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Seroepidemiologie von vernachlässigten viralen Erkrankungen in der Mbeya Region, Tansania

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Nina Weller
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Affidavit

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Publikationsliste

I. **Seroprevalence of alphavirus antibodies in a cross-sectional study in southwestern Tanzania suggests endemic circulation of chikungunya.**

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II. **High seroprevalence of Rift Valley Fever and evidence for endemic circulation in Mbeya region, Tanzania, in a cross-sectional study.**

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1 Beitrag zu den Publikationen

1.1 Beitrag zu Publikation I

Als Co-Autorin, der genannten Publikation wurde von mir zunächst eine weitreichende Literaturrecherche durchgeführt und ca. 2/3 der Einleitung verfasst.

Die aus Tansania bereits vorliegenden Serumproben der ausgewählten Studienteilnehmer wurden von mir wie im Material und Methoden Teil beschrieben prozessiert und analysiert. Der Abgleich durch einen zweiten Beobachter wurde im Wesentlichen durch Gerhard Dobler durchgeführt. Mein Anteil der Labordiagnostik beträgt 80%.

Die Datenanalyse erfolgte hauptsächlich durch den Bioepidemiologen Elmar Saathoff. Die Ergebnisse aus der Datenanalyse wurden von mir mit aufbereitet und beschrieben. Mein schriftlicher Anteil am Ergebnisteil der Publikation beträgt ca. 50%.

In einem Forschungsaufenthalt in Mbeya (Feb-April 2010) wurden von mir relevante Studienorte besichtigt und relevante Parameter wie Wasser, Höhe, Nutztiere usw. nochmals gezielt beobachtet. Mit vor Ort arbeitenden *medical officers* wurde über Auftreten/Diagnostik von Arbovirosen gesprochen.

Für Diskussion wurde wiederum eine ausgedehnte Literaturrecherche von mir durchgeführt. Die Interpretation der Ergebnisse erfolgt anhand dieser, sowie der gesammelten Erfahrungen aus dem Forschungsaufenthalt. Mein schriftlicher Anteil an der Diskussion beträgt 50%.

1.2 Beitrag zu Publikation II

Als Co-Autorin, der genannten Publikation wurde von mir zunächst eine weitreichende Literaturrecherche durchgeführt und ca. die Hälfte der Einleitung verfasst.

Die aus Tansania bereits vorliegenden Serumproben der ausgewählten Studienteilnehmer wurden von mir wie im Material und Methoden Teil beschrieben prozessiert und analysiert. Der Abgleich durch einen zweiten Beobachter wurde im Wesentlichen durch Gerhard Dobler durchgeführt. Mein Anteil der Labordiagnostik beträgt 80%.

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2 Einleitung

2.1 Die vernachlässigten Tropenerkrankungen

Die „vernachlässigten Tropenerkrankungen" (engl. Neglected tropical diseases NTDs) sind eine heterogene Gruppe von Infektionserkrankungen, die durch verschiedene Würmer, Parasiten, Bakterien und Viren hervorgerufen werden. Von der WHO wurden im Jahre 2020 20 Erkrankungen bzw. Gruppen von Pathogenen als wichtigste NTDs gelistet. Zu ihnen gehören: Buruli Ulcus (Mycobacterium ulcerans), Chagas Erkrankung (Trypanosoma cruzi), Denguefieber (Dengue Virus Serotyp 1-4) und Chikungunyafieber (Chikungunya Virus), Dracunculiasis „Guinea worm disease" (Dracunculus medinensis), Echinococcose (Echinococcus sp.), nahrungsbedingte („foodborne") Trematodeninfektionen (z.B. Schistosoma sp.), Afrikanische Schlafkrankheit (Trypanosoma brucei ssp.), Leishmaniose (Leishmania sp.), Lepra (Mycobacterium leprae), Lymphatische Filariasis (Wucheria sp., Brugia sp.), Mycetom „Madura-Fuß" (div. Pilzgattungen z.B. Madurella sp., Allescheria sp., Candida sp.), Chromoblastomykose (z.B. Cladosporium sp., Exophiala sp., Phialophora sp.) und andere Mykosen, Onchozerkose „Flußblindheit" (Onchocerca volvulus), Tollwut (Rabies Virus), Skabies (Sarcoptes scabiei) und andere Ektoparasiten, Boden assoziierte („soil transmitted") Helminthenerkrankungen (Enterobius vermicularis, Trichuris trichuria, Ancylostoma duodenale, Strongyloides stercoralis), Schlangenbisse/Vergiftungen, Zystizerkose (z.B. Taenia solium), Trachom (Chlamydia trachomatis Serotyp A-C), Frambösie (Treponema pallidum pertenue) und andere endemische Treponematosen.

(http://www.who.int/neglected_diseases/diseases/en/).

Weltweit sind von diesen Erkrankungen über eine Milliarde Menschen betroffen, nahezu alle gehören zu den 2,7 Milliarden Menschen, die in starker Armut leben, d.h. denen ein Tageseinkommen von weniger als zwei US-Dollar zur Verfügung steht (Savioli et al. 2010). Überproportional häufig sind Frauen und Kinder betroffen.

Der Ausdruck „vernachlässigt" wurde gewählt, um im Wesentlichen den Unterschied im öffentlichen Interesse (v.a. der Industrienationen) zu den „großen Drei" (Infektionskrankheiten) Aids, Tuberkulose und Malaria auszudrücken.

Dabei sind es gerade die vernachlässigten Erkrankungen, die der Verbesserung der Lebensbedingungen von weltweit verarmten, vorwiegend ländlichen Bevölkerungen durch ihre Krankheitslast im Wege stehen. Durch Helminthen verursachte Anämien führen bei Kindern zu Defiziten in der Entwicklung, bei Frauen entstehen Schwangerschafts- und Geburtskomplikationen. Durch Bakterien und Protozoen verursachte „Verunstaltungen" führen zu sozialer Stigmatisierung und Isolation. Virale Erreger verursachen oft chronische Arthritiden, die gerade Landarbeiter existentiell bedrohen und die Agrarproduktivität mindern. Dazu kommt der Verlust von Nutzvieh durch zoonotische virale und bakterielle Infektionen. Infektionen mit dem bakteriellen Erreger Chlamydia trachomatis führen zur Erblindung und konsekutiv zum Verlust der Selbständigkeit und der Produktivität. In den lateinamerikanischen Hochendemiegebieten von Chagas erkranken durch eine Autoimmunreaktion Jahre nach Infektion mit dem Parasit Trypanosoma cruzi vermehrt junge Erwachsene an schweren Herzinsuffizienzen und stehen der Gesellschaft als Leistungträger nicht mehr zur Verfügung (Hotez et al. 2010, Hotez und Kamath 2009, Mathers et al. 2007).

Klinisch verlaufen die NTDs in der Regel milder. Direkte Todesfälle sind fast ausschließlich bei den viralen hämorrhagischen Fiebern zu beobachten.

Um die Last der NTDs anzugeben, wird - ähnlich wie bei anderen chronischen Erkrankungen z.B. aus dem rheumatologischen Formenkreis- häufig der Begriff DALY disability adjusted life years verwendet.

Dabei berechnet sich DALY über die Formel DALY= years of life lost (YLL) + years of life lived with disability (YLD) (Mitra and Mawson, 2017). Hotez und Kamath schätzten in ihrer Übersichtsarbeit von 2009 die weltweiten DALYs der NTDs auf ca. 56 Millionen.

2.2 Globale Verbreitung der vernachlässigten Tropenerkrankungen

In einem Leitartikel der Fachzeitschrift PLOS „Neglected Tropical Diseases" benennt Peter Hotez (May 2014), die seiner Meinung nach zehn globalen hotspots für die vernachlässigten Tropenerkrankungen (siehe Abbildung 1). In Lateinamerika zählen dazu Brasilien und die Amazonasregion, die Region Gran Chaco (ein Gebiet mit ca. zehn Millionen Einwohnern auf dem Territorium von Boliven, Paraguay, Nordargentinien und zwei brasilianischen Bundesstaaten) und die Region Mesoamerica und Texas (südliche Staaten von Mexiko, Zentralamerika und Texas). In Asien ist die Zahl der NTDs weltweit am höchsten. Die hotspots sind dort Indonesien und Papua-Neuguinea, Indien und die südasiatischen Länder sowie China. Ein weiterer hotspot ist der Nahe Osten mit Nordafrika (v.a. Ägypten, Jemen, Iran, Algerien).

Im Afrika südlich der Sahara (SSA engl. Sub Saharan Africa) werden weitere drei hotspots beschrieben. Dazu gehört Nigeria, die Demokratische Republik Kongo mit den angrenzenden Gebieten Südsudan, Zentralafrikanische Republik, Norduganda und Angola sowie ein Gebiet bestehend aus Chad, Niger, Mali und angrenzenden Teilen der Sahelzone.

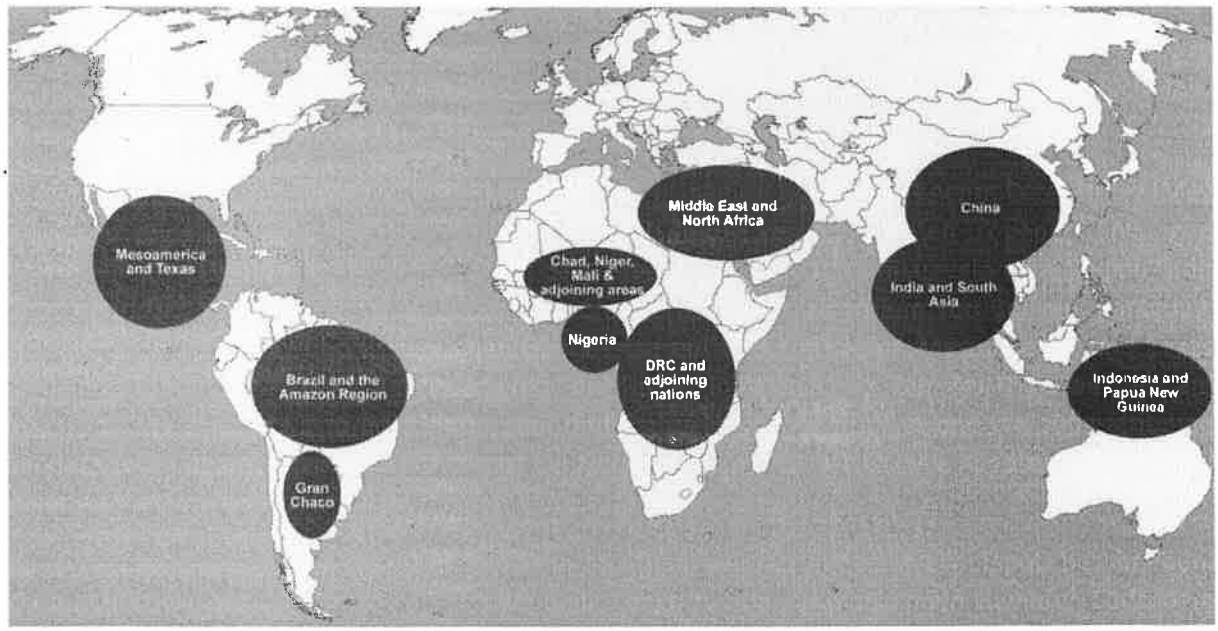

Abb. 1. aus Hotez PJ (2014): Die (vermuteten) zehn, am stärksten durch vernachlässigte (Tropen) Erkrankungen betroffenen Regionen der Erde.

2.3 Die vernachlässigten Tropenerkrankungen in Afrika südlich der Sahara

Die Regionen Afrikas südlich der Sahara beherbergen heute die weltweit ärmsten Bevölkerungen. Nach Analysen der Weltbank leben dort etwa 51% der Menschen von weniger als 1,25 US-Dollar täglich, 73% der Menschen von weniger als zwei US-Dollar. In der Literatur gibt es wenig Übersichtsarbeiten zu tatsächlichen Zahlen. 2009 veröffentlichten Hotez und Kamath einen Review zu Zahlen und Krankheitslast von NTDs in SSA. Die hier genannten Zahlen beziehen sich im Wesentlichen auf diesen Artikel. Aktuell geht die WHO von einer Krankheitslast aller NTDs in SSA zusammen von jährlich 19 Millionen DALYs aus.

Etwa 500 Millionen Menschen sind in SSA von NTDs betroffen. 85% davon entfallen auf Helminthen-Infektionen, wobei die häufigsten durch den Hakenwurm Necator americanus bzw. Ancylostoma duodenale (198 Millionen Infizierte) und den Pärchenegel Schistosoma mansoni und Schistosoma haematobium (192 Millionen Infizierte) verursacht werden. Man geht davon aus, dass die Hälfte der ärmsten 500 Millionen Menschen in SSA mit dem Hakenwurm infiziert sind, darunter ca. 40-50 Millionen Kinder im Schulalter sowie sieben Millionen schwangere Frauen. Hier ist durch das Auftreten von Anämien besonders mit komplizierten Verläufen und Entwicklungsstörungen zu rechnen.

Bei den Protozoenerkrankungen Leishmaniose und Schlafkrankheit geht man von zusammen ca. 100. 000 Fällen aus. Bei der Augenerkrankung Trachom, verursacht durch das Bakterium Chlamydia trachomatis, von ca. 30 Millionen Fällen. Schätzungen existieren ebenfalls für die Viruserkrankung Gelbfieber mit ca. 180.000 Fällen. Realistische Zahlen gibt es außerdem für Filarienerkrankungen oder Onchozerkose.

Von vielen NTDs existieren nicht einmal Schätzungen über Prävalenz und ihre regionale Verbreitung, so dass die Krankheitslast der NTDs in SSA möglicherweise noch deutlich unterschätzt wird. Grob geschätzt kann man davon ausgehen, dass alle NTDs zusammen ungefähr die Hälfte der Krankheitslast von Malaria ausmachen und über das Doppelte der Krankheitslast von Tuberkulose.

Da zudem viele Regionen durch anhaltende Krisen und mangelnde Infrastruktur nur schwer zugänglich sind, ist die Gewinnung von (Langzeit-)Daten schwierig. Eine Orientierung können Daten aus benachbarten, stabileren Ländern bieten (Hotez und Kamath 2009, LaBeaud 2008).

2.4 Arbovirosen- Vernachlässigte virale Erkrankungen

Bei dieser Gruppe der NTDs handelt es sich um Viren, die von Arthropoden (Gliederfüßern) übertragen werden (aus dem Englischen arthropod-borne). Die Eingruppierung erfolgt nach dem biologisch-ökologischen Infektionsmechanismus und richtet sich nicht nach taxonomisch-phylogenetischen Kriterien. Es sind derzeit etwa 400 Arboviren bekannt, viele davon sind verantwortlich für Tierseuchen. Zwischen 80 und 100 Spezies werden als humanpathogen angenommen. Die meisten Arboviren kommen in tropischen und subtropischen Gebieten der Erde vor, und haben ihren Ursprung vor allem auf dem afrikanischen Kontinent. Eine Ausnahme ist z.B. das FSME-Virus, das in weiten Teilen Mitteleuropas vorkommt und vom gemeinen Holzbock (Ixodes ricinus) über Infektionen durch Arboviren verlaufen klinisch oft inapparent und sind meist selbstlimitierend. In der Regel überwiegen Allgemeinsymptome wie Abgeschlagenheit, Fieber, Kopf- und Gliederschmerzen. Diese Symptome sind häufig begleitet von einem makulopapulösen Exanthem. Bei schwer verlaufenden Infektionen können Nephritiden, Hepatitiden, Retinitiden sowie Enzephalitiden und Meningitiden beobachtet werden. Zu hämorrhagischen Manifestationen kommt es lediglich beim Krim-Kongo-, dem Gelb- (ca. 20%), und dem Dengue-Fieber (ca. 5%) (Preiser 2010, Blümel et al. 2004, Weaver und Reisen 2010). Auch für das Rifttal-Fieber sind hämorrhagische Verläufe in ca. 1-2% beim Menschen beschrieben (Wright et al. 2019).

Arbovirosen sind in den Industriestaaten selten und werden in der Regel von Reiserückkehrern, Zugvögeln oder durch Handelsverkehr/güter verbreitet (Hubalek 2004, Reiter und Sprenger 1987). Eine Ausnahme stellt beispielsweise das West Nil Virus dar, das in Afrika endemisch ist, von Epidemien wird aber hauptsächlich aus den USA, Europa und dem Nahen Osten berichtet (Hayes et al., 2005, Bernkopf et al. 1953, Zeller und Schuffenecker 2004). In den tropischen und subtropischen Regionen der Erde sind Arboviren endemisch, wobei oben beschriebene Umstände vor allem in Afrika südlich der Sahara genauere Aussagen zu Inzidenz, Prävalenz und Krankheitslast in der lokalen Bevölkerung verhindern (Hotez und Kamath 2009). Zu Epidemien kommt es meist, wenn Klimaphänomene wie beispielsweise „El Nino", Fluten oder Dürren das biologische Gleichgewicht der

Überträgerpopulationen (vor allem Moskitos und Zecken) stören (Anyamba et al. 2001, Chretien et al. 2007, Logan et al. 1991). Am meisten geschädigt werden dadurch wiederum die ärmsten Bevölkerungsteile (Viehhirten, Feldarbeiter, Bewohner von Elendsvierteln), da die Krankheitssymptomatik oft zu längeren Arbeits- bzw. Schulunfähigkeiten oder dem Verlust von Nutzvieh führt, so dass ein Teufelskreis entsteht und sich die sozioökonomischen Verhältnisse der Betroffenen zusätzlich verschlechtern.

2010 wurde das Denguefieber als erste Arbovirose von der WHO auf die Liste der wichtigsten NTDs gesetzt (s.o.). 2020 kam das Chikungunyafieber hinzu. Weitere Erkrankungen, die in diesem Zusammenhang als bedeutend angesehen werden, sind das Gelbfieber, das West Nil Fieber und das Rifttalfieber. Auf den ersten Blick gibt es über die ursächlichen Viren bereits eine Fülle an Daten aus wissenschaftlichen Publikationen. Molekularbiologie, Genetik und Phylogenie sind gut erforscht. Auch sind einige pathophysiologische und immunologische Mechanismen der Infektion in Zellkultur und am Tiermodell aufgeklärt. Die meisten Publikationen stehen dabei im Zusammenhang mit größeren Epidemien und Pandemien.

Im Gegensatz dazu ist die Datenlage über die tatsächlichen Prävalenzen und die Auswirkungen auf die öffentliche Gesundheit und die Volkswirtschaften in den Endemiegebieten jedoch rar (Hotez et al. 2009, Hotez et al. 2014)

2.5 Das Rifttal Virus

Der erste Rifttalfieberausbruch wurde 1930 beschrieben (Pepin et al. 2010). Am Naivasha- See in Kenia im östlichen Teil des Rift Tals (Ostafrikanischer Grabenbruch) war es zu einer Tierseuche gekommen, die vor allem Schafe, aber auch Rinder befiel. Seit dieser Zeit wurde in regelmäßigen Abständen über Ausbrüche bei Wildtieren (v.a. Kaffern-Büffel) und Nutztieren (Rinder, Schafe, Ziegen, Kamele) berichtet, die zunächst ausschließlich südlich der Sahara im Osten des afrikanischen Kontinents auftraten und eng mit starken Niederschlägen vergesellschaftet waren. Bei diesen epizootischen Ausbrüchen erkranken vor allem Schafe und Rinder schwer. Es kommt zu einem sogenannten *abortion storm*, einer massiven Welle von Fehlgeburten, die sich deutlich unterscheidet von vereinzelten Fehlgeburten durch Infektionen mit *Coxiella burnetti*, *Listeria sp.*, *Chlamydia sp.* oder *Salmonella* sp. (Pepin et al. 2010).

Das infektiöse Agens, das diese Tierseuche (Epizootie) auslöst, ist ein Phlebovirus aus der Familie Bunyavirirdae. Das Rifttalfieber Virus (engl. Rift Valley Fever Virus, RVFV) ist ein umhülltes RNA (-)-Virus dessen Genom aus den drei Segmenten L, M und S besteht, die für verschiedene strukturelle und nicht-strukturelle Proteine codieren (Bird et al. 2009).

Linthicum et al. (1999) stellten beim Vergleich von klimatischen Daten, während Rifttalfieberausbrüchen (Epizootien) in den Jahren 1950-1998 fest, dass die Ausbrüche regelmäßig nach Perioden mit starken Niederschlägen auftraten, denen außerordentliche Trockenperioden vorausgegangen waren. Das Auftreten des Klimaphänomens ENSO (El Nino Southern Oscillation) an der ostafrikanischen Küste, führt zu unter anderem zu diesen regelmäßig wiederkehrenden heftigen Niederschlägen im Rifttal. Außerdem waren zu den Ausbruchzeiten die Wassertemperaturen im Pazifik und im Indischen Ozean sowie die Vegetation an der ostafrikanischen Küste stets ähnlich.

In den Jahren ohne die oben beschriebenen klimatischen Geschehen bleibt das Rifttal Virus enzootisch, d.h. es zirkuliert zwischen seinen etwa 30 Vektorspezies und Wild/Nutztieren in einem stabilen Gefüge, aufgrund der Herdenimmunität (Weaver and Reisen, 2010).

Eine Infektion von Menschen wird hauptsächlich während epizootischen Episoden beobachtet. Wie bei vielen Arbovirosen ist die Infektion häufig asymptomatisch, die Letalität beträgt zwischen 0,5 und 2%. Häufig sind Fieber und Kopfschmerzen, bei schwereren Verläufen kann es außerdem zu Hepatitis, Retinitis mit bleibender Sehbehinderung, Enzephalitis oder hämorrhagischem Fieber kommen (Pepin et al. 2010, Wright et al. 2019).

Neuere Daten aus Tansania legen nahe, dass es dort auch ein endemisches Zirkulieren des Erregers innerhalb der Allgemeinbevölkerung gibt (Budodo et al. 2020).

2.6 Die Gattung Alphavirus und ihr bekanntester Vertreter- das Chikungunya Virus (CHIKV)

Die Gattung Alphavirus aus der Familie der Togaviridae besteht aus etwa 40 verschiedenen Virustypen und Subtypen (Pialoux et al. 2007). Unter ihnen sind verschiedene tierpathogene Spezies wie beispielsweise das Venezuelanische Pferde Enzephalitis Virus (VEEV), aber auch humanpathogene Arten, wie z.B. das im tropischen Australien vorkommende Ross River Virus oder die auf dem Afrikanischen Kontinent beheimateten Vertreter O´nyong-nyong Virus und Chikungunya Virus.

Letzteres ist derzeit wohl aufgrund seiner weltweiten, epidemischen Ausbreitung der bedeutendste Vertreter. Es wurde 1952/53 in Tansania erstmalig isoliert und beschrieben. Der Name stammt von dem Makonde Wort *kungunyala*, was so viel bedeutet, wie „der gekrümmt Laufende" und bezieht sich auf die typischen starken Arthralgien, die im Rahmen der Infektion auftreten und oft chronifizieren. Korrelat dafür sind Virus-infizierte Monozyten, die in der Synovia verschiedener meist peripherer Gelenke überdauern und über IFN-Alpha und andere Chemokine eine starke Immunantwort und Entzündungsreaktion hervorrufen (Her et al. 2010).

Wie bei den meisten Arboviren handelt es sich um ein Einzelstrang (+) RNA-Virus mit einem linearen Genom von 11,8 kb Länge (Powers and Logue, 2007). Seit der Erstbeschreibung des Virus sind immer wieder Epidemien auf dem afrikanischen und asiatischen Kontinent beobachtet worden (Silva et al. 2018). Man geht heute davon aus, dass die Zahl der Epidemien und Erkrankten allerdings weit unterschätzt ist, da das Chikungunyafieber klinisch stark dem Denguefieber ähnelt, in den gleichen Regionen der Welt endemisch ist und außerdem die gleichen Vektoren (*Aedes aegypti* und *Aedes albopictus*) beteiligt sind (Carey 1971).

Es haben sich heute zwei Infektionszyklen - ein sylvatischer und ein urbaner- etabliert. Bei dem in West- und Zentralafrika vorherrschenden sylvatischen Zyklus sind Affen die Reservoirwirte und Moskitos der *Aedes furcifer-taylori* Gruppe die Vektoren. Es gibt ein sich alle drei bis vier Jahre wiederholendes zyklisches Muster von kleineren Ausbrüchen, das mit der Herdenimmunität der Reservoirtiere korreliert und vor allem nach starken Regenfällen mit steigender Vektorendichte einsetzt (Diallo et al. 1999). In Ostafrika und Asien gibt es zudem noch einen urbanen Zyklus mit den Hauptvektoren *Aedes aegypti* und *Aedes albopictus*. Das Virus bewegt sich vor allem zwischen Menschen und Moskitos. Die Rolle von Tieren ist hier vernachlässigbar. 2004 begann in Kenia eine große Epidemie, die sich rasch über den Indischen Ozean nach Indien und Südostasien ausdehnte. Zu dieser Zeit war das Klima an der kenianischen Küste besonders heiß und trocken (Chretien et al. 2007) und es wurde vermutet, dass das unregelmäßige Auffüllen von Wassertanks im Zusammenhang mit dem Infektionsrisiko stand. Andere Parameter, die mit der Verbreitung im Indischen Ozean assoziiert wurden, waren niedrige und moderate Höhen sowie ebenes Gelände mit darauf stehenden Oberflächenwassern (Bonn, 2006). Seit Oktober 2013 breitet sich das Chikungunya Virus außerdem epidemisch über die Karibik auf den amerikanischen Kontinent aus (Johannson et al. 2014, Mowatt und Jackson 2014).

2.7 Der Südwesten Tansanias und die EMINI Kohorte

Die EMINI Studie (2006-2011) hatte zum Ziel, in der Mbeya Region im Südwesten Tansanias eine Infrastruktur zu schaffen, um den Effekt von neuen Gesundheitsinterventionen zu beobachten und zu evaluieren (EMINI *engl. Evaluation and Monitoring the Impact of New Interventions*). Im Vorfeld der Studie wurden hierfür neun geographisch unterschiedliche Gegenden der Region ausgewählt und ein Zensus der dort ansässigen Gesamtbevölkerung durchgeführt. Dabei wurden Basisinformationen über die Haushalte wie z.B. Zahl der Bewohner, Alter, Geschlecht erhoben und die GPS-Position aufgezeichnet. 10% der Haushalte mit all ihren Bewohnern wurden im Anschluss durch ein geographisch geschichtetes Zufallsstichprobenverfahren ausgewählt, um an der für fünf Jahre ausgelegten longitudinalen Studie teilzunehmen (EMINI Kohorte, bestehend aus 4283 Haushalten mit 17872 Bewohnern). Die Teilnahme beinhaltete im Wesentlichen einen Besuch durch ein speziell geschultes lokales Studienteam pro Jahr (immer im gleichen Zeitraum), bei dem eine strukturierte Befragung und Probenentnahmen (Blut, Urin, Stuhl) durchgeführt wurden. Alle Proben wurden vor Ort in eine biologische Datenbank integriert.

2.8 Zielsetzung der vorliegenden Arbeit

Mittels einer serologischen Untersuchung einer bestehenden Kohorte im Südwesten Tansanias sollte eine Aussage über Prävalenzen von verschiedenen Arbovirosen in der Region getroffen werden. Alle Proben wurden auf Antikörper (IgG) gegen Rifttal Virus (RVV), Alphavirus (z.B. CHIKV), West Nil Virus (WNV), Dengue Virus Serotyp I-IV (DENV I-IV) und Gelbfieber Virus (YFV) getestet. Ziel war es, in einer von Epidemien bislang nicht betroffenen Region im Afrika südlich der Sahara, das endemische Vorkommen von Arboviren zu überprüfen und einen Eindruck über ihre unterschiedlichen Verbreitungsmuster zu bekommen. Dies sollte im Hinblick auf die Lebensverhältnisse der Studienteilnehmer und die Ökologie der entsprechenden Vektoren geschehen. Durch die detaillierten Kenntnisse der EMINI Haushalte und ihrer Bewohner, sollten Risikofaktoren für eine Infektion identifiziert werden.

3 Material und Methoden

Für die hier vorliegende Arbeit wurden 1228 Blutseren verwendet, die aus der zweiten Befragungsrunde (2007/2008) stammen. Die Probenauswahl erfolgte zufällig und stratifiziert nach Alter, Geschlecht, Haushaltshöhe über dem Meeresspiegel und Kontakt zu Nutzvieh. Die Mbeya Region ist eine politisch stabile, für Langzeitstudien geeignete Region.

Die stichprobenartig ausgewählten Blutproben der EMINI Studienteilnehmer wurden hitzeinaktiviert und vor der Testung 1:10 verdünnt. Ein, mit Virus infizierten Vero E6 Zellen beschichteter, kommerziell erhältlicher Biochip (Euro Immun, Lübeck, Deutschland) wurde für den Antikörpernachweis mittels indirekter Immunfluoreszenz Mikroskopie verwendet (verändert nach Swanepol et al. 1986). Die Prozessierung der Proben erfolgte nach einem Standardprotokoll. Es wurden von allen Seren Titerstufen zwischen 1:20 bis 1:640 hergestellt. Im Serum befindliche Antikörper gegen ein bestimmtes Virus, binden an die mit diesem Virus infizierten Vero E6 Zellen und können durch einen, aus Kaninchenserum gewonnenem, mit dem Fluoreszenzfarbstoff FITC (Fluoresceinisothiocyanat) markierten, anti-Mensch Antikörper (DAKO, Hamburg, Deutschland) markiert werden. Die Fluorezenzmikroskopie wurde im Anschluss durch zwei unabhängige Beobachter durchgeführt und die

Ergebnisse miteinander verglichen. Eine Probe wurde als Antikörper- positiv definiert, wenn in mehr als 20% der infizierten Vero E6 Zellen eine feingranuläre, cytoplasmatische, grünliche Fluoreszenz detektiert werden konnte. Außerdem musste per definitionem eine negative Negativ- und eine positive Positivkontrolle vorliegen. Unterschieden sich die Ergebnisse der beiden Beobachter um mehr als eine Titerstufe, so wurde die Probe erneut prozessiert und mikroskopiert.

Die Auswertung der Daten erfolgte mittels Stata statistics software (Version 12, Stata group, College Station, Tx, USA). Mögliche Risikofaktoren für das binäre Resultat „Positiv/Negativ" für anti-Alpha Virus/ anti- RVFV IgG Antikörper wurden durch uni- und multi-variable Poisson Regressions- Modelle identifiziert. Das Signifikanzniveau betrug <0,05.

4 Ergebnisse

Für die vorliegende kumulative Dissertation werden die bereits publizierten Daten über Rifttal Virus und Alphavirus verwendet. Anzumerken ist, dass auch für die untersuchten Flaviviren (Westnilfieber Virus, Denguefieber Virus Serotyp 1-4, Gelbfieber Virus) hohe Antiköpertiter in der Studienpopulation gefunden wurden. Aufgrund von serologischen Kreuzreaktionen, werden die Ergebnisse vor Publikation noch weiter verifiziert.

Mittels indirekter Immunfluoreszenzmikroskopie konnten 1228 Serumproben für die Rifttalfieber Virus Prävalenz eindeutig valide ausgewertet werden. Für die Untersuchung bzgl. der Alphavirus Prävalenz wurden 1215 valide Ergebnisse erhalten.

4.1 Ergebnisse Seroprävalenz Rifttalfieber Virus in der Mbeya Region, Tansania

In 5,2% der untersuchten Humanseren konnten Antikörper (IgG) gegen das Rifttal Virus (RVFV) nachgewiesen werden. Extrapoliert man dieses Ergebnis der stratifizierten Kohorte, ergibt das eine Seroprävalenz von 3,1%. Ein lokales Maximum von 29,3% zeigt sich für Kyela (der am niedrigsten gelegene Studienort am Malawisee, nochmals unterteilt in zwei Unterorte), und zwar für den direkt am Ufer des Sees gelegenen Unterort Bujonde- Kajunjumele (BK). Aufgrund des großen lokalen Unterschiedes der Seroprävalenzen wurden die statistischen Auswertungen sowohl für Kyela/BK allein, als auch für alle Orte zusammen durchgeführt.

Die Poisson Regression zeigt eine signifikant steigende Prävalenz von anti-RVF IgG mit zunehmendem Alter, sowohl in der uni- als auch in der multivariablen Analyse. In der multivariablen Analyse konnte außerdem gezeigt werden, dass Besitz von Rindern ein signifikanter Risikofaktor für eine Infektion mit dem Rifttal Virus ist. Weiterhin signifikant ist die Assoziation von hohen anti-RVF IgG Titern mit niedrigem sozialem Status. Dieser wurde über den SES (engl. *social economic situation status score*) abgeschätzt. Eigentum von Rindern wurde für die Bildung des SES nicht verwendet.

Die Betrachtung der Umweltfaktoren ergibt eine starke Assoziation mit der Vegetationsdichte (EVI engl. Enhanced vegetation index) und ebenso mit der Umgebungstemperatur. Vor allem sind hohe Seroprävalenzen mit hohen Minimum- und niedrigen maximalen Oberflächentemperaturen assoziiert. Jeder dieser Umweltfaktoren lässt sich in einem Modell durch einen der anderen ersetzen, so dass nicht genau klar ist, welcher hier am besten korreliert. Auffallend ist die Relevanz von nur wenigen Höhenmetern, die mit Abstand zum Seeufer, zu einem signifikanten Abfall der Seroprävalenzen führen. Der Nachweis von Antikörpern gegen das Rifttal Virus korreliert außerdem signifikant mit dem Nachweis von Antikörpern gegen Alphavirus sowohl in BK als auch insgesamt in den Regionen, im

jeweils gleichen Haushalt. In BK zeigte sich diesbezüglich weiterhin eine starke Assoziation mit dem Vorkommen von Filarien und *Plasmodium falciparum.*

4.2 Ergebnisse Seroprävalenz Alphavirus in der Mbeya Region, Tansania

Antikörper (IgG) gegen Alphaviren wurden in 18% der untersuchten Seren gefunden. Durch direktes Extrapolieren der stratifizierten Proben auf die Zusammensetzung der Gesamtbevölkerung, kommt man auf eine Gesamtprävalenz von 11,8% in den neun Regionen. Wieder zeigt sich eine signifikante, positive Korrelation mit dem Alter der Studienteilnehmer in der uni- und multivariablen Analyse. Negativ assoziiert ist die Prävalenz von Anti-Alphavirus IgG mit der Höhe des Wohnortes über dem Meeresspiegel, wobei die Seroprävalenz über 1198 m abnimmt. Die Steigung des Geländes am Wohnort korreliert ebenfalls negativ in der uni- und multivariablen Analyse, mit den höchsten Prävalenzen bei Steigungen unter 1,6 Grad Gefälle. Signifikante Korrelationen mit Sozialstatus, Populationsdichte, sowie Temperatur- und Vegetationsparametern bestehen jedoch nur in der uni-variablen Analyse, und waren in der multi-variablen Analyse nach Korrektur für Alter, Höhenlage und Neigung des Geländes nicht mehr signifikant assoziiert. Korrelationen mit anderen Krankheitserregern bestehen in der Uni-variablen Analyse für das Vorkommen von Plasmodium falciparum (RDT *engl. Rapid diagnostic test*), Antikörpern gegen Flaviviren (WNV, DENV 1-4, YFV), Antikörpern gegen Rickettsien der Fleckfiebergruppe (SFG engl. *Spotted fever group*) sowie Antikörpern gegen das Rifttalfieber Virus. Nach Korrektur für die Risikofaktoren Alter, Höhe über dem Meeresspiegel und Steigung des Geländes am Wohnort, war keine Assoziation mit dem Nachweis von Plasmodium falciparum-Antigen mehr vorhanden. Keine Korrelationen bestanden mit der Haltung von Nutzvieh.

5 Diskussion

In der vorliegenden Dissertation wurden mit Hilfe einer bestehenden Kohorte die Seroprävalenzen für verschiedene Arboviren in einem Gebiet Afrikas südlich der Sahara bestimmt, für das bis heute keine Epidemien registriert wurden. Es wurden unter den Studienteilnehmern, die einen Teil der Allgemeinbevölkerung repräsentieren (siehe EMINI- Kohorte), für alle getesteten Arboviren zum Teil hohe Titer nachgewiesen.

Der Studienort Kyela ergab für alle untersuchten Viren die höchsten Seroprävalenzen. Die Besonderheit an diesem Studienort ist seine nochmalige Unterteilung in die beiden Unterorte Bujonde-Kajunjumele (BK) und Katumba-Songwe (KS). BK liegt direkt am Ufer des Sees, KS auf der anderen Seite einer Straße ein paar Höhenmeter oberhalb und dementsprechend weiter vom See entfernt. Interessant ist vor allem der Vergleich der beobachteten Seroprävalenzen für Rifftal Virus und Alphavirus. Während die Gesamtprävalenz des Rifttal Virus für die neun Studienorte nur 3,2% beträgt, gibt es eindeutigen Spitzenwert von 29,8% in BK. Die Seroprävalenz im anderen Unterort KS beträgt dagegen nur 2,5%.

Auch für Alphaviren (a.e. Chikungunya Virus) ist Kyela der Ort mit der höchsten Seroprävalenz, allerdings gibt es für die beiden Unterorte keine signifikanten Unterschiede (BK 50%, KS 44,3%). Es scheint nun so, dass die direkte geographische Beziehung zum Seeufer einen deutlichen Einfluss auf das Vorkommen von Antikörpern gegen RVFV und damit auf eine Infektion mit dem Virus hat. Der Malawisee selbst scheint dabei nicht der entscheidende Faktor zu sein, da das Vorkommen von Antikörpern gegen RVFV nicht mit Infektionen durch Schistosoma haematobium assoziiert ist. Diese Trematodenerkrankung ist im Malawisee endemisch und der Zwischenwirt, eine Süßwasserschnecke, ist direkt auf ein permanent bestehendes Gewässer angewiesen.

Eine Erklärung könnten die jährlichen Überschwemmungen während der Regenzeit sein, die weite Teile der Uferzone betreffen. Diese nicht-permanenten Wasserflächen bieten ausgezeichnete Habitate für brütende Moskitos und sind bereits ein paar Höhenmeter weiter und in einigem Abstand zum See nicht mehr vorhanden. Das RVFV kann verschiedene Moskitogattungen als Vektoren benutzen. Darunter sind vor allem Moskitos der Gattungen Culex, Anopheles und Aedes. Diese Gattungen sind wiederum in ihrer Ökologie durchweg verschieden. Gerade Aedes sp. besitzen Eier, die extrem resistent gegenüber Austrocknung sind und in Böden überdauern können (Fontenille et al. 1995, Linthicum et al. 1985). Wurden diese bereits transovariell mit dem Virus infiziert, entsteht bei der nächsten Überflutung bereits eine neue Generation infizierter Moskitos (sog. Erhaltungsvektor). Horizontal kann sich das Virus dann wiederum in anderen Gattungen z.B. *Culex sp.* vermehren. Vor allem während Rifttalfieber-Epidemien wurde den *dambos* (Überflutungsgewässern) eine wesentliche Bedeutung zugestanden (Davies et al. 1985, Sang et al. 2010). Die Ergebnisse dieser Untersuchung scheinen deren Rolle auch für das endemische Vorkommen von RVFV zu bekräftigen und damit auch die Rolle von *Aedes sp.* als relevantem Vektor.

Weiterhin kann Wasser auch als proxy für Vegetation gesehen werden. Die Vegetationsdichte ist in uni- und multivariabler Analyse mit Anti-RVFV-IgG assoziiert. Ebenso gilt dies für eine geringe Temperaturspanne mit höheren Minimaltemperaturen nachts und niedrigeren Maximaltemperaturen tagsüber. Vereint man nun diese gemachten Beobachtungen zu einem Gesamtbild, so könnte man sich vorstellen, dass Blattwerk, Laub und Baumschatten im Oberflächen-/Überschwemmungswasser zu eben genau diesen niedrigeren Tagesmaximal- und höheren Nachtminimaltemperaturen führen, die für eine Vermehrung von RVFV in seinem Vektor nötig ist. Das auf den Böden am Seeufer stehende Wasser ist wiederum ideales Moskitohabitat und Rindertränke zugleich. Der Besitz von Rindern ist wiederum mit der Seroprävalenz korreliert (nicht aber mit der Dichte). In BK gibt es besonders viel Rinderhaltung. Aus Angst vor Diebstahl binden die Hirten ihr Vieh nachts gerne direkt bei den Wohnhäusern an, so dass ein unmittelbarer Kontakt zwischen Menschen und Rind besteht. Moskitos der Gattung *Culex* sind in menschlichen Behausungen häufig und verbreiten dort das Virus.

Alle wesentlichen Risikofaktoren für eine RVFV Infektion sind somit vereint und können den hohen Durchseuchungstiter, der in Kyela BK lebenden Studienteilnehmer gut erklären. Diese Beobachtungen decken sich auch mit anderen Studienresultaten, die eine RVFV Infektion mit verschiedenen Faktoren assoziiert sehen. Darunter sind Nähe zu Wasserflächen und Rinderhaltung (LaBeaud et al. 2008, Pourrut et al. 2010) sowie hohe Vegetationsdichte rund um Wasserstellen (Soti et al. 2013).

Obwohl das Rifttal Virus von vielen verschiedenen Vektorenspezies übertragen wird, scheint insgesamt die ökologische Plastizität so gering zu sein, dass eine weite geographische Verbreitung nicht stattfindet und es bereits in einem Umfeld von nur wenigen hundert Metern zu einem massiven Abfall der Seroprävalenzen kommt. Global betrachtet, passt dies gut zu der Tatsache, dass das RVFV bis heute neben dem Afrikanischen Kontinent lediglich auf der Arabischen Halbinsel epidemisch wurde (Jup et al. 2002).

Für die Verbreitung von Alphaviren (mit einem wesentlichen Vertreter, dem Chikungunya Virus) ergeben die Untersuchungsergebnisse ein völlig anderes Bild. Der Durchseuchungstiter der Studienteilnehmer liegt in beiden Unterorten von Kyela deutlich über 40% und auch in den anderen acht Regionen gibt es einen signifikanten Abfall der Seroprävalenz erst ab ca. 1200 Höhenmetern. Ökologische Faktoren wie Temperatur und Vegetation sind lediglich in der univariablen Analyse signifikant. Im multivariablen Modell verschwindet diese Signifikanz nach Adjustierung für die Variablen Alter, Höhe über dem Meeresspiegel und Steigung des Geländes am Wohnort. Die Hauptvektoren für die Übertragung von Alphaviren, insbesondere das Chikungunya Virus, sind *Aedes* (*Stegomyia*) *aegypti* und *Aedes albopictus*. Diese Moskitoarten sind beide anthropophil und in ihrer

Verbreitung im Wesentlichen durch die Höhenlage begrenzt (und konsekutiv durch die dadurch entstehenden Temperaturen). Anish et. al (2011) konnten nach der großen Chikungunyafieber-Pandemie 2006/2007 in Indien mit einer Prävalenz von 40% in Kerala (klinische Falldefinition, IgM ELISA) nachweisen, dass in 95,6% der Fälle Wasserbehälter um das Wohnhaus vorhanden waren. Es scheint, dass *Aedes* (*Stegomyia*) Mücken für ihre Brutreviere eher kleine Wasserreservoire nutzen. Dies können z.B. Pfützen sein, die sich auf ebenem Gelände leichter bilden, aber auch zivilisatorische Relikte wie Wassertonnen, Autoreifen, Plastikeimer, Blumenkübel usw., die auf ebenem Gelände leichter liegen bleiben und sich füllen können. Diese Anspruchslosigkeit mag auch die Erklärung für die seit Ende 2013 in der Karibik und auf dem amerikanischen Kontinent herrschende Epidemie sein (Johannson et al 2014., Mowatt und Jackson 2014), da moderate Temperaturen und kleine Wasserreservoire für den Hauptvektor *Aedes albopictus* und das Virus vermutlich die einzige Notwendigkeit sind.

Auch für die untersuchten Flaviviren, v.a. für das Dengue Virus (Serotyp 1-4) und das West Nil Virus wurden zum Teil sehr hohe Seroprävalenzen in verschiedenen Studienorten gefunden (noch nicht publizierte Daten), was ebenfalls daraufhin weist, dass diese Viren und ihre Vektoren ökologisch anpassungsfähiger sind als das Rifttal Virus.

Allen Viruserkrankungen gemeinsam ist jedoch die Tatsache, dass sie weder klinisch noch labortechnisch in der Mbeya Region bei fiebernden Patienten in Gesundheitseinrichtungen diagnostiziert wurden.

6 Ausblick

Alle untersuchten Arboviren zirkulieren endemisch in der Mbeya-Region. Es stellt sich nun die Frage, wie häufig akute Infektionen zu Arztbesuchen, Klinikaufenthalten und Arbeits- bzw. Schulunfähigkeit führen. Leitsymptome der Arbovirosen sind in der Regel Fieber und unterschiedlich stark ausgeprägte Knochenschmerzen. Gerade hohes Fieber wird im Afrika südlich der Sahara auch ohne Erregernachweis häufig als Malaria gedeutet und weitere Diagnostik bleibt nicht selten aus, auch weil die entsprechende Infrastruktur oft fehlt. In Tansania ist zwar ein Malariaschnelltest bei jedem fiebernden Patienten obligatorisch, aber gerade negative Ergebnisse werden oft angezweifelt und ignoriert. Das kann nicht nur dazu führen, dass Malariamedikamente unnötig eingesetzt werden, sondern auch dazu, dass notwendige andere Therapien, wie beispielsweise antibiotische Therapien bei bakteriellen Infekten unterbleiben. Außerdem tauchen die nicht diagnostizierten Erkrankungen nicht in Statistiken auf und ihr tatsächlicher Anteil an den akuten Fiebererkrankungen wird so zu niedrig eingeschätzt. In Guinea zeigten Jentes et al. (2010), dass in einer Studie 63% der Patienten mit Fieber mit Arboviren infiziert waren, davon am häufigsten mit West Nil Virus und dem Chikungunya Virus. Leider war die Fallzahl bei dieser Untersuchung sehr gering und die auf zwölf Monate angesetzte Studie musste aufgrund von politischen Unruhen bereits nach vier Monaten abgebrochen werden. Ähnliche Daten gibt es aus Kamerun (Fokam et al. 2010). Im Norden Tansanias wurden über 800 fiebernde Patienten untersucht, allein 8% hatten akute Chikungunya Virus Infektionen (Hertz et al. 2012).

Es gibt bereits zahlreiche Hinweise, dass in den afrikanischen Ländern der tatsächliche Anteil von Malaria an akuten Fiebererkrankungen überschätzt wird (D´Acremont et al. 2010) und der Anteil von gerade bakteriellen und viralen Erkrankungen unterschätzt wird. In einer aktuellen Veröffentlichung konnte ebenfalls gezeigt werden, dass in verschiedenen Regionen und Klimazonen Tansanias hohe Seroprävalenzen von Chikungunya Virus, Dengue Virus und Zika-Virus in der Allgemeinbevölkerung bestehen (Mwanyika et al. 2021), so dass ein differentialdiagnostisches Miteinbeziehen von Arbovirosen bei einem akuten fieberhaften Infekt -bei Ausschluss einer Malaria- mehr und mehr

sinnvoll erscheint. Eine Untersuchung unter Mitarbeitern des Gesundheitssystems im Norden Tansanias zum Wissen über Dengue- und Chikungunyafieber konnte zeigen, dass Denguefieber bereits einen Stellenwert in der klinischen Differentialdiagnose hat, während das Bewusstsein für Chikungunyafieber noch sehr gering ist (Saringe et al. 2019).

In der Mbeya-Region gibt es ebenso wie in den meisten Gegenden Afrikas südlich der Sahara, keine standardisierten Diagnostikmethoden, um virale oder bakterielle Erreger in Akutseren zu detektieren. Mit dem Wissen, dass sowohl vernachlässigte bakterielle als auch virale Erreger in der Region in nicht geringem Maße endemisch sind, ist der nächste Schritt, fiebernde Patienten auf diese Erkrankungen zu untersuchen, um einen Überblick über den Anteil an Akuterkrankungen zu bekommen- eine derartige Studie, die auf den Ergebnissen dieser Arbeit aufbaut, befindet sich aktuell in Vorbereitung. Außerdem wären neue und vor allem billige Diagnostikmethoden hilfreich, um eine schnelle Differentialdiagnostik vor Ort zu ermöglichen. Eine Herausforderung ist dies v.a. bei den Flaviviren aufgrund der ausgeprägten serologischen Kreuzreaktionen (Dobler 2008). DNA bzw. RNA basierte Verfahren sind genauer, aber auch teurer und an Orten ohne entsprechende Infrastruktur schwer zu realisieren

7 Zusammenfassung

Die vernachlässigten Tropenerkrankungen sind eine heterogene Gruppe von Infektionserkrankungen, die ihren Namen durch das, im Vergleich zu den „großen Drei" AIDS, Malaria und Tuberkulose, geringe öffentliche Interesse erhalten haben. Es werden nach Erregerart bakterielle, virale, durch Protozoen verursachte und Wurmerkrankungen unterschieden. Allen gemeinsam ist die Verbreitung vorwiegend in den ländlichen Armutsgebieten der Welt.

In dieser Arbeit wurde die Seroprävalenz von vernachlässigten, durch Arthropoden (=Gliederfüßer) übertragenen Viruserkrankungen („Arbovirosen") im Südwesten Tansanias untersucht, um ihre epidemiologische Relevanz abzuschätzen und Risikofaktoren für eine Infektion zu identifizieren.

Dazu wurden aus einer bestehenden Kohorte von über 17.000 Bewohnern an neun verschiedenen Orten der Region stichprobenartig ca. 1200 Serumproben ausgewählt und mittels indirekter Immunfluoreszenz-Mikroskopie auf Antikörper gegen Rifttal Virus und Alphavirus getestet. Keines dieser Viren hatte zuvor eine Epidemie in der Region ausgelöst bzw. war je bei einem sich in einer Gesundheitseinrichtung vorstellenden Patienten diagnostiziert worden.

Es wurden für die untersuchten Arboviren überraschend hohe Seroprävalenzen in der Allgemeinbevölkerung gefunden, die auf ein endemisches Vorkommen in der Mbeya Region hinweisen.

Für das Rifttal Virus ergab sich eine Gesamtprävalenz von 3,2 % an allen neun Studienorten und ein lokales Maximum von 29,8% am Unterort Bujonde-Kajunjumele in Kyela direkt am Ufer des Malawisees. Seropositivität war in der multi- variablen Analyse assoziiert mit höherem Alter, niedrigem sozialen Status, Besitz von Rindern, hohem Vegetationsindex und geringen Temperaturschwankungen. Obwohl das Rifttal Virus mehrere Gattungen von Moskitos als Vektoren nutzen kann, ist die ökologische Plastizität offensichtlich so gering, dass nur die regelmäßig durch saisonale schwere Regenfälle auftretenden Überschwemmungsgewässer der Uferzone die idealen Verbreitungsbedingungen für das Virus darstellen und zu einer hohen Durchseuchung der dort ansässigen Bevölkerung führen.

Dagegen sind die Seroprävalenzen der Bevölkerung an allen untersuchten Orten der Region gegen ein Alphavirus (a.e. Chikungunya Virus) mit 18% hoch und fallen erst ab einer Höhe von 1200 Metern signifikant ab. Die Seroprävalenz beträgt in Kyela, dem niedrigsten Studienort, weit über 40%, jedoch gibt es für die zwei Unterorte BK und KS keine signifikanten Unterschiede. Neben den direkten Risikofaktoren Alter und Höhe am Wohnort, zeigte sich außerdem, dass die Steigung des Geländes am Wohnort negativ mit dem Vorliegen von Antikörpern assoziiert ist. Am ehesten spielen demzufolge kleine Wasserreservoire wie z.B. Pfützen für die Verbreitung von Virus und Vektor eine Rolle.

Da es sich bei den Arbovirosen um akute Fiebererkrankungen handelt, sollten sie in der Mbeya Region in Zukunft in die entsprechende Differentialdiagnostik miteinbezogen, und eine Surveillance etabliert werden.

8 Abstract

The neglected tropical diseases are a heterogeneous group of infectious diseases, which are mainly defined by their public neglect in comparison to the Big Three infectious diseases AIDS, Malaria and Tuberculosis. The infectious agents range from helminths to protozoa, bacteria and viruses. All of them share the common feature to be particularly present in the worlds´ poorest (rural) regions. In this work, the seroprevalence of arthropod- borne viruses in South-western Tanzania was studied, in order to determine the epidemiologic relevance of these neglected diseases and identify possible risk factors for infection. Out of a study cohort with 17.000 Tanzanian people living in nine geographic distinct areas of the Mbeya Region, approximately 1200 blood sera were chosen by stratified random sampling and tested for antibodies against Rift valley virus and Alpha virus by indirect immune fluorescence microscopy.

None of these viruses had ever been detected as the causative agent of febrile disease with a sick patient presenting at a health facility, or was detected as the cause of an outbreak in the region.

There were, to some extent, found surprisingly high seroprevalences against the tested arboviruses in the normal population, suggesting an endemic circulation in the Mbeya Region.

For Rift valley virus, the overall prevalence of antibodies was 3,2% for all nine study sites with a unique local maximum of 29,8% at the Bujonde-Kajunjumele site of Kyela, at the shore of Lake Malawi. Age, low socio-economic status, cattle ownership, high vegetation indices and stable surface temperatures were identified to be direct risk factors for infection. Although RVFV is able to use different genera of mosquitoes as a vector, the ecological plasticity is apparently low. It seems that only the frequent emerging surface waters due to seasonal heavy rain fall at the littoral zone of the lake and its close surroundings, provide the ideal conditions for virus distribution and lead to high prevalences in the resident population.

In comparison, the general population seroprevalence of an Alphavirus (most probably Chikungunya Virus) of all study areas is high, at 18%. Spread oft the causative virus seems to be only limited by elevation of residence above 1200 meters. The seroprevalence within the study population of Kyela, namely the lowest study site, was well over 40% with no significant differences between the two sub-sites BK (lake shore) and KS (some hundred meters away from the lake side). Besides the two identified direct risk factors age and altitude of residence, slope of the terrain showed strong significant association with prevalence of antibodies against Alphavirus, leading to the assumption, that for distribution of vector and virus mostly small water reservoirs like puddles are of importance that form in even terrain.

Since fever is a common symptom of acute arboviral infections and seroprevalence of arboviruses in Mbeya Region is surprisingly high, differential diagnosis should be complemented by this finding and a surveillance system established.

9 Literaturverzeichnis

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10 Anhang

10.1 Publikation I

I. Seroprevalence of alphavirus antibodies in a cross-sectional study in southwestern Tanzania suggests endemic circulation of chikungunya.

Weller N, Clowes P, Dobler G, Saathoff E, Kroidl I, Ntinginya NE, Maboko L, Löscher T, Hoelscher M, Heinrich N (2014), PLoS Negl Trop Dis 8(7) e2979,

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PLOS | NEGLECTED TROPICAL DISEASES

Seroprevalence of *Alphavirus* Antibodies in a Cross-Sectional Study in Southwestern Tanzania Suggests Endemic Circulation of Chikungunya

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Abstract

Background: To date, *Alphavirus* infections and their most prominent member, chikungunya fever, a viral disease which first became apparent in Tanzania in 1953, have been very little investigated in regions without epidemic occurrence. Few data exist on burden of disease and socio-economic and environmental covariates disposing to infection.

Methods: A cross-sectional seroprevalence study was undertaken in 1,215 persons from Mbeya region, South-Western Tanzania, to determine the seroprevalence of anti-*Alphavirus* IgG antibodies, and to investigate associated risk factors.

Results: 18% of 1,215 samples were positive for *Alphavirus* IgG. Seropositivity was associated with participant age, low to intermediate elevation, flat terrain and with IgG positivity for Rift Valley fever, *Flaviviridae*, and rickettsiae of the spotted fever group. When comparing the geographical distribution of *Alphavirus* seropositivity to that of Rift Valley fever, it was obvious that *Alphaviruses* had spread more widely throughout the study area, while Rift Valley fever was concentrated along the shore of Lake Malawi.

Conclusion: *Alphavirus* infections may contribute significantly to the febrile disease burden in the study area, and are associated with several arthropod-borne infections. Their spread seems only limited by factors affecting mosquitoes, and seems less restricted than that of Rift Valley fever.

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Introduction

Alphaviruses form a genus in the family *Togaviridae*. About 40 different viruses including type and sub-type viruses are members of the genus. Among them are major human pathogens such as chikungunya virus (CHIKV) and viruses of veterinary importance, e.g. Venezuelan equine encephalitis virus. The currently most important *Alphavirus* of human pathogenicity is CHIKV, which causes significant morbidity and economic losses [1]. Although it has been described and isolated first in 1953 from a febrile person in Tanzania, East Africa [2], currently only few data on the distribution and medical importance of CHIKV and other *Alphaviruses* in Africa are available.

Since the 1960s, especially CHIKV was repeatedly isolated throughout African and Asian countries [3], and small outbreaks were frequently reported. The virus gained notoriety, when in the years 2004–2007 an outbreak was noticed of so far unknown dimension. Starting in Kenya, a severe epidemic hit the islands of the Indian Ocean in 2005/2006, with nearly 280.000 people infected on the island of La Reunion alone [1,4,5]. Transmission to the Indian sub-continent resulted in chikungunya fever in an estimated 1.3 million people [6]. The enormous scientific interest in this outbreak led to several new findings concerning viral molecular biology and ecology [3,7–9]. Investigations regarding the climatic conditions before the outbreak revealed unusual warm and dry conditions along the Kenyan coast in 2004 [10,11]. Infrequent replenishment of domestic water stores due to these dry conditions may have facilitated the transmission of the virus.

Despite this increased research interest, the role of CHIKV as well as other *Alphaviruses* in endemic regions, especially in sub-Saharan Africa, remains unclear. Recent studies concentrated mainly on areas of the latest CHIKV pandemic. The disease burden and the epidemiology in local populations not affected by the devastating outbreak in 2004–2007 is largely unknown. In a

Author Summary

The origin of febrile disease is often difficult to diagnose. In tropical countries, viral infections that are transmitted by arthropods include, among others, *Alphavirus* infections (e.g. chikungunya fever), dengue, West Nile, Yellow Fever and Rift Valley fever. In malaria endemic areas, these diseases are often mis-diagnosed and treated as malaria. Our study examined serum samples from 1,215 participants of a population survey from the Mbeya region, south-western Tanzania, for antibodies against *Alphaviruses* of the Semliki forest group as a sign of past infection. We found 18% of study participants positive, a surprisingly high number which points to a hitherto undetected circulation of *Alphaviruses* in the region. Among examined risk factors, even terrain, low to moderate elevation and participant age were associated with antibody positivity. Comparison with the distribution of Rift Valley fever seropositivity showed that *Alphaviruses* are more widely distributed throughout the study area, while Rift Valley fever seems to occur in a limited area close to Lake Malawi only.

small study in Guinea, arboviruses as causative agent for febrile disease were identified by neutralization assays in 63% of 47 patients [12]. 17% of these had acute CHIKV infections. In a clinical study conducted in Northern Tanzania with 870 febrile patients, PCR-confirmed acute CHIKV infections were diagnosed in 7.9% of all cases [13]. However, surveillance of other *Alphaviruses* is even less developed as most of these studies are targeting CHIKV using PCR. A serosurvey in rural Kenya revealed a seropositivity prevalence of 34% for anti-*Alphavirus* IgG, which was not associated with age, indicating frequently occurring smaller epidemics rather than endemic cycling [14]. Although CHIKV is expected as the main pathogen, other *Alphaviruses* cannot be excluded since a broadly cross-reactive ELISA was used.

With the recent outbreak of CHIKV in Italy, and detection of autochthonous transmission in southern France, it is clear that *Alphaviruses* and especially CHIKV have the potential to become endemic in areas in Europe where *Aedes albopictus* is already established [15,16].

In this study we aimed to assess the epidemiological patterns of *Alphavirus* infections in the Mbeya Region in Tanzania, by measuring seroprevalence in 1215 individuals participating in an epidemiological survey in the Region. This region was not affected by the 2004–2007 outbreak, and diagnosis or laboratory verification of acute chikungunya fever or other *Alphavirus* infection does not occur locally. The survey gave us the opportunity to study the role of this pathogen genus and its dependence on certain social and ecological factors in an endemic transmission cycle in a typical local setting.

Materials and Methods

Ethics statement

Both EMINI and this sub-study were approved by local and national Tanzanian ethics committees. Each EMINI participant had provided written informed consent before enrolment, with parents consenting for their children.

Study population and the EMINI survey

The EMINI population survey had the objective to create the infrastructure to **E**valuate and **M**onitor the **I**mpact of **N**ew Interventions in the Mbeya Region of south-western Tanzania. Financed by the European Union over five years (2006 to 2011), the strengthening of the local health infrastructure and the establishment of a cohort which could be followed up on an annual basis created a platform on which the impact of improved health care infrastructure and new interventions could be monitored and evaluated. Embedded into the EMINI project were several focused studies such as this sub-study, which determined seroprevalences for a number of tropical arthropod-borne diseases.

In preparation of the EMINI survey, a census of the entire population in nine geographically distinct and ecologically different sites of the Mbeya Region was carried out. Study sites were selected to reflect the wide range of different conditions within the region in terms of elevation, population density and development (urban versus rural). Basic information regarding the households and their inhabitants was collected and all household positions were recorded with handheld GPS receivers. Ten percent of the surveyed households were then chosen by geographically stratified random selection for inclusion into the EMINI survey, to obtain a representative sample of the population from each site. The resulting EMINI cohort included all consenting participants of 4,283 households. Over the following five years annual visits at the same time of the year were conducted, during which structured interviews with all household members were performed, and blood, urine and stool specimens collected.

For this sub-study, we stratified the 17,872 participants, who had provided a blood sample during the second EMINI survey in 2007/2008, by age, gender, altitude of residence and ownership of domestic mammals. To be able to assess factors of interest that were identified in the literature but might have been underrepresented in the study population, we employed disproportionate random sampling with equal participant numbers for each stratum to identify 1.226 samples from participants above the age of 5 years to be tested for IgG antibodies against *Alphaviruses* and other tropical arthropod-borne diseases.

Socio-economic status

To characterize the socio-economic situation of each household, the head of each household was asked for the following information during each annual EMINI visit: Presence/absence of different items in the household (clock or watch, radio, television, mobile telephone, refrigerator, hand cart, bicycle, motor cycle, car, savings account), sources of energy and drinking water, materials used to build the main house, number of persons per room in the household and availability and type of latrine used. Based on the provided information, a socio-economic-status (SES) score was established, using a modified method originally proposed by Filmer and Pritchett which has frequently been employed to characterize the SES of people living in developing countries [17–19].

Environmental data

Population- and livestock-densities were calculated using data and household positions collected during the initial population census. Elevation data were retrieved from the NASA Shuttle Radar Topography Mission (SRTM) global digital elevation model, version 2.1 [20,21].

Land surface temperature (LST) and vegetation cover (EVI = enhanced vegetation index) data for the years 2003 to 2008 were retrieved from NASA's Moderate-resolution Imaging Spectroradiometer (MODIS) Terra mission which "are distributed by the Land Processes Distributed Active Archive Center (LP DAAC),

located at the U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center (lpdaac.usgs.gov)" [22]. These data were used to produce long-term averages of day and night LST and EVI.

Population-, household-, and livestock-densities, LST, EVI, and elevation data were averaged for a buffer area within 1000 meter radius around each household in order to characterize the ecological situation around the household. This approach was preferred to using the respective spot values at the household position, because spot data are more prone to random error than averages for a wider area.

Serology

Detection of anti-*Alphavirus* IgG, anti-Yellow fever virus IgG, anti-dengue 1–4 virus IgG, and anti-West Nile virus IgG on biobanked samples were performed as described previously for Rift Valley fever virus (RVfV) IgG [23]. A commercially available biochip (Euroimmun, Lübeck, Germany), containing infected and non-infected Vero E6 cells or only non-infected Vero E6 cells (negative control), was used for indirect immunfluorescence testing (IIFT), following a standard protocol. All serum samples were heat-inactivated and diluted tenfold prior to testing. Further dilutions of positive sera were carried out in the range of 1:20 to 1:640. A rabbit anti-human IgG FITC-labelled antibody (DAKO, Hamburg, Germany) served as conjugate. To decrease the known subjectivity of reading IIFT results to the best objective level, fluorescence microscopy was carried out independently by two experienced observers. In case of discrepancies (positive vs. negative; difference >1 titer step) the testing was repeated. A sample was classified as positive, if at a screening dilution of 1:20 a typical fine granular cytoplasmatic fluorescence was detected in around 20% of the cells on the positive field of the biochip with a typical location and morphology of infected cells, while no signal was detectable in the negative field. IIFT was repeated in case of indeterminate results, i.e. in cases where samples differed clearly from the negative control but did not match the criterion "positive". Ultimately, 1,215 definitive results were available from the selected samples.

Testing for *P. falciparum* malaria

Fresh EDTA-blood was used for malaria testing using a rapid test (ICT, South Africa) for each participant.

Data analysis

Stata statistics software (version 12, Statacorp, College Station, TX, USA) was used for all statistical analyses, and Manifold System 8.0 Professional Edition (Manifold Net Ltd, Carson City, NV) was used for processing of geographical data and to produce maps. In order to identify possible risk factors for anti-*Alphavirus* IgG positivity, we analysed seropositivity as the binary outcome in uni- and multi-variable Poisson regression models with robust (or Huber-White) variance estimates adjusted for household clustering [24,25]. Initial uni-variable models for all factors that we deemed as possibly related to CHIKV infection were used to identify variables with a uni-variable p-value $<=0.1$ for further multi-variable evaluation. Stepwise backward and forward regression, the Akaike and Bayes information criterion and various assessments of model-fit were used to identify the best multi-variable model, where only variables with a multi-variable p-value <0.1 were retained.

Associations of anti-*Alphavirus* IgG positivity with other diseases were assessed in uni-variable Poisson regression models with anti-*Alphavirus* IgG as the binary outcome, and the respective disease as the only predictor variable. In addition we ran the same models adjusted for those risk factors that had been retained in the above described multi-variable models regarding risk factors for anti-*Alphavirus* IgG positivity.

In the analysis, positivity for at least one of dengue, West Nile and Yellow fever antibodies, was categorized as "*Flavivirus* IgG" positive.

Results

219 of 1,215 (18.0%) samples reacted positive for anti-*Alphavirus* IgG. The estimated overall population prevalence, predicted from our stratified sample by direct extrapolation, is 11.8% for the population of our 9 sites (fig. 1).

Seropositivity increased with participant age, both in uni- and in multi-variable analysis (table 1; prevalence ratio (PR) for a 10-year increase in multi-variable analysis: 1.26, 95% confidence interval (CI) 1.20 to 1.32, $p<0.001$). Gender was not significantly associated with seropositivity.

We found a significant association of anti-*Alphavirus* IgG status with elevation above sea level, with significantly higher seroprevalence in the strata below 1,198 m, both in uni-variable and multi-variable analysis (fig. 2). The median elevation of the Kyela site is 487 m (Interquartile range IQR 483 m–514 m), and that of Igurusi is 1,193 m (IQR 1,156–1,205 m). Not only elevation, but also slope of the terrain was negatively associated with seropositivity in uni- and multi-variable analysis, even when adjusted for age and elevation (PR 0.86 per degree, 95% CI 0.77 to 0.95, $p = 0.004$), with the highest anti-*Alphavirus* IgG prevalence occurring on terrain with a slope of less than ~1.6° (fig. 2).

Several social, economic and behavioural factors showed significant association in uni-variable analysis but were rendered non-significant in multi-variable analysis when adjusting for age, elevation and slope of terrain. Factors associated with higher anti-*Alphavirus* IgG prevalence in uni-variable analysis included a lower socio-economic status, lower population density, higher vegetation density and higher land surface temperatures, especially night temperatures. Also, bed net ownership and higher frequency of use, which occurred in areas with higher mosquito burdens, were associated with a higher seropositivity in uni-variable analysis. Anti-*Alphavirus* IgG status was not associated with animal ownership, including cattle, sheep, goats and chicken (data not shown).

Next, we analysed correlations between anti-*Alphavirus* IgG status and other infectious diseases throughout the survey. Uni-variable analyses showed significant positive associations of anti-*Alphavirus* IgG with *P. falciparum* malaria RDT positivity, antibody positivity for spotted fever group rickettsiae (SFG) and Rift Valley fever virus (RVFV) [23], and any of the tested *Flaviviridae* (table 2). In separate models for each of these pathogens, that were adjusted for age, elevation and slope of terrain, a significant positive association was retained for SFG IgG, *Flavivirus* IgG and RVFV IgG, while the association with *P. falciparum* disappeared. A comparison of the spatial distribution of RVFV IgG and anti-*Alphavirus* IgG shows that for both viruses, Kyela site has the highest seroprevalences, but a wider occurrence is seen for anti-*Alphavirus* IgG when compared to RVFV IgG (fig. 1).

HIV status was unrelated with individual anti-*Alphavirus* IgG status in uni- and multi-variable analysis (data not shown).

Discussion

In the current study we present high rates of IgG antibodies against an *Alphavirus*. Cross reactions mainly occur in IFAT between antibodies against CHIKV and other viruses of the

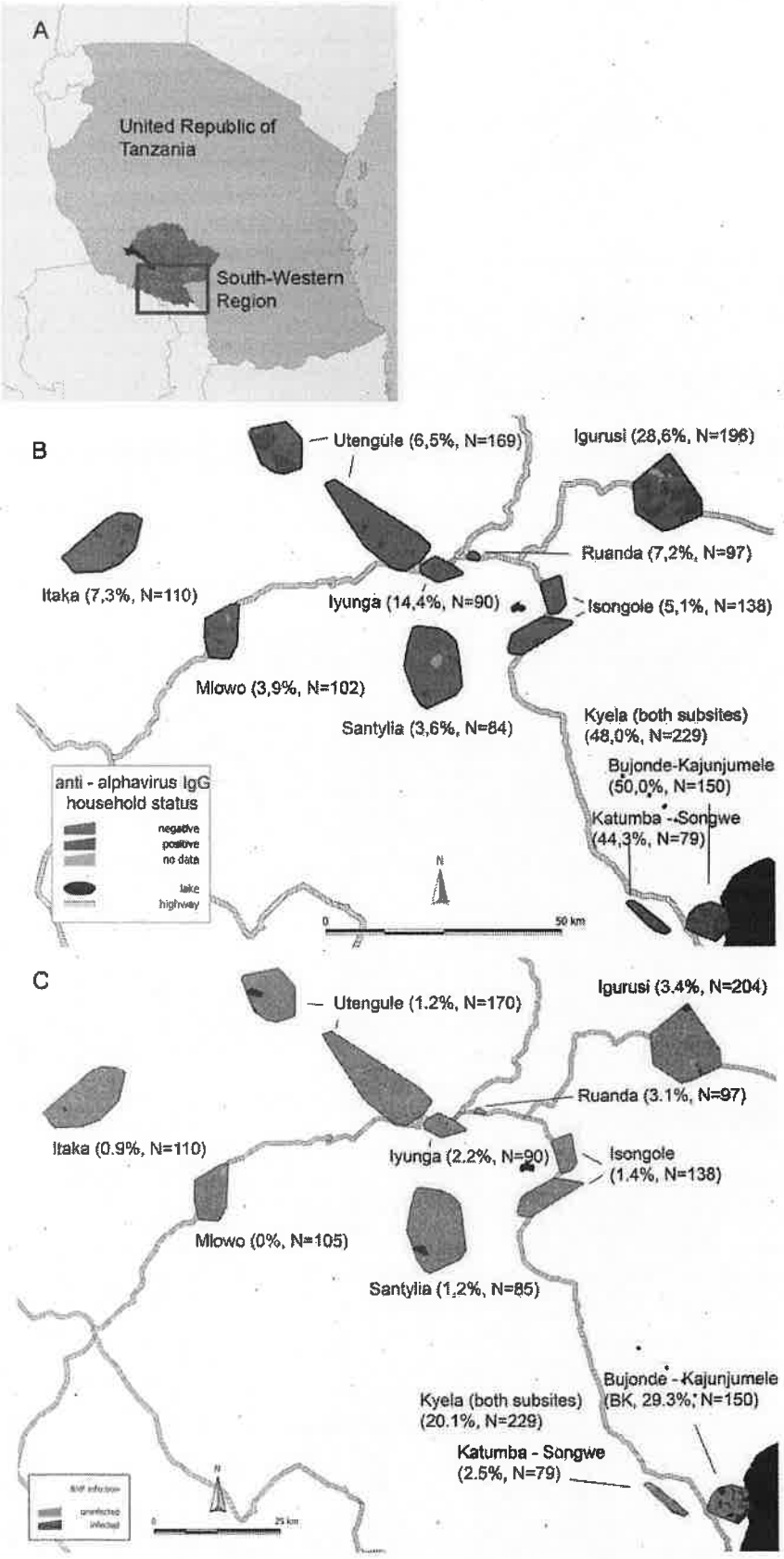
A
United Republic of Tanzania
South-Western Region
B
Utengule (6,5%, N=169)
Igurusi (28,6%, N=196)
Ruanda (7,2%, N=97)
Itaka (7,3%, N=110)
Iyunga (14,4%, N=90)
Isongole (5,1%, N=138)
Mlowo (3,9%, N=102)
Santylia (3,6%, N=84)
Kyela (both subsites) (48,0%, N=229)
Bujonde-Kajunjumele (50,0%, N=150)
Katumba - Songwe (44,3%, N=79)
anti - alphavirus IgG household status
negative
positive
no data
lake
highway
N
0
50 km
C
Utengule (1.2%, N=170)
Igurusi (3.4%, N=204)
Ruanda (3.1%, N=97)
Itaka (0.9%, N=110)
Iyunga (2.2%, N=90)
Isongole (1.4%, N=138)
Mlowo (0%, N=105)
Santylia (1.2%, N=85)
Bujonde - Kajunjumele (BK, 29.3%, N=150)
Kyela (both subsites) (20.1%, N=229)
Katumba - Songwe (2.5%, N=79)

Figure 1. Location of Households with positive participants for anti-*Alphavirus* IgG and Rift Valley fever virus IgG. Localisation of the study area in Tanzania (A). Location of households with (B) *Alphavirus* IgG-positive and (C) Rift Valley fever virus IgG-positive participants displayed as Voronoi polygons, with every polygon representing one household. Percent IgG-positives and total N examined in the site are displayed with site name. Households with one or more individuals positive for *Alphavirus* IgG are marked in red, all others in green. For Kyela site, both subsites Bujonde-Kajunjumele and Katumba-Songwe are displayed. Map created by use of Manifold System 8.0 software. (C) reproduced from [23] under creative commons license.
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Semliki-Forest virus complex of *Alphaviruses*, while cross reactivities against the Venezuelan equine, the Eastern equine and the Western equine encephalitis group are rare and low (≥4 titer steps; Dobler, unpublished observations). Therefore we assume that cross reactions may mainly occur between Semliki Forest complex viruses like O'nyong nyong virus or Semliki Forest virus. Other non-African Semliki-Forest virus complex viruses, like Ross River virus or Mayaro virus do not seem to be responsible for the antibodies as the inhabitants of the areas tested did not leave the region. However, we cannot exclude that a so far unknown *Alphavirus* of the Semliki Forest virus complex is circulating and may cause infection with or without clinical symptoms. The question can only be answered by virus detection by isolation or molecular detection and characterization of genome parts.

This analysis of the seroprevalence for *Alphaviruses* adds to the picture of arthropod-borne infectious diseases in our study population. Together with previous reports on RVFV, rickettsiae of the typhus group and spotted fever group, we are demonstrating comparably high seroprevalences which could be caused by considerable exposure of the population to arthropod-borne infections other than malaria [23,26,27]. Akin to RVFV, a near-linear correlation of anti-*Alphavirus* IgG prevalence with age suggests endemic exposure rather than single or few epidemic events.

Acute *Alphavirus* infections such as chikungunya fever are neither known nor regularly diagnosed in the health facilities in the region, and might be overlooked by medical staff as a possible causative agent for febrile illness, leading to presentation at the health facility. Febrile disease in the area is mostly regarded as malaria by treating clinicians, despite the fact that our survey showed a marked reduction of *P. falciparum* infection since the introduction of artemether - lumefantrine as first line therapy in 2006 [28]. Therefore, the awareness for zoonoses as a possible underlying cause of febrile illness should be increased.

Our analysis shows that anti-*Alphavirus* IgG prevalence is associated with geographical features related to favourable mosquito breeding conditions. These include low to moderate elevations and flat terrain, which disposes to the formation of surface water collections [4].

Climate has been consistently pointed out as one of the major determinants for the distribution of vector borne diseases. Although lower larval rearing temperatures result in increased likelihood of adult female mosquitoes becoming infected with CHIKV or other arboviruses in laboratory experiments [29,30], it is higher temperatures which are generally linked to more efficient disease transmission in laboratory and epidemiological investigations [31]. However, the temperature variables examined here dropped out of the multi-variable model due to lack of multi-variable significance, with the elevation variable obviously producing a better fit than the satellite-measured land surface temperatures. In La Reunion, the spread of *Ae. albopictus* has been found to be limited to elevations <1200 m in summer [32]; in Gharwal/India, spread was limited to <1.400 m. This corresponds well with the drop in seroprevalence in strata above 1197 m (table 1). We still assume that temperature is the causal limiting factor in higher elevations, not other elevation-dependent factors such as radiation or atmospheric pressure. It should thus be kept in mind that our elevation results should not be generalized to predict infection risk in other climatic settings.

Comparisons with our data on spread of other mosquito-borne infections, namely RVF, *Flaviviridae* and *P. falciparum* malaria, and the tick-borne spotted fever group (SFG) rickettsiae, produce interesting findings. The uni-variable association of *P. falciparum* to anti-*Alphavirus* IgG disappears when adjusting for age, elevation and slope, suggesting that this association was due to factors supporting the breeding of the different mosquito vectors alike. We did not test to distinguish between anti-CHIKV IgG and o'nyong'nyong virus IgG due to lack of capacities to perform the neutralization test, so it is possible that the seroprevalence is caused by more than one virus.

A similar association exists between bednet ownership and anti-*Alphavirus* IgG. Bednet ownership is not homogenous over the study area, but more frequent in areas of higher malaria transmission, which in our data are characterised by low elevation and even terrain, favouring standing surface water as mosquito breeding grounds. This not only supports *Anopheles* but also other mosquito species, so the use of bednets can be seen as a proxy for general abundance of mosquitoes – hence the positive association in uni-variable analysis. When corrected for elevation and slope of terrain, the association with bednets disappeared – showing that in malaria endemic areas, bednet ownership neither favours nor protects against *Alphavirus* infection. This may point towards a diurnally active vector such as *Aedes* spp., against which bednets do not protect.

The association of anti-*Alphavirus* IgG with RVFV and *Flavivirus* IgG, viruses sharing *Aedes* spp. as vector, is however retained in multivariable analysis. This shows that in addition to age, elevation and slope, additional relevant factors still influence the spread of these *Aedes* – borne infections which remain to be identified. Others have found higher seroprevalences in Camerroonians living under corrugated iron roofs vs. thatched grass roofs; furthermore, living in rural areas was associated with higher seroprevalences [33].

SFG rickettsiae and *Alphaviruses* are transmitted by completely different vectors (cattle ticks and mosquitoes respectively), therefore the reason for the observed association is not clear. Rural living conditions, defined by low population density and long distances to roads, was a risk factor in our analysis of SFG rickettsia IgG [27]. Others authors also found this to be a risk factor for anti-*Alphavirus* IgG [33], so it is possible that rural conditions are the factor which increases the risk for both of these diseases which do not have much else in common.

Interesting are the differences in geographical spread of anti-*Alphavirus* IgG versus RVFV IgG in our population. Anti-*Alphavirus* IgG is more evenly distributed in the two Kyela subsites, and is also common in other sites. RVFV IgG on the other hand concentrates along the shore of Lake Malawi and nearby watercourses. The preferred occurrence of RVFV along water

Table 1. Association of anti-*Alphavirus* IgG status with environmental and socio-economic factors.

				uni-variable[1]			multi-variable[2]		
Covariate	**stratum**	**N**	**% pos.**	**PR**	**95% conf.int.**	**p-val**	**PR**	**95%conf.int.**	**p-val**
Age									
	per 10 years	1215		1.24	(1.18 to 1.30)	<0.001	1.26	(1.20 to 1.32)	<0.001
Slope									
	per degree	1215		0.58	(0.50 to 0.66)	<0.001	0.86	(0.77 to 0.95)	0.004
Elevation (m)									
	479.1-	122	50.0	1	-	-	1	-	-
	487.5-	122	43.4	0.87	(0.66 to 1.14)	0.317	1.09	(0.85 to 1.41)	0.504
	973.7-	118	33.9	0.68	(0.49 to 0.94)	0.019	0.73	(0.54 to 0.98)	0.038
	1197.8-	120	15.8	0.32	(0.20 to 0.51)	<0.001	0.41	(0.25 to 0.66)	<0.001
	1290.9-	123	4.9	0.10	(0.04 to 0.22)	<0.001	0.16	(0.07 to 0.37)	<0.001
	1491.4-	120	4.2	0.08	(0.03 to 0.20)	<0.001	0.13	(0.05 to 0.34)	<0.001
	1578.0-	122	10.7	0.21	(0.12 to 0.37)	<0.001	0.27	(0.15 to 0.47)	<0.001
	1612.8-	123	9.8	0.20	(0.11 to 0.35)	<0.001	0.23	(0.13 to 0.40)	<0.001
	1724.5-	123	5.7	0.11	(0.05 to 0.24)	<0.001	0.19	(0.08 to 0.43)	<0.001
	2002.8-	122	2.5	0.05	(0.02 to 0.15)	<0.001	0.10	(0.03 to 0.34)	<0.001
Gender							*This and the following variables were not included into multi-variable analysis due to lack of multi-variable significance.*		
	female	667	17.1	1	-	-			
	male	540	19.3	1.13	(0.89 to 1.42)	0.318			
	missing data	8	12.5	0.73	(0.12 to 4.61)	0.739			
SES Rank									
	per unit	1215		0.90	(0.86 to 0.94)	<0.001			
Bednet owned									
	No	692	10.4	1	-	-			
	Yes	523	28.1	2.70	(2.07 to 3.53)	<0.001			
Frequency of bednet use									
	Never	694	10.2	1	-	-			
	Sometimes	55	16.4	1.60	(0.84 to 3.05)	0.153			
	Most times	21	23.8	2.33	(1.10 to 4.94)	0.028			
	Always	443	30.0	2.93	(2.25 to 3.84)	<0.001			
	missing data	2	50.0	4.89	(1.20 to 19.87)	0.027			
Persons/km^2									
	per unit	1215		0.92	(0.87 to 0.97)	0.002			

Table 1. Cont.

				uni-variable[1]			multi-variable[2]		
Covariate	**stratum**	**N**	**% pos.**	**PR**	**95% conf.int.**	**p-val**	**PR**	**95%conf.int.**	**p-val**
Enhanced Vegetation Index (Max.)									
	per 0,1 units	1215		2.03	(1.56 to 2.65)	<0.001			
Enhanced Vegetation Index (Avg.)									
	per 0,1 units	1215		2.53	(2.04 to 3.14)	<0.001			
Enhanced Vegetation Index (Min.)									
	per 0,1 units	1215		2.49	(1.92 to 3.23)	<0.001			
Average Land Surface Temperature									
	per 10°	1215		1.73	(1.02 to 2.93)	0.043			
Night Land Surface Temperature									
	per 10°	1215		9.70	(6.91 to 13.60)	<0.001			
Rainfall									
	per unit	1215		1.01	(1.01 to 1.01)	<0.001			

Results of Poisson regression models adjusted for household clustering using robust variance estimates.
N= number of observations; % pos. = percent anti-*Alphavirus* IgG positive in stratum; PR = Prevalence ratio; 95% conf.int = 95% confidence interval; SES rank = rank (from 0 for lowest to 10 for highest) according to socio-economic score.
1: results of separate models for each of the below covariates.
2: multivariable model including only age, elevation and slope of terrain as covariates.
doi:10.1371/journal.pntd.0002979.t001

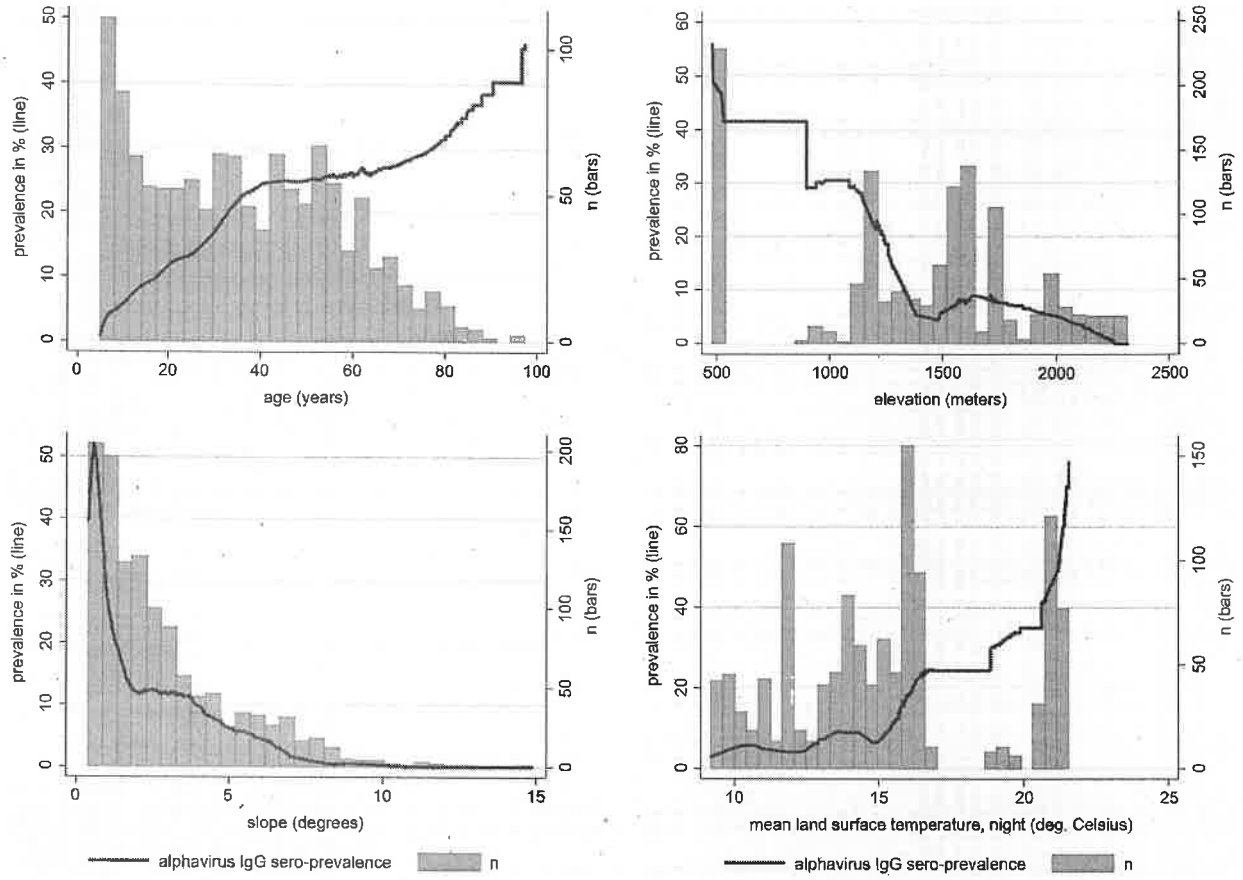

Figure 2. Anti-*Alphavirus* IgG prevalence in association with age, elevation, slope and land surface temperature. Lowess smoothed plots of anti-ChikV IgG-seroposivitivity over age, elevation, slope of terrain, and land surface temperature during the night. Red line: anti-*Alphavirus* seroprevalence. Grey bars: number of observations in stratum.
doi:10.1371/journal.pntd.0002979.g002

bodies has been demonstrated in other settings as well [34], but does not seem to apply to anti-*Alphavirus* IgG positivity in our setting. This observation may imply that the vectors of RVFV in Kyela region are floodwater mosquito species and therefore need the shore of Lake Malawi, whilst the vectors of the *Alphavirus* may show an anthropophilic behaviour. The strong affinity to water, which applies for RVFV, but not for the *Alphavirus*, may also be related to a higher density of cattle as RVFV reservoir hosts along the water, or with the transovarial transmission of RVFV in diapausing *Aedes* mosquitoes along waterbodies [35]. It is also possible that RVFV requires a temperature optimum as suggested by our previous work, with a direct correlation with higher minimum temperatures, lower maximum temperatures, and positive influence of dense vegetation, conditions that are fulfilled mainly at the lakeshore [23]. The causative *Alphavirus*, despite probably limited to a smaller number of vector species compared to RVFV, seems to be less specific in terms of ecological conditions and seems to show a more anthropophilic behaviour, leading to a wider spread of the virus throughout the study area.

In summary, our data suggest that CHIKV or a closely related *Alphavirus* like o'nyong'nyong virus is circulating in the study area. If this virus causes disease, it could be an important cause of febrile illnesses in the region, and may be currently underdiagnosed. The linear relation of seropositivity to age suggests endemic rather than epidemic cycling, opposed to a study from Kenya where seropositivity was linked by the authors to epidemic exposure [14]. Kenya was reportedly affected by past CHIKV outbreaks, while there are no reports of outbreaks from our study area. A study from northern Tanzania reported acute CHIKV infection in 7.9% of febrile hospitalized patients, demonstrating that CHIKV circulates between epidemics in the country and may well be responsible for the seroprevalences in our study [13].

The power of our statements is further limited by the stratified nature of our study cohort, which results in prevalence levels slightly different from the general population. Further, the serological method used does not allow distinguishing between different *Alphavirus* species, and only gives information on cumulative lifetime infection risk. Therefore, prospective studies are needed to establish the rate of acute fever caused by CHIKV or other *Alphaviruses* in febrile patients. If the infecting *Alphavirus* is shown to be CHIKV or another *Alphavirus* of human medical importance these results should lead to a re-assessment of the local diagnostic algorithm for febrile illnesses, to take into account the endemic presence of the causative *Alphavirus(es)* in the area. These studies would also have to answer the question whether endemic strains do have a reduced pathogenicity, and have evaded detection by not causing the typical symptoms.

Table 2. Association of anti-*Alphavirus* IgG status with other infectious diseases.

				uni-variable[1]			adjusted[2]		
Covariate	**stratum**	**N**	**% pos.**	**PR**	**95% conf.int.** ‡	**p**	**PR**	**95%conf.int.**	**p**
SFG Rickettsiae IgG status									
	neg.	392	9.4	1	-	-	1	-	-
	pos.	823	22.1	2.34	(1.67 to 3.28)	<0.001	1.51	(1.11 to 2.06)	0.008
Rift Valley fever IgG status									
	neg.	1151	15.2	1	-	-	1	-	-
	pos.	62	71.0	4.67	(3.78 to 5.76)	<0.001	1.68	(1.25 to 2.25)	0.001
	miss.[3]	2	0.0	ND	-	-	ND	-	-
Any *Flavivirus* IgG status									
	neg.	1049	13.3	1	-	-	1	-	-
	pos.	161	49.1	3.68	(2.95 to 4.59)	<0.001	1.34	(1.06 to 1.70)	0.013
	miss.[3]	5	0.0	ND	-	-	ND	-	-
***P. falciparum* status**									
	neg.	1195	17.8	1	-	-	1	-	-
	pos.	18	33.3	1.87	(0.96 to 3.64)	0.065	1.01	(0.53 to 1.94)	0.970
	miss.[3]	2	0.0	ND	-	-	ND	-	-

Results of Poisson regression models adjusted for household clustering using robust variance estimates.
N = number of observations; % pos. = percent anti-*Alphavirus* IgG positive in stratum; PR = Prevalence ratio; 95% conf.int = 95% confidence interval; SFG: spotted fever group rickettsiae; IgG = Immunogolbulin G; ND = not done.
1: results of separate uni-variable models for each of the above infections.
2: results of separate multi-variable models for each of the above infections adjusted for age, elevation and slope of terrain as covariates.
3: 95% confidence interval and p-value not calculated due to lack of variability of the outcome variable in this stratum.
doi:10.1371/journal.pntd.0002979.t002

Supporting Information

Strobe Checklist S1 The STROBE checklist for quality assurance in reporting of observational studies is attached. (PDF)

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Author Contributions

Conceived and designed the experiments: GD ES TL MH NH. Performed the experiments: NW NEN PC LM IK. Analyzed the data: ES NH NW GD. Contributed reagents/materials/analysis tools: GD. Wrote the paper: NW PC ES TL NH.

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10.2 Publikation II

II. High seroprevalence of Rift Valley Fever and evidence for endemic circulation in Mbeya region, Tanzania, in a cross-sectional study.

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PLoS NEGLECTED TROPICAL DISEASES

High Seroprevalence of Rift Valley Fever and Evidence for Endemic Circulation in Mbeya Region, Tanzania, in a Cross-Sectional Study

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Abstract

Background: The Rift Valley fever virus (RVFV) is an arthropod-borne phlebovirus. RVFV mostly causes outbreaks among domestic ruminants with a major economic impact. Human infections are associated with these events, with a fatality rate of 0.5–2%. Since the virus is able to use many mosquito species of temperate climates as vectors, it has a high potential to spread to outside Africa.

Methodology/Principal Findings: We conducted a stratified, cross-sectional sero-prevalence survey in 1228 participants from Mbeya region, southwestern Tanzania. Samples were selected from 17,872 persons who took part in a cohort study in 2007 and 2008. RVFV IgG status was determined by indirect immunofluorescence. Possible risk factors were analyzed using uni- and multi-variable Poisson regression models. We found a unique local maximum of RVFV IgG prevalence of 29.3% in a study site close to Lake Malawi (N = 150). The overall seroprevalence was 5.2%. Seropositivity was significantly associated with higher age, lower socio-economic status, ownership of cattle and decreased with distance to Lake Malawi. A high vegetation density, higher minimum and lower maximum temperatures were found to be associated with RVFV IgG positivity. Altitude of residence, especially on a small scale in the high-prevalence area was strongly correlated (PR 0.87 per meter, 95% CI = 0.80–0.94). Abundant surface water collections are present in the lower areas of the high-prevalence site. RVF has not been diagnosed clinically, nor an outbreak detected in the high-prevalence area.

Conclusions: RVFV is probably circulating endemically in the region. The presence of cattle, dense vegetation and temperate conditions favour mosquito propagation and virus replication in the vector and seem to play major roles in virus transmission and circulation. The environmental risk-factors that we identified could serve to more exactly determine areas at risk for RVFV endemicity.

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Introduction

The Rift Valley fever virus (RVFV), a member of the genus Phlebovirus in the family Bunyaviridae, was first isolated in 1930 during an outbreak in Kenya. Rift Valley fever (RVF) occurs endemically and epidemically in most parts of sub-Saharan Africa and epidemically in Egypt, Madagascar and the Comoros. In 2001 it was detected for the first time outside of Africa during an outbreak in Yemen and Saudi-Arabia [1,2,3,4,5].

The disease is mostly apparent in epizootic events with large numbers of sick cattle, and a high abortion rate in pregnant animals ("abortion storm"), with adverse economic consequences for cattle herders, including bans on animal trade [4]. Transmission to humans is common during such events. In the majority of cases, human infection is oligo- or asymptomatic, but may cause hepatitis, hemorrhagic fever, encephalitis and retinitis, with fatality rates of 0.5 to 2%, and permanent vision impairments after retinitis [4].

Contrary to the assumption of virus persistence and inactivity between outbreaks, some evidence for inter-epidemic circulation of RVFV has been reported from the Senegal and from northern Kenya, using a serology approach to detect antibodies in samples from children born after the last reported outbreak [6,7].

The most important vectors for RVFV are *Aedes* and *Culex* mosquitoes. However, RVFV has also been isolated from *Anopheles spp*, *Simulium* blackflies, sand flies and *Amblyomma* ticks [2,4,8], which may represent remnants of a blood meal rather than the

Author Summary

We describe a high seropositivity rate for Rift Valley fever virus, in up to 29.3% of tested individuals from the shore of Lake Malawi in southwestern Tanzania, and much lower rates from areas distant to the lake. Rift Valley fever disease or outbreaks have not been observed there in the past, which suggests that the virus is circulating under locally favorable conditions and is either a non-pathogenic strain, or that occasional occurrence of disease is missed. We were able to identify a low socio-economic status and cattle ownership as possible socio-economic risk factors for an individual to be seropositive. Environmental risk factors associated with seropositivity include dense vegetation, and ambient land surface temperatures which may be important for breeding success of the mosquitoes which transmit Rift Valley fever, and for efficient multiplication of the virus in the mosquito. Low elevation of the home, and proximity to Lake Malawi probably lead to abundant surface water collections, which serve as breeding places for mosquitoes. These findings will inform patient care in the areas close to Lake Malawi, and may help to design models which predict low-level virus circulation.

ability to transmit the pathogen. Direct transmission through infectious body fluids is of relevance mainly during epizootic/epidemic events [5,9]. As many competent vector species occur outside Africa, a high potential for further geographical spread is attributed to the virus, and RVF is classified as an emerging disease [4,10].

RVF outbreaks are known to occur predominantly after unusual flooding events. *Aedes* mosquito species are seen as vectors and reservoir, since their transovarially infected eggs withstand desiccation and larvae hatch when in contact with water [6,11]. Transovarial transmission is assumed as mechanism of virus persistence between epizootic events.

After flooding, the *Aedes* mosquito populations will multiply in the persisting water collections, and develop into infectious adult mosquitoes. The RVFV may amplify in wild and domestic ungulates and may reach epizootic and epidemic dimensions [8]. The presumed link between extraordinary flooding events and RVF outbreaks was validated, among others, by a successful prediction of the 2007 outbreak in Somalia, Kenya and northern Tanzania, using climate modelling [12].

A number of variables associated with higher likelihood for RVFV Immunoglobulin G (RVFV IgG) positivity have been identified. Among them are the proximity to perennial surface water bodies and proximity to ruminants [7,13].

Here we report a cross-sectional seroprevalence study that used samples from 1228 participants collected during a cohort study (EMINI) from the Mbeya region in Southwestern Tanzania, an area from which no RVF disease activity has been reported previously. The objective was to assess any RVFV circulation that had possibly remained undetected, and to describe infection patterns and factors associated with seropositivity.

Methods

Ethics statement

Both EMINI and this substudy were approved by Mbeya Medical Research and Ethics Committee, Tanzanian National Institute for Medical Research – Medical Research Coordinating Committee, as well as by the Ethical Commission of University of Munich. Each EMINI participant had provided written informed consent before enrolment. Parents consented for participation of their children.

Study population

Data and samples for this study were collected between June 2007 and June 2008 during the second annual survey of the EMINI (Evaluating and Monitoring the Impact of New Interventions) cohort study. Before the start of EMINI, a census of the complete population had been conducted in nine geographically distinct sites of the Mbeya Region in Southwestern Tanzania, which had been selected to represent a wide variety of environmental and infrastructural settings, including urban and rural sites, different proximity to main roads, elevation above sea-level etc (Figure 1). During the census we collected basic information regarding the households and their inhabitants, and recorded all household positions, using handheld GPS receivers. Ten percent of the census households and all their inhabitants were chosen by geographically stratified random selection to participate in the 5-year longitudinal EMINI cohort study, resulting in a representative sample of the population in the nine study sites. Every year, each participating household was visited to conduct structured interviews and to collect blood and other specimen from all household members. Blood samples were cryopreserved after cells were separated from serum.

For this substudy, we stratified the 17,872 participants, who had provided a blood sample in the second EMINI survey, by age, gender, altitude of residence and ownership of domestic animals (mammals), to be able to assess factors of interest that were identified in the literature but might have been underrepresented in the general population. We employed disproportionate random sampling with equal participant numbers for each stratum to identify 1228 samples from participants above the age of 5 years to be tested for RVFV IgG.

Socio-economic status (SES)

During the annual EMINI visits, we conducted interviews with the head of each household regarding the socio-economical and infrastructural setting in and around the household. With this information we constructed an SES score that characterizes the socio-economic situation of each household, employing a modification of a method originally proposed by Filmer & Pritchett that uses principal component analysis and has been widely applied to assess wealth and poverty in developing countries [14,15,16]. The score included the following information: Availability of different items in the household (clock or watch, radio, television, mobile telephone, refrigerator, hand cart, bicycle, motor cycle, car, savings account); sources of energy and drinking water; materials used to build the main house; number of persons per room in the household and availability and type of latrine used.

Environmental data

Population- and livestock-densities were calculated using data and household positions collected during the initial population census.

Elevation data were retrieved from the NASA Shuttle Radar Topography Mission (SRTM) global digital elevation model, version 2.1 [17,18].

Land surface temperature (LST) and vegetation cover (EVI = enhanced vegetation index) data were retrieved from NASA's Moderate-resolution Imaging Spectroradiometer (MODIS) Terra mission which "are distributed by the Land Processes Distributed Active Archive Center (LP DAAC), located at the U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS)

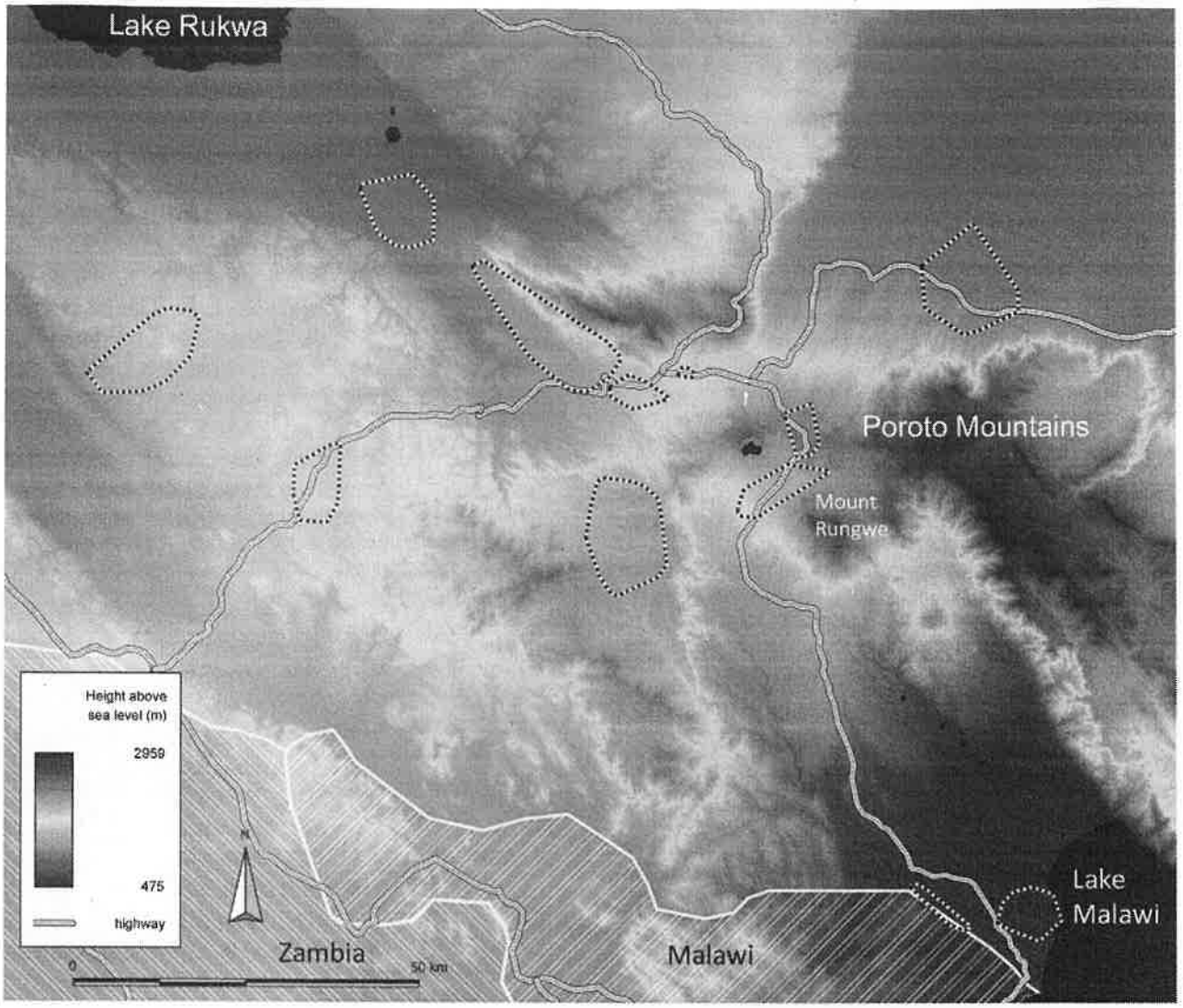

Figure 1. Location and Elevation of the Study Sites. A geographical map of the study area and sites.
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Center (lpdaac.usgs.gov)." [19]. LST data (Version MOD11A2) have 8 days temporal and ~1 km spatial resolution , EVI data (Version MOD13Q1) have 16 days temporal and 250 m spatial resolution. Both, LST and EVI data were processed in the following way to produce long-term averages: After download via FTP, data surfaces for every 8 day period for the years 2000 to 2008 (LST) and every 16 days period for the year 2007 (EVI) were reprojected to Universal Transverse Mercator projection (zone 36 South) using the MODIS reprojection tool (MRT) [20] and imported into Idrisi GIS software (version 32, Clark Labs, Worcester, MA, USA). In Idrisi, 8 year averages of annual average and maximum day-LST and average and minimum night-LST and 2007 EVI averages were calculated for each pixel utilising only those pixels that were "good quality" according to the quality assessment layers that are distributed together with the actual data. Then LST was converted to degrees Celsius and EVI was converted back to its native range between −1 and +1.

All above environmental data were then combined with the houshold position data in a GIS database using Manifold System 8.0 Professional Edition (Manifold Net Ltd, Carson City, NV). Population-, household-, and livestock-densities, LST, EVI, and elevation data were averaged for a buffer area within 1000 meter radius around each household in order to characterize the ecological situation around the household. This approach was preferred to using the respective spot values at the household position, because spot data are more prone to random error than averages for a wider area.

Serology

Anti-RVFV IgG was detected by indirect immunofluorescence assay (IIFA), following a methodology adapted from Swanepoel [21]. Each serum sample was screened for the presence of anti-RVFV IgG, using a commercial biochip with a mixture of infected and non-infected Vero E6 cells on one field (positive field) and non-infected Vero E6 cells on a negative control field (Euroimmun, Lübeck, Germany).

Sensitivity and specificity of the IIFA test were tested using 20 negative sera from German blood donors and five sera positive for IgG against Sandfly Toscana virus, Sandfly Naples virus, Sandfly Sicilian virus, Puumala virus, Tahyna virus and Bunyamwera

virus. No cross reactivities with other members of the genus Phlebovirus or the viruses of other genera of the family Bunyaviridae were detected.

Serum samples were screened in a dilution of 1:10, using standard procedures for IIFA. A rabbit anti-human IgG FITC-labelled antibody (DAKO, Hamburg, Germany) was used as conjugate. A sample was classified as positive if a typical fine granular cytoplasmatic fluorescence in some groups of cells on the positive field of the biochip was detected, with no detectable fluorescing cytoplasmatic signal in the negative field. Each sample was independently assessed by two experienced observers. Results were compared and re-tested if discrepant. A part of the positive sera was re-tested by titration, and all tested sera were found to have IgG titres between 1:20 and 1:640.

Data analysis

Stata statistics software (version 11, Statacorp, College Station, TX, USA) was used for all statistical analyses, maps were produced in Manifold System 8.0 Professional Edition (Manifold Net Ltd, Carson City, NV).

After exploratory data analysis, it became clear that RVFV seroprevalence in Bujonde-Kajunjumele (BK) subsite was much higher than in all other study locations. We therefore decided to first analyse data for BK separately before trying to develop models including the data for all sites.

Since none of the continuous variables that we examined was normally distributed according to the Shapiro-Wilk and Shapiro-Francia tests for normality, the median and interquartile range (instead of mean and standard deviation) of these variables are reported to characterize the study area and population in BK and in all other sites. This is also the reason why the non-parametric Wilcoxon ranksum test was used to assess differences between BK and all other sites regarding continuous variables. Differences between sites regarding binary variables (RVF seropositivity, gender and cattle ownership) were assessed by chi square testing. The association of binary RVFV IgG status with possible risk factors was examined using uni- and multi-variable poisson regression models with robust variance estimates adjusted for within household clustering [22,23]. Uni-variable regression models were used to identify possible risk-factors for inclusion into the multi-variable model for this site. Variables with a p-value$<$0.2 in uni-variable regression and other variables that did not fulfill this criterion, but where an association with RVFV IgG seemed likely due to biological reasons (gender, and all variables related to the presence of ruminants), were further evaluated in multi-variable regression models and were retained in the final multi-variable model if their p-value was $<$0.1. Because most variables characterizing the natural environment (LST, vegetation, elevation and distance to Lake Malawi) showed strong collinearity, they were not included into the same model but entered one by one into models adjusted for the other variables that were included into the final model.

Once the final multi-variable model for BK site was identified, we used the same approach to identify a multi-variable model where data for all sites including BK were pooled. Prevalence ratios (PR) and 95% confidence intervals for covariates mentioned in the text refer to multi-variate analysis within BK site, if not mentioned otherwise.

Results

Site characteristics and seroprevalence

Of the 1228 analyzed sera, 5.2% (64 sera) were positive for RVFV IgG. This translates into an estimated overall population prevalence of 3.1% when extrapolated from our stratified sample to the underlying population of the 9 study sites.

We found a unique local maximum of 29.3% (95% confidence interval (CI) 22.2–37.3) seroprevalence in Bujonde-Kajunjumele (BK), a subsite of the Kyela site, which is situated close to Lake Malawi. The prevalence in the other sites ranged from 0.0% to 3.4% (table 1, Figure 2, 3).

We thus decided to analyze covariates within the high-prevalence setting of BK site, and to compare BK to the low-prevalence sites, in order to better understand possible causes for this marked difference.

With an altitude range of 479 to 492 meters, BK is the lowest of our study sites, while the other sites range from 499 m to 2316 m (table 1, Figure 1). The two Kyela subsites BK and Katumba-

Table 1. Characteristics of Bujonde-Kajunjumele site (N = 150) and all other sites (N = 1078).

	BK-site Median (IQR) or % (N)		All other sites Median (IQR) or % (N)		p[a]
RVF IgG positive	29.3%	(44)	1.9%	(20)	<0.001
Female gender	55%	(82)	55%	(590)[b]	0.885
Age (years)	32.1	(17.8 to 53.2)	34.2	(16.9 to 51.9)	0.684
SES score	−0.81	(−1.19 to −0.42)	−0.06	(−0.54 to 0.55)	<0.001
Cattle owned	49%	(74)	28%	(307)	<0.001
Elevation (meters)	484	(481 to 487)	1570	(1207 to 1714)	<0.001
Vegetation Density (EVI*10)	3.83	(3.60 to 4.05)	2.87	(2.56 to 3.27)	<0.001
Max. LST (°C)	43.1	(39.5 to 45.4)	45.7	(42.9 to 46.5)	<0.001
Average Day LST (°C)	30.9	(29.3 to 31.8)	32.5	(30.2 to 33.2)	<0.001
Min. LST (°C)	14.9	(13.5 to 15.7)	11.0	(7.3 to 11.9)	<0.001
Cattle Density (cows/km^2)	165	(143 to 190)	68	(34 to 186)	<0.001
Distance to Lake Malawi (km, BK only)	2.9	(1.4 to 5.2)			

BK = Bujonde-Kajunjumele; IQR = interquartile range; SES = socio-economic status; EVI = enhanced vegetation index; LST = land surface temperature.
[a]p-value of chi square test (for binary variables) or Wilcoxon Ranksum test (for continuous variables) for difference between BK and all other sites.
[b]gender unclear for 11 participants.
doi:10.1371/journal.pntd.0001557.t001

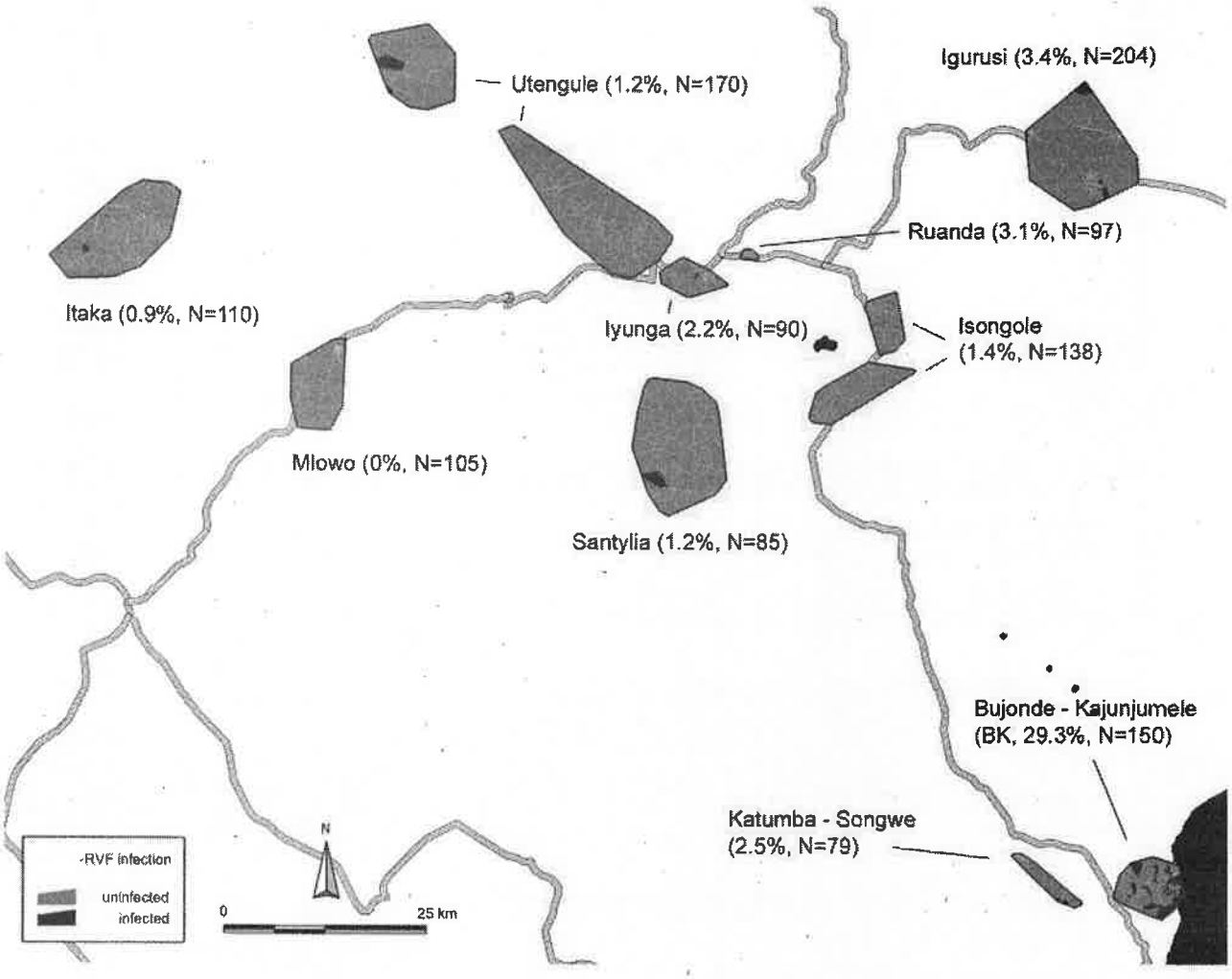

Figure 2. Location of Households with IgG-positive Participants in the entire Study Area. Location of households displayed in Voronoi polygons, with every polygon representing one household.
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Songwe are the only sites south of the Poroto mountain range, and receive the highest amount of annual rainfall (1956 and 2292 mm, respectively), whereas the average across all sites is 1473 mm.

Further characteristics of BK site, compared to all other sites, are listed in table 1. Of special relevance are denser vegetation, lower temperature variability (higher minimum and lower maximum land surface temperatures), higher cattle density and more frequent ownership of cattle, which is presumed to be the main animal host of RVFV. During the rainy season, wide areas close to Lake Malawi are flooded, especially where the terrain is marshy and barely above the Kiwira river's water level (Figure 4).

Analysis of potential risk-factors

As demonstrated in Figure 5, RVFV IgG prevalence rises with age in our study population. This is in agreement with the poisson regression results for BK (table 2; prevalence ratio (PR) 1.02 per year of age, 95% CI 1.01–1.03), and for the pooled results from all sites, where age is significantly associated with rising RVFV IgG prevalences, both in uni- and in multi-variable regression models (table 3; PR 1.02 per year of age, 95% CI 1.01–1.03). Increasing socio-economic status is associated with decreasing RVFV IgG prevalences (BK site: PR 0.60 per unit, 95% CI 0.40–0.90), whereas gender appears not to influence RVFV IgG prevalence in the study population (uni-variable PR for male gender as compared to female in BK, 1.0, 95% CI 0.61–1.65).

According to the multi-variable models, cattle ownership is significantly associated with RVFV seroprevalence, both in BK and in all sites (PR 1.81, 95% CI 1.15–2.85 for BK; PR 1.76, 95% CI 1.15–2.71 for all sites), although it's uni-variable association in BK is far from significant. Cattle density per square kilometer is a significant prognostic factor in all sites (PR 2.06 per 100/skm, 95% CI 1.64 to 2.59, multi-variable model), including BK, where mean cattle density is higher than in the other sites.

Due to collinearity between the examined environmental variables, these could not be simultaneously included into one model, but were entered one at a time into multi-variable models that were adjusted for age, SES, cattle ownership, and – for the all-sites pooled model – cattle density. Of these environmental variables, vegetation density (EVI) results in the model with the best fit, both in BK and in the pooled analysis. However, most other environmental factors are also strongly associated with RVFV IgG prevalence. It is noteworthy though, that maximum and average land surface temperature (LST) during the day have significant negative associations (PR 0.87 per °C, 95% CI 0.81–

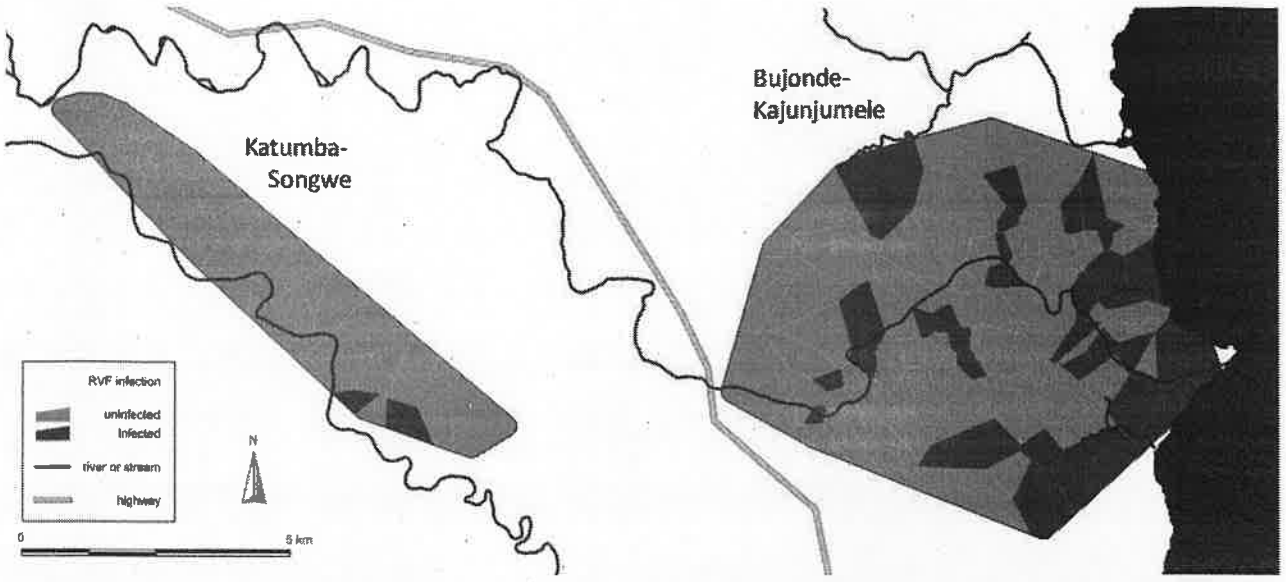

Figure 3. Location of households with RVF IgG-positive participants in Katumba-Songwe and Bujonde-Kajunjumele (BK) sites. Location of households with RVF IgG-positive participants displayed as Voronoi polygons.
doi:10.1371/journal.pntd.0001557.g003

0.94 for max. LST; PR 0.73, 95% CI 0.61–0.86 for average day LST, both multi-variable for BK), whereas average LST during the night has a positive association with RVFV seroprevalence (PR 2.51 per °C, 95% CI 0.94–6.70). Minimum LST was less strongly associated than the other LST variables in the pooled analysis and unrelated to RVFV IgG prevalence in BK site.

Other factors that we examined (population density and the ownership of livestock other than cattle) do not show any strong associations with RVFV infection in our study population (data not shown).

Association with other mosquito-borne and water-borne infections

Within the EMINI study population our group also collected data on chikungunya virus IgG, *P. falciparum* malaria (ICT Malaria P.f./P.v. ICT Diagnostics, Cape Town, South Africa) and presence of *W. bancrofti* filarial antigen (TropBio® Og4C3 serum ELISA, Townsville, Australia). We found that on a household level, RVFV IgG positivity was strongly associated with chikungunya virus IgG in BK site and in all other sites (PR = 4.3; 95% CI 2.3–8.1; PR = 5.3, 95% CI 2.1–13.5, respectively), and with filarial antigen (PR = 2.2; 95% CI 1.3–3.7) and P.falciparum malaria (PR = 4.2, 95% CI 3.3–5.5) in BK. No association was found with *Schistosoma haematobium* infection in BK, nor in the other sites.

Figure 4. Surface Water Collections in Bujonde-Kajunjumele Site. Surface water collections situated close to Lake Malawi. At the end of the rainy season in April, the high prevalence area close to Lake Malawi, with elevations barely above the Kiwira river water level, is characterized by abundant waterlogging, with surface water between homesteads.
doi:10.1371/journal.pntd.0001557.g004

Discussion

The presented analyses identify several socio-demographic and environmental factors that are strongly associated with RVFV seropositivity in our study population. As prevalences in all sites apart from BK are relatively low, we were unsure whether the few cases in the non-BK sites were autochthonous or imported cases and whether an overall analysis of all sites would really yield credible results. It is therefore reassuring that the results of the BK only analysis and those for all sites are similar.

Socio-economic variables

Figure 4 shows that RVFV seroprevalence increases with age, which is in line with the regression results for BK and for all sites. This suggests an endemic circulation of RVFV in our study area, rather than a single outbreak event as reason for the detected seroprevalence.

The inverse association of SES with RVFV IgG means that more affluent people are at lower risk of infection. This has been described for many different infectious diseases in a wide range of settings. Importantly, cattle ownership was not used for SES calculation, as it is a direct risk factor.

Environmental variables

Despite the strong associations that we found for age and SES, most of the examined environmental variables were still significantly associated with RVFV IgG prevalence, when adjusted for possible socio-economic confounding in the multi-

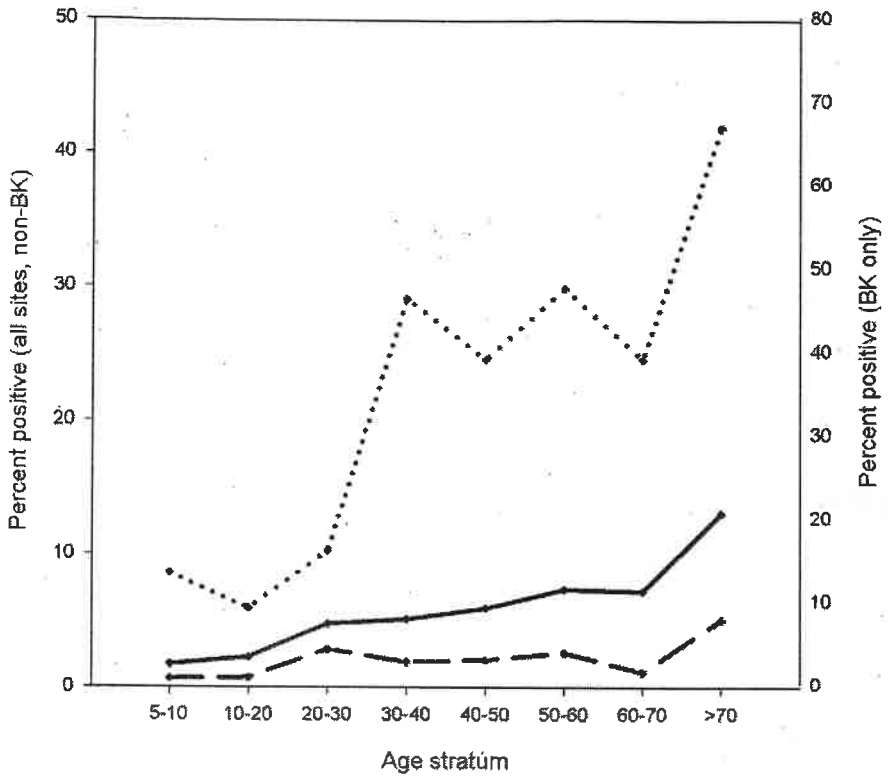

Figure 5. Seropositivity of RVFV IgG by Age. Dotted line: percent positives in BK site, N = 150. Solid line: percent positives in all sites including BK, N = 1228. Dashed line: percent positives in all sites other than BK, N = 1078. Please note the different scales for BK (right axis) and for all sites and non BK sites (left axis).
doi:10.1371/journal.pntd.0001557.g005

variable models showing that their association with RVF is independent.

Our findings that cattle ownership and density of cattle in the area are important factors for RVFV seropositivity were to be expected, since ruminants are the main animal host of RVFV [4]. Cattle owners in BK have the habit of tethering their animals on the doorsteps of their houses at night for fear of theft, providing an animal reservoir of RVFV in proximity of humans, and reportedly increasing the number of *Culex* mosquitoes in the house [24].

Some previously described risk factors for RVF were confirmed in our study: dense vegetation and proximity to perennial water bodies were found associated with RVFV seropositivity in ruminant herds in the Senegal and in humans in Gabon [13,25]. These and other risk factors seem to make the BK site uniquely favorable for human and animal RVFV infection.

In BK, only the low-lying areas close to Lake Malawi are subjected to regular flooding during the rainy season, whereas areas further away from the lake and at slightly higher elevation are not flooded. This phenomenon provides abundant mosquito breeding places in low-lying areas, and is a likely reason for the strong negative association of altitude with RVFV seropositivity, that – in BK site – is already visible on a per meter scale, and for the association with distance from the lake. Frequent waterlogging has lead to large areas of BK site being used for wetland rice cultivation. One report from the 2006–2007 outbreak in Kenya found that soils that retain water were more frequently found in RVF-affected areas than in other areas [26].

Over the entire study area with an altitude range of 479 to 2313 m, it seems obvious that higher elevation negatively affects mosquito breeding and survival.

The association of RVFV seropositivity with other mosquito-borne diseases transmitted by *Anopheles*, *Aedes* and *Culex* species is in agreement with above considerations regarding the role of mosquito favorable habitats as an important factor contributing to RVF prevalence in BK site. However, in our study, RVFV seropositivity is not associated with *S. haematobium*, a water-borne disease. *Bulinus* snails, the intermediate hosts of *S. haematobium*, require permanent water-bodies [27]. Thus, RVFV infection in BK does not seem to depend on proximity to Lake Malawi itself, which is a reservoir for *S. haematobium*, but is more likely a consequence of the seasonal surface water collections that are more common close to the lake. Although it is difficult to single out the predominant factors causing the observed difference between BK and the neighboring low-prevalence site Katumba-Songwe, we presume that the difference in altitude and distance to the lake, and their impact on surface water collections, are the most important reasons.

According to our results, RVFV seropositivity seems to be associated with an optimum temperature range. An adverse effect of low temperatures has been shown for RVFV replication and infectiousness, e.g. in the vector *Culex pipiens* [28,29,30], while higher temperatures above 27–32°C adversely affect hatching success and size of adult *Aedes aegypti* mosquitoes [31]. The correlation with EVI may be explained by dense vegetation protecting water pools from being heated in the sunlight, and from cooling off at night. Furthermore, vegetation density can be regarded as a proxy for the presence of water. Seasonal increases in vegetation are associated with RVF outbreaks on a larger scale and are used for predicition [32,33]. Our results confirm this association on a small scale.

Table 2. Socio-economic and environmental factors and association with RVFV IgG[a] in BK.

Covariate		univariable			multivariable[b d]		
	stratum	PR	(95%CI)	p	PR	(95%CI)	p
Gender[b]							
	female[c]	1					
	male	1.00	(0.61 to 1.65)	0.985			
Age							
	per year	1.02	(1.01 to 1.04)	<0.001	1.02	(1.01 to 1.03)	<0.001
SES score							
	per unit	0.53	(0.34 to 0.84)	0.006	0.60	(0.40 to 0.90)	0.014
Cattle owned							
	no[c]	1			1		
	yes	1.23	(0.74 to 2.05)	0.421	1.81	(1.15 to 2.85)	0.010
Cattle per sqkm[b]							
	per 100	0.84	(0.48 to 1.45)	0.522			
Vegetation (EVI)							
	per unit	3.98	(1.84 to 8.61)	<0.001	2.99	(1.34 to 6.65)	0.007
					Results for other environmental variables when included into the above model instead of vegetation:		
Dist. to Lake Malawi[d]							
	per km	0.74	(0.64 to 0.86)	<0.001	0.79	(0.69 to 0.90)	<0.001
Elevation[d]							
	per m	0.84	(0.77 to 0.93)	<0.001	0.87	(0.80 to 0.94)	0.001
LST maximum[d]							
	per °C	0.84	(0.77 to 0.92)	<0.001	0.87	(0.81 to 0.94)	<0.001
LST average day[d]							
	per °C	0.67	(0.56 to 0.80)	<0.001	0.73	(0.61 to 0.86)	<0.001
LST average night[d]							
	per °C	3.82	(1.28 to 11.45)	0.017	2.51	(0.94 to 6.70)	0.066
LST minimum[d]							
	per °C	1.18	(0.94 to 1.49)	0.149	1.07	(0.88 to 1.31)	0.473

N for BK site = 150.
PR = prevalence ratio; SES = socio-economic status; skm = square kilometre; EVI = enhanced vegetation index; LST = land surface temperature.
[a]results of uni- and multivariable poisson regression with robust variance estimates adjusted for clustering within household.
[b]gender and cattle per skm were not included into multivariable model due to lack of significance.
[c]reference stratum.
[d]to avoid collinearity problems, the environmental variables (vegetation, distance to lake, elevation and the four LST variables) were entered separately into models adjusted for age, SES, and cattle ownership. Multivariable results for these three adjustment variables are those for the model that included vegetation.
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It is a limitation of this study that only serologic findings were available for analysis, specific questioning regarding RVF-related symptoms and sequelae to assess clinical significance of serological findings was not possible because samples were analysed retrospectively. However, conduct of this study within the very well characterised EMINI cohort allowed for a detailed analysis of socio-economic, spatial and ecological covariates on a small scale. Given the persistence of IgG responses over several years, the actual date of infection cannot be deduced from these examinations, and socio-economic and environmental conditions at the time of infections may have differed from the time of participant assessment for EMINI. The presence of RVFV IgG in the younger age groups suggests an ongoing or recent virus circulation.

Public health significance

There are no previous reports of RVF in Mbeya region, and to our knowledge the disease was never diagnosed clinically in Kyela. Since no virus isolation has yet been done in our study, it remains to be elucidated whether the cycling virus is a less virulent RVFV strain such as the apathogenic "clone 13" from the Central African Republic [34,35], or whether acute cases of RVF have been overlooked in the past. Taking into account the relatively high prevalence for malaria and HIV in the area [36], RVFV encephalitis, retinitis and hemorrhagic fever would be comparatively rare events, which may have been misdiagnosed as malaria- or HIV related morbidity, as is often the case with febrile illnesses in malaria-endemic areas [37,38].

Conclusion

In conclusion, this study finds a relatively high RVFV IgG prevalence in an area without previous reports of RVF, and identifies several environmental factors that are associated with RVF infection, independently of age and socio-economic status.

Table 3. Socio-economic and environmental factors and association with RVFV IgG[a] in all sites, including BK.

Covariate		univariable			multivariable[b]		
	stratum	PR	(95%CI)	p	PR	(95%CI)	p
Gender[b]							
	Female[c]	1					
	male	1.12	(0.69 to 1.82)	0.643			
Age							
	per year	1.02	(1.01 to 1.04)	<0.001	1.02	(1.01 to 1.03)	<0.001
SES score							
	per unit	0.31	(0.20 to 0.48)	<0.001	0.51	(0.33 to 0.78)	0.002
Cattle owned							
	no[c]	1			1		
	yes	1.73	(1.05 to 2.86)	0.033	1.76	(1.15 to 2.71)	0.010
Cattle per sqkm							
	per 100	1.67	(1.39 to 2.00)	<0.001	2.06	(1.64 to 2.59)	<0.001
Vegetation (EVI)							
	per unit	6.31	(3.68 to 10.81)	<0.001	2.94	(1.87 to 4.63)	<0.001
					Results for other environmental variables when included into the above model instead of vegetation:		
Elevation[d]							
	per 100 m	0.79	(0.74 to 0.84)	<0.001	0.85	(0.79 to 0.90)	<0.001
LST maximum[d]							
	per °C	0.88	(0.83 to 0.92)	<0.001	0.87	(0.83 to 0.92)	<0.001
LST average day[d]							
	per °C	0.88	(0.82 to 0.94)	<0.001	0.83	(0.76 to 0.92)	<0.001
LST average night[d]							
	per °C	1.46	(1.32 to 1.63)	<0.001	1.31	(1.81 to 1.46)	<0.001
LST minimum[d]							
	per °C	1.45	(1.18 to 1.79)	<0.001	1.21	(0.99 to 1.47)	0.063

N for all sites = 1228.
PR = prevalence ratio; SES = socio-economic status; skm = square kilometre; EVI = enhanced vegetation index; LST = land surface temperature.
[a]results of uni- and multivariable poisson regression with robust varicance estimates adjusted for clustering within household.
[b]gender was not included into multivariable model due to lack of significance not included into multivariable model due to lack of significance.
[c]reference stratum.
[d]to avoid collinearity problems, the environmental variables (vegetation, elevation and the four LST variables) were entered separately into models adjusted for age, SES, cattle ownership and cattle density. Multivariable results for these four adjustment variables are those for the model that included vegetation.
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If confirmed in future studies, these findings have important implications in the areas close to Lake Malawi, where health facilities and their staff should be made aware of RVF as a possible diagnosis for their patients. The environmental risk-factors for RVF infection that we identified could serve to predict areas of RVFV endemicity, in addition to outbreak prediction which can be done based on rainfall and vegetation data. It would be interesting to do further studies in similar high risk areas, since it is likely that undetected endemic cycling of RVFV is occurring in many areas apart from our study site.

Supporting Information

Checklist S1 STROBE checklist.
(DOC)

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Author Contributions

Conceived and designed the experiments: NH ES PC TL GD MH. Performed the experiments: NW PC IK GD. Analyzed the data: NH ES NW GD MH. Contributed reagents/materials/analysis tools: ES PC EN HM LM. Wrote the paper: NH ES GD PC. Acquisition of funding: MH NH. Supervision of acquisition of data: NH ES PC EN HM LM GD.

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