communities as part of this study, will help facilitate the appropriate control of tungiasis with sustainable benefits to the community.
- If TTO gel effectively treats tungiasis, this would provide compelling evidence for a simple, affordable, and effective treatment for tungiasis, which does not require direct supervision by a trained health worker, essentially enabling the communities and/or individuals to manage their own health.

### Limitations

- Compliance to the treatment protocol is likely to be less than ideal in the targeted study settings in Kenya, and we also expect considerable attrition; however, regular follow up is likely to improve patient compliance and limit attrition.
- The identity of trial interventions could not be concealed to the study participants and caregivers.

## INTRODUCTION

Tungiasis (sand flea disease or jigger infestation), is a Neglected Tropical Disease (NTD) caused by penetration of an ectoparasite, female sand flea, *Tunga penetrans*.[1-3] It is rampant in resource-limited communities in Latin America, the Caribbean, and sub-Saharan Africa,[2] where children (aged 5-14 years) and the elderly (≥60 years) are most heavily affected, with prevalence data ranging from 50-85%.[4-6] No clear estimates of global burden of tungiasis exist,[7] but according to the World Health Organisation (WHO), 20 million people are estimated to be at risk of developing tungiasis in South America alone.[8] Based on Kenyan and Ugandan Ministries of Health,[9 10] about 4 million people suffer from the tungiasis, with another 16 million are at risk. A tungiasis infected person can harbour up to hundreds of parasites, usually on the feet and hands with toes, soles, and heels are the sites most frequently affected.[6 11] The infection results in intense inflammation and itching, and frequently occurs with secondary bacterial infections, resulting in abscesses, suppuration, cellulitis, lymphangitis, sepsis, tetanus, and post-streptococcal glomerulonephritis. Repeated infection can lead to deformation and loss

of nails, and disfigurement of the feet.[8 12-15] Tungiasis negatively impacts education (in children), quality of life, household economy, and wellbeing of the affected individuals.[8 9 16-19]

Currently, there is no standard treatment for tungiasis.[19] Parasiticides such as oral thiabendazole,[20] oral ivermectin,[21] and topical benzyl benzoate[22] and disinfectants like hydrogen peroxide,[9] have been explored for tungiasis treatment, but there is little conclusive evidence available on their safety or effectiveness. Our seminal systematic review on this topic (Abrha *et al*, The Lancet Infectious Disease, 2020) identified eight RCTs[23-30] investigated interventions for tungiasis. These included: coconut oil-based lotion (Zanzarin®) for prevention; and oral – niridazole and ivermectin; topical–ivermectin lotion, metrifonate lotion, thiabendazole lotion, thiabendazole ointment, dimeticones (NYDA®), and a neem seed and coconut oils-based mixture for treatment. Among these, the coconut oil-based lotion for prevention, and dimeticones for treatment of tungiasis displayed the most promise. However, the coconut oil-based lotion is no longer commercially available and dimeticones are expensive and currently not available in tungiasis endemic areas in sub-Saharan Africa including Kenya, thus limiting treatment options to surgical extraction of embedded fleas and bathing feet with 0.05% potassium permanganate ($KMnO_4$) solution.[30 31] In such settings, surgical extraction is frequently performed using unsafe procedures involving sharing of sharp instruments, leading to additional bacterial superinfections, and potential transmission of viral pathogens like HIV, Hepatitis B, and Hepatitis C.[12,32 33] Although bathing feet with 0.05% $KMnO_4$ solution is widely used in Kenya and is recommended by the country's Ministry of Health,[9] recent trials[24 30] have revealed that it was only marginally effective, killing less than 40% of embedded fleas.

Thus, there is a critical need for new, safe, effective, and affordable treatments for tungiasis. This trial aims to address this unmet critical need by trialling a novel 5% tea tee oil (TTO) gel-based skin formulation. Unlike current treatment agents used, TTO possesses a unique combination of potent insecticidal, acaricidal, antibacterial, anti-inflammatory, and wound healing properties. [34 35] It has long been used as a helpful topical treatment agent for a variety of epidermal parasitic skin diseases in Australia and Europe, with good safety and efficacy data.[36] The insecticidal and acaricidal effects of topical formulations of TTO for a range of medical ectoparasites/pests, including house dust mites, *Demodex* mites, scabies mites, ticks, headlice, and fleas, have been investigated in several *in vitro*, animal, and clinical studies, reporting an efficacy range of 70-100% against these parasites.[37-42] TTO is also effective at low concentrations (*in vitro*) as a bactericide (at 0.002–2%; including against MRSA [methicillin-

resistant *S. aureus*]), and as an anti-inflammatory agent (≤ 0.125%).[35] In sum, an ideal therapeutic candidate for tungiasis should be able to kill the embedded parasite, prevent inflammatory skin reactions, and block bacterial infection. The unique parasiticidal, antibacterial, and anti-inflammatory properties of TTO appear to hold tremendous potential in reducing the burden of tungiasis and its deadly sequalae. The aim of this RCT is to investigate the safety and efficacy of a 5% v/w TTO-proprietary gel formulation in comparison with the locally endorsed, 0.05% w/v $KMnO_4$ solution for tungiasis treatment in children.

## METHODS AND ANALYSIS

This protocol has been written in line with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (Supplemental file 1).[43]

### Study setting and design overview

The study will be conducted at eight selected primary schools (permission letters obtained from the respective directors of the schools) in Kisii and Nyamira counties, South-Western Kenya where tungiasis is endemic.[9] Schools have been selected based on the presence of students with tungiasis and willingness of the principals to collaborate in the study. Schools already have strong collaborative working relationships with our community collaborator, Global School Partners (GSP), a local non-government and not-for-profit charity organisation in Kenya (GSP).[44] This pre-existing network of the GSP with school directors and student parents will be utilised to facilitate the successful completion of this study.

The study is designed as an assessor-blinded, Phase II RCT. It will be conducted in the dry season as tungiasis peaks during this period.[14] Eighty-eight participants with tungiasis will be recruited and randomised in a 1:1 ratio to receive either the 5% TTO proprietary gel formulation or 0.05% $KMnO_4$ solution. TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. It contains 5% v/w pure and standard Australian TTO (ISO 4730: 2017 and AS 2782: 2017), approximately 14% poloxamer 407 gel, and other excipients such as formulation stabilisers, penetration enhancers, and preservatives. It will be prepared following the WHO's current Good Manufacturing Practice (Institute of Drug Technology (IDT) Limited, Australia). $KMnO_4$ solution contains 0.05g $KMnO_4$ in a litre of water. The selection of $KMnO_4$ solution as the active comparator in this study reflects its status as a local tungiasis treatment used in mass campaigns in children (and adults) in Kenya,[45] and its being the recommended tungiasis treatment by the Kenyan Ministry of Health.[9] Study participants' feet will be fully assessed as

more than 95% of embedded sand flea lesions are localised to this site (toes, soles, and heels),[6 7 46] with lesions staged according to Fortaleza classification system (Supplemental file 2).[47] The test and control interventions will then be applied twice-daily on days 1, 4, and 7. These treatment days are selected based on the lifecycle of the embedded sand flea. As a sand flea can take up to 1-2 weeks to develop from stage II/III (viable embedded lesions) to stage IV (dying or dead embedded sand flea),[27 47] the use of the 3 treatment doses is designed to ensure that any stage II or III embedded sand flea lesion would be killed by the treatments before they die due to their natural course. After the treatment, viability signs of embedded fleas in each participant will be monitored. The proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 will be determined and compared between test and control groups, as the primary outcome.

**Study personnel**

The trial will be conducted by a recruitment team and a study team in each school. These teams will be composed of staff members of GSP.[44] The recruitment team will consist of school nurses led by a recruitment officer. This team will be responsible for liaising with the school directors and caregivers to facilitate the participants' informed consent and allocation procedures. The school directors will be used as mediators to reach out to caregivers and potential participants. The members of the team will receive information and training about the trial particularly the recruitment procedure.

The study team will comprise clinical advisors and clinical assessors, led by one of the clinical advisors. The clinical advisors are experienced medical doctors working in hospitals located in the study areas. The clinical assessors are school nurses who will be responsible for collecting baseline demographic and disease characteristics, treating participants and performing outcome assessments. They will be trained on the overall trial and outcome assessment (viability assessment and staging of the embedded sand fleas), intervention application, and safety monitoring procedures. The clinical advisors will supervise the clinical assessors, particularly in outcome assessment procedures, and be consulted in any case of diagnostic uncertainty. The reports of clinical assessors will further be evaluated by a panel of infectious disease specialists or offsite clinical assessors by evaluating the photograph records of each participant.

## Sample size calculation

The sample size calculation is based on the primary outcome measure, assuming the 0.05% $KMnO_4$ solution will have a 40% efficacy[24], and the 5% TTO proprietary gel formulation will have a 70% efficacy at 10 days. There are no reports of clinical trials exploring TTO proprietary gel formulation for tungiasis treatment. Hence, the efficacy of TTO was estimated based on the existing observational studies on tungiasis, the clinical experience of our team members, findings from similar trials exploring other tungiasis treatments, and findings of studies (*in vitro* and *in vivo*) on TTO against other ectoparasites. To enable the detection of this 30% difference with at least 80% power at a significance level of 5%, a sample size of 40 participants per arm (88 in total accounting for 10% attrition, as seen in similar settings[26]) is required.

## Study participants

The study population will consist of school children aged 6–15 years from eight schools with a confirmed diagnosis of tungiasis. The age range of 6–15 years was selected because tungiasis is highly prevalent in this group.[48 49]

## Consent and assent

Before starting the study, face-to-face meetings with caregivers, participants, and school directors will be held to explain the objectives of the research and to facilitate an understanding of how the research aligns with community values. The overall procedure of the study, the nature of the disease, the preventive strategies, details of the treatments, and risks and benefits of participation will also be explained to caregivers and participants using instruction manuals containing coloured photographic images to ensure they fully understand the consequences of participation. A pictorial consent flipchart will be used and any study documents including information booklet will be translated into the locally spoken language to assist and facilitate the consent process After this explanation, the participant's legally responsible caregivers caregiver/parent will be provided a participant information sheet and asked to complete an informed consent with written assent (if aged 12-15 years) or verbal assent (if aged 6-11 years) provided by children (Supplemental files 3 and 4). If a subject and his/her caregiver are unable to read, an impartial witness must be present during the entire informed consent discussion. The signature of the impartial witness will certify the subject's consent. The participant's parent/caregiver subject will receive a signed and dated copy of the consent from.

**Recruitment and enrolment**

Potential participants with tungiasis will be identified in each school and recruited by the recruitment team over three months. Eligibility assessment (presence of viable embedded sand fleas) will be initiated by the clinical assessors under the supervision of the clinical advisor as per the inclusion and exclusion criteria. If a potential participant meets the study criteria, he or she will be invited to a room designated for study procedures, referred to as a study centre, for further examination.

Participants' must have at least one viable embedded sand flea lesion (stage II or Stage III) as inclusion criterium. Viable embedded sand flea lesions located at the tip of toes, soles, and rim of heels will be exclusively selected for this purpose.

Participants' exclusion criteria are 1) participants with cluster lesions (≥3 together) or manipulated lesions. 2) the presence of complicated sand flea lesions requiring antibiotic treatment (these children will be referred to nearby health facilities for appropriate management); 3) evidence that guardian/parent/caregiver intend to change their place of residence during the study period; 4) known history of allergy to any of the study medications (TTO or other essential oils and/or $KMnO_4$); and 5) the use of systemic or topical drugs or medications, particularly antibiotics, which may interfere with the study results.

Eligible participants will be instructed to come back to the study centre located in each school for randomisation, baseline assessment, treatment, and outcome assessments. An outline of the recruitment and enrolment process with study timeline is given in **Figure** 1.

Figure 1: Overview of the study process. *BID- twice daily

**Randomisation and treatment allocation**

Participants will be allocated to either the test (5% TTO gel) or control (0.05% $KMnO_4$ solution) group in a 1:1 ratio using a predetermined, computer-generated randomisation schedule developed by an independent statistician who will not be directly involved in the study. All participants in each school will be allocated in the study with participant from new schools included to the study until the minimum sample is reached. The randomisation schedule will be kept secure (password-protected) by the statistician. The randomisation schedule will be concealed from trial participants, clinical assessors, and data assessors (who will be analysing the data) until the participants have been assigned into the trial.

**Blinding**

Foot bathing with the $KMnO_4$ solution may change the colour of the skin to dark purple. As a result, the trial participants and onsite clinical assessors cannot be blinded to the trial interventions. However, a blind assessment of photographs of tungiasis lesions by an expert panel of clinicians (offsite clinical assessors) during the data analysis phase will prevent any likelihood of investigator bias in the outcome assessments. To keep the offsite clinical assessors and data assessors blind, they will not be involved in the clinical trial procedures or have any contact with trial participants. Given the primary efficacy outcome will be measured three days after the last treatment, we do not consider that the colour of $KMnO_4$ solution on the feet would compromise the blinding of offsite clinical assessors. The onsite team will carefully assess the skin surrounding the targeted lesions and ensure the absence of an any trace of purple staining prior to taking the photographs. In any case that the blinding is broken, the study team will document the date and reason for breaking.

**Study participant treatment**

The randomised participants will receive either the test (5% TTO-proprietary gel formulation) or control (0.05% $KMnO_4$ solution) intervention. They will be required to attend the study centre in each school twice-daily (AM and PM) on days 1, 4 and 7 for the treatments. At each visit the feet of the participants will be washed with water and soap, dried with a clean towel, and toenails clipped as necessary to enable the easier application of the products. The allocated treatments will be applied by the clinical assessors. The test product will be applied by smearing the required amount (up to 8g/day) of the product on the palms and spreading it over the skin surface of the feet up to the ankle including the soles, and interdigital areas (between toes). The treated feet will then be left for about 15 minutes to allow the test products to dry. In contrast, the comparator product will be applied by immersing and bathing the feet up to the ankle in a bucket containing a 0.05% KMnO4 solution (up to 2.5 litres) for 15 minutes. After sun drying the feet, a thin layer of petroleum jelly, fully covering the treated surface, will be applied for the purpose of softening the roughness on the skin caused by the KMnO4 treatment.

After the initial treatment (day 1 AM), all participants will be given pairs of closed shoes to be worn throughout the study period and to be kept after the study participation. This will help the treatments remain on the feet and protect the feet from contamination with dirt and water. Also, wearing closed shoes may decrease reinfestation. Participants will be advised to avoid using or mixing any other tungiasis treatments with trial medications during the study period. They will

also be advised at each visit to regularly wear the provided pair of shoes throughout the study period. Dates and times of start and end of treatment application, as well as any noncompliance with the trial protocol will be documented in the CRF.

**Outcome assessment**

*Primary outcome*

The primary and secondary efficacy end points are the proportion of observed viable embedded sand fleas that have lost viability (non-viable lesions) by day 10 (9 days after first treatment) and by day 5 (4 days after the first treatment), respectively. Participants will be required to attend the study centre in each school once daily (AM or PM) at baseline, days 5 and 10 for the outcome assessment. At baseline, viability of the embedded sand flea lesions located in the feet will be assessed using a handheld digital video microscope, assisted with pictorial flipcharts. Sites of all viable (stage II – III) lesions will be recorded on the foot diagram sheets and the entire feet and appearance will be photographed to document the baseline characteristics of the embedded sandflea lesions. Two viable embedded sand flea lesions will be selected as target lesions and will then be observed for their viability at each outcome assessment visit. All the information collected at baseline, such as the number of viable embedded sand flea lesions, non-viable lesions, manipulated lesions, SSAT, itching, pain, and pain-related and itching-related sleep disturbance, will be documented and recorded in each participant's case report form (CRF). The photographs will also be linked to the participant's CRF (Supplemental file 5). At each follow up visit, the entire feet of participants will also be thoroughly examined and the two target lesions per participant selected during bassline assessment, will be observed for their viability on days 5 and 10. The number of target lesions that become non-viable after the interventions will be recorded for each study participant at each follow-up visit. Photographs will be recorded and reviewed during the analysis phase to confirm observations recorded in the CRF

A panel of blinded offsite clinical assessors will evaluate photographs of the targeted embedded sand flea lesions taken at baseline, days 5 and 10 independently of the onsite clinical assessors and the primary outcome measure will be determined by the blinded photograph assessment of the offsite clinical assessor. Any discrepancy in the assessment results will be adjudicated by a third person. An empirical evaluation of the onsite versus offsite agreement will be performed using the kappa coefficient to determine reliability of the assessment. To evaluate the efficacy

of the test intervention, the proportions of non-viable lesions in the test group will be compared with the control groups at day 10.

*Secondary outcomes*

The secondary outcomes are severity score for acute morbidities (SSAT), itching, pain, pain-related and itching-related sleep disturbance, safety, and participant acceptability of the trial intervention/s. The SSAT, which includes typical signs of local inflammation (erythema, oedema and warmness) and the presence of suppuration, ulcers and fissures, will be evaluated by the clinical assessors at baseline, days 5, and 10, using a validated scoring system designed for tungiasis morbidity assessment.[50] The entire feet and appearance will be photographed and recorded in the CRF to evaluate this outcome measure. The itch-man scale for pain,[51] and 4 point tungiasis pictorial scales[18] for pain, and pain-and itching-related sleep disturbance will be used to evaluate these outcomes.

Safety will be assessed through adverse events (AEs) and evaluations of the skin irritation during each visit (days 1, 4, 5, 7, and 10). Participants/caregivers (in-person or on the phone) will be asked at each follow-up visit by the study team about the occurrence of local (stinging/burning, irritation, and itching) or systemic AEs (nausea and headache). Children will be physically examined for evidence of local swelling, erythema and fever. The severity of the AEs will be categorised as mild (Grade 1), moderate (Grade 2), severe (Grade 3), and life-threatening (Grade 4) according to the common terminology criteria for adverse events (CTCAE) v5.0 guideline (Supplemental file 2).[52] Acceptability of the treatments will be assessed at the end of the study (day 10) by asking the participants to rate the treatment in terms of effectiveness, side effects, convenience, suitability, and overall satisfaction. Responses to these questions will be recorded in the CRF.

**Adherence and retention**

Continuous motivation and advice will be given by the clinical assessors to the participants at each visit throughout the study to promote study retention. Participants and/or carers will also be asked to complete a treatment diary recording the daily progression of the condition – which will reinforce the need for optimum treatment compliance. Community home visits will be organised, if required (e.g. in case of absenteeism from school).

**Monitoring and reporting of adverse events (AEs)**

If AEs occur, the clinical advisors will determine the relationship between the AEs and the trial medication. AEs considered related to the trial medication will be followed up until either

resolution, or the event is considered stable. All Grade 1 and 2 AEs reported spontaneously by the subject or observed by the study team will be recorded in the AE form and documented in each participant's CRF. The following information about each AE will also be recorded where available: description, onset and end date, severity, expectedness, assessment of relatedness to trial medication, what action was taken afterwards, and whether the participant was withdrawn from the trial.

A Serious Adverse Events (SAEs) will also be reported to the Human Ethics Committees and regulatory bodies as per the reporting schedule stipulated in their guidelines. The following information will be documented in the SAE form: description, classification, start date, status/outcome, relatedness to study intervention, therapy given, and any actions taken to study intervention.

**Statistical analysis**

All data will be reported following the Consolidated Standards of Reporting Trial (CONSORT) guidelines (Supplemental file 6).[53] A detailed analysis plan will be approved by all investigators before any data analysis. The data will be analysed by the study statistician who will be blinded to the treatment allocation. Statistical analyses will be performed for both the intention to treat (ITT) and per-protocol (PP) populations. The ITT population will include all randomised participants treated or not, and any participants who withdraw prematurely or poorly comply with the protocol. The PP population will be all subjects who are enrolled in the study, randomly assigned to the treatment regimen, received three doses (twice daily) and did not deviate from the study protocol in a clinically significant manner. Results will be considered significant if $p \leq 0.05$.

Baseline characteristics collected on each patient will be reported and compared between randomisation group including age, sex, number of viable embedded sand flea lesions, SSAT, as well as scores for pain, itching and sleep disturbance. Categorical (qualitative) variables will be summarised by frequency and percentage. Continuous variables will be summarised as mean and standard deviation in case of normal distribution and as median and interquartile range in case of non-normal distribution. The Shapiro-Wilk test will be used to assess the normality of the distribution of outcome variables for both groups. Independent student's t or Mann-Whitney tests will be used to investigate differences in continuous variables, and chi-squared tests will be used to identify significant variations in proportions across treatment groups.

Based on the change in primary outcome, the efficacy of test and comparator products will be compared at each follow up visit. The difference in proportion of non-viable lesions between the test and control groups will be compared using student's t-test or Mann-Whitney tests depending on the distribution and presented as relative and absolute risk reductions with their respective 95% confidence intervals and P values. Further, within-group differences will be assessed using paired t-test in case of normally distributed data and a Wilcoxon signed-rank test in case of non-normally distributed data. Secondary outcomes will be compared in the same fashion as the primary outcome.

### Study management

Quality assurance audits of the clinical trial and related documentation will be performed during and after this study in accordance with the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines and recommendations.[54 55] The quality assurance will also consider the Kenyan Good Clinical Practice (GCP) guideline and the Pharmacy and Poisons Board (PPB) requirements. Trial SOPs will be used to ensure that the trial will be conducted, and data are generated, documented (recorded), and reported in compliance with the latest approved protocol, ICH-GCP, Declaration of Helsinki, Kenyan GCP, PPB and National Commission for Science Technology and Innovation (NACOSTI) requirements. The data monitoring committee will involve a medical practitioner, toxicologist and pharmacist.

### Patient and public involvement

Patients and/or the public were not involved in the study design, or conduct, or reporting, or dissemination plans of this research. Study results and feedback will be disseminated to patients by local trial team in formats that are useful and understandable, such as community meetings, presentations, websites, and social media.

## ETHICS AND DISSEMINATION

### Ethical approval

The trial protocol has been approved on August 29, 2019 by the University of Canberra Human Research Ethics Committee (HREC20192114) and registered with WHO accredited registries (Supplemental files 7–9). Further, the investigators will secure ethical approvals from one of the National Commission for Science Technology and Innovation (NACOSTI, Kenya) accredited ethics review committees and will seek letters of support from both the Kenyan Ministry of Health and Ministry of Education.

### Confidentiality and access to data

The privacy of participants will be protected by appropriate collection and storage of data. Participants will be identified only by initials and a participant ID number on the CRFs and in any electronic databases. Data collection forms will be stored in locked filing cabinets in a locked office at the participating schools until the end of the study period, which will then be transferred to the University of Canberra and handled as per the university's recommended data storage guideline for clinical trials. All documents will only be accessible by trial staff and authorised personnel. Documents containing participant's identifying information will not be stored electronically and will be anonymised as soon as practical. Participants will be advised their records may be examined by lawful authorities but will be treated with strict confidentiality and will not be made publicly available.

**Dissemination**

Study results and feedback will be disseminated to end-users (participants and community members counties' health bureaus and other relevant government organisations) in formats that are useful and understandable, such as community meetings, presentations, websites, social media, and radio announcements. The findings of this study will also be disseminated through peer-reviewed journals and national and international scientific meetings.

## DISCUSSION

In endemic communities, tungiasis morbidity is caused by the parasite and associated inflammatory skin reactions and secondary bacterial infections. Thus, proposed treatment options should have the potential to address the morbidities caused by the parasite and treat secondary bacterial complications. In this vein, TTO is a strong fit for tungiasis treatment as it possesses a unique combination of parasiticidal, antibacterial, anti-inflammatory, and wound healing properties.[35] There has been a claim that TTO causes skin irritation or allergic contact dermatitis.[56] In a suitable pharmaceutical base at concentrations ≤25%, multiple clinical studies[57-63] have shown that TTO has no or low risk of adverse skin reactions. While potential toxicity in children is yet to be extensively evaluated, a report from a RCT[64] in children (mean age 6.3+5.1 years) with viral molluscum contagiosum demonstrated that 75% (v/v) TTO was well tolerated in the 30-day treatment period. TTO's sensitising potential is largely due to elevated levels of peroxides and other degradation products from oxidised oil.[65] When correctly stored in amber glass bottles with polypropylene caps, TTO has no appreciable degradation for up to 12 months.[35 56] Due to its high volatility, 90% of the applied TTO rapidly evaporates, minimising the potential for components to permeate the dermis and bloodstream.

Nevertheless, key active components (terpinen-4-ol, α-terpineol, and 1,8-cineole) have sufficient epidermal penetration to provide antimicrobial, anti-inflammatory and potentially insecticidal and acaricidal effects.[34]

If TTO gel effectively treats tungiasis, this trial will provide compelling evidence for a simple, affordable and effective treatment for disadvantaged populations with a significant health burden. This will lead to a significant change in the treatment of this neglected condition. While the tungiasis-affected children in selected Kenyan villages are intended as the primary beneficiaries of this research, the pattern of tungiasis and associated bacterial complications among children is analogous to that observed in resource-poor and underprivileged endemic communities in many parts of the world, especially in sub-Saharan Africa. Thus, the results from this study have the potential to provide evidence for a global health role of TTO in managing tungiasis and its associated complications in children.

**Authors' contributions:** SA and JT conceived the study. GMP, AB, JKC, JH, JM and SC contributed to the study design. SA and JT drafted the manuscript. JKC, JM, WT, SVN, AYC TS, SK, GK, SC, JH, HF, AB, MD, and GMP assisted in developing the protocol and have reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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**Competing interests' statement:** None

**Patient consent for publication**: Not required.

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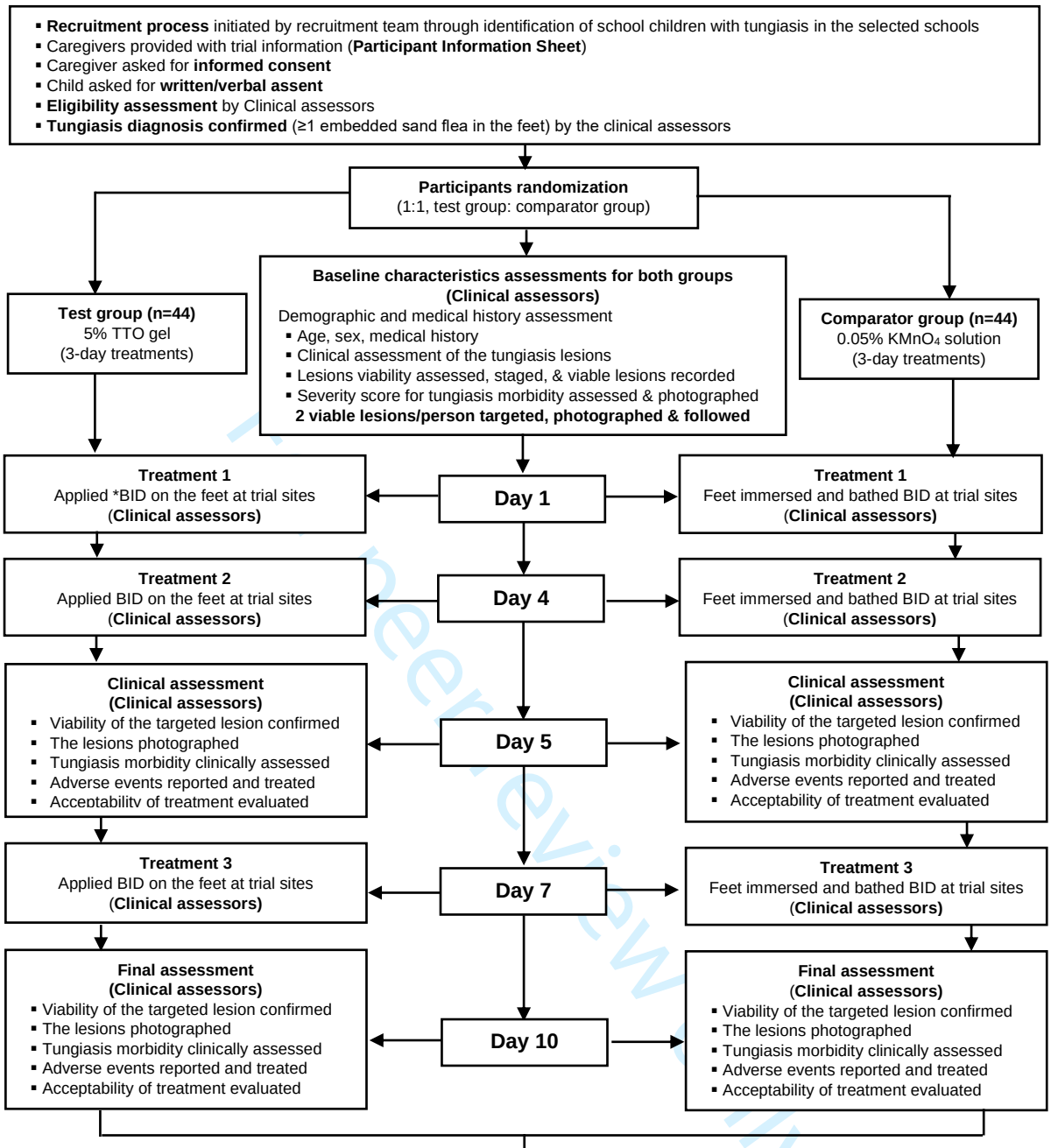


Figure 1: Overview of the study process. *BID- twice daily


SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Page NO |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 2 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | Attached as supplement |
| Protocol version | 3 | Date and version identifier | Attached as supplement |
| Funding | 4 | Sources and types of financial, material, and other support | 15 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1 &15 |
| | 5b | Name and contact information for the trial sponsor | 1 & 15 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | None |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see item 21a for data monitoring committee) | 6 |

| | | | |
|---|---|---|---|
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 3-5 |
| | 6b | Explanation for choice of comparators | 5-6 |
| Objectives | 7 | Specific objectives or hypotheses | 5 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 5 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 5-6 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 8 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 5-6 & 8-9 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 12-13 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 11 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 8 |

| | | | |
|---|---|---|---|
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 10-11 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 7-11, Figure 1 and Supplemental file 2 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 7 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 8 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: | | | |
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 8 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 8 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 8 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 9 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 9 |
| **Methods: Data collection, management, and analysis** | | | |

| | | | |
|---|---|---|---|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10-11 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 10-11 & 12-13 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 13-14 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 12-13 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 12-13 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 12-13 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 13 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 13-14 |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 11-12 |

| | | | |
|---|---|---|---|
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 13 |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 13 |
| Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | Attached as supplement |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 6-7 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 13-14 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 15 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 13-14 |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 11-12 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 14 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 14 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 13-14 |
| **Appendices** | | | |

| | | | |
|---|---|---|---|
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Attached as supplement |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

# Supplemental file 2

## Supplemental file 2.1: Fortaleza classification system

Embedded sand flea lesions are stratified into different developmental stages, as per the Fortaleza classification system[1] .

**Table 1:** Fortaleza classification system

| Stages | Appearance/phases | Symptoms | Time span |
|---|---|---|---|
| Stage I | Penetrating flea (penetration) | Erythema, and itching | 30 min–several hours |
| Stage II | Brownish-black dot (beginning of hypertrophy) | Erythema surrounding a central black dot, unpleasant itching, and pain | 1–2 days after penetration |
| Stage $III_a$ | White (tender) halo with black dot at the centre (hypertrophy) | Eggs expulsion, faecal coil, brownish-watery secretion, pulsation, severe itching, pain, and tenderness | 2– 6 days after penetration |
| Stage $III_b$ | White (non-tender) halo with caldera formation, discoloration, and skin peeling around lesion (hypertrophy) | Eggs (white and shining) expulsion, faecal coil, pulsation, watery secretion, severe pain while walking, and loss of tenderness | 6 days–3 weeks after penetration |
| Stage $IV_a$ | Brownish-black wrinkled lesion (involution) | Rare egg expulsion and pulsation, sporadic faecal expulsion, and watery secretion | 3–4 weeks after penetration |
| Stage $IV_b$ | Brownish-black, necrotised, desiccated lesion (crust) (involution) | No vital signs (pulsation, egg, faeces, and watery secretion), (dead flea) | 4–6 weeks after penetration |
| Stage V | Circular depression in the stratum corneum (residue) | No flea | 6 weeks–several months after penetration |

Stage II and III lesions can be classified as viable embedded sand flea lesions, whereas stage IV is classified as a lesion with either a dying ($IV_a$) or dead ($IV_b$) embedded flea. An embedded sand flea is considered to be viable when any of the viability signs (expulsion of eggs, excretion of faecal threads, excretion of faecal liquid, and/or pulsations/contractions) are observed using diagnostic tools (hand held digital microscope).[1]

## Supplemental file 2.2: Study schedule

**Table 2**: Study schedule of enrolment, interventions, and assessments.

| Study procedures | Time points | | | | | |
|---|---|---|---|---|---|---|
| | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 |
| | Day 0* | Day 1 | Day 4 | Day 5 | Day 7 | Day 10 |
| **Recruitment and enrolment** | | | | | | |
| Training clinical recruitment and study team | X | | | | | |
| Identifying potential participants with tungiasis | X | | | | | |
| Participant information sheet | X | | | | | |
| Informed consent/assent | X | | | | | |
| Subject demographics / medical history | X | | | | | |
| Inclusion/exclusion criteria - review | X | | | | | |
| Concomitant medications - review | X | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Subjects instructions | X | | | | | |
| Subject randomisation | X | | | | | |
| Baseline assessment-lesion viability & staging | X | | | | | |
| Baseline assessment-acute tungiasis morbidity | X | | | | | |
| **Study intervention** | | | | | | |
| Distribution of intervention products | X | X | X | | X | |
| Application of test intervention | | X | X | | X | |
| Application of control intervention | | X | X | | X | |
| **Outcome assessment** | | | | | | |
| Efficacy outcome-viability of embedded sand flea | | | | X | | X |
| Acute morbidity outcome-SSAT, itching & sleep disturbance | | | | X | | X |
| Safety outcome-monitoring AEs | | X | X | X | X | X |
| Product acceptability outcome | | | | X | | X |
| Study compliance confirmation | | X | X | X | X | X |

## Supplemental file 2.3: Adverse events grading

**Table 3**: Grading severity of adverse events.

| Grade | Type | Description |
|---|---|---|
| Grade 1 | Mild | Signs or symptoms which are easily tolerated, does not interfere with the subject's usual function; clinical or diagnostic observations only; intervention not indicated |
| Grade 2 | Moderate | Signs or symptoms causes interference with usual activity or affects clinical status; minimal, local or non-invasive intervention indicated |
| Grade 3 | Severe | Signs or symptoms affect clinical status and likely requires medical intervention and/or close follow-up |
| Grade 4 | Life-threatening | Sign or symptom results in a potential threat to life; urgent intervention indicated<br>This grade will be considered as SAE |

## Supplemental file 2.4: Parasiticidal and repellent effects of tea tree oil (TTO)

**Table 4:** Summary of studies on the insecticidal, acaricidal, and repellent effects of TTO.

| Study setting | Study design | TTO concentration or volume tested | Ectoparasite (insect or arachnid) | Treatment outcome |
|---|---|---|---|---|
| Akkad *et al*. 2016,[2] Egypt | *In vitro* | 5% TTO Head Lice Gel | Louse (*Pediculus humanus capitis*) | 96.7% mortality |
| Alver *et al*. 2017,[3] Turkey | *In vivo* | 10% TTO eye shampoo with 4% gel | Mite (*Demodex folliculorum* & *D. brevis*) | 82.1% improvement in blepharitis |
| Barker & Altman 2010,[4] Australia | RCT | 10% w/v TTO and 1% w/v lavender oil NeutraLice Lotion® (TTO/LO) | Louse (*Pediculus humanus capitis*) | 97.6% cure rate |
| Benelli *et al*. 2013,[5] Italy | *In vitro* | 1.5-3 µL oil/cm2 TTO | Mediterranean fruit fly (*Ceratitis capitate*) | >60% mortality |
| Callander & James 2012,[6] Australia | *In vitro* | 2.5-3% TTO | Blow fly (*Lucilia cuprina*) | 100 % ovicidal and larvicidal (1st instar) & 100% repellent effect for 7hrs |
| De Wolff 2008,[7] USA | *In vitro* | 20% TTO | Fleas (*Siphonaptera*) | 78% mortality(in1hr) and |

| | | | | |
|---|---|---|---|---|
| | | | | 100% mortality (in day) |
| Di Campli *et al*. 2012,[8] Italy | *In vitro* | 1-8 % TTO | Louse (*Pediculus humanus capitis*) | 100 % mortality |
| Ellse *et al*. 2013,[9] UK | *In vitro*<br>*In vivo* | 5% & 10% TTO<br>5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | >80% mortality |
| Ellse *et al*. 2016,[10] UK | *In vivo* | 5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 78% mortality |
| Fitzjarrell 1995,[11] USA | *In vivo* | 2–10% v/v TTO | Fleas (*Siphonaptera*) | 100% mortality |
| Gao *et al*. 2005,[12] USA | *In vitro* and *in vivo* | 50–100% TTO | Mite (*Demodex folliculorum*) | 100% mortality |
| Iori *et al*. 2005,[13] Italy | *In vitro* | 8 -10µl TTO | Tick (*Ixodes ricinus*) | >80% mortality |
| James & Callander 2012,[14] Australia | *In vitro* | 1–20% TTO | Sheep louse (*Bovicola ovis Schrank*) | 100% mortality (adult lice and eggs) |
| James & Callander 2012,[15] Australia | *In vivo* | 1–2% TTO | Sheep louse (*Bovicola ovis Schrank*) | 100% mortality |
| Klauck *et al*. 2014,[16] Brazil. | *In vitro* | 5.0% TTO | Houseflies (*Musca domestica* & *H. irritans*) | 100% mortality |
| Maher 2018,[17] United Arab Emirates | *In vivo* | 5% TTO eyelid scrub | Mite (*Demodex folliculorum*) | 100% improvement in symptoms |
| Nicholls *et al*. 2016,[18] Australia | *Case series (in vivo)* | 5 % TTO | Mites (*Demodex folliculorum* & *D. brevis*) | 91% improvement in symptoms |
| Pazinato *et al*. 2014,[19] Brazil | *In vitro* | 1–10 % TTO & 0.075–0.75 % TTO nanoparticles | Tick (*Rhipicephalus (Booophilus) microplus*) | 100 % reproductive inhibition<br>70 % mortality |
| Sands *et al*. 2016,[20] UK | *In vitro* | 5% TTO | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 100% mortality |
| Talbert & Wall 2012,[21] UK | *In vitro* | 0.5–10% *TTO* | Donkey chewing louse (*Bovicola (Werneckiella) Ocellatus*) | 100% mortality |
| Walton *et al*. 2004,[22] Australia | *In vitro* | 5% TTO | Scabies mite (*S scabiei var hominis*) | 100% mortality |
| Walton *et al*. 2000,[23] Australia | *in vitro* | 5% TTO | Scabies mite (*S scabiei var hominis*) | 100% mortality |
| Williamson *et al*. 2007,[24] UK | *In vitro* | 10% TTO | House dust mites **(***Dermatophagoides pteronyssinus* & *D. farinae*); Louse (*Pediculus humanus capitis*) | 100% immobility<br>100% mortality |
| Yim *et al*. 2016,[25] Australia | *In vivo* | 2–5% TTO | Cattle tick (*Rhipicephalus australis*) | 78–100% repellent effect for 2 days |

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UNIVERSITY OF CANBERRA

# PARTICIPANT INFORMATION SHEET AND ASSENT FORM

## Tea tree oil gel for Tungiasis (Jiggers) Treatment

***What the study is about?***

We are testing whether tea tree oil (TTO)-based gel can kill the jiggers in your feet without causing you any pain or discomfort compared to the purple medicine called potassium permanganate, in a10-day treatment period.

***What would I have to do?***

If you agree to be a part of the study, you will be asked to sign this form and to:

1. Allow the study team to wash and carefully examine your feet using a handheld digital microscope
2. Allow the study team to take photographs of your feet
3. Allow the study team to apply the treatment on days 1, 4, and 7
4. Wear a pair of new closed shoes throughout the study period (which we will be provided on day 1)
5. Attend the clinic for treatment and examination on Days 1,4, 5, 7 and 10
6. Avoid applying any other medicine or skin products on the jiggers affected skin area during the study period (1-10 days).
7. Avoid cutting your jiggers affected skin during the study period
8. Follow the study instructions explained to you by the study team

***What are the side effects of taking part?***

TTO has long been used as a medicine by indigenous communities in Australia and internationally and the likelihood for developing sever skin reactions are minimal. However, the trail medication may have some side effects. It may cause skin discomfort with an allergic or irritant reaction. If you suffer from these or any other symptoms you should report them immediately to the study team. If you are concerned in any way, you can speak to study team at the school. As for the purple medicine, it will not hurt you, but it will change the colour of your feet. This colour will go away after a few days. However, the provided closed footwear will adequately mask this skin colouration – and this is likely to prevent other students from giving you a hard time.

***What happens if something goes wrong during the trial?***

The risk of serious side effects is small compared to the risk you face as a result of having jiggers. If you do experience side effects as result trial medications, you will be referred to the nearby health facility for appropriate treatment and medical care.

***What would I benefit from the participation?***

We hope that the TTO gel will help you, but this cannot be guaranteed. The information we get from this study may help us to improve the treatments available for jiggers in the future.

***Will my taking part in this study be kept confidential?***

The information gathered about you by the investigator or obtained during the study will be held by the investigators in strict confidence. All the people who handle your information will adhere to traditional standards of confidentiality and will also comply with all relevant privacy legislation, in Australia and Kenya.

If needed, summary data without your name attached will be made available, to government regulatory bodies in Kenya and Australia.

***Do I have to take part?***

You do not have to be in this study if you do not want to be, even if your parents and teachers said it is okay for you to be in the study. If you decide to stop after we begin, that's okay too. Your parents know about the study too.

# PARTICIPANT INFORMATION SHEET AND ASSENT FORM

UNIVERSITY OF CANBERRA

## Consent approval

1. I have been given clear information, both verbally and in writing, about this study and, having had time to consider it, am able to make an informed decision to participate.
2. I have read and understood the Patient Information Sheet and have retained a copy of it.
3. I have been given the opportunity to ask the investigator questions about the study.
4. I have been told about the possible benefits and risks of taking part and I understand what I am being asked to do.
5. I understand that I may withdraw from the study at any time without affecting any future medical treatment, or the treatment of the condition which is the subject of the trial.
6. I agree to take part in this research and for the data obtained to be published provided that my name or other identifying data is not used.
7. I understand that if I leave the study for any reason, the information and samples collected will still be used unless I specifically ask for them to be removed from the study at the time I leave.
8. I understand that the investigators of the trial will adhere to usual standards of confidentiality in the collection and handling of my personal information.

I, _____, agree to participate in the above study. I have read and understood the Participant Information Sheet and I have been given a copy of it. I have been given the opportunity to ask questions about the study. I understand that I may withdraw from the study at any time without affecting my future medical treatment, or the treatment of the condition which is the subject of the trial.

Participant Name: _____ Signature: _____

Date: _____/_____/_____

Investigator Name: _____ Signature: _____

Date: _____/_____/_____

**Thank you for your interest in the study.**

# PARTICIPANT INFORMATION SHEET AND CONSENT FORM

## Tea tree oil gel for Tungiasis (Jiggers) Treatment

You are being invited to take part in this research study because your child has been identified with jiggers in his/her feet. We are asking for your willingness to allow your child to take part in this study. Please take time to carefully read the following information. Ask us if there is anything that is not clear or if you would like more information. Consider carefully before you make your decision whether or not you wish to take part. You may also wish to discuss the study with a relative, friend or your friendly clinical staff at the school.

***What is the objective of this study?***

This study aims to evaluate whether tea tree oil (TTO 5% v/w) gel can kill the embedded jiggers better than the locally recommended potassium permanganate solution followed by Vaseline® application (within a 10-day study period). The study also aims to determine whether the TTO gel can reduce skin inflammation, pain and itching caused by the jiggers better than the potassium permanganate/ Vaseline® treatment. If the proposed treatment is effective, this study might help us improve the treatment outcomes for jiggers.

***What would I have to do?***

We are asking for your willingness to allow your child to take part in this study. If you agree to proceed, you will be given this information sheet to keep and be asked to sign a consent form.

***If we choose to participate, will our participation be kept confidential?***

The information gathered about you child during the study will not be shared. All of the people who handle your information will maintain confidentiality and will also comply with NHMRC clinical trial guidelines and local privacy laws.

***What will happen to my child if we take part?***

Your child will be randomly allocated to either the TTO gel or potassium permanganate treatment. Treatments will be given twice daily on days 1, 4, and 7. In addition, your child will be given a pair of new closed shoes as part of the study. The clinical investigator will then make careful observations about the jiggers on days 5 and 10. The doctor will also ask your child about how much pain and itching he/she is feeling. In summary, your child will be asked to attend the clinics at the school 6 times during the treatment phase (i.e. AM and PM on days 1, 4, and 7), and 2 follow up visits on days 5 and 10. Each clinic-visit will take about 30 mins.

***What would be expected from us during study period?***

It is **VERY** important that you and your child, **DO NOT** cut out any jiggers from the child's foot during this time.

You should not use any other jigger medicine or any other skin products on the affected skin area during this time (days 1-10). We would like you to maintain the daily diary of events during study participation (1- 10 days).

If your child develops a reaction to the trial medication, you should notify the study clinical team as soon as possible.

***What information would be collected?***

The study will not be collecting any samples from your child. We will only make observations of the jiggers. If your child is found to have any other disease, we will advise you on the best way to manage it. The information we collect from your child will be entered into a computer system along with information from other study participants. The study team based at University of Canberra, will analyse the data and prepare a report with findings from this study and necessary recommendations. These findings will be communicated with other organizations, the Kisii, and Nyamira counties and National Ministry of Health, Kenya.

***What would be the risks of participation for the child?***

Tea tree oil (TTO) has been documented as a topical antiseptic (nationally and internationally) for over 90 years and even longer in the indigenous communities in Australia as a bush medicine. The treatment is unlikely to pose any serious health risk to your child. However, the trail medication may have some side effects. It may cause skin discomfort with an allergic or irritant reaction. If your child suffers from these or any other symptoms you should report them immediately to the study team. If you are concerned in any way, you can speak to study team at the school.

# PARTICIPANT INFORMATION SHEET AND CONSENT FORM

UNIVERSITY OF CANBERRA

***Your Right to Refuse or Withdraw from the study***

The decision to participate in the study is entirely voluntary. Clinical examination and treatment will be conducted in the school. This research study has received support and endorsement from the participating school. And you are free to withdraw at any time and without giving a reason.

***What is the contact for further information?***

If you need any further information or have any concerns, you can speak to the school health officer or study team or Doctor Stanislous Misati (GSP: +254 710 521804).

**Consent approval**

The undersigned _____ (full name) testifies that she/he is the legal guardian of _____ (name of child) and that she/he has read and understood the consent form which was also read aloud and explained by _____ _____.

I understand the objectives, the necessities, the potential risks and benefits regarding the participation of my child in the study, including the time commitment during the treatment, assessment and follow-up period.

I agree that any living sand fleas remained at the end of the study will be treated with the local government/medical recommendations.

I am aware of the fact that all information which could lead to an identification of my child will be kept strictly confidential. I have the right to withdraw my child from the study at any time without giving any justification for the removal. I voluntarily agree for my child to participate in this study based on these conditions.

School _____ Date: _____/_____/_____
Subject Study ID-No: _____
Parent/Caregiver
Name: _____ Signature: _____
Date: _____/_____/_____
Investigator who provided the information:
Name: _____ Signature: _____
Date: _____/_____/_____
Witness:
Name: _____ Signature: _____
Date: _____/_____/_____

**Thank you for your interest in this study.**

Version 2.0 dated 20/08/2019

UNIVERSITY OF CANBERRA

# Case Report Form (CRF)

## Treatment of tungiasis using a 5% v/w tea tree oil (TTO) gel: A randomised, controlled, proof-of-principle trial

## Subject Study ID:





Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

UNIVERSITY OF CANBERRA

## A. Recruitment Form

Please complete this form for every child who is identified as a potential participant in the TTO (5% v/w) gel tungiasis Trial

Investigator: _____ Date: ___/___/_____ [dd/mm/yyyy]

School: _____

| **Question** | **Response** (tick one) |
|---|---|
| 1. *Has the child been identified as having active embedded jiggers?*<br>- *If No, excuse participant*<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 2. *Is the child aged between 6 and 15 years?*<br>- *If No, excuse participant*<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 3. Explain the study protocol to the caregiver (and the child if appropriate) with the aid of the Participant Information Sheet.<br>- *Once done, tick 'Done' and proceed* | ☐ Done |
| 4. Is the caregiver able and willing to provide written informed consent for the child to take part in the study?<br>- *If No, record reason (if given) and excuse participant*<br>_____<br>- *If Yes, proceed* | ☐ Yes ☐ No |
| 5. Is the caregiver able and willing to be contacted by telephone (voice call and SMS) after the initial assessment? | ☐ Yes ☐ No |
| 6. Is the child willing to participate in the study?<br>- *If No, excuse participant*<br>- *If Yes, ask child to fill in Written Assent if aged ≥12 years, then proceed* | ☐ Yes ☐ No |
| 7. *Ensure that the child's caregiver has signed informed consent for the child to participate in the study*<br>- *If 'Done', proceed to Eligibility Assessment Form*<br>- *If consent was not given, provide reason below (if given) and excuse participant*<br>_____ | ☐ Done<br>☐ Consent not given |



Investigator signature_____

## B. Eligibility Assessment Form

Please complete this form for every participant who is recruited to the TTO (5% v/w) gel tungiasis trial. This form is used to assess whether the participant meets the criteria to be eligible for enrolment into the study.

Investigator: _____ Date assessed: ___/___/_____ [dd/mm/yyyy]

School: _____

### Study Eligibility Criteria

### Inclusion criteria

Please tick 'Yes' or 'No' for each item.

Both 2 items must be marked 'Yes' for the child to be eligible for enrolment.

| Inclusion criteria | Yes | No |
|---|---|---|
| 1. Is the child aged from 6–15 years with at least 1 viable (stage II and Stage III) lesion according to the Fortaleza classification on the child's feet? *Perform clinical examination of the lesions and confirm their viability based on the four viability signs using the handheld digital microscope. Refer to Figure 1 and Figure 2 on page 10 and 11 of Case Report Form.* | ☐ | ☐ |
| 2. Is the caregiver able and willing to provide written informed consent for the child to take part in the study? | ☐ | ☐ |

### Exclusion Criteria

Please tick 'Yes' or 'No' for each item.

All items must be marked 'No' for the child to be eligible for enrolment

| Exclusion criteria | Yes | No |
|---|---|---|
| 1. Are there any cluster lesions (more than 3 lesions together) and manipulated lesions? | ☐ | ☐ |
| 2. Are there any complicated lesions (severe) requiring antibiotic treatment? | ☐ | ☐ |
| 3. Do the caregivers intend to change their place of residence during the study period? | ☐ | ☐ |
| 4. Has the child used any medication (systemic or topical drugs medication) in the past week? This could include antibiotics, prescription or non-prescription medications, creams, ointments, medicated wash products, etc.<br>If Yes, please tick all that apply and provide name of medication (if known).<br>☐ Oral medication (specify) _____<br>☐ Cream/ointment (specify) _____<br>☐ Anti-itch preparation, e.g. steroid (specify) _____<br>☐ other (specify) _____ | ☐ | ☐ |
| 5. Does the child have a known history of allergy to any of the study medications listed below?<br>If Yes, please tick all that apply.<br>☐ Potassium permanganate<br>☐ Tea tree oil or other essential oils | ☐ | ☐ |



Investigator signature_____

**Eligibility outcome**

The child is eligible for enrolment into the TTO (5% v/w) gel Tungiasis Trial only if they meet all of the inclusion criteria and do not meet any of the exclusion criteria.

For an eligible child, the answer must be 'Yes' to question 1 and 'No' to question 2 below.

| | Yes | No |
|---|---|---|
| 1. Does the child meet all the **Inclusion Criteria** (answered 'Yes' to both 2 questions on page 1)? | ☐ | ☐ |
| 2. Does the child meet any of the **Exclusion criteria** (answered 'Yes' to any of the 5 questions on pages 2)? | ☐ | ☐ |

The participant is

☐ **Not eligible** for the trial

- Please excuse child and caregiver

☐ Eligible for the trial but will not be randomized due to other reasons

- Please specify reason: _____

_____

☐ **Eligible** for the trial and will be randomized

- Proceed to Baseline Assessment form

Form completed by: _____ Date: ___/___/_____ [dd/mm/yyyy]

Signature: _____

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## C. Baseline Assessment

Please complete this assessment form at the participant's first visit (Day 0, Week 0).
Investigator: _____ Date assessed: ___/___/_____ [dd/mm/yyyy]
School: _____

**Participant details**

**Demographics**

| Clinical Assessment 1 - Demographics | Response |
|---|---|
| Age | |
| Sex | ☐ Male ☐ Female |
| Date of Informed Consent from legal caregiver (dd/mm/yyyy) | ___/___/_____ |
| School | |
| Usual place(s) of residence | ☐ Rural ☐ Remote |
| Usual place(s) of residence (name of suburb, town or community) | _____ _____ |

**Physical Examination**

Please record any existing medical conditions (e.g. diabetes), allergies, illnesses (e.g. gastroenteritis). Provide further detail in 'comments' below if necessary.

| Clinical Assessment 2 – Physical examination | | | | | Response |
|---|---|---|---|---|---|
| Height (cm) | | | | | __ __ __ . __ cm |
| Weight (kg) | | | | | __ __ __ . __ kg |
| Date assessed | Study day | BP | Pulse | Temp. | Comment |
| ___/___/_____ | Day 1 | | | | |
| ___/___/_____ | Day 4 | | | | |
| ___/___/_____ | Day 5 | | | | |
| ___/___/_____ | Day 7 | | | | |
| ___/___/_____ | Day 10 | | | | |



Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

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## Medical history

| **Medical condition/illness/allergy** | **Start date** (dd/mm/yyyy) | **End date** (dd/mm/yyyy) | **Condition ongoing** (Y/N) |
|---|---|---|---|
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |
| | ___/___/_____ | ___/___/_____ | |

Please record any medications taken by the child in the last 1 week.

| **Medication name** | **Indication** | **Dose** | **Start date** (dd/mm/yyyy) | **End date** (dd/mm/yyyy) | **Ongoing** (Y/N) |
|---|---|---|---|---|---|
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |
| | | | ___/___/_____ | ___/___/_____ | |

Comments:

_____

_____

_____

_____

_____

## History of Jiggers

Please record answers to these questions about jiggers in the child and their community.

| Clinical Assessment 1 - History of jiggers | Response |
|---|---|
| 1. How long ago did the child's jiggers start? (tick one) | ☐ < 1 week<br>☐ 1-3 weeks<br>☐ 3-6 weeks<br>☐ > 6 weeks |
| 2. Has the child previously been diagnosed with jiggers by a health worker or doctor? | ☐ Yes ☐ No |

| Clinical Assessment 1 - History of jiggers | Response |
|---|---|
| 3. How many times in the past has the child had jiggers? (tick one) | ☐ 0<br>☐ 1-2 times<br>☐ 3-5 times<br>☐ >5 times<br>☐ unknown |
| 4. Has the child been treated with any jigger's medication <u>at any time in the past</u>?<br><br>If Yes, please tick all that apply<br>☐ Potassium permanganate (KMNo4) and Vaseline<br>☐ Vaseline<br>☐ Neem extracts<br>☐ Coconut oil<br>☐ Other (specify) _____<br>☐ Skin cream/ointment, name unknown<br>☐ Oral medication, name unknown<br>If Yes, how long ago did the most recent treatment end?<br>☐ < 1 week<br>☐ 1-2 weeks<br>☐ > 2 weeks | ☐ Yes ☐ No |
| 5. Has the child been treated with any antibiotics in the last 1 week?<br><br>If Yes, what is the name and indication of the antibiotic?<br><br>Name: _____ ☐ Unknown<br><br>Indication: _____ ☐ Unknown | ☐ Yes ☐ No |

## D. Study drug administration

Please record the type of intervention and time of application in this form.

| **Treatment applied** | **Amount applied (g)** | **Date of application** dd/mm/yyyy | **Time of application** 24-hr time |
|---|---|---|---|
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |
| ☐ Yes ☐ No | | ___/___/_____ | ____:____ |



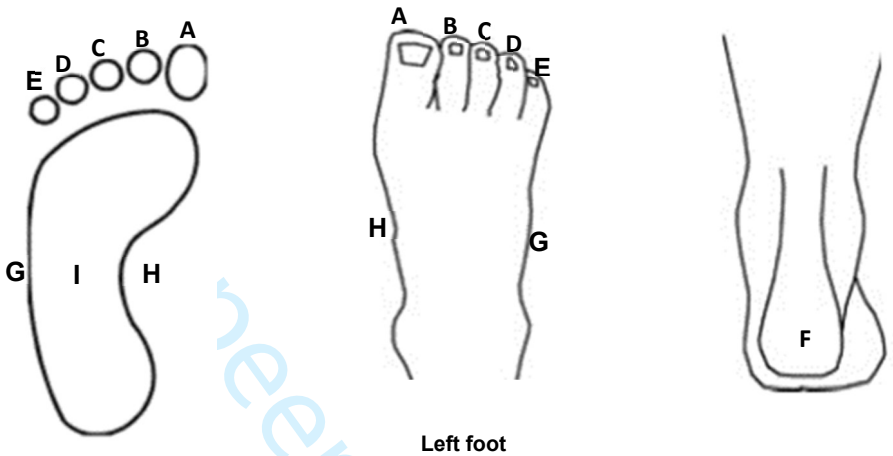
Investigator signature_____

## E. Clinical and symptomatic assessment -1

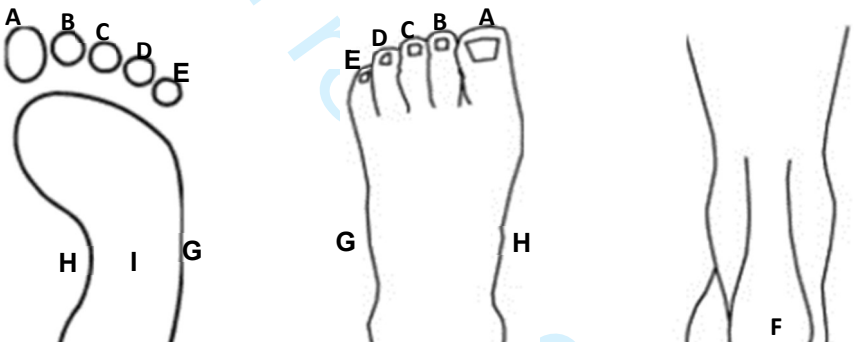
1. Please assess each of the following foot parts for typical jigger lesions (tick if present).

| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side- **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side- **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole- **I** |

**Right foot**




**Left foot**




| | |
|---|---|
| 2. Are any viable lesions present on the child's feet? | ☐ Yes ☐ No |
| 3. Mark all sites of viable lesions on the feet diagrams on pages 9 and 10. | ☐ Done |

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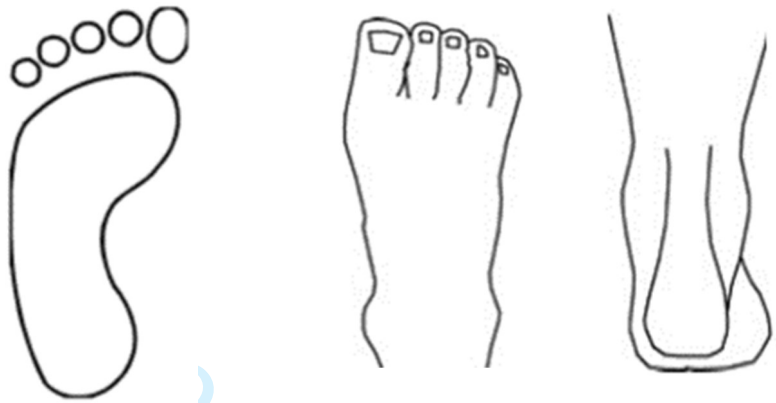
## E. Clinical and symptomatic assessment -1

**Feet diagram – Full**

Mark all sites of active jigger lesions with an X. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **E. Clinical and symptomatic assessment -1** | |
|---|---|
| 4. Select and record 2 target lesion in 2 target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 5. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 6. Photograph each of the 2 viable lesions together with their target sites | ☐ Done |
| 7. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |
| 8. Assess the viability of 2 target lesions. Tick all that apply for each site. | |

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

| | |
|---|---|
| 9. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 10. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***numbers of viable lesions*** (Stages II & III, total) are there on the child's feet? | |
| 12. How many ***numbers of manipulated lesions (total)*** are there on the child's feet? | |
| 13. How many ***numbers of cluster lesions (total)*** are there on the child's feet? | |

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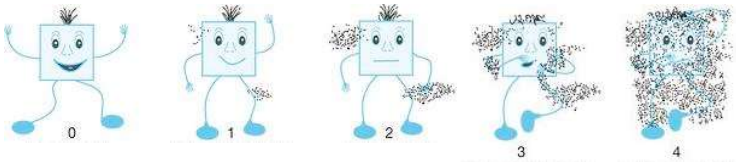
## E. Clinical and symptomatic assessment -1

14. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



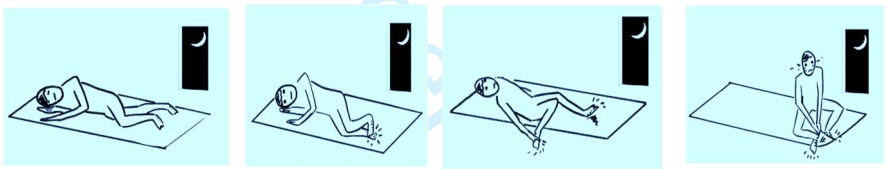

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

16. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

**Acute pathology examination and scoring**

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

| | | Sites on the left foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

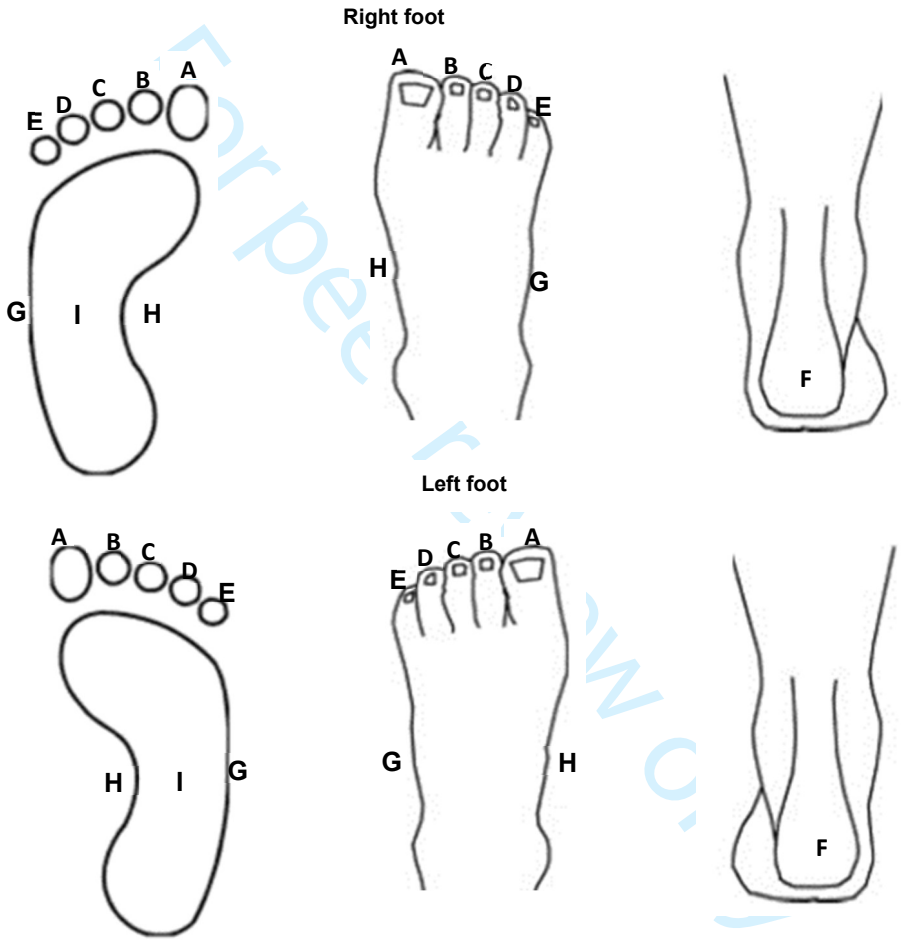


Investigator signature_____

## F. Clinical and symptomatic assessment- 2

1. Please assess each of the following foot parts for new and existing jigger lesions (tick if present).

| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side- **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side- **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole- **I** |




| | |
|---|---|
| 2. Are any new embedded jiggers present on the child's feet? | ☐ Yes ☐ No |
| 3. How many numbers of **newly embedded sand fleas** since the last examination? | |

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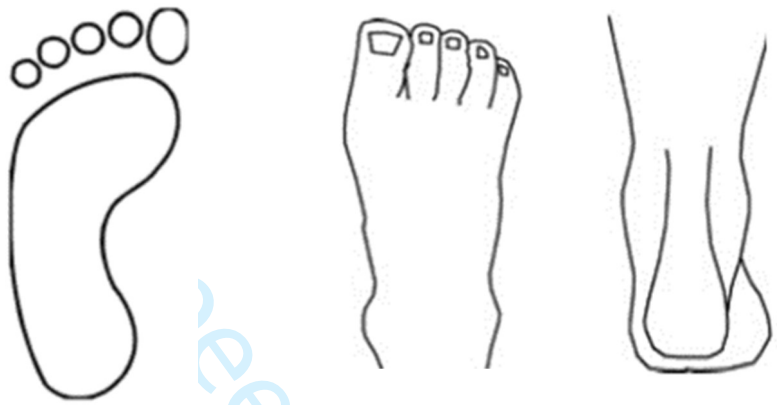
## F. Clinical and symptomatic assessment- 2

| | |
|---|---|
| 4. Mark all sites of new embedded jiggers and existing viable lesions on the feet diagrams on pages 13 and 14. | ☐ Done |

**Feet diagram – Full**

Mark all sites of active jigger lesions with X and newly embedded jiggers with Y. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **F. Clinical and symptomatic assessment- 2** | |
|---|---|
| 5. Follow the selected viable lesions together with their target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 6. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 7. Photograph the 2 target lesions together with their target sites | ☐ Done |
| 8. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |
| 9. Assess the viability of 2 target lesions. Tick all that apply for each site. | |

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

| | |
|---|---|
| 10. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 12. How many ***total numbers of viable lesions*** (sage II & III) are there on the child's feet | |

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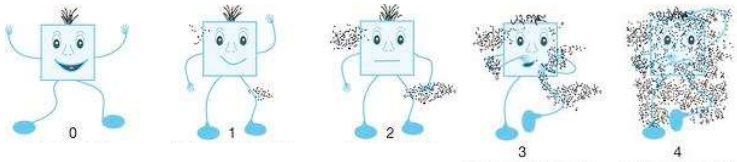
## F. Clinical and symptomatic assessment- 2

13. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



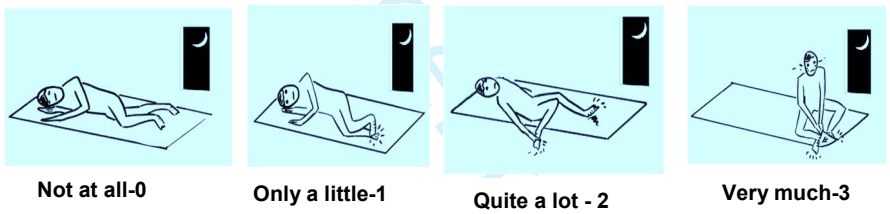

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

14. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

### Acute pathology examination and scoring

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |

| | | Sites on the left foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |



Investigator signature_____

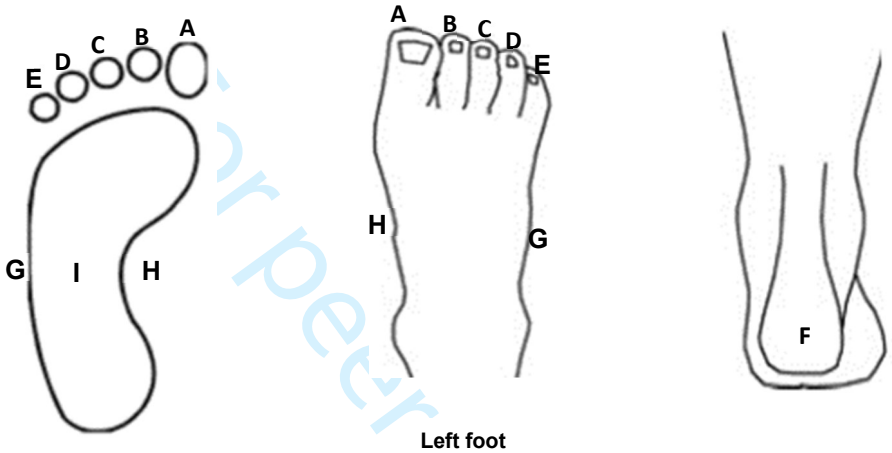
## G. Clinical and symptomatic assessment - 3

1. Please assess each of the following foot parts for new and existing jigger lesions (tick if present).

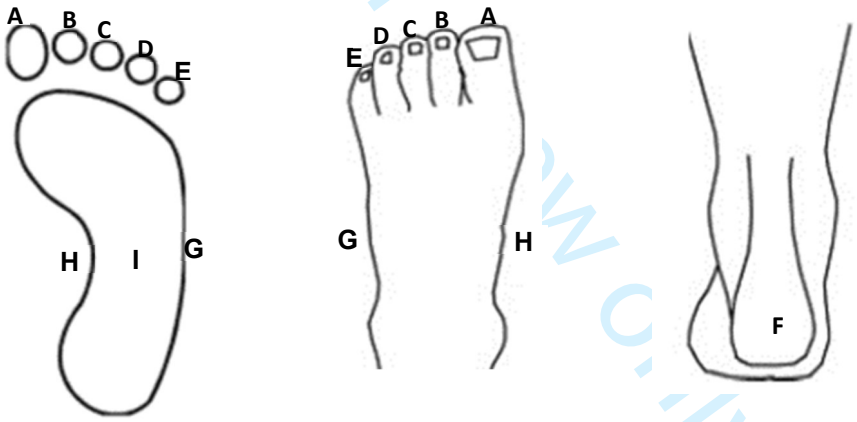
| | | |
|---|---|---|
| ☐ Toe 1- **A** | ☐ Toe 4- **D** | ☐ Lateral side - **G** |
| ☐ Toe 2- **B** | ☐ Toe 5- **E** | ☐ Medial side - **H** |
| ☐ Toe 3- **C** | ☐ Heel- **F** | ☐ Sole - **I** |

_____

**Right foot**




**Left foot**




| | |
|---|---|
| 2. Are any new embedded jiggers present on the child's feet? | ☐ Yes ☐ No |
| 3. How many numbers of **newly embedded sand fleas** since the last examination? | |

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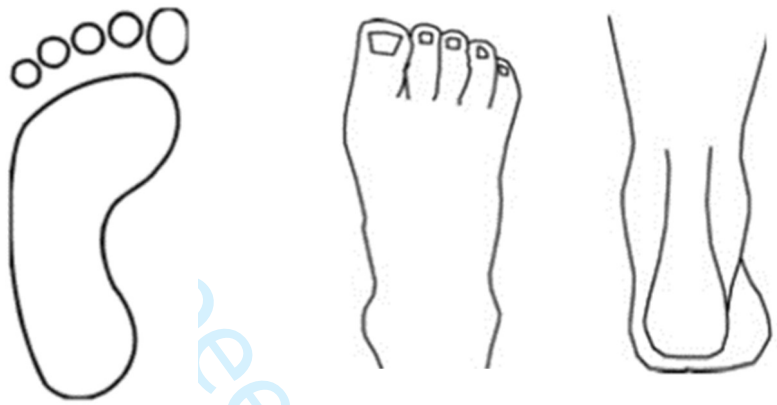
## G. Clinical and symptomatic assessment - 3

| | |
|---|---|
| 4. Mark all sites of new embedded jiggers and existing viable lesions on the feet diagrams on pages 13 and 14. | ☐ Done |

**Feet diagram – Full**

Mark all sites of active jigger lesions with X and newly embedded jiggers with Y. Clearly label the 2 target sites (see question 4) on the diagrams (e.g. "Target Site 1").

**Right foot**



**Left foot**

Additional comments:

_____

_____

_____

_____



Investigator signature_____

| **G. Clinical and symptomatic assessment - 3** | |
|---|---|
| 5. Follow the selected viable lesions together with their target sites.<br>*These should be indicated in the sites that are easily accessible for taking photograph and viability assessment using handheld video microscope. Use a descriptive name like "Toe 1" or "heels "as per question number 1. Mark the location of these sites on the feet diagrams on pages 2.*<br>**Target site 1:** _____<br>**Target site 2:** _____ | ☐ Done |
| 6. Record the names of the target sites on the last page of each of the *Clinical Assessment Forms 1, 2* & *3* for future reference. | ☐ Done |
| 7. Photograph the 2 target lesions together with their target sites | ☐ Done |
| 8. Record the photograph number using stickers on the last page of each of the Clinical Assessment Forms 1, 2 & 3 for future reference. | ☐ Done |

9. Assess the viability of 2 target lesions. Tick all that apply for each site.

| **Lesion characteristics** | **Lesion 1** | **Lesion 2** |
|---|---|---|
| Localization | | |
| Excretion of faeces (threads) | | |
| Excretion of faeces (liquid) | | |
| Expulsion of eggs | | |
| Pulsation of the flea | | |
| Stage of the lesion | | |

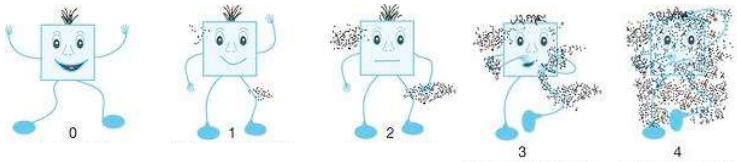
| | |
|---|---|
| 10. How many ***Stage II*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 11. How many ***Stage III*** jigger lesions are there on the child's feet (both right (R) and left (L) foot)? | R ☐<br>L ☐ |
| 12. How many ***total numbers of viable lesions*** (sage II & III) are there on the child's feet | |

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

UNIVERSITY OF CANBERRA

## G. Clinical and symptomatic assessment - 3

13. Ask the child to rate their itching over the last day (24 hours) based on the 'itch man' picture scale (tick one).



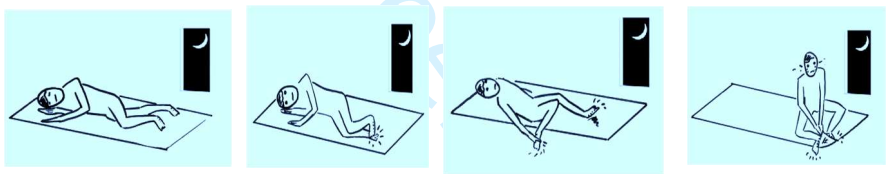

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

14. Ask the child to rate their pain over the last day (24 hours) based on the 'itch man' picture scale (tick one).

Not at all-0 Only a little-1 Quite a lot -2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

15. Ask the child to rate their sleep disterbance over the last day (24 hours) based on the following picture scale (tick one).



Not at all-0 Only a little-1 Quite a lot - 2 Very much-3

☐ 0
☐ 1
☐ 2
☐ 3
☐ 4

**Acute pathology examination and scoring**

| | | Sites on the right foot | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |
| | | Sites on the left foot | | | | | | | | |
| | | Toe 1 | Toe 2 | Toe 3 | Toe 4 | Toe 5 | Heel | Lateral side | Medial side | Sole |
| Acute pathology | Erythema | | | | | | | | | |
| | Warmness | | | | | | | | | |
| | Edema | | | | | | | | | |
| | Desquamation | | | | | | | | | |
| | Fissure | | | | | | | | | |
| | Suppuration | | | | | | | | | |
| | Ulcer | | | | | | | | | |
| | Abscess | | | | | | | | | |



Investigator signature_____

TTO (5% v/w) gel Tungiasis Trial – CRF Subject Study ID-no: _____

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## H. Adverse Event Log

| Date of entry | Adverse Event | Grade/ Severity | Serious | Date/time of Onset | Date/time of Resolution | Relation to study drugs | Action taken | Treatment given | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| dd/mm/yyyy | Diagnosis (if known) or Signs/symptoms (list one per line) | 1=mild 2=moderate 3=severe | (Y/N) * | dd/mm/yyyy 24-hr time | dd/mm/yyyy 24-hr time | 1=related 2=not related 3=other Specify | 1=none 2=interrupted 3=patient withdrawn 4=medication discontinued 5=other??? | (Y/N) | 1=resolved 2=resolved w sequelae 3=ongoing 4=death 5=unknown |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |
| ___/___/_____ | | | | ___/___/_____ ____:____ | ___/___/_____ ____:____ | | | | |

*For any Serious Adverse Events, participant must be immediately referred to nearby healthcare facility for medic



Investigator signature_____

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# I. Final Study Outcome

| Question | Response (tick one) |
|---|---|
| Has the subject completed the study? | ☐ Yes ☐ No |
| If yes, indicte the completion date dd/mm/yyyy | ___/___/_____ |
| If NO, specify last follow up date dd/mm/yyyy | ___/___/_____ |
| What are the reasons for not completing the study? | ☐ Significant non-compliance |
| | ☐ Drug-related AE |
| | ☐ Treatment failure |
| | ☐ Consent withdrawn |
| | ☐ Lost to follow-up |
| | ☐ Other (specify) |
| | |
| | |
| | |
| Remarks | |
| | |
| | |
| | |
| ***Investigator's Statement*** ***I have reviewed the data recorded in this CRF and confirm that the data are complete and accurate*** | |
| Investigator (Full name) | |
| Investigator signature | |
| Signature Date /dd/mm/yyyy/: | ___/___/_____ |



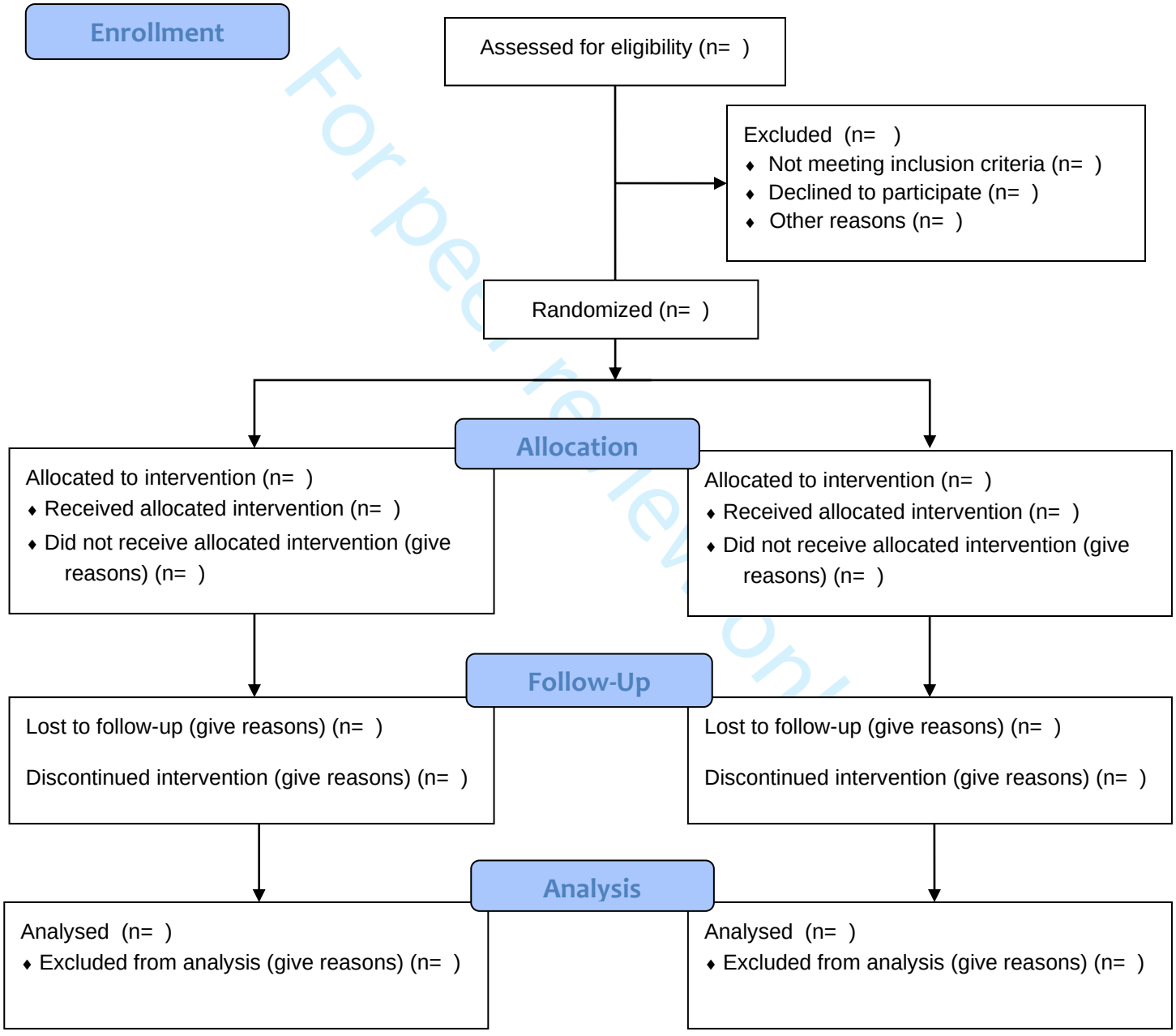


Investigator signature_____

CONSORT

TRANSPARENT REPORTING of TRIALS

# CONSORT 2010 Flow Diagram



# CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | |
| | 2b | Specific objectives or hypotheses | |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | |
| Participants | 4a | Eligibility criteria for participants | |
| | 4b | Settings and locations where the data were collected | |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | |
| Sample size | 7a | How sample size was determined | |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | |
| Randomisation: Sequence | 8a | Method used to generate the random allocation sequence | |

| | | | |
|---|---|---|---|
| | generation | 8b | Type of randomisation; details of any restriction (such as blocking and block size) |
| | Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned |
| | Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions |
| | Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how |
| | | 11b | If relevant, description of the similarity of interventions |
| | Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes |
| | | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses |
| **Results** | | | |
| | Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome |
| | | 13b | For each group, losses and exclusions after randomisation, together with reasons |
| | Recruitment | 14a | Dates defining the periods of recruitment and follow-up |
| | | 14b | Why the trial ended or was stopped |
| | Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group |
| | Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups |
| | Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) |
| | | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended |
| | Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory |

| | | | |
|---|---|---|---|
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.




## Main

*Note: This record shows only 22 elements of the WHO Trial Registration Data Set. To view changes that have been made to the source record, or for additional information about this trial, click on the URL below to go to the source record in the primary register.*

| | |
|---|---|
| **Register:** | ANZCTR |
| **Last refreshed on:** | 10 December 2019 |
| **Main ID:** | ACTRN12619001610123 |
| **Date of registration:** | 21/11/2019 |
| **Prospective Registration:** | Yes |
| **Primary sponsor:** | University of Canberra |
| **Public title:** | Exploring a tea tree oil (TTO)-based skin treatment for tungiasis in children |
| **Scientific title:** | Treatment of tungiasis using a proprietary tea tree oil (TTO)-gel formulation in children: Protocol for a randomised, controlled, proof-of principle trial |
| **Date of first enrolment:** | 03/02/2020 |
| **Target sample size:** | 88 |
| **Recruitment status:** | Not yet recruiting |
| **URL:** | https://anzctr.org.au/ACTRN12619001610123.aspx |
| **Study type:** | Interventional |
| **Study design:** | Purpose: Treatment; Allocation: Randomised controlled trial; Masking: Blinded (masking used);Assignment: Parallel;Type of endpoint: Safety/efficacy; |
| **Phase:** | Phase 2 |

## Countries of recruitment

Kenya

## Contacts

| | | | |
|---|---|---|---|
| **Name:** | A/Prof Jackson Thomas | **Name:** | A/Prof Jackson Thomas |
| **Address:** | Faculty of Health University of Canberra Building 12 Level D Office 36 Kirinari Street Bruce ACT 2601 Australia | **Address:** | Faculty of Health University of Canberra Building 12 Level D Office 36 Kirinari Street Bruce ACT 2601 Australia |
| **Telephone:** | +61 2 62068928 | **Telephone:** | +61 2 62068928 |
| **Email:** | Jackson.Thomas@canberra.edu.au | **Email:** | Jackson.Thomas@canberra.edu.au |
| **Affiliation:** | | **Affiliation:** | |

## Key inclusion & exclusion criteria

Inclusion criteria: 1. Children aged 6-15 years with at least 1 viable (stage II and Stage III) lesions according to the Fortaleza classification and a maximum of 2 viable sand flea lesions will be targeted.
2. Children whose legal guardians are willing to give informed written consents after having been oral and written informed about benefits and potential risks of the trial
Exclusion criteria: 1. Children with cluster lesions and manipulated lesions.
2. Children with complicated lesions requiring antibiotic treatment. They will be referred to the nearby health facilities for appropriate clinical management.
3. Children whose guardian/parents intend to change their place of residence during the study period
4. Children with known history of allergy to any of the study medications (Tea Tree Oil or other essential oils and potassium permanganate)
5. Individuals have/had systemic or topical drugs or medications, including systemic antibiotics, which may interfere with the study results (based on clinical team's assessment).

Age minimum: 6 Years
Age maximum: 15 Years
Gender: Both males and females

## Health Condition(s) or Problem(s) studied

Infection - Other infectious diseases

Public Health - Other public health

Skin - Dermatological conditions

Tungiasis (sand flea disease) ;
Tungiasis (sand flea disease)

**Intervention(s)**

Test group- treatment of tungiasis with a 5% (v/w), proprietary tea tree oil (TTO) gel

The feet of the participants will be washed with water and non-medicated soap, dried with a clean towel, and the participants' toenails will be clipped to enable easier application of the test medication. Then, the test medication will be applied twice daily on days 1, 4 and 7 by trained study personnel (concerned case officers from participating schools). The mode of administration of the test medication is by taking the required amount of the gel on the palms (up to 8g/day) and spreading it over the infested skin areas until it provides a full coverage of the affected area (skin surface of the feet up to the ankle) and the feet will then be left for 15 minutes to allow the medication to dry.

**Primary Outcome(s)**

Proportion of non-viable fleas

Determination of viability of the sand flea lesions will be performed using a handheld digital video microscope, assisted with pictorial flipcharts. Expulsion of eggs, excretion of faecal threads, excretion of faecal liquid, and pulsations/contractions in the abdomen of the embedded flea will be considered as four viability signs and lesions with 2 out of 4 viability signs will be recorded viable. Lesions will be considered dead (non-viable) if their viability signs are not detected during the 10 min follow-up examinations. Differences in the proportion of non-viable lesions between test and control groups will be compared and presented with their respective confidence intervals at 95% and p-values. [Day 10 (9 days after the first treatment).]

**Secondary Outcome(s)**

Acute morbidity evaluation

The severity score for acute morbidities (SSAT; which includes typical signs of local inflammation, the presence of suppuration, ulcers and fissures) will be assessed using a validated scoring system designed for tungiasis morbidity assessment.
In addition to SSAT, a visual analogue scale (VAS) called the 'Itch-man scale'-- a 5-point pictorial Likert scale, validated for paediatric burn survivors, will be adopted to evaluate itching. Finally, a 4 point pictorial scale, validated in paediatric tungiasis patients will be adopted to assess the pain, as well as pain-related and itching related sleep disturbances (QoL assessment).
[Days 0 (baseline), 5 and 10 (post treatment)]

Participant acceptability of the trial intervention/s

Participants/caregivers will be asked to rate the acceptability of the treatment in terms of effectiveness, side effects, convenience, and overall satisfaction on a 0-5 visual analogue scale. [Day 10 (9 days after the first treatment).

]

Proportion of participants with side effects (adverse events)

Safety will be assessed through evaluation of treatment related adverse events and skin irritation.
Participants/caregivers (in person or on the phone) will be asked about the occurrence of any solicited or unsolicited adverse reactions to the treatment during each follow-up visit. The trial team (clinical officer and health officers) will also carefully follow-up the trial participants on a regular basis at the trial site, until the end of trial period. This will be done using a pre-specified list of possible AEs, including local adverse reactions (swelling, stinging/burning, itching, induration, erythema) and systemic adverse reactions (fever, nausea and headache). Caregivers of participants will also be given a diary card to record ongoing solicited adverse events. The severity of the adverse events will be categorized as mild, moderate and severe according to common terminology criteria for adverse events (CTCAE) v5.0 guideline[Days 1 (PM), 4, 5, 7 and 10 (post-treatment)

]

**Secondary ID(s)**

None

**Source(s) of Monetary Support**

University of Canberra

**Secondary Sponsor(s)**

**Ethics review**

Status: Approved
Approval date:
Contact:
University of Canberra Human Ethics Research Committee

Results

**Results available:**
**Date Posted:**
**Date Completed:**
**URL:**

# Pan African Clinical Trials Registry

South African Medical Research Council, South African Cochrane Centre
PO Box 19070, Tygerberg, 7505, South Africa
Telephone: +27 21 938 0506 / +27 21 938 0834 Fax: +27 21 938 0836
Email: pactradmin@mrc.ac.za Website: www.pactr.org

| | | | |
|---|---|---|---|
| **Trial no.:** | PACTR202003651095100 | **Date registered:** | 26/02/2020 |
| **Trial Status:** | Registered in accordance with WHO and ICMJE standards | | |

## TRIAL DESCRIPTION

| | |
|---|---|
| **Public title** | Exploring a tea tree oil (TTO)-based skin treatment for tungiasis in children |
| **Official scientific title** | Treatment of tungiasis using a proprietary tea tree oil (TTO)-gel formulation in children: Protocol for a randomized, controlled, proof-of-principle trial |
| **Brief summary describing the background and objectives of the trial** | Tungiasis is a neglected parasitic skin disease caused by the female sand fleas (Tunga penetrans), which is highly prevalent in central and south America, the Caribbean, and Sub-Saharan Africa. The disease inflicts pain and suffering on millions of people, particularly children, and yet it is neglected by donors, governments, the scientific community, and health care providers. Left untreated, tungiasis can lead to substantial human consequences including impaired sleep, school absenteeism social isolation, difficulty in walking, auto-amputation, childhood disability, and immobility in severe cases. There is no approved drug treatment for tungiasis, and the available treatment options are very limited. There is a clear need for new, safe, effective, affordable and culturally acceptable tungiasis treatment options. Topical treatment is most ideally suited in endemic settings and the treatment should be simple, enabling self-administration, and should be started as soon as symptoms appear so that it can kill the embedded parasite at an early stage, prevent secondary bacterial complications, and substantially reduce the occurrence of acute and chronic morbidities. This trial aims to investigate the safety and efficacy of a proprietary tea tree oil gel (TTO) formulation (5% v/w) in comparison with an active comparator (i.e. 0.05% w/v potassium permanganate solution) for the treatment of tungiasis in children, over a 10-day period. TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. Unlike other tungiasis treatments, the TTO proprietary treatment offers a unique combination of parasiticidal, antibacterial, wound-healing, anti-inflammatory and anti-itch properties. |
| **Type of trial** | RCT |
| **Acronym (If the trial has an acronym then please provide)** | |
| **Disease(s) or condition(s) being studied** | Paediatrics,Skin and Connective Tissue Diseases |
| **Sub-Disease(s) or condition(s) being studied** | |
| **Purpose of the trial** | Treatment: Drugs |
| **Anticipated trial start date** | 01/06/2020 |
| **Actual trial start date** | |
| **Anticipated date of last follow up** | 04/09/2020 |
| **Actual Last follow-up date** | |
| **Anticipated target sample size (number of participants)** | 88 |
| **Actual target sample size (number of participants)** | |
| **Recruitment status** | Not yet recruiting |
| **Publication URL** | |

| Secondary Ids | Issuing authority/Trial register |
|---|---|
| ACTRN12619001610123 | Australian New Zealand Clinical Trial Registry, ANZCTR |
| U111112432294 | World Health Organization, Universal Trial Number |
| HREC20192114 | University of Canberra Human Research Ethics Committee |

## STUDY DESIGN

| Intervention assignment | Allocation to intervention | If randomised, describe how the allocation sequence was generated | Describe how the allocation sequence/code was concealed from the person allocating the participants to the intervention arms | Masking | If masking / blinding was used |
|---|---|---|---|---|---|
| | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Parallel: different groups receive different interventions at same time during study | Randomised | Simple randomization using a randomization table created by a computer software program | Sealed opaque envelopes | Masking/ blinding used | Outcome Assessors |

## INTERVENTIONS

| Intervention type | Intervention name | Dose | Duration | Intervention description | Group size | Nature of control |
|---|---|---|---|---|---|---|
| Experimental Group | Tea tree oil gel | Up to 8g/day | Twice daily for three days (Days 1, 4 and 7) | TTO-gel is a water-based, transparent, skin formulation with excellent spreading properties and pleasing aesthetic characteristics. It contains 5% (v/w) tea tree oil as an active ingredient. It will be applied by taking the required amount of the gel on the palms (up to 8g/day) and spreading it over the infested skin areas until it provides a full coverage of the affected area (skin surface of the feet up to the ankle) and the feet will then be left for 15 minutes to allow the medication to dry. | 44 | |
| Control Group | Potassium permanganate solution | Up to 2.5 liters of 0.05% potassium permanganate solution solution | Twice daily for three days (Day 1, 4 and 7) | Potassium permanganate solution contains 0.05g of potassium permanganate in a liter of water. It will be applied by immersing and bathing the feet of the participants in a bucket containing a required volume of 0.05% potassium permanganate solution for 15 minutes. After air-drying the feet (for about 15 mins), petroleum jelly will be applied to soften the skin, which may get rough and irritated after bathing with potassium permanganate solution. | 44 | Active-Treatment of Control Group |

## ELIGIBILITY CRITERIA

| List inclusion criteria | List exclusion criteria | Age Category | Minimum age | Maximum age | Gender |
|---|---|---|---|---|---|
| Children aged 6–15 years with at least 1 viable stage II and Stage III embedded sand flea lesions according to the Fortaleza classification. A maximum of 2 viable sand flea lesions will be targeted and the lesions must be the sum of the feet and lesions located at the tip of toes, soles, and rim of heels will be selected properly. Children whose legal guardians are willing to give informed written consents after having been oral and written informed about benefits and potential risks of the trial | Children with cluster lesions and manipulated lesions. Children with complicated lesions requiring antibiotic treatment. They will be referred to the nearby health facilities for appropriate clinical management. Children whose guardian/parents intend to change their place of residence during the study period Children with known histories of allergy to any of the study medications (Tea Tree Oil or other essential oils and potassium permanganate) Children have/had systemic or topical drugs or medications, including systemic antibiotics, which may interfere with the study results (based on the clinical team's assessment). | Adolescent: 13 Year-18 Year,Child: 6 Year-12 Year | 6 Year(s) | 15 Year(s) | Both |

## ETHICS APPROVAL

| Has the study received appropriate ethics committee approval | Date the study will be submitted for approval | Date of approval | Name of the ethics committee |
|---|---|---|---|
| Yes | | 28/08/2019 | University of Canberra Human Ethics Research Committee |

**Ethics Committee Address**

| Street address | City | Postal code | Country |
|---|---|---|---|
| Kirinari Street | Canberra | 2617 | Australia |

## OUTCOMES

| Type of outcome | Outcome | Timepoint(s) at which outcome measured |
|---|---|---|
| Primary Outcome | The proportion of non-viable embedded sand fleas | Day 10 or 9 days after the first treatment |
| Secondary Outcome | Acute morbidity evaluation | Days 0 or baseline, 5 and 10 or post treatment |
| Secondary Outcome | The proportion of participants with adverse events | Days 1 at PM, 4, 5, 7 and 10 |
| Secondary Outcome | Participant acceptability of the trial intervention/s | Day 10 or 9 days after the first treatment |

## RECRUITMENT CENTRES

| Name of recruitment centre | Street address | City | Postal code | Country |
|---|---|---|---|---|
| Riamajeshi Bright Start Academy | Sotik Ikonge Road | Nyamira | 0800 | Kenya |

## FUNDING SOURCES

| Name of source | Street address | City | Postal code | Country |
|---|---|---|---|---|

## SPONSORS

| Sponsor level | Name | Street address | City | Postal code | Country | Nature of sponsor |
|---|---|---|---|---|---|---|
| Primary Sponsor | University of Canberra | Kirinari St | Canberra | 2617 | Australia | University |

## COLLABORATORS

| Name | Street address | City | Postal code | Country |
|---|---|---|---|---|
| Global School Partners Kenya Chapter | Sotik Ikonge Road | Nyamira | 0800 | Kenya |
| Global School Partners Australia Chapter | Deakin | Canberra | 2603 | Australia |

## CONTACT PEOPLE

| Role | Name | Email | Phone | Street address |
|---|---|---|---|---|
| **Principal Investigator** | Solomon Abrha Bezabh | Solomon.Bezabh@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | PhD student | |
| **Role** | **Name** | **Email** | **Phone** | **Street address** |
| **Public Enquiries** | Jackson Thomas | Jackson.Thomas@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | Academic staff and researcher | |
| **Role** | **Name** | **Email** | **Phone** | **Street address** |
| **Scientific Enquiries** | Jackson Thomas | Jackson.Thomas@canberra.edu.au | +61262068928 | Kirinari Street |
| **City** | **Postal code** | **Country** | **Position/Affiliation** | |
| Canberra | 2601 | Australia | Academic staff and researcher | |

## REPORTING

| Share IPD | Description | Additional Document Types | Sharing Time Frame | Key Access Criteria |
|---|---|---|---|---|
| Yes | The ethical approval for this study requires the individual participant data to be kept confidential. However, the deidentified pooled data per intervention will be made | Informed Consent Form,Statistical Analysis Plan,Study Protocol | The findings of the trial will be available through peer-reviewed journals as well as national and international scientific conference meetings once the primary outcome paper is published. | Through open access research publications, with no restriction. |

| | | | | |
|---|---|---|---|---|
| | available through open access research publications. | | The additional document types will also be shared during the publication. | |
| **URL** | **Results Available** | **Results Summary** | **Result Posting Date** | **First Journal Publication Date** |
| | No | | | |
| **Result URL Hyperlinks** | **Baseline Characters** | **Participant Flow** | **Adverse Events** | **Outcome Measures Description** |
| **Link To Protocol** | | | | |

**Changes to trial information**

| Section Name | Field Name | Date | Reason | Old Value | Updated Value |
|---|---|---|---|---|---|
| Reporting | Plan to share IPD | 06/03/2020 | it was indicated 'No' in the previous submission but modified to elaborate on how and where results will be stored and how they will be available for the public good. | No | Yes |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD description | 06/03/2020 | It was not included in the previous submission. | | The ethical approval for this study requires the individual participant data to be kept confidential. However, the deidentified pooled data per intervention will be made available through open access research publications. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD-Sharing time frame | 06/03/2020 | It was not included in the previous submission. | | The findings of the trial will be available through peer-reviewed journals and national and international scientific meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | Key access criteria | 06/03/2020 | It was not included in the previous submission. | | Through open access research publications, with no restriction. |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | Study protocol document | 06/03/2020 | It was not included in the previous submission. | | Study Protocol, Statistical Analysis Plan, Informed Consent Form |
| **Section Name** | **Field Name** | **Date** | **Reason** | **Old Value** | **Updated Value** |
| Reporting | IPD-Sharing time frame | 06/03/2020 | It was not included in the previous submission. | The findings of the trial will be available through peer-reviewed journals and national and international scientific meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. | The findings of the trial will be available through peer-reviewed journals as well as national and international scientific conference meetings once the primary outcome paper is published. The additional document types will also be shared during the publication. |

Supplementary Table S3: Protocol Amendment History – Tea Tree Oil tungiasis Trial

| Amendment No | Protocol version No | Date issued | Author(s) of changes | Details of changes made |
|---|---|---|---|---|
| 1 | Version 1 | 2019 | Solomon Abrha Bezabh | Original version |
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