Aus dem Institut für Mikrobiologie und Infektionsimmunologie
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DISSERTATION

Epidemiologie, Klinik und Therapie der Hakenwurm-assoziierten Larva migrans cutanea in Manaus, Brasilien.

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Zusammenfassung

Abstrakt

Einführung: Die Hakenwurm-assoziierte Larva migrans cutanea (HrCLM) ist eine parasitäre Hauterkrankung, die durch die Migration von tierpathogenen Hakenwurmlarven in der Epidermis ausgelöst wird. Diese führt zu einer kutanen Entzündungsreaktion, die sich als erhabener, meist serpiginiöser Larvengang präsentiert und von starkem Juckreiz begleitet ist. Da Umweltfaktoren wie Temperatur und Feuchtigkeit die Überlebensdauer der Eier und Larven im Boden beeinflussen, ist sie nur in tropischen und subtropischen Gebieten endemisch. Bevölkerungsbasierte Daten zu Epidemiologie und Klinik der HrCLM existieren nur aus dem Nordosten Brasiliens mit einem semiariden Klima. Therapiestudien wurden bislang nur an Reiserückkehrern durchgeführt.

Methoden: Im tropischen Monsunklima von Manaus in Brasilien wurden in einem Armutsviertel eine Quer- und eine prospektive Längsschnittstudie durchgeführt. Für die Querschnittuntersuchung wurden Prävalenz, Risikofaktoren und Klinik der HrCLM in der Gesamtbevölkerung untersucht. Anschließend wurden über insgesamt 6 Monate mit einer Kohorte von Kindern die Risikofaktoren prospektiv überprüft und monatlich Inzidenz und klinische Merkmale der HrCLM bestimmt. Die Wirksamkeit von Ivermectin wurde anhand einer offenen Therapiestudie in insgesamt 7 Armutsvierteln in Manaus untersucht.

Ergebnisse: Die Prävalenz in der Gesamtbevölkerung betrug 8.2% (95% Konfidenzintervall [KI], 6.3–10.1%), die Inzidenz in der Kohorte 0.52 Infektionen/Personenjahr (95% KI, 0.42-0.65). Monatliche Schwankungen der Prävalenz korrelierten mit der Niederschlagsmenge, ebenso wie die Anzahl der Läsionen. Auch Lokalisation und bakterielle Superinfektionen zeigten eine Saisonalität. Als unabhängige Risikofaktoren wurden Alter von 10-14 Jahren, männliches Geschlecht, Armut und Barfußlaufen identifiziert. Das bevölkerungsbezogene attributable Risiko von Barfußlaufen betrug 45%. Nach Therapie mit Ivermectin per os reduzierten sich Schweregrad und Anzahl der Läsionen innerhalb von zwei Wochen signifikant.

Schlussfolgerung: Auch im feuchten tropischen Monsunklima von Manaus findet sich eine Saisonalität von Erkrankungshäufigkeit und Klinik der HrCLM, die mit der monatlichen Niederschlagsmenge korreliert. Es wurde die weltweit höchste bisher bekannte Prävalenz und Inzidenz festgestellt. Der einzige Risikofaktor, der einer direkten Intervention zugänglich wäre, ist das Barfußlaufen. HrCLM ist eine Armuts-assoziierte Erkrankung. Ivermectin per os zeigte auch in diesem Setting eine gute Wirksamkeit.

Abstract

Introduction: Hookworm-related cutaneous larva migrans (HrCLM) is a neglected parasitic skin disease, caused by the migration of canine or feline hookworm-larvae in the human epidermis. Environmental factors such as temperature and soil moisture influence survival of hookworm eggs and larvae. HrCLM is endemic in subtropical and tropical regions, though population-based data on morphology and epidemiology in a tropical climate is lacking. Therapy has never been evaluated in an endemic area.

Methods: Epidemiology, morphology and clinical effectiveness of ivermectin were investigated by a cross-sectional, a prospective longitudinal study and an open clinical trial in the tropical monsoon climate of Manaus, Brazil. For the cross-sectional study the entire population of a resource-poor neighbourhood was examined to determine prevalence, risk factors and clinical characteristics. A cohort was formed out of all children and observed for further 5 months in order to verify the identified risk factors prospectively and investigate seasonal variation of morbidity. The observational therapy study was conducted in different resource-poor neighbourhoods. Effectiveness on morphology and associated morbidity was assessed 2 and 4 weeks after a single dose of oral ivermectin (200 µg/kg).

Results: Prevalence among the total population was 8.2% (95% confidence interval [CI], 6.3–10.1%). Incidence among children was 0.52 cases per person-year (95% CI, 0.42-0.65). Seasonal variations in prevalence and infection intensity correlated with the amount of monthly precipitation. Seasonal variation also existed regarding the lesions´ localisation and the frequency of bacterial superinfection. Male sex, walking barefoot on sandy ground, poverty and age between 10 and 14 years were independent predictors of HrCLM. The Population Attributable Fraction (PAF) related to walking barefoot on sandy ground was 45%. A single-dose of oral ivermectin reduced amount and severity of the lesions significantly.

Conclusion: Even in the tropical monsoon climate of Manaus a seasonal variation of morbidity peaking in the rainy season exists. Nowhere else has been described a higher prevalence/ incidence. Walking barefoot is the only risk factor amenable to intervention. HrCLM is a poverty-related disease. As ivermectin showed high effectiveness in this endemic area it would be desirable to make it available to the poorest part of population, which has to carry the highest disease burden.

Einführung

Die Hakenwurm-assoziierte Larva migrans cutanea (HrCLM) ist eine parasitäre Hauterkrankung, die durch tierpathogene Hakenwurmlarven wie *Ancylostoma braziliense [1,2], Ancylostoma caninum[3,4]* und *Uncinaria stenocephala [5]* ausgelöst wird. Wirtstiere sind Katzen und Hunde, die die Wurmeier über die Fäzes ausscheiden. Unter günstigen Umweltbedingungen schlüpfen daraus Larven, die nach 2 Häutungen in der Lage sind, einen Wirt durch perkutane Penetration zu infizieren [6]. Regen fördert die Verteilung und sandiger Boden die Migration der Larven [7]. Die Infektion erfolgt über Hautkontakt mit von Larven kontaminiertem Boden, selten über textile Oberflächen, welche zuvor Kontakt mit Sand oder dem Boden hatten [8]. Der Mensch ist ein Fehlwirt, da die Larven nicht die Basalmembran der Epidermis durchdringen können. Die Larven migrieren in der Epidermis, verursachen eine Entzündungsreaktion und sterben dort innerhalb von Wochen bis Monaten ab [9–11]. Pathognomonisch ist ein erhabener, meist schlangenförmiger Larvengang, der mit einer Geschwindigkeit von wenigen Millimetern am Tag fortschreitet [12] und von einem starken Pruritus begleitet ist. Die vorherrschende Lokalisation sind die Füße [12,13]. Als Folge des Juckreizes kommt es zu Schlaf- und Konzentrationsstörungen sowie bakteriellen Superinfektionen mit Staphylokokken und Streptokokken sowie deren Folgeerkrankungen [14,15]. HrCLM geht mit einer deutlichen Einschränkung der Lebensqualität einher [14,16]. Die therapeutische Wirksamkeit der systemischen Therapie mit Ivermectin oder Albendazol ist bei Reiserückkehrern belegt [17–20]. Zur Wirksamkeit bei Einwohnern aus Endemiegebieten lagen hingegen keine Studien vor. Ivermectin ist in der Therapie der Onchocerciasis millionenfach eingesetzt und gut verträglich [21,22], jedoch erst ab einem Körpergewicht von 15 kg zugelassen [23].

Die HrCLM ist eine sogenannte vernachlässigte Tropenkrankheit [24,25]. Obwohl die verursachenden Spezies weltweit prävalent sind, ist die Erkrankung nur in tropischen und subtropischen Gebieten endemisch. Aus Europa sind nur sporadische Infektionen bekannt [26–31]. Daten über die Verbreitung gibt es fast ausschließlich aus Studien über Reiserückkehrer aus Endemiegebieten [13,32,33]. Untersuchungen über die Prävalenz in endemischen Gebieten sind bislang nur in Nordost-Brasilien durchgeführt worden [12,34–36]. Im dortigen semi-ariden Klima wurde eine starke saisonale Schwankung der Prävalenz mit einem positiven Zusammenhang mit der Niederschlagsmenge beobachtet [12,34]. Als Risikofaktoren wurden Barfußlaufen, männliches Geschlecht, junges Alter und die Abwesenheit eines soliden Hausbodens ermittelt, ein Zusammenhang mit Armut wurde vermutet [34,36,37]. Ein kausaler Zusammenhang konnte aufgrund des Studiendesigns nicht belegt werden.

Die Zielsetzung der vorliegenden Dissertation ist:

1. In einer für die Transmission ganzjährig förderlichen Umgebung Prävalenz- und Inzidenzschwankungen zu erheben.
2. Eine gründliche Analyse von Risikofaktoren durchzuführen und mittels eines geeigneten Studiendesigns auf Kausalität zu überprüfen.
3. Das Krankheitsbild und seine Abhängigkeit von Prävalenz bzw. Umweltfaktoren zu erfassen.
4. Die Wirksamkeit von Ivermectin in einem Endemiegebiet zu evaluieren und, basierend auf den epidemiologischen Merkmalen, Möglichkeiten der Kontrolle aufzuzeigen.

Die Dissertation ist Teil eines Forschungsprojektes zur Epidemiologie, Morbidität, Immunologie, Pathologie und Soziologie der Hakenwurm-assoziierten Larva migrans cutanea.

Methodik

Studiengebiete und –populationen

Die Studiengebiete befinden sich in Manaus, der Hauptstadt des brasilianischen Bundesstaates Amazonas. Dort herrscht nach der Klimaklassifikation von Köppen und Geiger ein tropisches Monsunklima [38,39] mit einer durchschnittlichen Jahrestemperatur von 26,7°C und einem mittleren Jahresniederschlag von 2307mm [40,41]. Die Trockenzeit mit weniger als 60mm Niederschlag im Monat ist normalerweise auf den August begrenzt, im Jahr der Datenerhebung umfasste sie jedoch auch den Juli und September [42].

Das Armutsviertel *Nova Vitoria*, eine sogenannte *Invasão,* ist exemplarisch für neue, ohne behördliche Genehmigung errichtete Wohngebiete, die kontinuierlich in den Randgebieten von Manaus entstehen. Auf vom Regenwald gerodeter Fläche befanden sich 412 improvisierte Hütten und Häuser, bewohnt von über 1000 Bewohnern, von denen die meisten nur einen gesetzlichen Mindestlohn (2009 waren das 465 R$, etwa 150€) oder weniger zur Verfügung hatten, um ihre Familie zu ernähren. Die Straßen waren nicht befestigt und wurden durch Regen und undichte Schläuche, die der Zuführung von Trinkwasser in die Häuser dienten, regelmäßig überflutet. Es fehlte jegliche städtische oder staatliche Infrastruktur, wie Gesundheitsversorgung, Kindergärten oder Schulen. Hunde und Katzen streunten frei herum. Kinder spielten häufig unbeaufsichtigt im Garten, Hof und auf den Straßen. In diesem Viertel wurden die Querschnitt- und Längsschnittuntersuchungen durchgeführt.

Für die Therapiestudie wurden Patienten in 6 weiteren Armutsvierteln rekrutiert, die zentral gelegen entlang von Zuflüssen zum Amazonas (sogenannten *Igarapés*) entstanden waren [14]. Dort waren die Lebensverhältnisse ähnlich prekär. Da die *Igarapés* in der Regenzeit viel Wasser führen, werden die Häuser auf Stelzen (*Palafitas*) erbaut. Abfall und Abwasser werden direkt in

die *Igarapés* oder unter den Häusern entsorgt. Dies wiederum lockt Hunde und Katzen an, die dort nach essbaren Überresten suchen und ihre - potenziell mit Hakenwurmeiern kontaminierte - Fäzes absetzen.

Studiendesign

Querschnittuntersuchung

Nach einem Zensus aller Haushalte und Bewohner wurde während der Regenzeit im April 2009 eine Querschnittsuntersuchung in *Nova Vitoria* durchgeführt.
Dabei wurden alle Haushalte des Studiengebietes aufgesucht und mithilfe des globalen Positionsbestimmungssystems (GPS) kartographiert. Um in die Studie eingeschlossen zu werden, mussten die Teilnehmer seit mindestens 2 Monaten in dem Studiengebiet wohnen und der Teilnahme schriftlich zustimmen. Ein vorgetesteter strukturierter Fragebogen wurde zur Erhebung von demographischen, sozioökonomischen, verhaltens- und umweltbezogenen Risikofaktoren eingesetzt. Das Grundstück wurde auf Vorhandensein von Tierfäzes untersucht. Die klinische Untersuchung der Teilnehmer erfolgte in einem Raum, der ausreichend Privatsphäre garantierte. Die Diagnose einer HrCLM wurde bei Vorliegen einer pathognomonisch leicht erhabenen, meist schlangenförmig gewundenen Läsion, die sich über Tage langsam ausdehnt und stark juckt, klinisch gestellt. Anzahl, Lokalisation und Superinfektion der Läsionen wurden erfasst.

Längsschnittuntersuchung

Da die Prävalenz unter Kindern besonders hoch ist, wurde aus den untersuchten Kindern eine offene Kohorte gebildet, welche über 6 Monate monatlich auf HrCLM untersucht wurde. Die Querschnittsuntersuchung diente dafür als Basisuntersuchung. Einschlusskriterien waren: ein fester Wohnsitz im Studiengebiet, Alter unter 18 Jahre und eine Einwilligungserklärung eines Erziehungsberechtigten. Patienten, bei denen eine Infektion mit HrCLM festgestellt wurde, wurden erst nach Behandlung in die Kohorte aufgenommen. Um die hohe Ausfallsquote aufgrund von Migration zu kompensieren, wurden neue Teilnehmer unter Geschwistern und aus neu angetroffenen Familien für die Kohorte rekrutiert.

Therapiestudie

Die Patienten wurden vor und 2 sowie 4 Wochen nach Therapie mit einer Einmaldosis Ivermectin oral (200 µg/kg) untersucht und mithilfe eines vorgetesteten strukturierten Fragebogens befragt. Der Schweregrad der Infektion wurde mit einem Index (1-10 Punkte) erfasst, in den Anzahl der Läsionen, bakterielle Superinfektion, lokale Entzündungszeichen und

lokale Lymphadenopathie einflossen [14]. Des weiteren erfolgte die Einteilung der Läsionen in komplexe Läsionen (mit Exkoriationen, bakterieller Superinfektion oder ausgeprägten lokalen Entzündungszeichen) und einfache Läsionen bei Abwesenheit dieser Charakteristika [14]. Juckreiz, Juckreiz-assoziierte Schlafstörungen und Schmerzen wurden mit einer visuellen Analogskala erfasst. Die Rekrutierung erfolgte über lokale Gesundheitszentren (*casinhas de saúde*) und Mund-zu-Mund-Propaganda. Einschlusskriterien waren Alter über 5 Jahre und mindestens eine HrCLM-Läsion. Patienten mit weiteren juckenden Dermatosen und Patienten, die bei den Nachuntersuchungen neue Infektionen zeigten, wurden von der Analyse ausgeschlossen.

Datenanalyse

Die Datenanalyse erfolgte mit PASW Statistics Version 18.0 (SPSS Inc., Chicago, USA).
Mit Hilfe einer Hauptkomponentenanalyse wurde ein sozio-ökonomischer Index („Wohlstandindex“) geformt, in den Vermögenswerte, monatliche Ausgaben fürs Mobiltelefon und die Art der Stromversorgung einflossen [43]. Die Haushalte wurden danach klassifiziert und in Tertilen eingeteilt, entsprechend einem hohen, mittleren und niedrigen sozioökonomischen Status.
Für die Querschnittsstudie wurde zunächst eine bivariable Risikofaktorenanalyse durchgeführt und Odds Ratios (OR) sowie 95% Konfidenzintervalle (95% KI) berechnet. Relative Häufigkeiten wurden bei dichotomen Variablen mit einem Chi-Quadrat- oder Fisher-Exakt-Test und bei polytomen Variablen mit einer Logistischen Regression verglichen. Anschließend wurden für die multivariable Analyse alle Variablen, die zumindest eine schwache Assoziation mit dem Auftreten einer HrCLM zeigten ($p<0.1$), in eine schrittweise Logistische Regression eingefügt. Bei Auftreten von Multikollinearität wurde die Variable wieder entfernt.
Für die Auswertung der Längsschnittdaten wurden Hazard Ratios und 95% KI mit Hilfe einer Cox Regression bestimmt. Die Proportionalitätsannahme wurde mit Hilfe eines Log-Minus-Log-Plots überprüft. Analog zum Vorgehen bei den Querschnittsdaten, erfolgte zunächst eine bivariable und anschließend eine multivariable Analyse. Zur Darstellung der infektionsfreien Intervalle wurden Kaplan-Meier-Kurven erstellt.
Wir berechneten das bevölkerungsbezogene attributable Risiko für unabhängige Risikofaktoren, die einer direkten Intervention zugänglich waren. Da dies nur für einen zutraf, benutzten wir Levins Gleichung: $\{p_e(RR-1)\}/\{p_e(RR-1)+1\}$. Dabei entspricht p_e dem Anteil der Bevölkerung, der gegenüber dem Risikofaktor exponiert war und RR der Ratio der Inzidenzraten [44,45].

Um saisonale Schwankungen darstellen zu können, wurden Inzidenz und Prävalenz monatlich berechnet. Mithilfe von Spearmans Rangkorrelationskoeffizient wurde der Zusammenhang zwischen Prävalenzen, klinischen Charakteristika (Anzahl der Läsionen, Anzahl der Kinder mit Superinfektionen) und der monatlichen Niederschlagsmenge überprüft. Aus der Subgruppe der initial infizierten Kinder, wurde eine Reinfektionsrate berechnet.
In der Therapiestudie wurde zur Bestimmung eines Zusammenhanges zwischen ordinalskalierten Variablen der Rangkorrelationskoeffizient nach Spearman berechnet. Da die Daten nicht normalverteilt waren, wurde zum Vergleich der klinischen Parameter vor und nach Therapie der Wilcoxon-Test für verbundene Stichproben verwendet.

Ethik

Die Studien wurden von der Ethikkommission des Tropeninstituts in Manaus (*Fundação de Medicina Tropical-Amazonas*) genehmigt. Alle Teilnehmer sowie die gesetzlichen Vertreter der Kinder wurden in brasilianischem Portugiesisch über die Ziele und Maßnahmen der Studie aufgeklärt und anschließend deren schriftliches Einverständnis eingeholt. Unabhängig von der Teilnahme an der Studie wurde allen Patienten eine kostenlose Therapie angeboten.

Ergebnisse

Die Ergebnisse wurden sämtlich in den untenstehenden Publikationen [46–48] veröffentlicht, welche deshalb im Folgenden nicht explizit aufgeführt werden.

An der Querschnittsuntersuchung nahmen 262 der 285 Haushalte teil (92%). Die übrigen erfüllten nicht die Einschlusskriterien (6%) bzw. lehnten eine Teilnahme ab (2%). Insgesamt wurden diese Haushalte von 1104 Personen bewohnt, von denen allerdings nur 806 (73%) angetroffen wurden und eingeschlossen werden konnten.

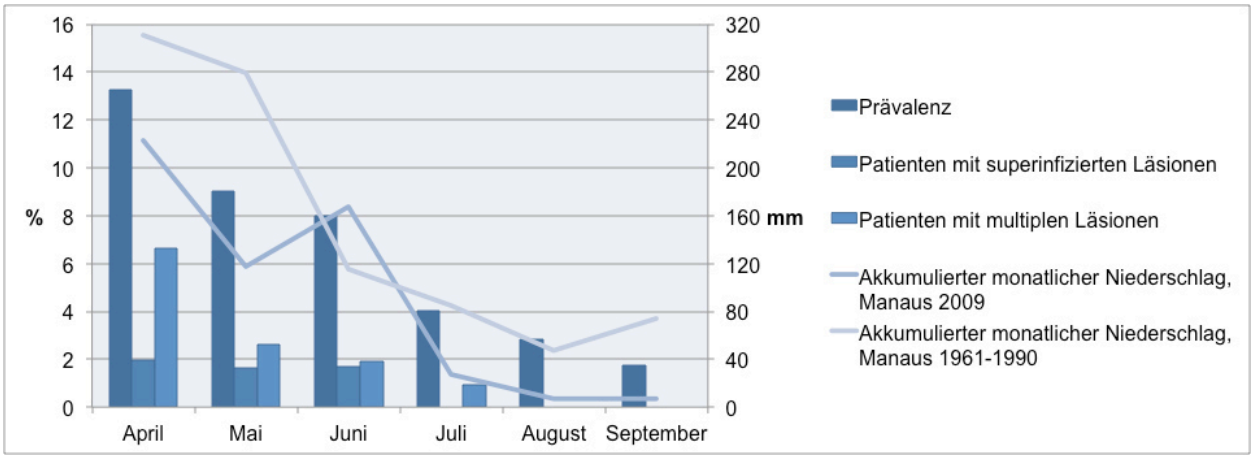
In die Längsschnittstudie wurden 476 Kinder mit einer Gesamtbeobachtungszeit von 54938 Tagen eingeschlossen.

Die Therapiestudie wurde an 92 Patienten mit insgesamt 441 HrCLM-Läsionen durchgeführt.

Prävalenz und Inzidenz

Die Prävalenz in der Gesamtbevölkerung während der Regenzeit betrug 8.2% (95% KI, 6.3–10.1%). In der Altersgruppe der 10-14-Jährigen erreichte sie 18.2% (95% KI, 9.3–27.1%). Innerhalb der Kohorte zeigte sich eine monatliche Abnahme der Prävalenz, die mit der akkumulierten Niederschlagsmenge korrelierte ($\rho = 0.928$; $p=0.008$; siehe Abbildung 1).

Abbildung 1: Prävalenz, klinische Charakteristika und Niederschlag pro Monat (Abbildung aus [47]).



Die monatliche Inzidenz schwankte zwischen 1.05 pro Personenjahr (95% KI, 0.76-1.48) in der Regenzeit und 0.21 (95% KI, 0.11-0.46) am Ende der Trockenzeit. Die Gesamtinzidenz betrug 0.52 pro Personenjahr (95% KI, 0.42-0.65). Die Reinfektionsrate lag bei 1.08 pro Personenjahr (95% KI, 0.71-1.73).

Risikofaktoren

In der Querschnittuntersuchung konnten in der Multivariablen Risikofaktorenanalyse folgende unabhängige Risikofaktoren identifiziert werden:
Barfußlaufen auf sandigem Boden, Vorhandensein von Tierfäzes auf dem Grundstück, ein niedriger Wohlstandsindex, männliches Geschlecht und ein Alter von weniger als 14 Jahren (Tabelle 1). Beim stärksten Risikofaktor „Barfußlaufen auf sandigem Boden“ war zudem eine Gradierung des Risikos je nach Häufigkeit der Benutzung von Schuhwerk zu erkennen.

Tabelle 1: Multivariable Risikofaktorenanalyse der Querschnittsuntersuchung mit 779 Teilnehmern (Tabelle aus [46]).

Charakteristika		Häufigkeit	Adjustierte Odds Ratio (95% KI)	2-seitiger p-Wert
Demographie				
Männlich		319	2.30 (1.30-4.08)	0.004
	Weiblich	460	1	
Alter	≤ 4 Jahre	163	2.55 (1.11-5.90)	0.028
	5-9 Jahre	155	2.80 (1.26-6.23)	0.012
	10-14 Jahre	87	2.98 (1.23-7.21)	0.015
	15-19 Jahre	44	0.37 (0.05-3.03)	0.354
	≥20 Jahre	330	1	
Sozioökonomische Charakteristika				
Wohlstandsindex*				
	Niedrig	318	2.53 (1.10-5.82)	0.028
	Intermediär	260	1.76 (0.73-4.22)	0.209
	Hoch	201	1	
Umwelt				
Tierfäzes auf Grundstück				
	Ja	92	2.66 (1.34-5.29)	0.005
	Nein	687	1	
Verhalten				
Laufen auf sandigem Boden				
	Immer barfuß	107	14.39 (4.62-44.85)	<0.001
	Manchmal barfuß	406	4.76 (1.63-13.90)	0.004
	Nie barfuß	266	1	

** Bezüglich der Definition siehe Methoden.*

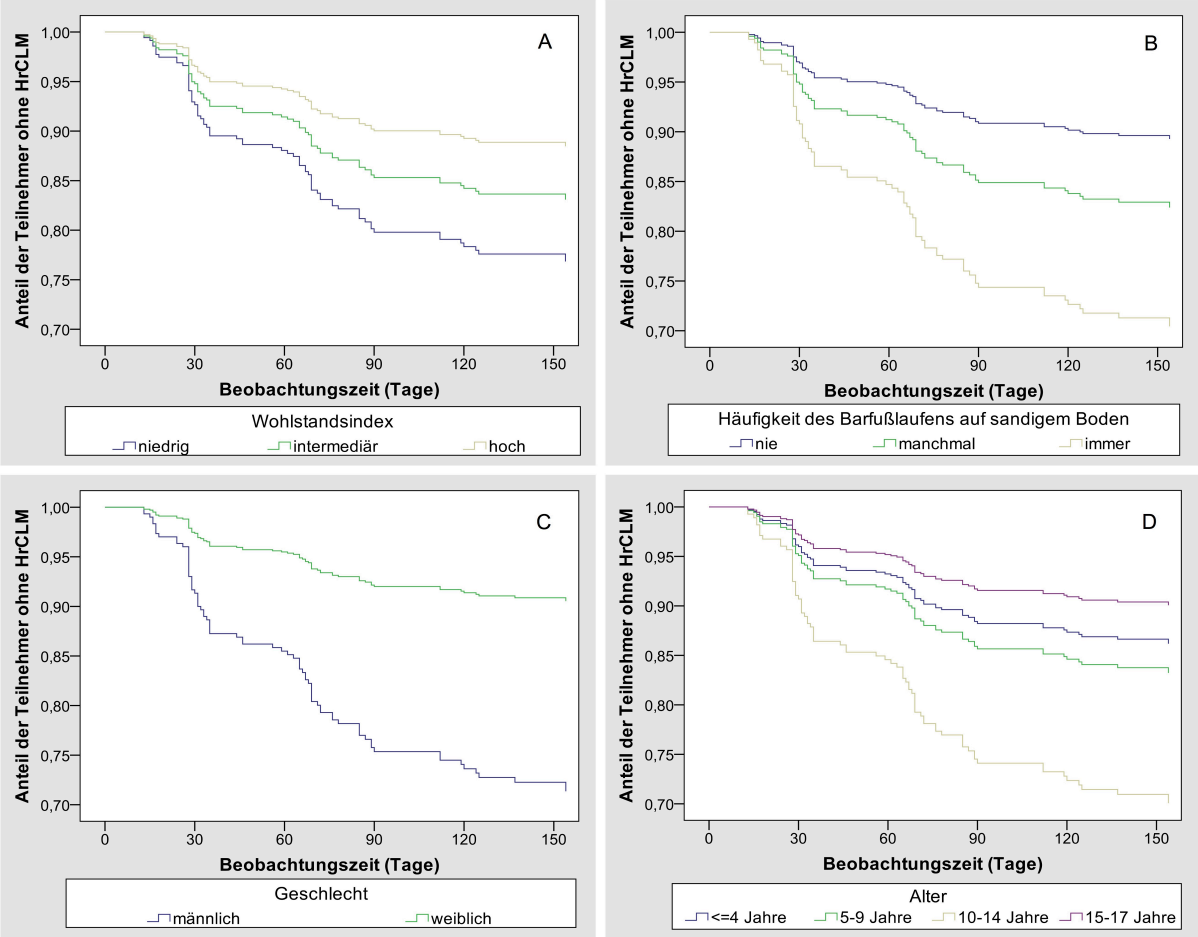
Sämtliche Risikofaktoren, die auf eine Transmission innerhalb des Hauses hindeuten könnten (Barfußlaufen zu Hause, Fußboden aus gestampfter Erde oder Sand, Halten eines Hundes oder einer Katze) waren statistisch nicht signifikant beziehungsweise abhängige Risikofaktoren.
In der prospektiven Kohortenstudie konnten Barfußlaufen, ein niedriger Wohlstandsindex, männliches Geschlecht und Alter in der Cox Regression als Risikofaktoren bestätigt werden (Tabelle 2, Abbildung 2).

Tabelle 2: Multivariable Risikofaktorenanalyse der Kohortenstudie mit 476 Kindern (Tabelle aus [47])

Charakteristika		Personenjahre	HrCLM*	Adjustierte Hazard Ratio (95% KI)	2-seitiger p-Wert
Demographie					
Männlich		65.90	57	3.29 (1.95-5.56)	<0.001
	Weiblich	84.62	21	1	
Alter	≤ 4 Jahre	55.87	21	1	
	5-9 Jahre	60.48	28	1.07 (0.59-1.94)	0.824
	10-14 Jahre	30.24	28	1.87 (1.01-3.46)	0.045
	15-18 Jahre	3.92	1	0.65 (0.09-4.91)	0.674
Sozioökonomische Charakteristika					
Wohlstandsindex†, §					
	Niedrig	60.51	41	2.13 (1.09-4.17)	0.027
	Intermediär	48.81	23	1.47 (0.71-3.04)	0.294
	Hoch	35.79	11	1	
Verhalten					
Laufen auf sandigem Boden †					
	Immer barfuß	27.90	25	2.30 (1.03-5.16)	0.043
	Manchmal barfuß	89.48	44	1.70 (0.81-3.56)	0.158
	Nie barfuß	31.22	9	1	

**Anzahl der Kinder mit Hakenwurm-assoziierter Larva migrans cutanea.*
†Fehlende Beobachtungen.
‡Mindestlohn 2009: 465 R$ ≈ 150€.
§Bezüglich der Definition siehe Methoden.

Abbildung 2: Kaplan-Meier-Kurven stratifiziert nach Wohlstandsindex (A), Häufigkeit des Barfußlaufens auf sandigem Boden (B), Geschlecht (C) und Alter (D) (Abbildung aus [47]).



Das bevölkerungsbezogene attributable Risiko für Barfußlaufen betrug 45%.

Klinik

In der Gesamtbevölkerung war der Großteil der HrCLM-Läsionen (62%) am Fuß lokalisiert. Superinfektionen traten bei 11% der Teilnehmer mit HrCLM auf.

In der Kohorte hatten während der Regenzeit bis zu 50% der infizierten Kinder multiple Läsionen. Die Läsionen befanden sich ebenfalls überwiegend am Fuß, bis zu 43% der Läsionen waren an weniger typischen Stellen wie Hand, Arm, Thorax, Gesäß, Kopf oder Bein lokalisiert. Zeichen einer bakteriellen Superinfektion waren bei bis zu 21% der infizierten Kinder zu finden. Die monatliche Niederschlagsmenge korrelierte nicht nur mit der Prävalenz, sondern auch mit der Anzahl der Läsionen ($\rho = 0.941$; $p<0.001$). Atypische Lokalisationen sowie bakterielle Superinfektionen waren in der Regenzeit überproportional häufig. Am Ende der Studie, während der Trockenzeit, traten nur noch singuläre Läsionen an Füßen oder Händen ohne Zeichen einer Superinfektion auf (Abbildung 1 und Tabelle 3).

Tabelle 3: Klinische Charakteristika in der Kohorte zu den verschiedenen Untersuchungszeitpunkten (übernommen von [47]).

	Regenzeit						Trockenzeit					
	April (N=407)		Mai (N=421)		Juni (N=414)		Juli (N=424)		August (N=427)		September (N=400)	
Charakteristika	n	%	n	%	n	%	n	%	n	%	n	%
Kinder mit HrCLM*	54	13.3	38	9.0	33	8.0	17	4.0	12	2.8	7	1.8
Kinder mit superinfizierten Läsionen†,‡	8	14.8	7	18.4	7	21.2	0	0	0	0	0	0
Anzahl Läsionen pro Kind‡												
1	27	50.0	27	71.1	25	75.8	13	76.5	12	100	7	100
2	18	33.3	3	7.9	3	9.1	3	17.7	0	0	0	0
≥3	9	16.7	8	21.1	5	15.2	1	5.9	0	0	0	0
Betroffene Körperregion §,#			¶						¶			
Hand	5	7.7	2	4.7	3	8.3	1	5.6	1	9.1	1	14.3
Arm	6	9.2	1	2.3	1	2.8	1	5.6	0	0	0	0
Stamm	5	7.7	6	14.0	1	2.8	1	5.6	0	0	0	0
Gesäß	6	9.2	4	9.3	3	8.3	1	5.6	0	0	0	0
Kopf	1	1.5	1	2.3	0	0	0	0	0	0	0	0
Bein	5	7.7	2	4.7	3	8.3	0	0	1	9.1	0	0
Fuß	35	53.9	23	53.5	21	58.3	12	66.7	8	72.7	5	71.4
Zeh	2	3.1	4	9.3	4	11.1	2	11.1	1	9.1	1	14.3

**Hakenwurm-assoziierte Larva migrans cutanea.*
†Pusteln oder Eiterbildung.
‡Prozent der Kinder mit HrCLM.
§Mehrfachnennung möglich.
¶Fehlende Beobachtungen.
#Prozent aller betroffenen Körperregionen.

Therapie

In der Therapiestudie wurde der Schweregrad der Infektion im Mittel als moderat eingeschätzt (Median 4 Punkte, Minimum- Maximum 0-6 Punkte). Dabei wurden 68.5% (n=302) der Läsionen als komplex klassifiziert. Juckreiz, Juckreiz-assoziierte Schlafstörung und Schmerz wurden von 92%, 70.5% bzw. 30.5% der Patienten angegeben. Der Schweregrad korrelierte mit der Intensität des Juckreizes ($\rho = 0.274$; $p=0.010$), der Schlafstörung ($\rho = 0.283$; $p=0.008$) und des Schmerzes ($\rho = 0.449$; $p< 0.001$).

Alle Teilnehmer der Studie sprachen auf die Behandlung mit Ivermectin an. Nach zwei Wochen war die Anzahl aller Läsionen von 441 auf 197 zurückgegangen ($p< 0.001$), nach 4 Wochen waren alle komplexen Läsionen abgeheilt ($p< 0.001$). Der Schweregrad war nach 2 und 4 Wochen im Median auf einen Punkt rückläufig (Minimum- Maximum 0-7; $p< 0.001$). Signifikant nahm auch der Anteil der Patienten mit Juckreiz (nach 2 Wochen 33%, nach 4 Wochen 3%), Juckreiz-assoziierte Schlafstörungen (24 und 0%) und Schmerzen (9.2 und 0%, alle $p< 0.001$) ab.

Diskussion

Prävalenz und Inzidenz

In einem Armutsviertel in Manaus wurde während der Regenzeit eine Prävalenz von 8.2% in der Gesamtbevölkerung dokumentiert. Dies ist die höchste jemals dokumentierte Prävalenz von HrCLM und fast doppelt so hoch wie im Nordosten Brasiliens (dort maximal 4.4%) [12,34–36]. Obwohl im tropischen Monsunklima von Manaus nur eine kurze Trockenzeit herrscht und der Bodenwassergehalt ganzjährig relativ hoch ist [49], zeigten sich identisch zum semiariden Klima von Brasiliens Nordosten signifikante saisonale Prävalenzschwankungen. Das bestätigt experimentelle Erkenntnisse, dass die Feuchtigkeit des Bodens zwar wichtig für die Überlebensdauer der Eier und Larven ist [50], für die Verteilung der Larven und damit die Vergrößerung der Fläche, auf der Exposition stattfindet auch Oberflächenwasser notwendig ist [7]. Die Korrelation der Prävalenz mit der monatlichen Niederschlagsmenge konnten wir erstmals nachweisen.

Zur Prävalenz außerhalb Brasiliens existiert nur eine Studie aus Sri Lanka mit einer Prävalenz von 58.2% unter hinduistischen Mönchen [51,52]. Diese kann jedoch nicht als Vergleich herangezogen werden, da sich die Mönche zuvor, einem religiösen Ritual folgend, über den Boden rollen mussten.

Zur Inzidenz unter Kindern gibt es weltweit keine Vergleichsdaten. Bei 0.52 Fällen/ Personenjahr ist theoretisch innerhalb eines Jahres jedes zweite Kind betroffen, was zusammen

mit der Häufigkeit von Juckreiz-assoziierten Schlafstörungen und Einschränkung der Lebensqualität [14,16] die Relevanz dieser vernachlässigten Erkrankung für die betroffene Bevölkerung verdeutlicht. Da die Re-Infektionsrate mit 1.08/ Personenjahr mehr als doppelt so hoch ist, können wir zudem davon ausgehen, dass sich die Krankheitslast nicht homogen in der Bevölkerung verteilt, sondern in Risikogruppen akkumuliert, die mehrmals im Jahr unter einer HrCLM leiden.

Risikofaktoren

HrCLM ist eine Armuts-assoziierte Erkrankung. Ärmliche Lebensbedingungen mit unbefestigten Straßen, fehlender Müll- und Fäkalienentsorgung, streunenden Hunden und Katzen sowie Kindern, die ohne Schuhe spielen, bieten ideale Bedingungen für das Auftreten von HrCLM. Wir konnten zeigen, dass der Grad der Armut, selbst innerhalb des Armutsviertels *Nova Vitoria*, das Risiko einer Infektion signifikant beeinflusst. Niedriges Familieneinkommen wurde in einer vorherigen Studie schon als Risikofaktor vermutet [36]. Der in unserer Analyse verwendet Wohlstandsindex bildet den sozio-ökonomischen Status eines Haushalts besser ab, als das in einer früheren Studie benutzte Monatseinkommen [36], das teilweise starken Schwankungen unterworfen ist [53].

Jungen hatten ein dreifach erhöhtes Risiko für eine HrCLM-Infektion im Vergleich zu Mädchen. Dies stimmt mit Studien aus Nordostbrasilien überein, wo bereits ein Zusammenhang mit geschlechtsspezifischen Verhalten vermutet wurde [12,34,36]. Während Jungen weitgehend unbeaufsichtigt draußen spielen dürfen, waren Mädchen häufiger zu Hause anzutreffen. Das traf insbesondere für die Altersgruppe der 10-14-Jährigen zu, die das höchste Risiko für eine Infektion zeigten.

Die Infektion mit tierpathogenen Hakenwurmlarven geschieht durch Hautkontakt mit infektiösen Larven auf dem Boden. Da Barfußlaufen eine häufige Exposition gegenüber dem Boden darstellt, konnte diese sowohl bei Touristen als auch bei Bewohnern von Endemiegebieten als Risikofaktor identifiziert werden [36,37]. Aus experimentellen Studien ist bekannt, dass die Migration der Larven im Boden und damit die Vergrößerung der kontaminierten Fläche durch eine sandige Bodenbeschaffenheit begünstigt wird [7]. Diese Erkenntnis konnten wir nun epidemiologisch bestätigen, da Barfußlaufen sich nicht nur als Risikofaktor zeigte, sondern die Höhe des Risikos von der Häufigkeit der Verwendung von Schuhen auf sandigem Boden abhing. Wichtig ist, dass der protektive Effekt schon mit den typischen brasilianischen Plastiksandalen erreicht wurde.

Ein unbefestigter Boden im Haus scheint eher ein Proxy für Armut als ein direktes Risiko zu sein [36]. Barfußlaufen im Haus trotz unbefestigtem Boden war in der Querschnittsstudie kein unabhängiger Risikofaktor. Möglicherweise ist die Bodenfeuchtigkeit im Haus nicht hoch genug, so dass die Eier nicht zu infektiösen Larvenstadien heranreifen können, zudem wird Tierfäzes im Haus in der Regel schneller entfernt als auf dem Hof. Die Transmission scheint also weitestgehend außerhalb des Hauses stattzufinden.

Klinik

Das morphologische Bild der HrCLM, die Häufigkeit von bakteriellen Superinfektionen, sowie die Lokalisation und die Anzahl der Läsionen variieren in den bislang durchgeführten Studien weit und sind von zuvor unbekannten Faktoren abhängig.

Zunächst müssen Infektionen bei Reisenden von Infektionen der Bevölkerung endemischer Gebiete unterschieden werden. Reisende in Endemiegebiete infizieren sich häufig am Strand und haben seltener superinfizierte Läsionen (0-8%)[13], was mit einer anderen, regelrechten Wundbehandlung [16] zu erklären ist.

Innerhalb der Bevölkerung endemischer Gebiete ist das klinische Bild heterogen [12,34–36,46–48], was offensichtlich mit unterschiedlichem Risikoverhalten begründet ist. So waren Kinder im Vergleich zu Erwachsenen häufiger an atypischen Lokalisationen und von bakteriellen Superinfektionen betroffen. Das deckt sich mit der Beobachtung, dass Kinder beim Spielen häufig nicht nur mit den Füßen Bodenkontakt haben und bei der Untersuchung exkorierte Läsionen selten abgedeckt bzw. sauber gehalten waren. Im Falle von spezifischem Risikoverhalten wie dem oben erwähnten hinduistischem Ritual, treten auch unter Erwachsenen mehr Infektionen am Körperstamm und den proximalen Extremitäten auf [51].

Erstmalig konnten wir einen Zusammenhang zwischen den klimatischen Bedingungen und dem klinischen Bild herstellen. In regenreichen Monaten war die Intensität der Infektionen mit multiplen Läsionen signifikant höher. Die gleiche Tendenz ließ sich auch für die Proportion der Superinfektionen und der atypischen Lokalisationen erkennen. Mit der Abnahme der Regenmenge nimmt die fäkal kontaminierte Fläche ab [7,50], wodurch HrCLM- Infektionen im Allgemeinen und Infektionen an selten exponierten Lokalisationen im Speziellen seltener werden. Eine höhere Prävalenz der Pyodermie unter warmen und feuchten Bedingungen ist schon lange beobachtet worden [54,55], wobei die geringen Schwankungen der medianen Temperatur in Manaus (1,5°C zwischen April und September) [41] offensichtlich keine Rolle gespielt haben.

Kontrollmöglichkeiten

Kontrollmöglichkeiten bestehen in der Eliminierung der Risikofaktoren oder der Behandlung der infizierten Tiere, um den biologischen Kreislauf zu durchbrechen. Eine Behandlung der Menschen kann die Inzidenz nicht senken, da der Mensch Fehlwirt ist. Eine konsequente anthelminthische Therapie von Katzen und Hunden kann sehr wirksam sein [52], ist aber in Brasilien, wo nicht einmal die betroffene Bevölkerung adäquat therapiert wird [16], praktisch nicht realisierbar.

Aufgrund des errechneten bevölkerungsbezogenen attributablen Risikos, sind 45% der Infektionen auf Barfußlaufen zurückzuführen und hätten somit verhindert werden können, falls alle Kinder der Studienpopulation konsequent Schuhe (bzw. die typischen brasilianischen Plastiksandalen) getragen hätten. Einfache Interventionen wie die Verteilung von Schuhen sind bezahlbar, führen allerdings nicht zum konsequenten Tragen derselben und zeigen isoliert wenig Wirkung, wie im Rahmen der Tungiasis gezeigt werden konnte [56]. Die im Rahmen dieser Promotion durchgeführten Studien zeigen, dass die Ursachen für eine hohe HrCLM-Inzidenz multifaktoriell sind und ärmliche Lebensbedingungen eine entscheidende Rolle spielen. Solange sich diese Lebensverhältnisse und das Risikoverhalten nicht ändern, sind die Betroffenen auf eine wirksame Therapie angewiesen.

Therapie

Therapiestudien an Reiserückkehrern zeigten bis zu 100% Heilungsraten für Albendazol und Ivermectin per os [18–20,57,58]. In der einzigen randomisierten Studie, zeigte sich bei kleiner Fallzahl eine Überlegenheit von Ivermectin gegenüber Albendazol [17]. In unserer Therapiestudie, in der erstmals Patienten aus endemischen Gebieten behandelt wurden, zeigte sich ein ähnlich gutes Ansprechen auf die orale Therapie mit Ivermectin. Die Studie ist die Größte bisher durchgeführte und der Verlauf wurde bei zweimaligen ärztlichen Folgeuntersuchungen sehr detailliert dokumentiert. Zudem hatte die Mehrheit der Patienten mehrere Läsionen, welche größtenteils als komplex eingestuft wurden. Dies erklärt möglicherweise die vergleichsweise lange Persistenz der Symptome. Bei Studien an Reisenden mit durchschnittlich weniger Läsionen beobachteten Bouchaud et al. ein Verschwinden des Pruritus im Median nach 3 Tagen und die Abwesenheit von lokalen Entzündungszeichen im Median nach 7 Tagen [59], Caumes et al. sogar schon nach 1 Tag [17]. Van der Emden et al. berichten von einer schnellen Heilung bei 94% der Patienten innerhalb von 5 Tagen, allerdings wurde Heilung nicht exakt definiert [20]. Unterschiedliche Nachbeobachtungsintervalle und

Definitionen des Therapieerfolgs sowie fehlende morphologische Daten schränken die Vergleichbarkeit unserer mit früheren Studien deutlich ein.
Einschränkungen der Therapie mit Ivermectin sind vor allem die fehlende Zulassung für Kinder unter 15 Kilogramm Körpergewicht, für die auch Albendazol p.o. keine Alternative ist [23,60]. Problematisch ist, dass der ärmste Teil der Bevölkerung, der gleichzeitig die größte Krankheitslast trägt, keinen Zugang zu adäquater Therapie hat. Die Betroffenen greifen deshalb auf potenziell toxische Therapieversuche mit Insektiziden oder scharfen Gegenständen zurück [16].

Zusammenfassung:

1. Im tropischen Monsunklima von Manaus konnte die bisher höchste Prävalenz und erstmals Inzidenzraten dokumentiert werden. Trotz der kurzen Trockenzeit können auch dort Prävalenzschwankungen beobachtet werden, die mit der akkumulierten Niederschlagsmenge korrelierten.
2. Die Evidenz für eine Kausalität der bekannten Risikofaktoren Barfußlaufen, männliches Geschlecht und Alter < 14 Jahren konnte durch eine prospektive Kohortenstudie erhöht werden. Erstmals konnten wir einen Zusammenhang mit dem sozio- ökonomischen Status zeigen.
3. Das Krankheitsbild im Sinne von Anzahl der Infektionen, Anteil von bakteriellen Superinfektionen und atypischen Lokalisationen zeigt saisonale Schwankungen.
4. Durch konsequentes Tragen von offenen brasilianischen Plastiksandalen könnten 45% der Infektionen im Studiengebiet verhindert werden. Die orale Therapie mit Ivermectin zeigt auch im Endemiegebiet gute Wirksamkeit.

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Eidesstattliche Erklärung

„Ich, Felix Reichert, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Epidemiologie, Klinik und Therapie der Hakenwurm-assoziierten Larva migrans cutanea in Manaus, Brasilien“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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

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

Datum _____ Unterschrift

Anteilserklärung an den erfolgten Publikationen

Felix Reichert hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Reichert F, Pilger D, Schuster A, Lesshafft H, Guedes de Oliveira S, Ignatius R, Feldmeier H. Epidemiology and morbidity of hookworm-related cutaneous larva migrans (HrCLM): Results of a cohort study over a period of six months in a resource-poor community in Manaus, Brazil. PLoS Neglected Tropical Diseases. 2018.
Beitrag im Einzelnen: 80%
Planung der Studie [Auswahl des Studiengebiet, Festlegung des endgültigen Studiendesigns, Zusammenstellung des Studienteams, Erstellung und Pilotierung der Fragebögen]: gemeinsam mit AS unter Supervision von DP und HF.
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Manuskripterstellung samt Tabellen und Grafiken: alleinig mit anschließender Korrektur durch Co-Autoren.

Publikation 2: Reichert F, Pilger D, Schuster A, Lesshafft H, Guedes de Oliveira S, Ignatius R, Feldmeier H. Prevalence and Risk Factors of Hookworm-Related Cutaneous Larva Migrans (HrCLM) in a Resource-Poor Community in Manaus, Brazil. PLoS Neglected Tropical Diseases. 2016.
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Durchführung der Studie/ Datenerhebung: alleinig abgesehen von 5 Tagen Unterstützung durch DP.
Dateneingabe und –analyse: alleinig nach methodischer Besprechung mit HF und DP.
Manuskripterstellung samt Tabellen und Grafiken: alleinig mit anschließender Korrektur durch Co-Autoren.

Publikation 3: Schuster A, Lesshafft H, Reichert F, Talhari S, de Oliveira SG, Ignatius R, Feldmeier H. Hookworm-related cutaneous larva migrans in northern Brazil: resolution of clinical pathology after a single dose of ivermectin. Clinical Infectious Diseases. 2013.
Beitrag im Einzelnen: 20% (Teile der Datenerhebung, Manuskriptkorrektur)

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

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Unterschrift des Doktoranden

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Druckexemplare der ausgewählten Publikationen

Publikation 1: Epidemiology and morbidity of hookworm-related cutaneous larva migrans (HrCLM): Results of a cohort study over a period of six months in a resource-poor community in Manaus, Brazil.

Reichert F, Pilger D, Schuster A, Lesshafft H, Guedes de Oliveira S, Ignatius R, Feldmeier H. PLoS Neglected Tropical Diseases. 2018.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Epidemiology and morbidity of hookworm-related cutaneous larva migrans (HrCLM): Results of a cohort study over a period of six months in a resource-poor community in Manaus, Brazil

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Abstract

Background

Hookworm-related cutaneous larva migrans (HrCLM) is a neglected parasitic skin disease, widespread in resource-poor communities in tropical and subtropical countries. Incidence and risk factors have never been investigated in a cohort study.

Methodology/Principal findings

To understand the seasonal epidemiology of HrCLM, an open cohort of 476 children in a resource-poor community in Manaus, Brazil was examined for HrCLM monthly over a period of 6 months. Monthly prevalence and intensity of infection were correlated with the amount of monthly precipitation. Multivariable Cox regression analysis indicated male sex (hazard ratio [HR] 3.29; 95% confidence interval [CI] 1.95–5.56), walking barefoot on sandy ground (HR 2.30; 95% CI 1.03–5.16), poverty (HR 2.13; 95% CI 1.09–4.17) and age between 10 and 14 years (HR 1.87; 95% CI 1.01–3.46) as predictors of HrCLM. Monthly incidence rates ranged between 0.21 and 1.05 cases per person-year with an overall incidence of 0.52 per person-year.

Conclusions/Significance

HrCLM is a frequent parasitic skin disease in this resource-poor community. Every second child theoretically becomes infected during one year. Boys, 10 to 14 years old,

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belonging to the poorest households of the community, are the most vulnerable population group. Even in the tropical monsoonal climate of Amazonia there is a considerable seasonal variation with monthly incidence and number of lesions peaking in the rainy season.

Author summary

Hookworm-worm related cutaneous larva migrans (HrCLM) is a parasitic skin disease caused by hookworm larvae of cats and dogs occurring in many countries with a tropical or subtropical climate. Humans are a biological impasse for these helminths as the larvae cannot pass the basal membrane of the epidermis and hence migrate haphazardly in the skin causing local inflammation and intense itching. In scientific literature HrCLM is generally described as a disease of travellers returning from endemic areas. In contrast, epidemiological data is scanty. In a previous study, we had examined an entire resource-poor neighbourhood in Manaus (Brazil) and showed, that HrCLM is an important individual and public health problem, affecting up to 8% of the population, in particular children. In this study, we followed a cohort of children for six months. We found a significant seasonal variation in incidence and morbidity between dry and rainy season. Extrapolated, every second child in this population will be affected at least once within one year. The longitudinal study design enabled us to validate previously identified risk factors. Children aged 10–14 years, in particular boys, and those walking barefoot on sandy ground had the highest infection rates. Children from the poorest families in the resource-poor community were most vulnerable to HrCLM.

Introduction

Hookworm-related cutaneous larva migrans (HrCLM) is a neglected tropical skin disease caused by hookworm larvae of cats and dogs such as *Ancylostoma braziliense*, *Ancylostoma caninum* and *Uncinaria stenocephal*a [1]. In humans, these larvae are unable to cross the basal membrane of the epidermis and hence cannot continue their normal development to adult worms. By consequence they haphazardly migrate in the epidermis, producing an elevated linear or serpiginous track. The intense itching leads to important pruritus-associated morbidity such as excoriations and bacterial superinfection of the lesions [2–5].

HrCLM is endemic in many tropical and subtropical countries worldwide [6–9]. Prevalence reached up to 8% in population-based studies with significant variation between sexes and age-groups [2]. Children are affected in particular [2,3]. In semi-arid climates such as in North-eastern Brazil, there is significant seasonal variation in prevalence from 0.2% in the middle of the dry season to 3.1% in the rainy season [4].

Known risk factors are male sex, young age, barefoot walking, poverty and presence of animal faeces on the compound [2,4,10]. In order to determine hazard ratios for previously identified risk factors and verify whether there is seasonal variation of incidence and morbidity in the tropical monsoonal climate of Amazonia, we conducted a longitudinal study with a cohort of 476 children living in a resource-poor community in the outskirts of Manaus, the largest city in Amazonia.

Methods

Study area and population

The study was conducted in the resource-poor neighbourhood Nova Vitoria in Manaus, capital of Amazonas State, Brazil. Manaus is situated at 03˚06' south latitude and has a tropical monsoonal climate following the Köppen-Geiger classification with a dry season (less than 60 mm precipitation/month) usually in August and heavy monsoon rains during the rest of the year [11]. As many other resource-poor communities in Brazil, the study area was built up without permission of public authorities. This explains the lack of public infrastructure such as health facilities, childcare, paved streets or a sewage disposal system. In consequence many stray dogs and cats roamed through the streets and children were playing unattended on the sandy ground. The population was poor, one third of the households experienced food shortage in the past 12 months. Most of the families had several children. The study area and population have been described in detail previously [2].

Study design

At baseline all households in the study area were visited. Based on stringent inclusion criteria 92% of the households were admitted to the cross-sectional study. Methods of the cross-sectional study have been published previously [2]. In brief, all household members were examined clinically for HrCLM and environmental, socio-economic and behaviour-related risk factors were documented using pre-tested, structured questionnaires.

All children of the 262 households were assessed for eligibility for the cohort and then monitored monthly for the presence of new HrCLM (Fig 1). The examination took place in a room where privacy was guaranteed. The whole body surface was examined, only the genital area was spared in case itching was absent. HrCLM was diagnosed clinically when the characteristic slow-progressing, elevated linear or serpiginous track was present [12–14].

Inclusion criteria for the cohort were: residence in the study area, absence of HrCLM-infection at baseline, age under 18 and provision of an informed written consent. Children who were found infected at baseline were included on the day they received treatment with ivermectin or topical thiabendazole. In all but one child, HrCLM lesions had resolved by the next follow-up.

Due to an expected important drop out rate caused by out- and in-migration of whole families and temporary residence of family members in the countryside outside Manaus, we chose an open cohort design. Siblings of participants and children of newly encountered families replaced children who were lost for follow-up in order to keep the number of cohort members stable (Fig 1). Participants who entered the cohort after baseline were examined and interrogated in an identical manner. In case of temporary absence, only the time of presence in the study area between two consecutive follow-ups was analysed.

Participants lost to follow-up before the second examination (n = 29) were excluded from the analysis. To avoid selection bias 12 individuals, who were not identified by active case finding but presented to the team for procurement of treatment, were excluded. One patient was excluded because he refused to be treated with ivermectin (Fig 1).

In order to gather information on reinfections, individuals censored after HrCLM diagnosis were observed further after treatment (S1 Fig). These data were included in the analysis of monthly incidence and prevalence, and for analysis of clinical characteristics.

Episodes of HrCLM at recruitment were documented and included in the analysis of clinical characteristics and monthly prevalence, if the prerequisite of residency for more than 2 months in the study area was fulfilled.

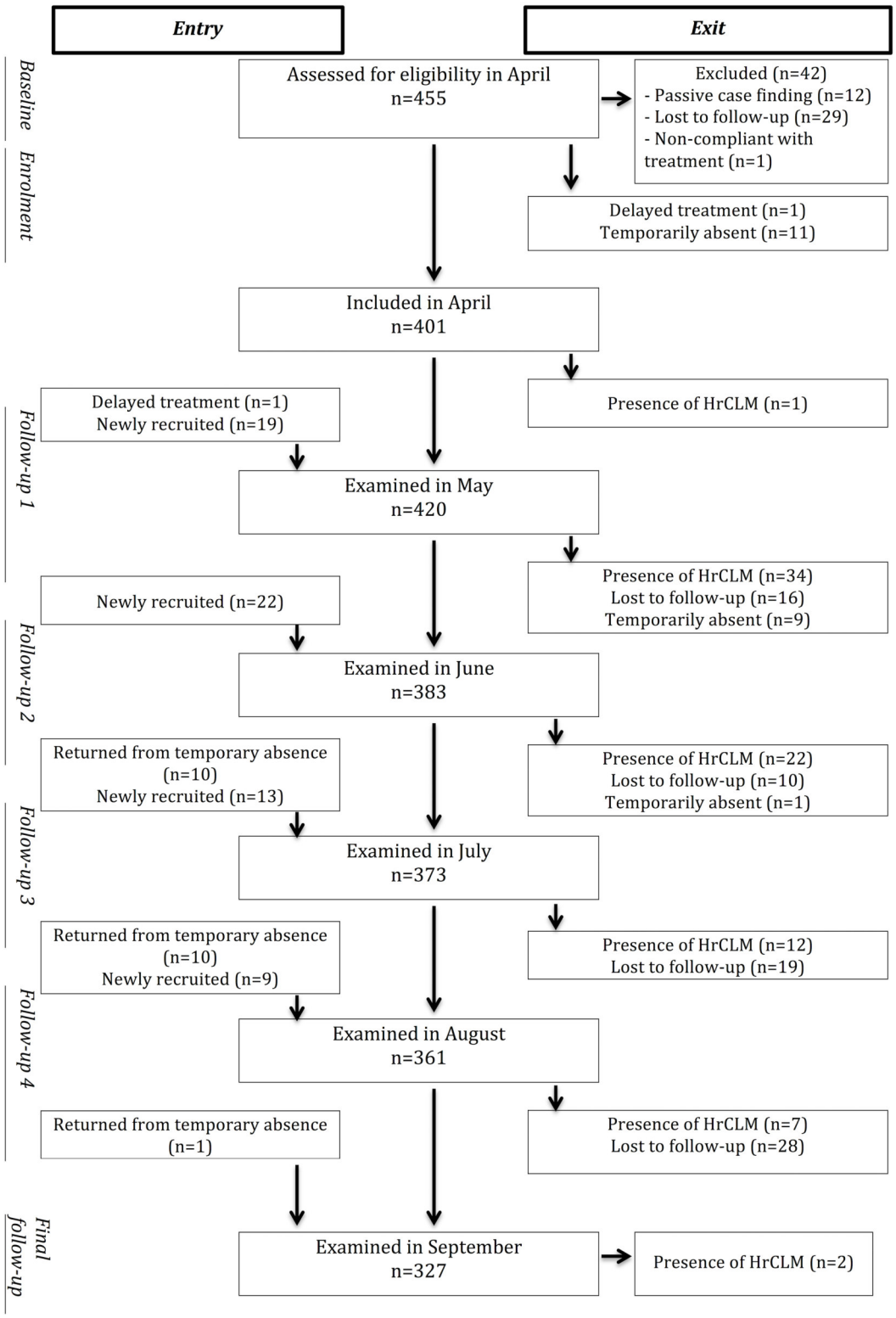

Fig 1. Flowchart of participants in the cohort.

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For the calculation of monthly incidence rates only participants without HrCLM or participants that had been treated in the preceding month were included.

The study started in April 2009 (rainy season) and ended in September 2009 (which in 2009 was still part of the dry season).

Ethical considerations

The study was approved by the Ethical Committee of the Fundação de Medicina Tropical–Amazonas (FMT-AM). Informed written consent was obtained from each participant's legal guardian. Every individual with HrCLM was offered free treatment independently whether he or she participated in the study. Treatment consisted of ivermectin (Ivermec, Uci-farma, São Paulo, Brazil) given as single oral dose (200 μg/kg). In the case of children <5 years or <15 kg, thiabendazole 5% (Tiadol, Bunker Indústria Farmacêutica Ltda., São Paulo, Brazil) was topically applied 3 times a day for one week [2].

Statistics

Data were entered in Microsoft Office Access 2007, cleaned for entering errors and analysed with PASW Statistics Version 18.0 (SPSS Inc., Chicago, USA). Missing data were assumed to be missing at random and were marked in the analysis. A wealth score was formed out of household assets using principal component analysis as previously described [2,15]. Income and age were categorized similar to previous population-based studies to allow comparison [2,4,10,16].

Time to infection was defined as the time between the date of inclusion in the cohort and the date of diagnosis of HrCLM, date of last examination before lost to follow-up, or date of last examination at the end of the study (right censoring), whichever occurred first. In case of temporary absence, only the time between consecutive follow-ups was included. Lost to follow-ups were compared to those who were censored by event or end of the study using chi-square-test or likelihood ratio where appropriate.

For bivariable risk factor analysis, hazard ratios (HR) were calculated together with 95% confidence intervals (95% CI) using a Cox proportional hazards model.

For multivariable risk factor analysis, all variables that showed weak evidence of an association with HrCLM ($p<0.1$) were entered into a stepwise Cox regression. Only significant variables ($p<0.05$) remained in the model. We calculated standard errors and 95% CI to identify multicollinearity and removed variables where necessary. We used log-minus-log function to check if proportional hazard assumption was satisfied.

Kaplan-Meier curves were used to visualize event-free periods and differences in incidence between sexes, age groups, frequency of barefoot walking and wealth strata.

We calculated Population Attributable Fraction (PAF) for independent risk factors practically amenable to intervention. As only one risk factor was amenable to intervention, we used Levins unadjusted equation: $\{p_e(RR-1)\}/\{p_e(RR-1)+1\}$ with p_e being the proportion of the population, which is exposed to the risk factor [17,18]. RR was calculated as a rate ratio.

Incidence rate and prevalence were determined monthly. Seasonal changes in prevalence, clinical presentation (superinfection, number of lesions, site of infection) and total monthly precipitation in Manaus were correlated using Spearman´s rank correlation with a two-tailed significance level of $p<0.05$. Climate data were obtained from the International Institute of Meteorology of Brazil (INMET).

The overall incidence rate was extrapolated from the number of patients divided by the accumulated time to HrCLM infection. Correspondingly, reinfection rate was calculated for the subgroup of patients who were infected at the first examination and were included after

treatment. Monthly incidence rates were obtained by dividing the number of HrCLM-infections by the number of followed children during the corresponding time period multiplied by 12 assuming an exact 1-month-period between two follow-ups. Corresponding 95% CI were calculated by a Poisson regression.

Results

During the 6 months of the study, a total of 476 children living in 209 households were included with a median time of follow-up of 149 days (range 13–166), which amounted to 54,938 person-days at risk. The median age at entry was 6 years (range 0–15) and the majority of the children were girls (52%). After a median time of 65.5 days (range 22–132), 68 children (14.3%) were lost to follow-up. Comparing their characteristics, there was no significant difference except for the proportion of barefoot walking on sandy ground (Table 1).

The Kaplan-Meier estimated HrCLM-free proportion after 90 days and after 166 days (at the end of the study) was 84% and 82%, respectively (Fig 2). Mean time to HrCLM infection was 146.4 days (95% CI 142.3–150.5). There was no difference in mean time to HrCLM infection between recruitment at baseline or later (145.6 days, 95% CI 141.1–150.0 days and 139.3 days, 95% CI 128.5–150.1 days, respectively, p = 0.462).

A total of 161 episodes of HrCLM were identified. Only 27 children (5.7%) accounted for 66 (41.0%) of all episodes by having several (up to 4) episodes of HrCLM. During follow-up, 78 children developed HrCLM resulting in an overall incidence rate of 0.52 per person-year.

Reinfection rate of children who were infected before inclusion in the cohort (n = 64) was more than twice as high with 1.08 per person-year. Monthly incidence rates and prevalence decreased every month from the rainy to the dry season (Table 2; Fig 3).

Clinical characteristics showed seasonal differences. During the rainy season in April, every second affected child had multiple lesions and lesions were found all over the body. During the dry season in contrast, only single lesions were encountered and lesions predominantly occurred on hands and feet. Superinfected lesions were only found from April to June (Table 3 and Fig 3).

The decreases in prevalence and infection intensity (multiple infections per person) were correlated with the decreasing amount of monthly precipitation (rho = 0.928, p = 0.008, and rho = 0.941, $p<0.001$ respectively).

Bivariable risk factor analysis revealed 10–14 years of age, a low wealth score, practicing soccer (soccer is usually played barefoot), walking barefoot on sandy ground and HrCLM-infection at recruitment as predictors for HrCLM-infection during the follow-up period. The most potent predictor was male sex (Table 4).

In the adjusted multivariable model age, sex, low wealth and barefoot walking remained as independent risk factors visualized in Kaplan-Meier curves (Fig 4). The PAF of walking barefoot on sandy ground was 45%.

Discussion

In this longitudinal study on the occurrence and determinants of HrCLM, we could show that higher infection rates correlate with larger amounts of precipitation even in a tropical monsoonal climate where soil remains wet the whole year round.

Development and transmission of nematode larvae depend on a favourable climate. High soil moisture and atmospheric humidity enhance development and survival of eggs and larvae [19]. Rain furthermore disperses larvae and eggs over a larger soil surface [20]. Consequently, climate defines the endemic areas and may cause seasonal variation. Seasonal variation in

Table 1. Comparison between participants lost to follow-up and censored by event or end of study (N = 476).

Characteristic	Total no. (%)	Lost to follow up (%)	Censored by event or end of study (%)	2-sided p-value *
Demography				
Male	228 (47.9)	26 (38.2)	202 (49.5)	0.083
Female	248 (52.1)	42 (61.8)	206 (50.5)	
Age ≤ 4 years	174 (36.6)	32 (47.1)	142 (34.8)	0.147
5–9 years	188 (39.5)	22 (32.4)	166 (40.7)	
10–14 years	102 (21.4)	11 (16.2)	91 (22.3)	
15–18 years	12 (2.5)	3 (4.4)	9 (2.2)	
Socioeconomic characteristics				
Income < 1 minimum wage †, ‡	133 (28.5)	18 (26.5)	115 (28.9)	0.130
= 1 minimum wage	169 (36.3)	19 (27.9)	150 (37.7)	
> 1 minimum wage	164 (35.2)	31 (45.6)	133 (33.4)	
Wealth score †, §				
Low	197 (43.2)	33 (48.5)	164 (42.3)	0.623
Intermediate	151 (33.1)	20 (29.4)	131 (33.8)	
High	108 (23.7)	15 (22.1)	93 (24.0)	
Behaviour				
Walking always/regularly †				
Barefoot outdoors	61 (12.9)	5 (7.5)	56 (13.8)	0.154
With sandals/shoes outdoors/ not walking at all (babies)	431 (87.1)	62 (92.5)	351 (86.2)	
Walking on sandy ground †				
Always barefoot	94 (20.0)	7 (10.6)	87 (21.5)	0.001
Sometimes barefoot	281 (59.8)	35 (53.0)	246 (60.9)	
Never barefoot	95 (20.2)	24 (36.4)	71 (17.6)	
Practicing soccer †	172 (36.5)	19 (27.9)	153 (38.0)	0.208
Other sport	39 (8.3)	8 (11.8)	31 (7.7)	
No sport	260 (55.2)	41 (60.3)	219 (54.3)	
Sport barefoot on sand †	170 (36.2)	18 (26.5)	152 (37.8)	0.105
Never barefoot/not on sand	40 (8.5)	9 (13.2)	31 (7.7)	
No sport	260 (55.3)	41 (60.3)	219 (54.5)	
Environment				
Faeces found on compound †	65 (14.7)	5 (7.8)	60 (15.8)	0.094
No faeces found	378 (85.3)	59 (92.2)	319 (84.2)	
Health				
Infection at first examination	64 (13.4)	6 (8.8)	58 (14.2)	0.228
No infection	412 (86.6)	62 (91.2)	350 (85.8)	

*For comparison of „Lost to follow-up"and „Censored by event or end of the study"group.

†Missing observations.

‡Minimum wage in 2009: 465 R$ ≈ 220$.

§For definitions see methods.

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prevalence with positive correlation to precipitation has been shown for the semi-arid climate of Northeast Brazil [4].

Additionally, variations in climate may have an indirect influence on prevalence through a change in behaviour of the population e.g. regarding sports or use of protective footwear.

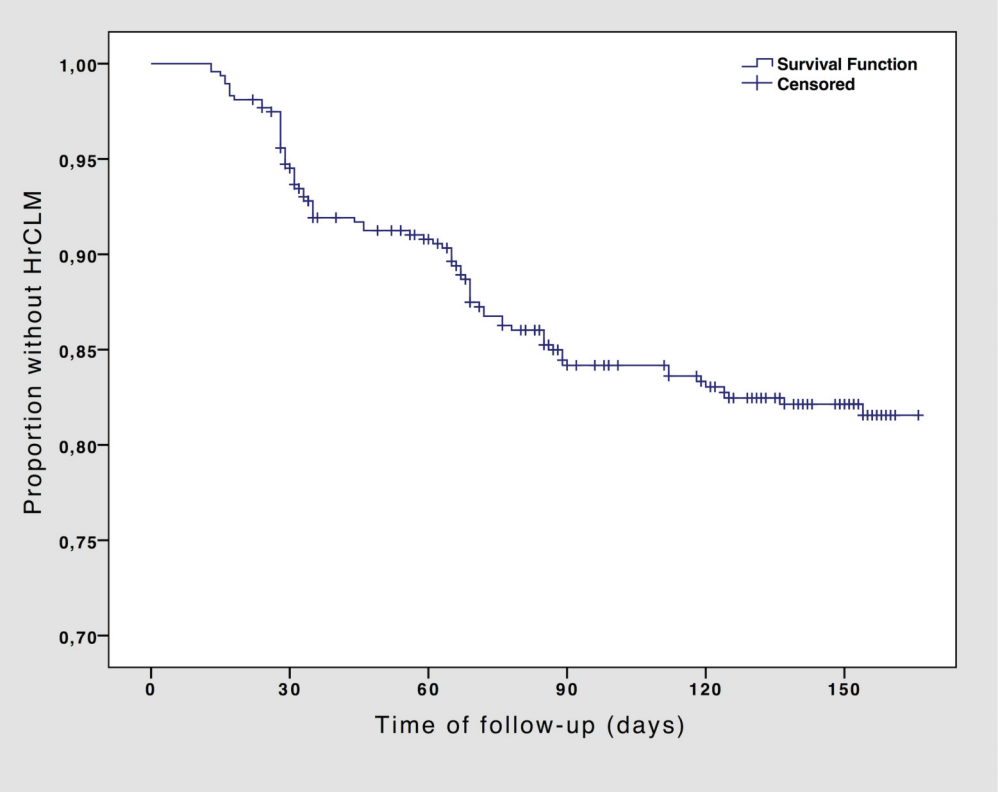

Fig 2. Kaplan-Meier-curve for overall HrCLM-free proportion.

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For the first time, our study showed that precipitation correlated with disease intensity. This is not surprising as more infective stage larvae do not only increase infection risk on the population but also on the individual level.

Interestingly, seasonal variation extended to the topographic localisation of infection. While untypical localisations such as the trunk, buttock or head were found in up to one fourth of infections during the rainy season, only feet and hands were affected at the last examination during dry season. These body areas are often uncovered, have most direct contact with the soil and therefore get infected the whole year round. Body areas with less exposure to the soil

Table 2. Incidence of hookworm-related cutaneous larva migrans (HrCLM).

	Person-years at risk	Patients with HrCLM	Incidence per person-year	(95% CI)
By month				
April-May	33.42 *	35	1.05	0.76–1.48
May-June	32.67 *	27	0.83	0.58–1.22
June-July	34.25 *	16	0.47	0.30–0.78
July-August	34.83 *	12	0.34	0.21–0.63
August-September	33.33 *	7	0.21	0.11–0.46
Overall	150.5	78	0.52	0.42–0.65
Reinfection Rate †	17.53	19	1.08	0.71–1.73

* Approximated data, assuming an exact 1-month-period between two follow-ups.

† Calculated with patients who were infected at baseline and were included after treatment; see study design.

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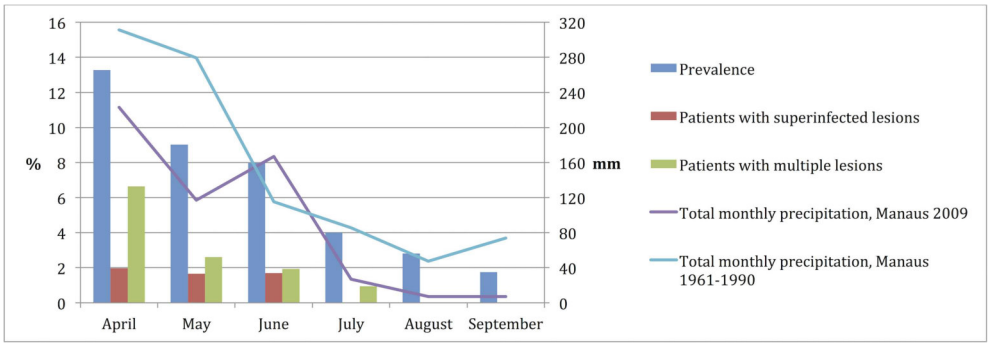

Fig 3. Precipitation, prevalence and clinical characteristics by month.

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seem to get infected regularly only in case of a higher larval burden in the soil. Similar results were seen in semi-arid Northeast Brazil where untypical localisations accounted for more than half of the lesions during rainy season [4,16].

Incidence

So far, an incidence rate of HrCLM has never been determined in an appropriately designed study. Calculated monthly it showed a seasonal variation between a maximum of 1.05 per person-year during rainy season and a minimum of 0.21 per person-year during dry season.

Table 3. Clinical characteristics of study participants at different times of examination.

	April (N = 407)		May (N = 421)		June (N = 414)		July (N = 424)		August (N = 427)		September (N = 400)	
Characteristic	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Children with HrCLM*	54	13.3	38	9.0	33	8.0	17	4.0	12	2.8	7	1.8
Children with superinfected lesions †	8	14.8 ‡	7	18.4 ‡	7	21.2 ‡	0	0	0	0	0	0
Number of lesions per child												
1	27	50.0 ‡	27	71.1 ‡	25	75.8 ‡	13	76.5 ‡	12	100 ‡	7	100 ‡
2	18	33.3 ‡	3	7.9 ‡	3	9.1‡	3	17.7 ‡	0	0	0	0
≥3	9	16.7 ‡	8	21.1 ‡	5	15.2 ‡	1	5.9 ‡	0	0	0	0
Affected body areas §,#			¶						¶			
Hand	5	7.7	2	4.7	3	8.3	1	5.6	1	9.1	1	14.3
Arm	6	9.2	1	2.3	1	2.8	1	5.6	0	0	0	0
Trunk	5	7.7	6	14.0	1	2.8	1	5.6	0	0	0	0
Buttock	6	9.2	4	9.3	3	8.3	1	5.6	0	0	0	0
Head	1	1.5	1	2.3	0	0	0	0	0	0	0	0
Leg	5	7.7	2	4.7	3	8.3	0	0	1	9.1	0	0
Foot	35	53.9	23	53.5	21	58.3	12	66.7	8	72.7	5	71.4
Toe	2	3.1	4	9.3	4	11.1	2	11.1	1	9,1	1	14.3

*Hookworm-related cutaneous larva migrans.

†Pustules or suppuration.

‡Percent of children with HrCLM.

§Multiple topographic affection possible.

¶Missing observations.

#Percent of affected body areas.

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Table 4. Predictors of hookworm-related cutaneous larva migrans in a cohort of 476 children.

Characteristic	Person-years at risk	HrCLM*	Incidence per person-year	Crude Hazard Ratio (95% CI)	2-sided p-value	Adjusted Hazard Ratio (95% CI)	2-sided p-value
Demography							
Male	65.90	57	0.86	3.41 (2.04–5.69)	<0.001	3.29 (1.95–5.56)	<0.001
Female	84.62	21	0.25	1 †		1 (reference)	
Age ≤ 4 years	55.87	21	0.38	1 †		1 (reference)	
5–9 years	60.48	28	0.46	1.28 (0.72–2.27)	0.40	1.07 (0.59–1.94)	0.824
10–14 years	30.24	28	0.93	2.28 (1.27–4.08)	0.006	1.87 (1.01–3.46)	0.045
15–18 years	3.92	1	0.26	0.78 (0.11–5.83)	0.811	0.65 (0.09–4.91)	0.674
Socioeconomic characteristics							
Income < 1 minimum wage ‡, §	43.46	22	0.51	1.27 (0.70–2.32)	0.429		
= 1 minimum wage	51.85	34	0.66	1.62 (0.94–2.78)	0.084		
> 1 minimum wage	51.95	21	0.40	1 †			
Wealth score ‡, ¶							
Low	60.51	41	0.68	2.15 (1.11–4.18)	0.024	2.13 (1.09–4.17)	0.027
Intermediate	48.81	23	0.47	1.51 (0.74–3.10)	0.259	1.47 (0.71–3.04)	0.294
High	35.79	11	0.31	1 †		1 (reference)	
Behaviour							
Walking always/regularly ‡							
Barefoot outdoors	17.89	14	0.78	1.57 (0.88–2.80)	0.126		
With sandals/shoes outdoors/ not walking at all (babies)	132.09	64	0.48	1 †			
Walking on sandy ground ‡							
Always barefoot	27.90	25	0.90	3.24 (1.51–6.94)	0.002	2.30 (1.03–5.16)	0.043
Sometimes barefoot	89.48	44	0.49	1.66 (0.81–3.42)	0.168	1.70 (0.81–3.56)	0.158
Never barefoot	31.22	9	0.29	1 †		1 (reference)	
Practicing soccer ‡	51.39	38	0.74	1.86 (1.17–2.95)	0.009		
Other sport	11.30	5	0.44	1.08 (0.42–2.74)	0.88		
No sport	86.14	34	0.39	1 †			
Sport barefoot on sand ‡	50.39	38	0.75	1.89 (1.19–3.00)	0.007		
Never barefoot/not on sand	11.88	5	0.42	1.04 (0.41–2.65)	0.939		
No sport	86.14	34	0.39	1 †			
Environment							
Faeces present on compound ‡	21.02	11	0.52	1.01 (0.53–1.92)	0.972		
No faeces present	123.45	63	0.51	1 †			
Health							
Infection at first examination	17.53	19	1.08	2.34 (1.40–3.93)	0.001		
No infection	132.99	59	0.44	1 †			

*Number of children with Hookworm-related cutaneous larva migrans.

† Reference category.

‡ Missing observations.

§ Minimum wage in 2009: 465 R$ ≈ 220$.

¶ For definitions see methods.

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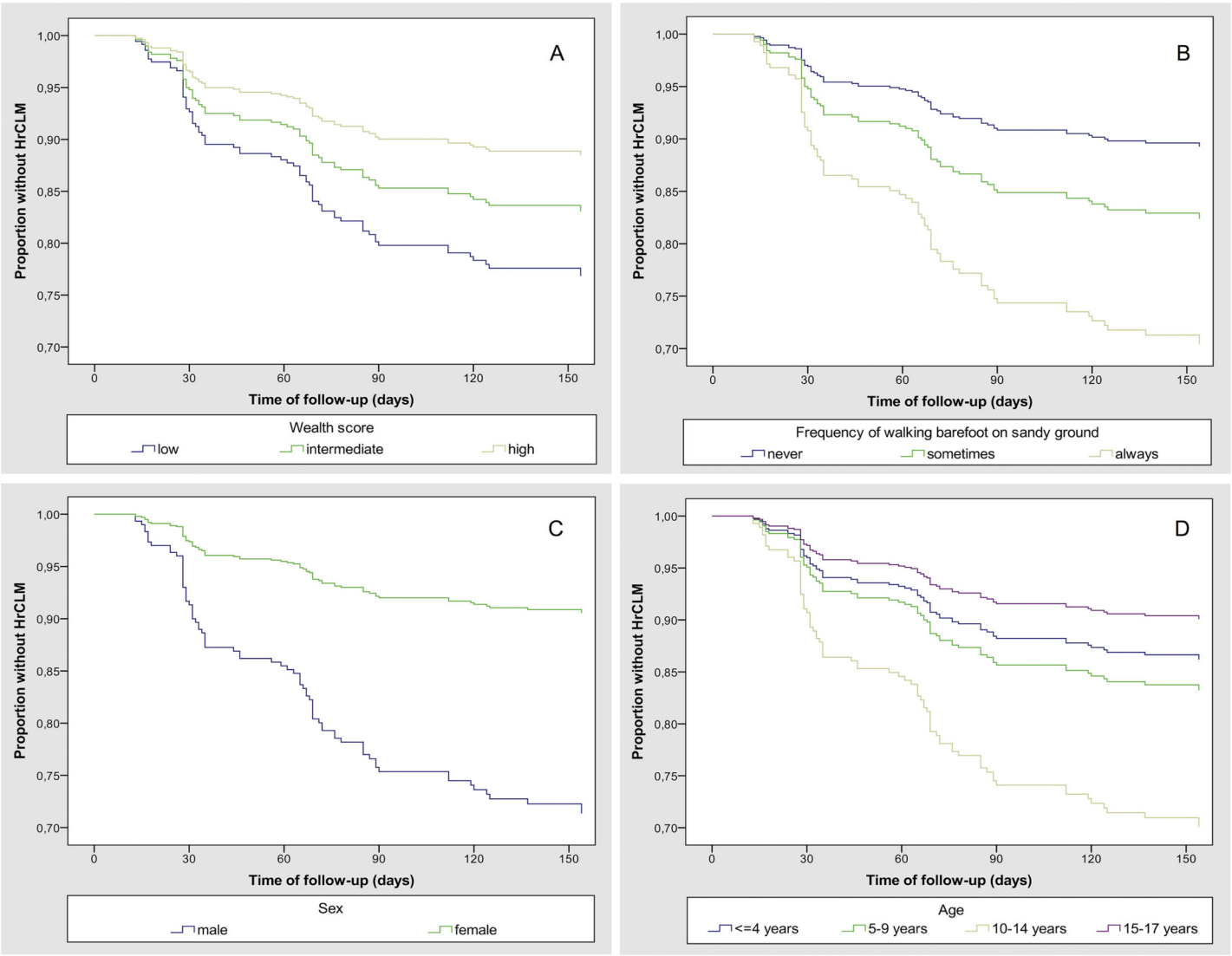

Fig 4. Stratified Kaplan-Meier curves for HrCLM-free proportion. (A) wealth (B) barefoot walking (C) sex (D) age.

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The overall incidence rate was 0.52 cases per person-year. That means that on the average every second child will get infected with HrCLM within one year, if the observed six months are representative for the whole year.

The reinfection rate was even higher (1.08 per person-year). HrCLM-infections are not distributed homogenously throughout the child population; but obviously, children with a certain high-risk profile get infected several times a year.

Considering the possible implications of such an infection including pruritus, sleep disturbance, impaired school performance and superinfection, this is a massive problem within the child population[5,21].

Risk factors

Most of the known risk factors identified in our previous cross-sectional study in Manaus and other studies in Northeast Brazil, could be confirmed by the present cohort study [2,4,10].

Male sex was the strongest predictor for HrCLM-infection with a more than three times higher risk in boys than in girls. Children between 10 and 14 years had a nearly two-fold higher risk to get infected than children of the youngest age group. Both might be due to gender-related behaviour or the extent of parental surveillance, as hypothesised earlier [4]. Generally, boys may spend more time outdoors, have more contact with the soil when playing and are less attended by their parents. These characteristics may be more present when children grow older, which might explain in part the age-related differences in hazard ratios.

Walking barefoot especially on sandy ground may also be a risk factor in travellers [6]. In endemic areas, the frequency of using protective footwear clearly influences the risk of infection [2]. The estimated PAF related to barefoot walking on sandy ground was 45%, meaning that nearly half of the infections with HrCLM could have been prevented if all children wore shoes [17]. Even plastic sandals, which are the typical footwear in this area, can prevent infection [2].

HrCLM is a poverty-related disease. Poverty-related living conditions with stray dogs and cats, unpaved streets and many unattended children playing in the streets create a beneficial environment for the transmission of hookworm larvae. More than half of the study households had only one minimum wage or less to their disposition [2]. But even within this poor community, poverty was an independent predictor for the acquisition of HrCLM. This corroborates the results of our cross-sectional study as well as observations from other neglected tropical diseases [2,22,23].

The presence of animal faeces on the compound was the only independent risk factor identified in the previous cross-sectional study that could not be confirmed by this study. This might be due to the different composition of the study population with only children, who might spent more time outside the compound while playing with other children.

Policy recommendations

Accordingly, the only deducible measure for disease control in the study area is prevention of barefoot walking. However, just providing shoes might not be sufficient similar to what we previously observed in a study on tungiasis control [24]. The underlying causes are much more complex. Even good parental knowledge about disease etiology couldn´t prevent HrCLM-infection [2,21]. Mothers feel unable to look out for their children [21]. Incidence was highest in boys aged 10–14 who probably are the less attended children in a poor household. Furthermore, the disease burden was highest among the poorest of the poor. In this way, HrCLM is a neglected disease acquired by the most neglected parts of the population. Only by changing social circumstances and health education of parents and children, preventive behaviour may be established.

Limitations

There may have been a selection bias in favour of younger children because of school attendance and work during visiting hours in the daytime. For security reasons visiting hours could not be extended beyond 6 p.m. Due to an exhaustive sampling strategy and high participation rate, we did obtain, however, a representative sample of the paediatric daytime population, which most likely is predominantly exposed in the study area.

Although children carry the biggest part of disease burden, the results cannot be translated to the whole population as no adults participated. [2,4,10].

We observed a differential loss of participants (Table 1). This might have led to an overestimation of events in the group exposed to "walking barefoot on sandy ground". Otherwise we have no reasons to believe that missing data biased the results.

The absence of treatment in the study area may have led to more HrCLM-infections in April and biased therefore the changes in disease intensity (multiple lesions per person) and severity (superinfection). However, the decrease in disease intensity continued over the whole study period and the correlation with monthly precipitation was significant.

Incidence rate was calculated with person-time and referred to a whole year even though the probability of disease is not constant during the study period. The observation period, however, included three months of the dry and three months of the raining season and may therefore be representative for the whole year.

Conclusion

In conclusion, the prevalence of HrCLM showed seasonal variation and was correlated with precipitation and disease intensity. Overall incidence rate among children was as high as 0.52 per person-year. Independent risk factors were male sex, age, walking barefoot on sandy ground and extreme poverty.

Supporting information

S1 Fig. Flowchart of participants in the cohort extended by treated and further observed cases.
(TIFF)

S1 Database. Study database.
(SAV)

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Author Contributions

Conceptualization: Felix Reichert, Daniel Pilger, Angela Schuster, Hannah Lesshafft, Hermann Feldmeier.

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Writing – review & editing: Daniel Pilger, Angela Schuster, Hannah Lesshafft, Ralf Ignatius, Hermann Feldmeier.

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RESEARCH ARTICLE

Prevalence and Risk Factors of Hookworm-Related Cutaneous Larva Migrans (HrCLM) in a Resource-Poor Community in Manaus, Brazil

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Abstract

Background

Hookworm-related cutaneous larva migrans (HrCLM) is a neglected tropical skin disease associated with significant clinical pathology. Little knowledge exists about prevalence and risk factors of HrCLM in endemic regions.

Methodology/ Principal Findings

To understand the epidemiology of HrCLM in Amazonia, we conducted a cross-sectional study in a resource-poor township in Manaus, Brazil. HrCLM was diagnosed in 8.2% (95% CI, 6.3–10.1%) of the study population (N = 806) with a peak prevalence of 18.2% (95% CI, 9.3–27.1%) in children aged 10–14. Most of the tracks (62.4%) were located on the feet, and 10.6% were superinfected. HrCLM was associated independently with age under 15, male sex, presence of animal faeces on the compound, walking barefoot on sandy ground and poverty.

Conclusions/ Significance

HrCLM is common in resource-poor communities in Amazonia and is related to poverty. To reduce the disease burden caused by HrCLM, living conditions have to be improved.

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: RI is employed by a commercial company, Labor Enders. Labor Enders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Summary

Hookworm-related cutaneous larva migrans (HrCLM) is a parasitic skin disease caused by the penetration of animal hookworm larvae into the human skin. In this compartment the larvae cannot pass the basal membrane and reproduce, but migrate in the outer skin layer for several weeks, causing skin inflammation and intense itching. Thus, humans are a biological impasse. Although HrCLM is a common skin disease in tropical and subtropical regions, studies on prevalence and risk factors are scarce. We clinically examined the population of a resource-poor neighbourhood in Manaus, capital of Amazonas State, Brazil, and investigated HrCLM-associated risk factors. HrCLM was very common with an overall prevalence of 8.2%. Children in general, and boys in particular, were most frequently infected. We could confirm that walking barefoot on sandy ground is a significant risk factor, and we identified the presence of animal faeces on the compound as another important predictive factor. Clearly, HrCLM was associated with low income and poverty-related living conditions. The poorest of the poor were identified as the most vulnerable population group.

Introduction

Hookworm-related cutaneous larva migrans (HrCLM) is a parasitic skin disease caused by the penetration of feline or canine hookworm larvae into the human epidermis. The most frequent species are *Ancylostoma braziliense*, *Ancylostoma caninum* and *Uncinaria stenocephal*a [1–3]. In humans, the larva is unable to cross the basal membrane of the epidermis and migrates in the compartment of the epidermis until it dies spontaneously after a few weeks to several months [1,4,5]. The migration of animal hookworm larvae causes a typical elevated erythematous linear or serpiginous track known as "creeping eruption" [6]. HrCLM is associated with intense pruritus and significantly impairs the quality of life [7]. The resulting scratching leads to denudation of the skin, which facilitates bacterial superinfection of the lesion [1,8,9]. Additional skin injury may be caused by inappropriate surgical manipulation of the lesion and treatment with toxic substances [10].

Whereas animal hookworm species parasitize dogs and cats worldwide [11], HrCLM is mainly seen in tropical and subtropical areas in South America, the Caribbean, Africa and South-East Asia [11–14]. Sporadic cases have been reported for Europe [15–20]. In semi-arid north-eastern Brazil, prevalence ranged from 0.2% to 4.4% in the general population and from 0% to 14.9% in children <5 years [21–23]. No population based data exists for other endemic areas.

Known risk factors are male sex, young age, living in a house without a solid floor and barefoot walking [8,23]. An association with low income has been suspected [23].

In order to investigate the epidemiology of HrCLM in Amazonia and to develop sustainable means of control, in a first step we determined prevalence and risk factors in a resource-poor community in the outskirts of Manaus. Data of a spatial analysis will be published separately.

Methods

Study area and population

The study was conducted in Manaus, capital of Amazonas State, North Brazil. Manaus is situated at 03°06' south latitude and has a hot humid climate. The average annual precipitation is

2307mm and the mean annual temperature is 26.7°C (International Institute of Meteorology of Brazil, http://www.inmet.gov.br/portal/index.php?r=clima/normaisclimatologicas).

The study area is part of Nova Vitoria, a resource-poor neighbourhood at the outskirts of Manaus. The boundaries of the study area are defined on three sides by an *igarapé*, a small affluent of the Amazon River. On the fourth side a paved road separates the study area from Grande Vitoria, another resource-poor community. The study area is characterized by unpaved roads, absence of public health facilities, kindergartens or public schools. There was no sewage disposal system at the time of the study. Electricity was available but only half of the households were legally connected to the grid; the other half used hand-made wire connections. Drinking water was distributed via rubber hoses, which often flooded the streets. Many cats and dogs strayed around in the streets and gardens. Children usually played on the compound of the house, in the streets or on improvised football fields. Hence, the study area was representative for the innumerable poor neighbourhoods at the periphery of Manaus.

Study design

As a first step into a comprehensive series of investigations on the epidemiology of HrCLM in Amazonia, we conducted a cross-sectional study in Nova Vitoria in April 2009, at the end of the rainy season. First, a census of all households and inhabitants was performed. During a door-to-door survey, households were GPS-mapped and environmental, socio-economic and behaviour-related risk factors were documented using a pre-tested, structured questionnaire. Inclusion criteria were residency in the study area for more than two months and provision of an informed, written consent.

All participants were examined clinically for HrCLM. The examination took place in the house where the family lived, in a room where privacy was guaranteed. The genital area was spared in case of absence of symptoms such as itching. HrCLM was diagnosed clinically by two investigators (DP and FR) when the characteristic slow-moving, elevated linear or serpiginous tracks were present [1,6,7,11–13,24]. Lesions were counted and the appearance and location of the tracks were documented. Each track was defined as a single lesion. Bacterial superinfection was diagnosed when pustules or suppuration were visible.

Ethical considerations

The study was approved by the Ethical Committee of the Fundação de Medicina Tropical-Amazonas (FMT-AM). Informed, written consent was obtained from each participant or in the case of minors from their legal guardian. Each affected inhabitant of Nova Vitoria was offered free treatment independently of the participation in the study. Treatment consisted of ivermectin (Ivermec, Uci-farma, São Paulo, Brazil) given as single oral dose (200 μg/kg) or—in the case of children <5 years or <15 kg and women with suspected or confirmed pregnancy—of topically applied thiabendazole (5%; Tiadol, Bunker Indústria Farmacêutica Ltda., São Paulo, Brazil) 3 times a day for one week.

Statistics

Data were entered in Microsoft Office Access 2007, cleaned for entering errors and analysed using PASW Statistics Version 18.0 (SPSS Inc., Chicago, USA). Missing data were assumed to be missing at random and flagged up in the analysis. Only complete cases were analysed.

An asset index was formed using principal component analysis (PCA) to categorize households according to socio-economic status. First, a set of assets that reflect wealth was identified. From this set of assets, we selected items with a high inequity in distribution among the

households and a high eigenvalue [25]. Included assets were presence of a car, television, fridge, type of house construction, legal connection to electricity and monthly mobile phone costs. Using these assets, an index ("wealth score") was built based on the respective value of each item in the PCA [25]. Households were ranked and divided into tertiles representing a high, intermediate or low socio-economic status. Income was categorized into three categories with the official minimum wage (R$ 465 per month in 2009) as a reference.

A knowledge score was derived out of six questions concerning the etiology of HrCLM. Every correct answer added one point to the score. The knowledge score values were categorized in tertiles representing households with little knowledge (0–3 correct answers), moderate knowledge (4 correct answers) and high knowledge (5–6 correct answers). Age groups were formed similar to previous population-based studies on HrCLM to allow comparison of the results [8,21,23].

For bivariable risk factor analysis, odds ratios (OR) were calculated together with 95% confidence intervals (95% CI). Statistical analysis consisted of χ^2-test or Fisher-exact-test to compare relative frequencies and logistic regression for non-binary variables.

For multivariable risk factor analysis, all variables that showed weak evidence of an association with HrCLM ($p<0.1$) were entered into a stepwise logistic regression. We observed standard errors and 95% CI to identify multicollinearity and removed variables where necessary. A random effects model was used to control for clustering on household level.

Results

According to the census 412 households existed in the study area, 127 of which were found without a resident present. Of the remaining 285 households, 5 (2%) did not match the inclusion criteria and 18 (6%) refused to participate. The remaining 262 households (92%) were inhabited by a total of 1104 people out of whom 806 (73%) were present during sampling and were included in the study.

Seventy-eight per cent of the adults were unemployed or working in the informal sector. Fifty-eight per cent of the households had one minimum wage (R$ 465 per month) or less at their disposition. The proportion of illiteracy in adults was at least 27%. Only 11.5% of the households had been visited by a community health worker within the last 12 months. Thirty-one per cent of the households stated that at least one case of HrCLM had occurred in household members within the last 12 months. (Table 1)

The median age was 13 years (range 0–72). The majority of the participants were females (59.3%). Sixty-six persons (8.2%; 95% CI, 6.3–10.1%) had HrCLM with a total of 117 lesions. Clinical characteristics of the infected study participants are presented in Table 2. Children aged 10–14 had the highest prevalence (18.2%; 95% CI, 9.3–27.1%; Fig 1). In all age groups of children, boys were significantly more affected than girls ($p<0.001$). The feet were the most common localisation of HrCLM.

Previous episodes of HrCLM were remembered of 18.7% of the participants. Following anamnestic information 39.7% had suffered of pediculosis capitis, 26.8% of tungiasis and 5.7% of scabies in the past year.

Bivariable risk factor analysis showed that male sex, age younger than 15, low family income, a low wealth score, playing football, practicing sport barefoot and presence of animal faeces on the compound were significantly associated with a high risk of HrCLM (Table 3). Those who reported to have had HrCLM in the last year had a significantly higher risk to be diagnosed with HrCLM in the cross-sectional study (OR = 15; 95% CI, 8.5–26.7). The highest risk was associated with the habit of always walking barefoot on sandy ground or soil (OR = 23.4; 95% CI, 8.0–68.6).

Table 1. Demographic, socio-economic and environmental characteristics of study households (N = 262).

Characteristic	n	%
Demography		
Persons per household: median (range)	4 (1–11)	
Children per household: median (range)	2 (0–8)	
Economy		
*Monthly per capita-income in R$: median (range)**	116 (0–1500)	
*Monthly income per household**		
< 1 minimum wage†	74	28.2
1 minimum wage†	78	29.8
> 1 minimum wage†	107	40.8
*Reported food shortage experienced in the last 12 months**		
Yes	82	31.3
No	179	68.3
Education		
Highest educational level in the household		
Secondary school or higher	85	32.4
Only primary school	122	46.6
No education at all	55	21.0
Number of households with $\geq$ 1 child aged 6–15 not going to school	27	22.0‡
Knowledge about HrCLM§¶*		
Little	84	32.1
Moderate	127	48.5
High	41	15.6
House construction		
House constructed of		
Plastered masonry	37	14.1
Non-plastered masonry	100	38.2
Wood and/or plastic foils	125	47.7
*Floor made of**		
Sand or soil	16	6.1
Wood	19	7.3
Concrete or tiles	226	86.3
*Compound not fenced in**	161	61.5
Compound fenced in with	93	35.5
Barbed wire	22	8.4
Paling	65	24.8
Bricks	6	2.3
Animals		
*Household kept cat or dog**		
Yes	148	56.5
No	112	42.7
*Stray cats or dogs on the compound**		
Yes	244	93.1
No	13	5.0
*Presence of animal faeces on the compound**		
Yes	31	11.8
No	230	87.8

*Missing observations.

†Minimum wage in 2009: 465 R$ $\approx$ 220$.

‡ Percent of households with children aged 6–15.

§For definitions see methods.

¶Hookworm-related cutaneous larva migrans.

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Table 2. Clinical characteristics of study participants with HrCLM (Hookworm-related cutaneous larva migrans) (N = 66).

Characteristic	n	%
*Persons with superinfected lesions**	7	10.6
Number of lesions per person:		
1	36	54.5
2	21	31.8
≥3	9	13.6
Topographic distribution of the lesions (n = 117) †, ‡		
Foot	72	62.4§
Leg	9	7.7§
Trunk	9	7.7§
Arm	9	7.7§
Buttock	8	6.8§
Hand	6	5.1§
Head	1	0.9§

*Pustules or suppuration.

†Missing observations.

‡Multiple topographic affection occurred in 21.2% of persons with HrCLM.

§Percentage of all lesions (n = 117).

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Multivariable risk factor analysis (Table 4) revealed that always walking barefoot on sandy ground or soil was the most important independent risk factor. Male sex, young age and presence of animal faeces on the compound remained independent risk factors for the presence of HrCLM. Obviously, HrCLM was significantly associated with poverty: A low wealth score of a household showed an adjusted odds ratio of 2.5 (95% CI, 1.1–5.8).

Discussion

HrCLM is a neglected tropical disease associated with significant clinical pathology [26]. From a global perspective it is one of the most common parasitic skin diseases—and not primarily a health problem in returning travellers as publications in journals of travel medicine may make believe [11–13,27–29]. Only few epidemiological studies have been performed in endemic areas and population-based data exists exclusively from north-eastern Brazil. To understand the epidemiology of HrCLM in the Amazonas region, we conducted a cross-sectional study in the outskirts of Manaus and reported findings on prevalence, risk factors and clinical pathology.

Clinical pathology

Clinical features were similar to those reported by others [12,13]. Most of the tracks (62.4%) were located on the feet, which reflects the fact that many people walked barefoot. This is consistent with our previous population-based study in rural Northeast Brazil [22]. The percentage of superinfected tracks was 10.6%. Previous studies in endemic areas by us and others reported similar proportions between 8 and 28% [8,21,22,30]. Unhygienic living conditions and practices as well as limited access to healthcare may explain the higher proportion of superinfected HrCLM in our study than usually seen in travellers [10,11].

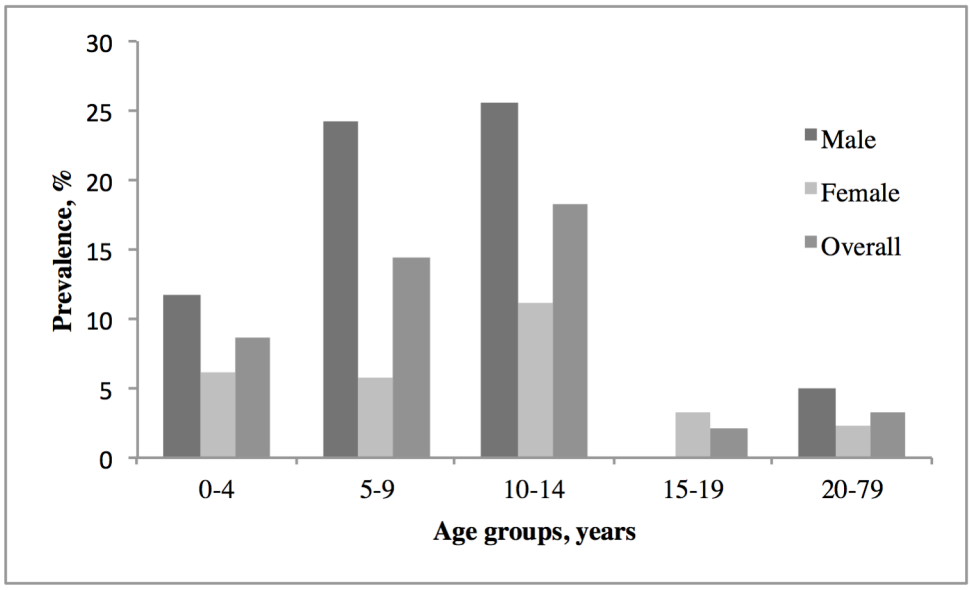

Fig 1. Prevalence of HrCLM (Hookworm-related cutaneous larva migrans) by age group and sex.

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Prevalence

The overall prevalence of 8.2% (95% CI 6.3–10.1%) found in this study is the highest ever documented in a population-based study. Previous population-based studies in Northeast Brazil showed an overall prevalence between 0.2% and 4.4% during the dry and the raining season, respectively [8,21,23]. Similar to previous studies, the prevalence differed by age group and sex with a peak prevalence of 25.6% in 10–14 year old boys (Fig 1) [8,23]. Whether there is a seasonal variation in HrCLM prevalence in the Amazonas region, where the climate is hot and humid throughout the whole year, remains to be clarified. Outside Brazil only one prevalence study has been conducted on devotees of a temple in Sri Lanka. Fifty-eight per cent of the devotees were found to have HrCLM; however, it is doubtful whether this finding reflects the true overall prevalence in that area since the participants were examined after a special religious ritual increasing the odds for exposure [30].

The extremely high prevalence found in our study indicates excellent conditions for the completion of the off-host cycle of animal hookworm in Nova Vitoria. First, many stray dogs and cats roam in the community and act as animal reservoirs. There is no public veterinary service at all and pets are not treated against intestinal helminths. Animal faeces were present on 11.8% of all compounds, and faecal material littered many public areas. Second, hookworm eggs require an environment that protects them from desiccation to evolve into infective third stage larvae [31]. Manaus is located in the middle of the Amazon basin. The precipitation in the month preceding the study was around 230 mm with 20 days of rain (International Institute of Meteorology of Brazil (INMET)). All streets and most of the compounds in Nova Vitoria were unpaved and became muddy after heavy rainfall. Furthermore, the average temperature never falls below 25°C. This means that the environmental conditions are exceptionally favourable for the propagation of animal hookworm larvae [5]. And third, risky behaviour with prolonged contact to contaminated soil was frequent. Many children did not go to school but roamed through the streets and compounds the whole day, the majority walking barefoot at least part of the time.

Table 3. Bivariable analysis (N = 806).

Characteristic	No.	HrCLM* (%)	Crude Odds Ratio (95% CI)	2-sided p-value
Demography				
Male	328	44 (13.4)	3.21 (1.89–5.47)	<0.001
Female	478	22 (4.6)	1 (reference)	
Age				
≤ 4 years	174	15 (8.6)	2.80 (1.26–6.25)	0.012
5–9 years	160	23 (14.4)	4.99 (2.37–10.52)	<0.001
10–14 years	88	16 (18.2)	6.61 (2.94–14.83)	<0.001
15–19 years	46	1 (2.2)	0.66 (0.08–5.24)	0.695
≥20 years	338	11 (3.3)	1 (reference)	
Socioeconomic characteristics				
Income				
< 1 minimum wage †, ‡	232	21 (9.1)	2.14 (1.05–4.38)	0.036
= 1 minimum wage‡	270	31 (11.5)	2.79 (1.43–5.46)	0.003
> 1 minimum wage‡	293	13 (4.4)	1 (reference)	
Wealth score †, §				
Low	321	37 (11.5)	3.16 (1.44–6.93)	0.004
Intermediate	263	21 (8.0)	2.10 (0.91–4.86)	0.081
High	202	8 (4.0)	1 (reference)	
Education				
Knowledge about HrCLM †, §				
Little	259	22 (8.5)	0.69 (0.35–1.36)	0.286
Moderate	384	25 (6.5)	0.52 (0.27–1.00)	0.051
High	135	16 (11.9)	1 (reference)	
Behaviour				
Walking always/regularly †				
Barefoot outdoor	58	14 (24.1)	4.16 (2.14–8.07)	<0.001
With sandals/shoes outdoor	731	52 (7.1)	1 (reference)	
Walking on sandy ground †				
Always barefoot	111	29 (26.1)	23.43 (8.00–68.60)	<0.001
Sometimes barefoot	420	33 (7.9)	5.65 (1.98–16.13)	0.001
Never barefoot	269	4 (1.5)	1 (reference)	
Walking indoor				
Walking barefoot and absence of solid floor †	103	15 (14.6)	2.18 (1.17–4.03)	0.019
Not walking barefoot or presence of solid floor	702	51 (7.3)	1 (reference)	
Sports				
Practicing football †	212	33 (15.6)	3.38 (1.99–5.76)	<0.001
Other sport	49	5 (10.2)	2.09 (0.77–5.67)	0.15
No sport	542	28 (5.2)	1 (reference)	
Sport barefoot on sand †	193	36 (18.7)	4.74 (1.41–15.95)	0.005
Sport never barefoot/not on sand	65	3 (4.6)	1 (reference)	
Environment				
Animal faeces on compound †	103	17 (16.5)	2.63 (1.45–4.78)	0.001
No faeces on compound	702	49 (7.0)	1 (reference)	
Cat/dog ownership †	469	36 (7.7)	0.99 (0.59–1.69)	0.983
No cat/dog ownership	324	25 (7.7)	1 (reference)	
Stray cats/dogs on compound †	753	65 (8.6)	3.78 (0.51–27.94)	0.243

(*Continued*)

Table 3. (*Continued*)

Characteristic	No.	HrCLM* (%)	Crude Odds Ratio (95% CI)	2-sided p-value
No stray cats/dogs	41	*1 (2.4)*	1 (reference)	

*Hookworm-related cutaneous larva migrans.

†Missing observations.

‡Minimum wage in 2009: 465 R$ ≈ 220$.

§For definitions see Methods.

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Risk factors

The multivariable model showed a complex pattern of risk factors with walking barefoot on sandy soil being most significant. This corroborates our previous findings from a semi-arid area of Brazil, where the lacking use of footwear was an independent risk factor [23]. For the first time we could show that the odds differed by the frequency protective footwear was used. Participants who always used shoes ran a lower risk of acquiring HrCLM than those wearing shoes sometimes (Table 4). Even the commonly used flip-flops (plastic sandals, which consist of a thin rubber sole with a single string) provided significant protection. However, closed shoes were worn regularly only by seven individuals.

Obviously, HrCLM was predominantly acquired outdoors. Neither walking barefoot indoors, even if the floor consisted of sand or soil, nor owning a cat or dog were identified as independent risk factors. Assumedly, animal hookworm larvae were unable to complete the life cycle indoors because the floors were usually dry and accidentally dropped animal excrements were rapidly removed.

Table 4. Multivariable regression analysis.

Characteristic	Frequency (N = 779)	Adjusted odds ratio (95% CI)	2-sided p-value
Sex			
Male	319	2.30 (1.30–4.08)	0.004
Female	460	1 (reference)	
Age			
≤ 4 years	163	2.55 (1.11–5.90)	0.028
5–9 years	155	2.80 (1.26–6.23)	0.012
10–14 years	87	2.98 (1.23–7.21)	0.015
15–19 years	44	0.37 (0.05–3.03)	0.354
≥20 years	330	1 (reference)	
Wealth score			
Low	318	2.53 (1.10–5.82)	0.028
Intermediate	260	1.76 (0.73–4.22)	0.209
High	201	1 (reference)	
Faeces found on compound			
Yes	92	2.66 (1.34–5.29)	0.005
No	687	1 (reference)	
Walking on sandy ground			
Always barefoot	107	14.39 (4.62–44.85)	<0.001
Sometimes barefoot	406	4.76 (1.63–13.90)	0.004
Never barefoot	266	1 (reference)	

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It remains uncertain whether the infections predominantly took place peridomestically or in public areas, such as parks, as suspected in some outbreak investigations [32–34]. Our findings that the presence of faeces on the compound was an independent risk factor and that playing football on improvised playgrounds was not an independent risk factor indicate that peridomestic transmission is important.

This study shows for the first time that low income and poverty-related living conditions are crucial risk factors for HrCLM. Hitherto, a low family income has been identified as a risk factor but didn´t reach statistical significance in the mulitvariate analysis. The concept of an asset index as a long-term indicator of the socio-economic status of the household has never been applied in earlier studies [8,23].

Even within a poor population, as in the community of Nova Vitoria, the relative level of poverty predicted the risk of acquiring HrCLM. A household income of one minimum wage or less was associated with a high risk of acquiring HrCLM. Also, a low wealth score was an independent risk factor. Hence, the poorest of the poor are the most vulnerable part of the population, which corroborates our hypothesis that occurrence of HrCLM is a proxy of the economic situation in a country [35]. Many neglected tropical diseases are considered to be associated with poverty [36,37] but HrCLM is particular in the sense that it affects the poorest of the poor.

Policy recommendations

In contrast to other soil-transmitted helminths, HrCLM has a pure animal reservoir and thus treating the human population cannot influence the incidence of HrCLM. Veterinary anthelmintic therapy can be effective [38] but is hard to realise in areas lacking basic infrastructure even for human health. Therefore, disease control strategies have to point towards improvement of living conditions, environmental factors and protective behaviour. Preventing access of cats and dogs to playgrounds and informing the public about pet-associated health risks and protective shoewear will be essential to reduce the parasite burden in humans as long as infrastructure remains precarious [32–34,39,40].

Limitations

For safety reasons Nova Vitoria could only be visited during daylight hours. Thus, there may have been a selection bias in favour of women and children staying at home versus adult males being at work. By means of an exhaustive sampling strategy, we still obtained a high participation and a representative sample of the daytime population. We have no reasons to believe that study participants with missing data differed from those without missing data and hence any missing observation reduced statistical power but is unlikely to have biased the results [41].

Confusion of HrCLM with other conditions that present as a creeping skin eruption such as gnathostoma, *Strongyloides stercoralis* (larva currens), fly maggots (migratory myiasis) and scabies is theoretically possible [1,6,24]. However, a slightly elevated linear or serpiginous track and the slow velocity of progression with several millimetres to few centimetres per day are pathognonomic [6,42]. We therefore assume that all participants were correctly diagnosed.

Conclusion

The study revealed the highest prevalence of HrCLM in a representative population sample known to date and showed transmission in peridomestic areas. We could prove that HrCLM is a disease of the poorest of the poor. It is therefore plausible that for elimination of HrCLM as a public health threat, it is necessary to improve the living conditions.

Supporting Information

S1 Checklist. STROBE checklist.
(DOCX)

S1 Database. Study database.
(SAV)

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Author Contributions

Conceived and designed the experiments: HF DP FR AS HL. Performed the experiments: FR DP. Analyzed the data: FR DP. Contributed reagents/materials/analysis tools: SGdO RI AS HL. Wrote the paper: FR. Provided infrastructural support: SGdO.

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Publikation 3: Hookworm-related cutaneous larva migrans in northern Brazil: resolution of clinical pathology after a single dose of ivermectin.

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Lebenslauf

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

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