Characterising the endemicity and disease burden of chikungunya virus infections among children in coastal Kenya

A thesis submitted for the degree of *Doctor of Philosophy*

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Abstract

Chikungunya fever (CHIKF) is a viral illness of significant public health concern characterised by acute, often chronic, debilitating joint pain and polyarthritis. CHIKF is caused by the mosquito-borne chikungunya virus (CHIKV) that was first discovered in Tanzania in 1952. CHIKV re-emerged in coastal Kenya in 2004 causing the largest CHIKF epidemic on record. The epidemic affected millions of people, with rapid geographic spread to other countries in Africa, India, southeast Asia and Europe. Descriptions of this and other more recent epidemics outside Africa have informed most of what we know about CHIKF. However, very little is known regarding the burden, distribution, risk factors and clinical manifestation of CHIKV infections in Africa especially among the paediatric population. In this DPhil project I aimed to estimate the burden of CHIKF among children in coastal Kenya over a five-year period, 2014-2018.

First, this thesis presents a comprehensive review of available literature on CHIKF, CHIKV and epidemiology (Chapter 1) and a report on the methods used (Chapter 2). It then describes a systematic literature review and meta-analysis of data on childhood CHIKV infections published between 1983-2021 (Chapter 3). I show that CHIKF is a significantly under-recognised and underreported health problem among children globally, with infants under 1 year of age being at highest risk of severe disease. To determine the prevalence and incidence of CHIKV infections among children in coastal Kenya, I used RT-PCR to test: i) children presenting with fever at two primary health facilities in Kilifi county (described in Chapter 4), ii) children admitted with neurological illness at the referral Kilifi County Hospital (KCH; Chapter 5), iii) newborns at the KCH maternity ward (Chapter 6).

During the five-year study period, I found that CHIKF was endemic in coastal Kenya, associated with 12.7% (95% CI 11.6, 13.8) of all febrile presentations at the primary healthcare facilities, and accounted for 9.2% (95% CI 8.3, 10.2) of children admitted with neurological

illness. A community survey of 435 asymptomatic children in the same study location estimated a prevalence of 0.7% (95% CI 0.2, 2.1) asymptomatic CHIKV infections in 2016 (a year when a CHIKF epidemic was reported in the country). CHIKF incidence was highest in young children and infants, implying acquisition of immunity. However, recurrent CHIKF episodes, associated with fever and viraemia, were observed among 19 of 170 children with multiple febrile episodes during the study period, suggesting imperfect immunity. This is the first time that recurrent CHIKV infections have been observed.

Among children aged <5 years, the incidence of CHIKV-associated neurological disease was 77 per 100,000 person-years, compared with 20 per 100,000 for cerebral malaria and 7 per 100,000 for bacterial meningitis during the study period, suggesting that CHIKV may now be the single most important pathogen associated with neurological illness in coastal Kenya.

In summary, this thesis reveals that CHIKF is endemic in coastal Kenya, a common cause of acute febrile illness and neurological disease among children. Sub-clinical CHIKF is rare and recurrent infections do occur. Maternal transmission of CHIKV to the neonate occurs in coastal Kenya and should be considered in obstetric and paediatric protocols.

Further studies to enhance knowledge on the geographical distribution of CHIKF, transmission dynamics and associated clinical outcomes should help determine the wider public health significance of CHIKF and inform development of effective prevention and control measures.

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Publications from thesis research

Three chapters presented in this thesis (Chapters 3, 4, and 5) have been published, or submitted for publication in peer-reviewed academic journals.

Chapter 3: The global burden of Chikungunya fever among children: A systematic literature review and meta-analysis

Status: Submitted to PloS Global Public Health

Authors: Doris K. Nyamwaya (DN), Samuel M. Thumbi (ST), Philip Bejon (PB), George M. Warimwe (GW), Jolynne Mokaya (JM)

Authors' contributions: DKN, JM, SMT and GMW conceived the idea of the study and developed the protocol. DKN and JM did the literature search, selected studies, and extracted relevant information. DKN and JM synthesised the data and wrote the first draft of the manuscript. DKN, JM, PB, GMW and SMT revised successive drafts of the paper. All authors have read and agreed to the published version of the manuscript

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Authors' contributions GMW and PB designed study. DKN, DOO, GG, HKK, JNG, ZL, JRO, RS, EK, and CNA performed experiments. MO, SC and EO managed and analysed data.

GMW and SMT supervised work. All authors contributed interpretation of the data and writing of the manuscript.

Reference: Nyamwaya DK, Otiende M, Omuoyo DO, Githinji G, Karanja HK, Gitonga JN, et al. Endemic chikungunya fever in Kenyan children: a prospective cohort study. BMC Infect Dis. 2021;**21**(1): 186. Epub 2021/02/20. doi: 10.1186/s12879-021-058755. PMID: 3602147 ; PubMed Central PMCID: PMCPMC7889702

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Reference: Nyamwaya DK, Otiende M, Mwango L, Kariuki SM, Otieno B, Omuoyo DO, et al. Incidence of chikungunya virus infections among Kenyan children with neurological disease, 2014–2018: A cohort study. von Seidlein L, editor. PLOS Med. 2022;19: e1003994. doi:10.1371/JOURNAL.PMED.1003994

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Abbreviations

BCS	Blantyre coma score
CFR	Case fatality rate
CHIKV	Chikungunya virus
CHIKF	Chikungunya fever
CI	Confidence interval
CSF	Cerebrospinal fluid
ECSA	Eastern, Central and Southern Africa
ELISA	Enzyme-linked immunosorbent assay
HDU	High dependency unit
IOL	Indian ocean lineage
IRR	Incidence rate ratio
Ig	Immunoglobulin
KCH	Kilifi County Hospital
KEMRI	Kenya Medical Research Institute
KHDSS	Kilifi Health and Demographic Surveillance System
KWTRP	KEMRI Wellcome Trust Research Programme
LMICs	Low and middle-income countries
MAR	Maternal admission record
MTCT	Mother to child transmission
ONNV	Onyong'nyong virus
OR	Odds ratio

PYO	Person years of observation
PID	Personal identifier
PCR	Polymerase chain reaction
RT PCR	Reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
VLP	Virus like particles
WA	West Africa

Chapter 1: Background & literature review

1.1 Chikungunya fever

Chikungunya fever (CHIKF) is caused by chikungunya virus (CHIKV) first isolated from a febrile patient in 1952. The term 'chikungunya' is from Makonde dialect of Tanzania, translating to 'that which bends up' or 'become contorted' [1,2]. Epidemics of CHIKF have been reported in Africa, Asia, Europe, the Americas, and have been associated with substantial morbidity. Unlike other arboviral infections, more than 80% of patients [3,4] develop symptoms during a viraemic acute phase characterized by sudden onset of fever after an incubation period of approximately 1-12 days [5]. CHIKF commonly manifests with bilateral polyarticular joint pain, inflammation and oedema affecting mostly the peripheral joints (ankles, phalanges and wrists), shoulders, elbows and knees [6,7]. Other symptoms reported include headache, backache, maculopapular rash, tachycardia, myalgia, lymphadenopathy, and pinnae erythema [8–10]. These manifestations usually resolve after approximately 7 days except for articular defects that could persist longer [8].

Chronic musculoskeletal symptoms due to CHIKV infection occur in more than 50% of patients and could last months or years as episodic joint pathology [8,11,12]. The time between consecutive episodes of joint pain is estimated to be approximately 8 weeks. During relapse, there is incapacitating, symmetrical polyarthralgia associated with local swelling, depression or asthenia of the same joints. It has been demonstrated that rheumatic manifestations are more likely due to local rather than systemic inflammation [11]. Predisposing risk factors to long-term CHIKV disease are poorly understood though some studies have reported increased age, comorbidities like diabetes and hypertension, gender, acute phase viral load and pre-existing joint pathology as factors [11,13].

About 25% of seropositive individuals do not exhibit any symptom upon infection [4,14–17]. Although this status is not detrimental to the individual, it plays a key role as a reservoir for viral transmission [17]. Asymptomatic people develop a CHIKV viral load that is not

significantly different from symptomatic individuals, and are capable of infecting mosquito vectors [4]. Host factors and inoculated CHIKV dose have been demonstrated to influence clinical progression of the infection [4]. Studies using macaque models have revealed a species-specific response and shown a positive correlation between inoculum size and symptom development [4,18].

Atypical clinical forms of the disease are reported in less than 2% of cases that could lead to hospitalization and/or mortality [19–21]. This is prevalent mostly in infants, the elderly, immuno-compromised individuals and among those with underlying co-morbidities including systemic lupus, hypertension and diabetes [21]. Complications associated with severe CHIKV infections include neurologic, cardiovascular, ocular, respiratory, renal, hepatic and cutaneous defects [22]. Direct invasion of the nervous system seems to occur in infants and elderly patients while autoimmune responses occur in middle-aged and healthy patients. Previously reported neurological manifestations include encephalitis, myelitis, cognitive deficits, Guillain Barre syndrome (GBS), seizures, acute flaccid paralysis, retro-bulbar neuritis, neuro-psychiatric behavioural disturbances and Horner's syndrome [23].

Studies during the 2005/2006 epidemic in Reunion island reported mortality rate among CHIKF severe cases ranging 3% to 35% [6,24,25] with a higher incidence among the elderly. A case fatality of 6% was observed among children with CHIKV-associated neurological disease [26].

Infection during pregnancy can follow a severe course leading to either vertical transmission, preterm births or stillbirths [19,27]. Intrapartum transmission, congenital infection and infectious mosquito bites at birth can lead to severe neonatal CHIKV infection [19,28]. Neonates develop symptoms after a median incubation period of 4 days characterized by a higher viral load compared to older children [29,30]. Complications associated with neonatal infections include fever, pain, prostration, difficulty in breastfeeding, neurological anomalies,

cardiac and haemorrhagic manifestations [27,29,31]. Exposure to the infection early in life can lead to poor neurocognitive outcomes and lifelong "CHIKV-driven disability" [27]. Neonatal predisposition to severe disease has been demonstrated using mouse models where susceptibility to severe infection decreased with age [32]. Prolonged viral persistence in breastmilk has been demonstrated but the infectivity risk to the infant remains unknown [33]. Clinical laboratory features of CHIKF patients have been reported to include lymphopaenia, moderate thrombocytopaenia, neutrophilia, hypocalceamia, anaemia and elevated levels of C-reactive protein, glucose, urea, aspartate aminotransferase and alanine aminotransferase [6,21,34–37]. However, these features are not specific to CHIKV infection and cannot be used for formal differential diagnosis against other causes of febrile illness [38].

1.2 Immunology to CHIKV

A robust immune response is generated following CHIKV infection that is characterized by production of type I IFNs, recruitment of innate and adaptive immune cells and development of neutralizing Abs [39,40]. In some cases however, the virus can persist in the host either due to active replication or incomplete elimination of infected cells triggering chronic inflammation [39]. CHIKF severity and pathogenesis has been associated with production of specific cytokines. Increased levels of IL-1b and IL-6 and a decrease in RANTES is linked to severe disease [41] while high viremia was correlated with increased levels of IL-6, IL-12, IL-15, CXCL10, CCL2, IFN-a, and IL-1Ra [42]. Chronic joint pathology has been associated with increased levels of IL-6 and GM-CSF [42]. Increased levels of CXCL10 and CXCL9 were reported among cases with mild-to-severe disease compared asymptomatics [43].

Protective immune response against CHIKV consists predominantly of IgG3 antibodies [44]. However, the virus can sometimes escape the impact of neutralising antibodies by direct cell-to-cell transmission [45]. Introduction of CHIKV into naïve populations results into

significantly high levels of seroconversion consistent with the rapid spread and high attack rate as everyone is susceptible [46,47]. The acquired antibodies are reported to offer long-lasting protection against re-infection though the exact duration of immunity conferred by CHIKV infection remains unknown [48]. In endemic regions, the risk of infection has been observed to decrease with age due to high sero-prevalence rates among the older age-groups [49].

1.3 Chikungunya virus

1.3.1 Classification, Structure and strain diversity

CHIKV belongs to the genus alphavirus under the Togaviridae family within the Semliki Forest antigenic complex [50] that also includes Mayaro, O'nyong nyong (ONNV), Semliki Forest and Ross River viruses which are all pathogenic to humans [2]. The virion is small, spherical (slightly pleomorphic) and enveloped, containing a single-stranded, linear, positive sense RNA genome of approximately 11 kilobases (kb; **Figure 1.1~~Figure 1.1~~)**. Two thirds of the genome encode four non-structural proteins (nsp1, nsp2, nsp3 and nsp4) that are translated to virus replication enzymes [51]. One third encodes five structural proteins (nucleocapsid protein, envelope glycoproteins (E1 and E2) and small peptides (E3 and 6K) [2].

Phylogenetic studies have indicated a common ancestor for CHIKV in Africa that diverged into 2 branches including West African (WA) and East/Central/Southern African (ECSA) genotypes [38,52,53]. The strains of the WA lineage are maintained in a sylvatic cycle in western Africa countries with small focal spillover outbreaks of human infections reported [9,54]. The ECSA strains are enzootic in East, Central and Southern Africa but they have been detected in samples from a broader geographical range [55]. These strains are thought to be the source of the 2004 CHIKV epidemic that began in coastal Kenya in 2004 and spread to the Indian Ocean islands, during which a monophyletic Indian Ocean Lineage (IOL) was identified [56]. The ECSA strain emerged in Asia and evolved into a distinct Asian genotype [55,57] that

has exhibited a remarkable spatial and temporal branching pattern spreading throughout southeast Asia [55]

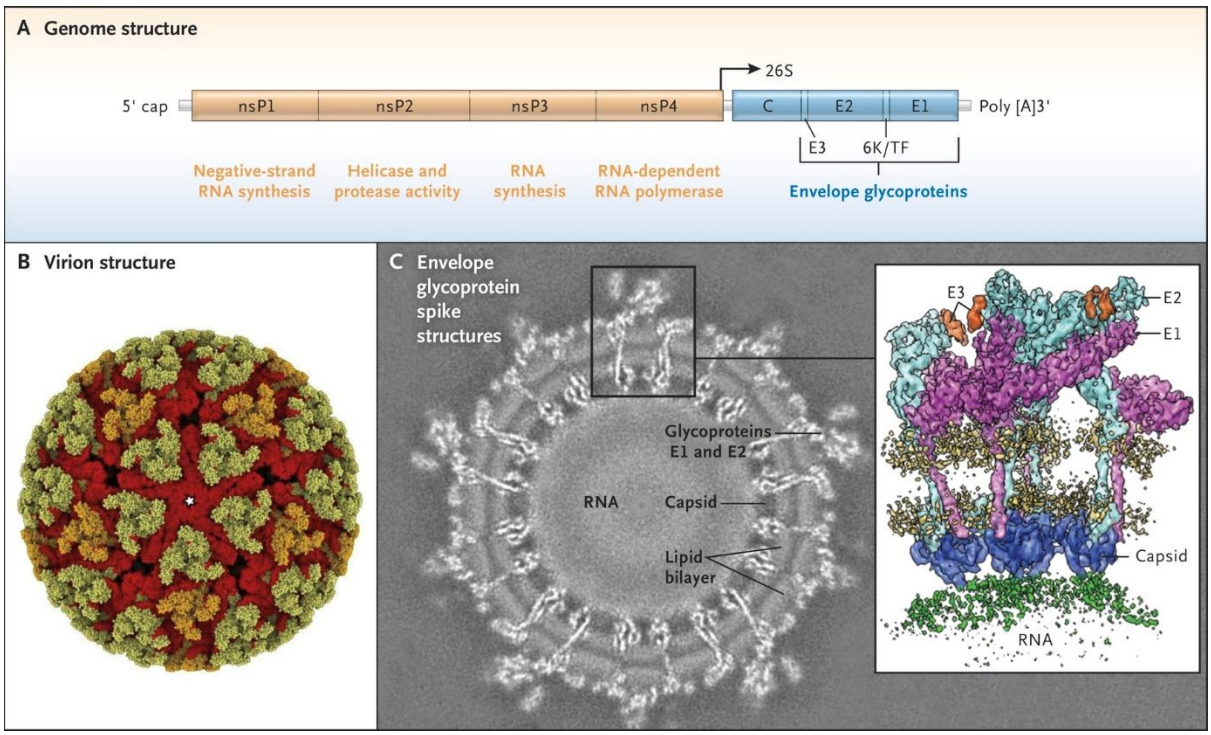

Figure 1.1. The physical and genomic structure of CHIKV. Panel A shows the organization of the chikungunya virus genome. Panel B is the structure of the virion. Panel C shows spike-protein predicted structures based on atomic resolution structures of the envelope glycoproteins and high-resolution cryo-electron microscopic reconstructions of chikungunya virus and other alphavirus particles. (*Adapted from Weaver et al., 2015. NEJM* [51])

1.3.2 Replication

CHIKV can replicate in both invertebrate and vertebrate hosts. In humans, it exhibits broad tropism in replication sites including joints, muscles, skin and less frequently the kidneys, liver, eye and the central nervous system [58,59]. Infectivity is limited to specific cell lines like fibroblasts, monocyte-derived macrophages, muscle satellite cells, endothelial and some epithelial cells depending on expression of certain surface receptors that are yet to be identified [37,58,59]. With the facilitation of E2 glycoprotein, the virus binds to a susceptible host cell

then gains entry by a clathrin-dependent endocytic pathway. The virus-host cell membrane fusion is mediated by E1 peptide and culminates in delivery of the viral nucleocapsid to the cytoplasm where replication takes place. Disassembly of the nucleocapsid liberates the genomic RNA from which non-structural proteins are translated and together with some host proteins form a short-lived viral replication complex. These complex catalyses synthesis of a negative-strand viral RNA that acts as a template for amplification of both genomic and sub-genomic positive-sense RNA. The sub-genomic RNA drives expression of the structural polyprotein (C–pE2–6K–E1) that is further processed by auto proteolysis. The capsid (C) is cleaved out and the remaining polypeptide is transited to the plasma membrane through a secretory pathway while being cleaved into E1, E2, E3 glycoproteins. The capsid binds to genomic RNA, then recruits the membrane-associated envelope glycoproteins to generate the virion particle. The particle is then released from the host cell by budding [37,58,60,61].

1.3.3 Infection route

The virus is inoculated by an infected mosquito bite either on human resident dermal cells i.e fibroblasts and macrophages, or directly unto the blood circulatory system [59,62]. Initial replication takes place in these skin cells triggering an immune response. It then disseminates to draining lymph nodes for further replication, and spread to other peripheral organs including muscle, peripheral joints, and tendons [60]. In severe cases, the virus invades the brain and liver [60,63]. Replication in the peripheral tissues results into the viremic phase of infection. CHIKV immune evasion and replication has been shown to be enhanced by autophagy and apoptosis of infected cells [37,64]. Innate immunity forms the first line of body defence against infection.

1.3.4 Transmission

Before spillage to human populations, CHIKV circulated primarily in Africa within a forest cycle involving primates and sylvatic *Aedes.spp* mosquitoes e.g. *A. furcifer tayleri*, *A.*

luteocephalus, A.africanus [65] Figure 1.2~~Figure 1.2~~. However, human encroachment of forest ecosystems and environmental factors promoting upsurge of vector densities has seen the virus spread and amplify within an urban cycle involving *A. aegypti* and *A. albopictus* Figure 1.2~~Figure 1.2~~. This cycle has been responsible for widespread epidemics in most parts of the globe. *A.aegypti* is preferentially a human feeder that acquires a complete single blood meal by biting multiple persons [65]. Additionally, it can breed in small water pools around human habitation hence explaining its CHIKV vectoral efficiency. Unlike *A.aegypti* that is restricted to tropical and subtropical regions, *A.albopictus* is more aggressive in invading new niches including temperate areas enabling the virus to establish in its areas of distribution [66]. Cases of maternal-fetal transmission of CHIKV have been documented and, in most instances, they result into severe clinical outcomes in the neonate [27,31]. Transmission can also occur through organ transplantation as highlighted by Couderc *et al* in the case of corneal grafts or blood transfusion.

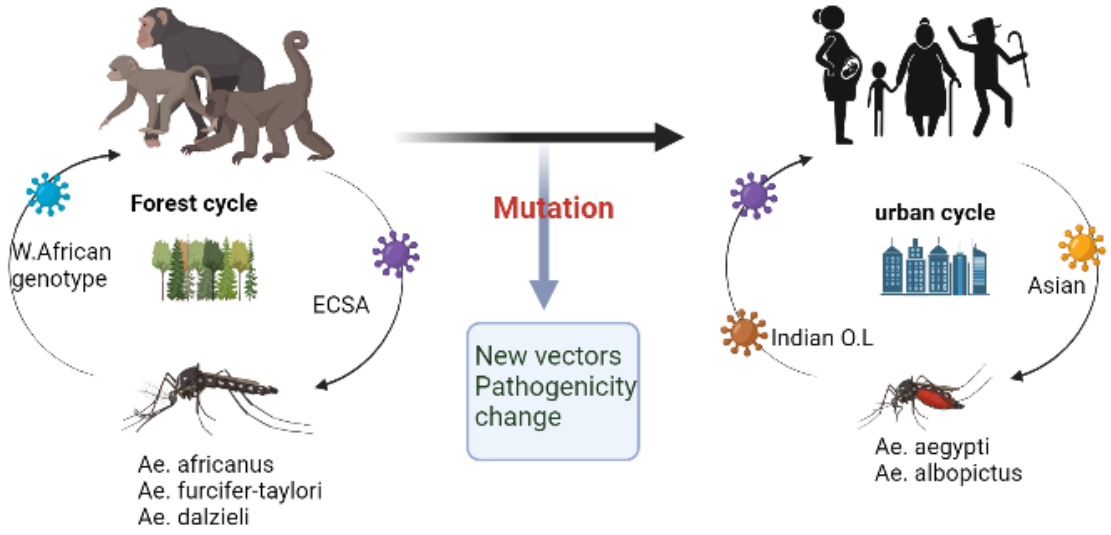

Figure 1.2. The transmission cycle of CHIKV. The forest cycle involves transmission between primates and Aedes.spp mosquitoes. The urban cycle is between humans and *Ae. Aegypti* or *Ae. albopictus*

1.3.5 Diagnosis, treatment and prevention

CHIKF diagnosis can be achieved through viral isolation, detection of the viral genome or anti-CHIKV IgM and IgG antibodies in blood, CSF, blister fluid, synovial fluid , breast milk or urine [67–70]. The virus can be successfully isolated in cell culture up to 3 days after the onset of symptoms [71]. RT-PCR is useful during the acute viremic phase lasting 7 days after the incubation period [72]. Anti-CHIKV IgM antibodies are detectable by ELISA or indirect immunofluorescent assay, from approximately the third day following infection and persist for about 3 months [71,72]. In convalescent samples, anti-CHIKV IgG antibodies that persist for years, can be detected using ELISA, hemagglutination inhibition assay or neutralisation [22]. A wide array of protocols with varying specificity and sensitivity for molecular and serological testing have been developed. Serological screening is hampered by cross reactivity with closely related viruses like Onyong'nyong' virus that could lead to false positive reactions [73].

There is no specific established therapeutic regime available for CHIKF patients. The WHO guidelines on clinical management of CHIKF focuses on alleviation of the symptoms by administering analgesics, anti-pyretics, fluids, and rest [74]. The synergistic effect of anti-viral agents ribavirin and interferon gamma inhibits in vitro CHIKV replication while Doxycycline and ribavirin reduce viremia levels and inflammation.

Effective prevention measures involve enhancement of community awareness, avoiding mosquito bites either by using repellants, insecticide-treated bed nets or wearing clothing that reduces skin exposure. Vector control can be achieved by elimination of mosquito breeding sites and spraying insecticides. Intensive surveillance and monitoring of new cases is key for early detection of outbreaks.

There are no licensed vaccines for CHIKV, but various vaccine candidates have been developed [75,76]. The measles-vectored CHIKV [77], live attenuated CHIKV [76] and protein-based CHIKV virus-like particle (VLP) [78] have been evaluated in phase 2 clinical

trials. VLA1553 is a live-attenuated, single dose vaccine that is designed by deleting a part of the chikungunya virus genome. It is the most advanced candidate in phase 3 clinical trial. It was observed to elicit an excellent immunogenicity profile with 96.3% of participants showing protective CHIKV neutralizing antibody titers six months after receiving a single vaccination. It was well tolerated among the subjects evaluated for safety [79].

1.4 Epidemiological history of Chikungunya

CHIKV has caused many well-documented outbreaks in Africa, Asia, America, Europe and the pacific islands [80–83]. The first outbreak was reported in Tanganyika (present day Tanzania) in 1952 and was followed by several sporadic and major epidemics in over 40 countries globally [3,57,84]. Epidemics were mostly experienced in 1960s and 1990s but the 2004 CHIKV epidemic that emerged in coastal Kenya is the largest on record reporting over 1.3 million cases [9,46]. It spread along the Kenyan coast from Lamu to Mombasa and islands on the Indian Ocean [85,86], India and subsequently to Southeast Asia and Europe [9,57,87]. The rapid spread and geographical expansion was facilitated by the prevalence and high vectoral capacity of *Aedes* mosquito vectors, as well as travel-associated importation of the virus into naïve populations [57]. Descendant strains from the ECSA lineage including the Asian and Indian Ocean Lineage [65,88], are commonly implicated in these outbreaks while the West African lineage tends to remain in sylvatic circulation with occasional spill over to humans causing sporadic outbreaks [84,89]. Epidemics are described to occur cyclically with an intermittent period of approximately 7 to 20 years [22]. Initially, the outbreaks were associated with high morbidity characterized by joint pathology but recent severe clinical manifestations have been proposed to involve more virulent virus strains [47,90–92].

1.5 CHIKV in Africa

CHIKV was first discovered in the Tanzania in 1952 then spread to other parts of the globe [9]. The virus was maintained in a forest cycle mostly involving primates and sylvatic *Aedes* mosquitoes [9]. The dynamics of the enzootic cycle including other possible reservoirs remain poorly understood. The virus emerged in the human population and successfully sustained an urban cycle involving *Aedes aegypti* mosquitoes, that has been associated with epidemics and sporadic outbreaks. The first outbreak was reported in Newala and Masisi districts of Tanzania in 1952 when the virus was first isolated from a febrile patient [93]. To date, presence of CHIKV has been demonstrated in various African countries including; South Africa, Malawi, Zimbabwe, Uganda, Kenya, Sudan, Democratic republic of Congo, Central African republic, Senegal, Benin, Guinea, Ivory Coast, Burundi, Cameroon, Gabon, Equatorial Guinea and Nigeria [9,27,84,94,95].

The description of CHIKV epidemiology and associated clinical manifestation in the continent is limited. The few available studies indicate continuous circulation of the virus both in epidemic and nonepidemic periods [96–98]. The exact prevalence and incidence of CHIKF remains unknown. The accuracy of seroprevalence estimates is impaired by cross-reactivity with co-circulating alphaviruses like Onyong'nyong' virus [99]. The observed seroprevalence variance in various studies could be attributed to differences in analytical methods and sample sizes [100–104].

An increased risk of exposure to CHIKV infections in the continent is associated with nomadism [105], lowland terrain [99], flooding and proximity to water bodies [106]. Pregnancy could be a predisposing factor with studies reporting seroprevalence rate ranging 24-40% among expectant women [107,108].

There is low public awareness about CHIKV in Africa [102]. Most cases of CHIKV remain undiagnosed or misdiagnosed due to non-specific clinical symptoms and lack of diagnostic

capacity in terms of resources and infrastructure. In a prospective study of febrile admissions in a Tanzanian hospital, Crump *et al.* found that approximately 8% of all febrile admissions were due to CHIKV. Most of these were clinically diagnosed as malaria [109].

Following the introduction of *Aedes albopictus* in Africa, CHIKV dissemination could increase. There is therefore need to strengthen surveillance strategies, and improve on epidemic preparedness and response activities [110].

1.6 Chikungunya in Kenya

Transmission and circulation of CHIKV in Kenya was first demonstrated in 1966 [111]. Previous serosurveillance studies indicate differential distribution of CHIKV in the country with increased risk of exposure in Western and Coastal Kenya [112–115]. Seropositivity is estimated to range from less than 1% to 80% [46,116] depending on calendar year and location. Presence of active infections outside epidemic periods suggests that CHIKV is endemic in the region [117]. However, due to lack of an established CHIKV surveillance system both in the community and referral hospital level, most cases remain unrecognized and misdiagnosed [118]. Investigations around CHIKV are mostly done during outbreaks and little attention is paid during the interepidemic periods. Moreover, reports of CHIKV outside epidemics are incorporated within studies aiming to understand arboviruses in general [113,115]. This limits generation of comprehensive data on characterization of CHIKV in the country.

The relative vector competence of *Ae. aegypti* mosquito for CHIKV in Kenya has been ascertained with susceptibility dependent on viral levels in the blood-meal, prevailing temperature and the feeding behaviour [119]. Transmission efficiency is positively associated with drought conditions. High temperatures promote virus development in the mosquitoes while massive domestic water storage enhance breeding and human contact [120]. Evidence

of enzootic circulation has been documented with an approximate seroprevalence of 13% among non-human primates [121].

The epidemiological history of CHIKV in Kenya was first documented during its re-emergence in the Lamu island in 2004 [122]. Then, in 2016 an outbreak was reported in Mandera, located in the North-east region bordering Somalia [123]. The virus spread south to coastal Kenya and re-emerged in Mombasa in 2017-2018 [124]. This outbreak caused a major disease wave against which the local county government responded by strengthening vector control activities like fogging and indoor residual spraying to eliminate mosquito breeding sites [125]. In early 2022 , CHIKV cases were detected in Wajir also located in the North-eastern region [123].

However, the real prevalence of CHIKV, associated risk factors, clinical manifestations as well as the transmission pattern remain unknown in the country.

1.7 Research problem and scope of dissertation

Re-emergence of CHIKV, rapid spread and disease burden is a public health concern. The evolutionary history of CHIKV reveals genetic mutations associated with increased transmissibility and deadly clinical manifestations. Its exclusion from general screening of undifferentiated febrile illness leads to underdiagnosis of CHIKF and, consequently, inappropriate management. For instance, administration of antibiotics and anti-malarial drugs to CHIKF patients aggravates the challenge of drug resistance. Fatalities due to atypical clinical manifestations of CHIKF commonly involving the nervous system are increasingly being reported especially among children [126–128]. In most LMICS, such as Kenya, routine surveillance for CHIKV is absent and the virus is only given attention during epidemics while its activity during the interepidemic period remains unknown.

High CHIKV seropositivity rates in coastal Kenya have been observed in various cross-sectional studies [46,129–131]. However, information on the prevalence of acute CHIKV

infections and associated clinical manifestations is lacking. This is critical epidemiological knowledge that would inform policy on CHIKF surveillance and management.

In this dissertation, I determined the frequency of acute CHIKV infections, the associated disease burden and clinical manifestation in children resident in Kilifi, coastal Kenya. I focused on children as they are more at risk of new infections compared to adults that have acquired immunity over time.

Specifically, I aimed to:

1. Determine the burden of CHIKV infections among children globally through a systematic literature review and meta-analysis
2. Estimate the incidence of acute CHIKV infections among children visiting local dispensaries in Kilifi, coastal Kenya
3. Estimate the incidence of atypical CHIKF among children admitted to Kilifi County Hospital with neurological disease
4. Determine the prevalence of CHIKV mother-to-child transmission in Kilifi

Chapter 2:

General methods

2.1 Study site and population

This research project was conducted in Kilifi County located along the Kenyan Indian ocean coast **Figure 2.1**~~**Figure 2.1**~~. The region typically experiences two main rainy seasons; the 'long rains' during April to June and the short rains from October to November each year [132]. The local economy is based on subsistence farming of maize, cassava, cashew nuts, coconuts and livestock rearing. The healthcare system in the County consists of a network of public and private health facilities, with Kilifi County Hospital (KCH) as the main referral centre registering approximately 4000 paediatric admissions annually. KCH lies within the catchment area of an established Kilifi Health Demographic Surveillance System (KHDSS) ran by Kenya Medical Research Institute (KEMRI)-Wellcome Trust Research Programme (KWTRP) [133] **Figure 2.1**~~**Figure 2.1**~~. The KHDSS covers an area of 891km^2 within 50km north, 50km south, and 30km west of KCH with approximately 290,000 residents that are enumerated every four months at the household level [134]. Residents of the KHDSS area are served by 21 public health facilities operating under the Kenya Ministry of Health guidelines. This includes KCH, Pingilikani and Ngerenya health facilities where my study was based. Kilifi creek divides the KHDSS into north and south regions that have been shown to have different rates of mosquito-borne pathogen exposure as inferred from previous malaria studies [132]. Malaria transmission intensity is low in Ngerenya area to the north of the creek and moderate in Pingilikani area to the south [132] **Figure 2.1**~~**Figure 2.1**~~. The surveillance area is bordered by Arabuko Sokoke forest that is home to a wide range of wildlife including monkeys.

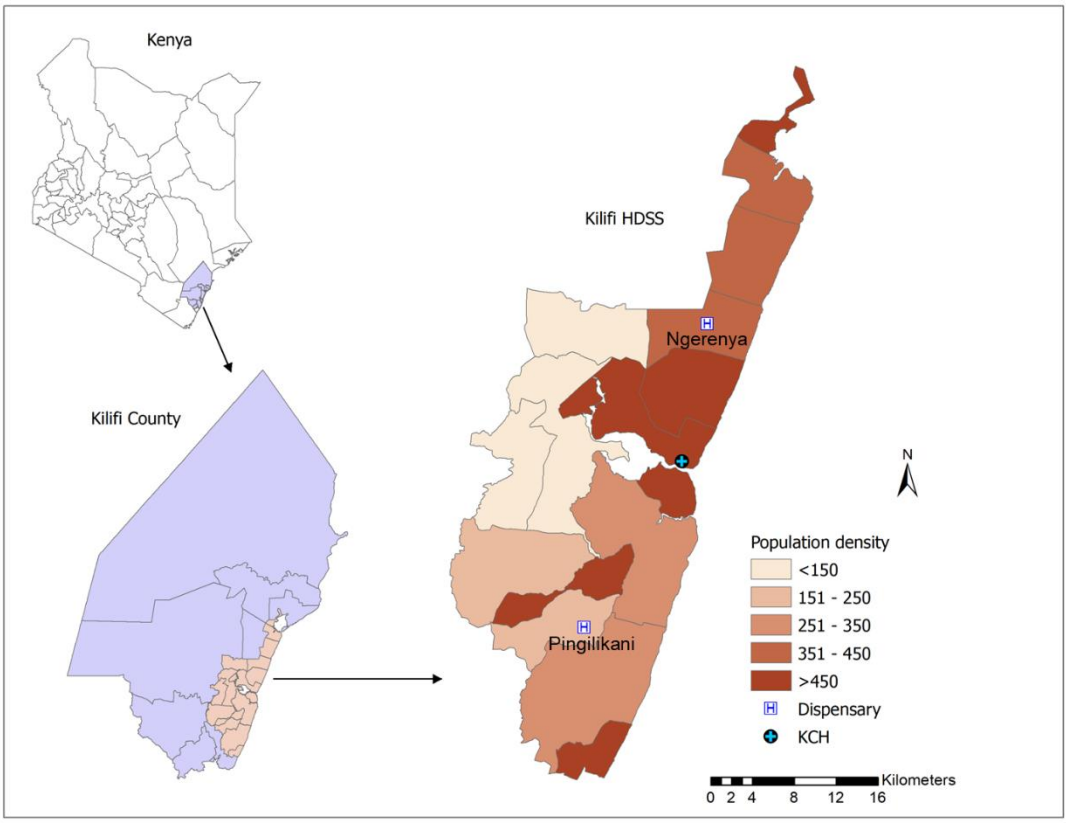

Figure 2.1. A map of kenya showing the location of Kilifi county and the demarcation of Kilifi health and demographic surveillance system (KHDSS). Ngerenya and pingilikani dispensaries located within KHDSS have an ongoing surveillance for febrile illness and part of my research project was based here.

2.2 Ethical approval

Ethical approval to undertake this study was provided by the Kenya Medical Research Institute Scientific and Ethics Review Unit (SSC No. 3296) and a written informed consent was provided by parents or guardians of all study participants.

2.3 Sampling

2.3.1 Community surveillance study

Stored EDTA blood samples in the KEMRI-Wellcome Trust Research Programme biobank were used. These samples were collected during an ongoing passive surveillance project aimed at monitoring malaria cases in children presenting to local dispensaries in Ngerenya and Pingilikani, Kilifi, in the period March 2014 – March 2018. These dispensaries lie within KHDSS **Figure 2.1**~~**Figure 2.1**~~ where each inhabitant is described by their family relationships and their homestead of residence with geospatial coordinates and assigned a unique personal identifier (PID). Febrile children (axillary temperature ≥ 37.5°C or fever reported by parents) visiting these dispensaries were identified using their PID and finger-prick blood samples were collected and examined for malaria parasites. This formed a good sample set for describing acute cases in the community for CHIKV infection as fever is a key symptom of CHIKF [1].

For this project, a random sample of 3,500 children out of 5,669 children who visited the two dispensaries with febrile illness during the study period was used. Random sampling was done using the *runiform* function in Stata™ version 15 with the PID as input. Based on previous studies on childhood malaria [135], it was expected that some children would present to the dispensaries with fever on multiple occasions during the study period. Therefore, a choice to limit selection of samples to the earliest febrile episode for each of the 3,500 children was considered. Subsequently, to identify either reinfections or recrudescent infections, samples from subsequent febrile illnesses among children that tested positive for infection at the index febrile episode were further selected.

2.3.2 Hospital surveillance for CHIKV-associated neurological illness

Neurological manifestations are outlined as common forms of atypical CHIKF [91]. To determine prevalence and incidence of CHIKV-associated neurological illness in Kilifi, cerebrospinal fluid (CSF) samples obtained from an established clinical surveillance in KCH paediatric ward were used. The pediatric service at KCH includes two wards; a 70-bed general ward and a 15-bed high dependency unit (HDU) staffed by research clinicians and nurses. The HDU admits children with serious illness requiring more intensive monitoring and management but has no ventilation facilities or renal replacement therapy. Electronic case records are kept for all admissions including demographics, vital signs, clinical history and examination, as well as routine laboratory investigations such as malaria blood slides, and blood and CSF culture. Each patient is assigned a discharge diagnosis by the attending clinician following review of the notes and results. All data are linked to the KHDSS database in real time by means of unique person identifiers.

For this study, all children aged <16 years whose illness required sampling and analysis of CSF were eligible for inclusion. The decision to collect CSF was made by clinicians. The same senior clinicians oversaw care throughout the period of surveillance. Where acute coma was present, the medical team considered the risks of lumbar puncture versus clinical benefits of diagnostic information from a clinical management perspective without reference to research considerations. Where clinically appropriate, delayed lumbar punctures were conducted after a period of treatment. Neuroimaging was not available on site during the period of surveillance. After investigations done for immediate clinical care, an aliquot of CSF was stored at -80ºC in the KEMRI- Wellcome Trust biobank and was available for use.

2.3.3 CHIKV mother-to-child transmission study

KCH provides comprehensive obstetric and neonatal care. Clinical care within the maternity department is fully integrated into clinical surveillance. All mothers who present in labour are registered on admission by research assistants based in the maternity ward. Specified clinical data are documented by nurses through completion of a structured maternal admission record (MAR). The MAR includes antenatal history, delivery details, partogram (the national standard) and maternal problems before discharge as well as neonate outcomes. Examination is structured and includes routine observations (respiratory rate, heart rate, temperature, blood pressure) as well as symphyseal-fundal height, cervical assessment, and general examination. Routine investigations for clinical care on admission include a full blood count and two point of care tests: a rapid test for malaria infection and a urine dipstick for proteinuria and nitrites. Rapid HIV testing is offered if the woman has not been tested in the current pregnancy, according to national guidelines.

At birth, following an informed consent, baseline information including age of the mother, number of previous pregnancies, antenatal clinic attendance, gestation period, birth weight and gender of the neonate are obtained. In addition, a sample of cord blood is collected and stored in the KEMRI-Wellcome Trust Research Programme biobank for research purposes.

For this study, I retrieved cord plasma samples collected in 2016 (a year when CHIKF epidemic was reported in Kenya) and screened them for CHIKV infection through molecular diagnostics (see section below).

2.4 Molecular diagnostics

2.4.1 RNA extraction

To determine a technique to use for viral RNA extraction, I explored the difference in yield from serially diluted CHIKV spiked serum using automated QIAcube® HT system, manual QIAamp® Viral RNA Mini kit (Qiagen) and TRIzol® method. The quantity of extracted RNA was determined by screening using real-time PCR and comparing the cycle threshold (ct) values. The automated system gave lower yields, as indicated by higher Ct values, but there was no significant difference between the manual methods **Figure 2.2**~~**Figure 2.2**~~.

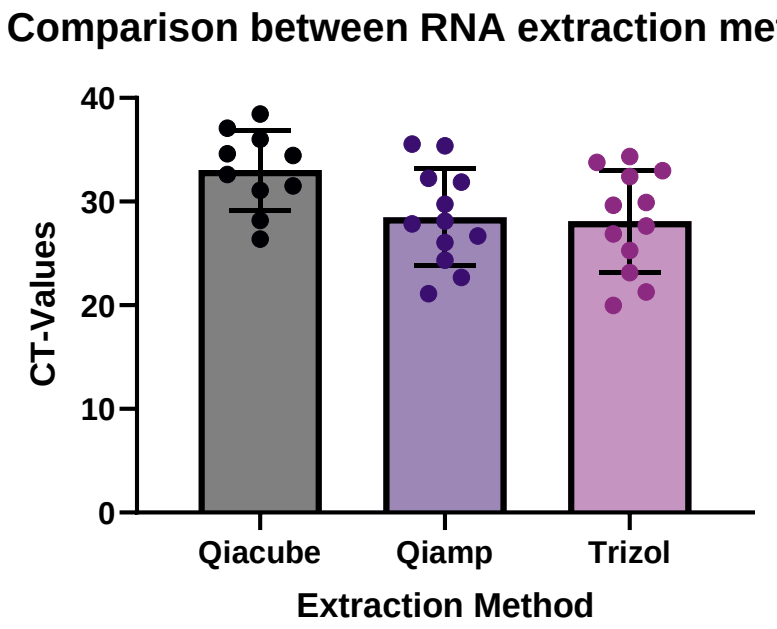

Figure 2.2. The difference in RNA yield from serially diluted CHIKV spiked serum using automated QIAcube® HT system, Manual QIAamp® Viral RNA Mini kit (Qiagen) and TRIzol method .

In this study, I chose to use the manual methods as follows because of higher RNA yields and availability of reagents. TRIzol® method was used on whole blood samples for community surveillance for acute CHIKF and CSF samples used to assess the incidence of CHIKV-associated neurological disease. Briefly, 1ml TRIzol® Reagent (Invitrogen ™, ThermoScientific), which is a monophasic solution of phenol and guanidine isothiocyanate was

added to 100 µl of whole blood or 100 µl CSF sample. The lysate was homogenized by pipetting then incubated at room temperature for 5 minutes to allow complete dissociation of the nucleoprotein complex. The integrity of the RNA is well maintained as the reagent effectively inhibits RNase activity. Chloroform (0.2 ml) was then added to the homogenate, briefly vortexed and incubated at room temperature for 5minutes before centrifuging for 15 minutes at 12,000 × g at 4°C. The mixture separates into a lower organic phase, an interphase and a colourless upper aqueous phase. The aqueous phase that contains RNA was transferred to a sterile Eppendorf tube then a co-precipitant (glycol blue, 1 µl) was added alongside isopropanol (500 µl) and incubated at room temperature for 15 minutes to enable RNA precipitation. This was then centrifuged for 10 minutes at 12,000 × g at 4°C. RNA formed a pellet at the bottom of the tube that was left after discarding the supernatant. The pellet was then washed by resuspending in 1ml ethanol, briefly vortexed and centrifuged for 5 minutes at 7,500× g at 4°C. The supernatant was again discarded and the pellet allowed to air dry before eluting in 60µl RNase- free water. This was incubated at 60°C to dissolve the pellet. The extracted RNA was then quantified using a nanodrop spectrophotometer.

The QIAamp® Viral RNA Mini kit (Qiagen) that was used to extract RNA from cord blood, combines the selective binding properties of a silica-based membrane with the speed of microspin or vacuum technology. The sample is first lysed under highly denaturing conditions provided by buffer AVL to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp membrane by addition of absolute ethanol. The sample is then loaded onto the QIAamp Mini spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in 2 steps using 2 different wash buffers. High quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases and other contaminants and inhibitors [136].

2.4.2 Real-time polymerase chain reaction (qPCR)

Samples were assayed by a quantitative, fluorescent, real-time probe-based qPCR to detect presence of CHIKV RNA. Previously published primer/probe sets targeting different regions of CHIKV genome were obtained [68,137,138]

Table 2.1

~~Table 2.1~~. The sensitivity of these sets was compared using a serial dilution of spiked serum with cultured CHIKV as template and QuantiFast Multiplex RT-PCR Kit (Qiagen) in a 7500 Real-Time PCR System (Applied Biosystems) Figure 2.2~~Figure 2.2~~**.**

Table 2.1. A list of the previously published primer-probe sets that were obtained and considered for use in CHIKV qPCR

Author	Forward primers	Reverse primers	Probe	Target
	Sequence	Sequence	Sequence	
Baudin *et al.* [137]	5'-CATGCAAAACAGAATTTGC-3'	5'-TAGGCAGTTACAGTGATG-3'	FAM-5'-CTCATACCGCATCTGCATCA- TAMRA-3'	E1 Envelop gene
Lanciotti *et al.* [138]	5′-AAAGGGCAAACTCAGCTTCAC-3′	(5′-GCCTGGGCTCATCGTTATTC-3′)	FAM-5′-CGCTGTGATACAGTGGTTTCGTGTG-TAMRA-3′	Non-structural protein 1
Pabbaraju *et al.* [68]	5'-TGTACTGGCWGCAGCCACG-3'	ATAGGGCTGGCAGCAAATTC	VIC-5'-AACGTCACACAGATGAG-3'	Non-structural protein 4
Giry *et al.* (Pan-Alpha) [139]	5'-AATGATGAARTCIGGIATGTTYYT-3'	ATYTTIACTTCCATGTTCATCCA ATYTTIACTTCCATRTTCARCCA ATYTTIACTTCCATGTTGACCCA	ATTO425- AT + GTT + GTC + GT + CN + CC-BHQ1/LNA™-3'	Non-structural protein 4

The primer-probe sets by Baudin., *et al* amplified at the earliest cycle thresh-hold Figure 2.4~~Figure 2.4~~. However, the obtained amplicons produced non-specific bands on visualization in gel electrophoresis when used to screen human blood samples Figure 2.3~~Figure 2.3~~. This was an indication of either reagent contamination, or presence of a profound primer-dimer issue. This necessitated a series of optimization assays to determine the optimum conditions that could produce reliable results.

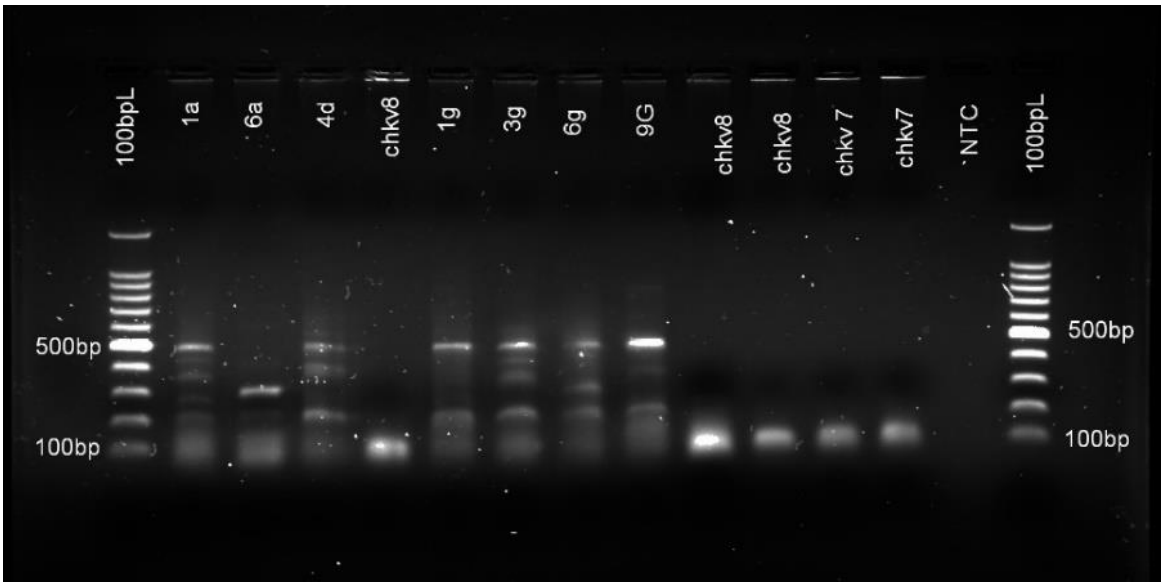

Figure 2.3. Gel electrophoresis image of amplicons produced by the primer-probe set in Baudin *et al*., 1a, 6a, 4d, 1g, 3g, 6g, 9G were human samples that were positively amplified in qPCR; chkv 7, chkv8 were positive controls including cultured CHIKV samples as PCR templates; NTC was a negative control that included RNase-free water as PCR template

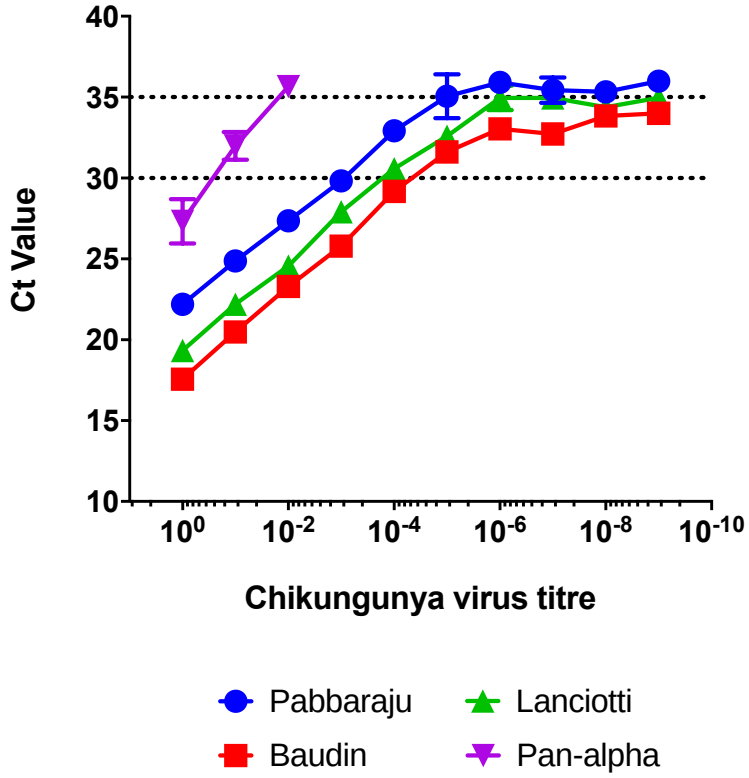

Figure 2.4. Efficiency of different primer-probe sets to amplify CHIKV. The earlier the CT-Value at a given concertation, the more effective the assay. Pan-alpha assay was the least sensitive while there was no significant difference among the other assays.

To rule out exogenous contamination, an assay using a new sealed kit and observing all laboratory standard operating protocols was performed using primers by Baudin *et al.* and Lanciotti *et al.* All the wells had nuclease-free water as a template. Amplification was observed in all the wells with a mean CT-value of 35 (range 31 – 38). Probability of endogenous contamination was assessed by excluding human DNA that could be present in the extracted RNA through DNase treatment. There was no difference in qPCR amplifications between DNase treated and untreated samples . Moreover, the negative controls were amplified in both cases **Figure 2.5**~~**Figure 2.5**~~.

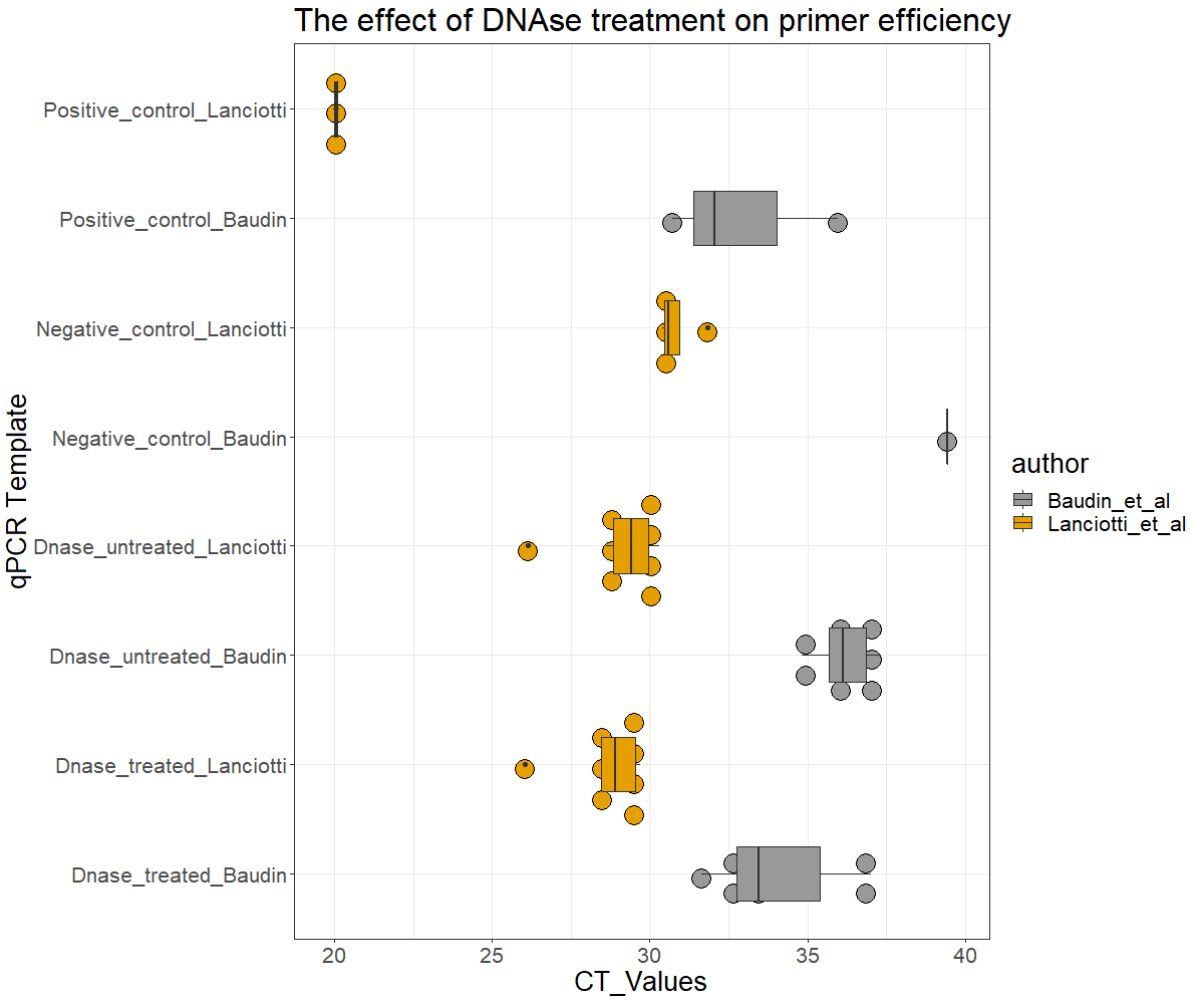

Figure 2.5. The effect of DNase treatment on real-time PCR amplification using primers by Lanciotti *et al.* and Baudin *et al*. There was no difference in qPCR amplifications between DNase treated and untreated samples. Negative controls were amplified in both cases

To assess the probability that primer-dimers could be producing non-specific amplification, experiments to determine the optimum probe concentration and primer concentration were done.

I. Primer concentration

The primers (in Baudin *et al* and Lanciotti *et al*) were diluted into 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.02 Mm concentrations. Each dilution was used in a 2-replicate SYBR green-based qPCR assay with RNAse-free water as the template. Amplification was observed in duplicate with 0.5 Mm – 0.2 Mm primer concentrations

Table 2.2

~~**Table 2.2**~~.

Table 2.2. Ct values of amplifications observed in a no-template control at different CHIKV primer (by Lanciotti *et al*.) concentrations

Replicates	**Primer Concentration**						
	0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	**0.02**
R1	31.98	33.13	34.54	35.5	-	-	-
R2	32.93	33.09	33.09	35.29	-	38.03	-

The Melt curves showed better consistency in amplification with Lanciotti *et al.* primers as compared to those by Baudin *et al*. **Figure 2.6**~~**Figure 2.6**~~. This informed the choice to do further optimization using primers by Lanciotti *et al*.

Baudin assay **Lanciotti assay**

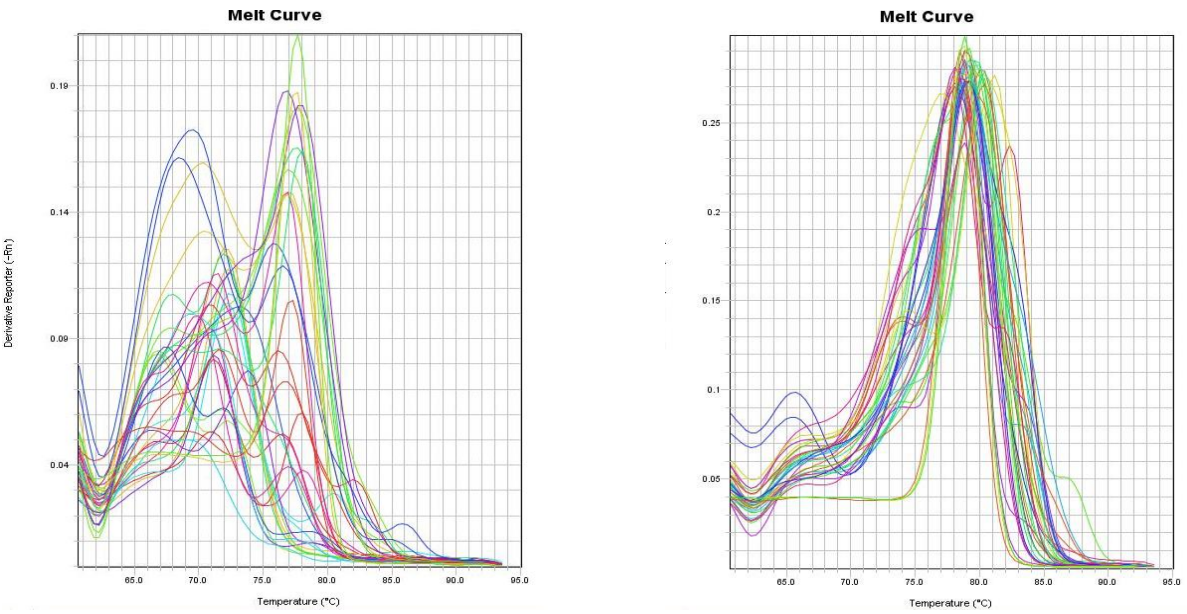

Figure 2.6. Melting curves illustrating the (T_m) temperature at which generated amplicons dissociate. Similar amplicons should dissociate at the same temperature range.

To assess amplification performance at 0.1 Mm, 0.05 Mm and 0.02 Mm primer concentration, a SYBR green-based qPCR assay was set up with serially diluted positive control (CHIKV culture) and a negative control as templates. The working primer concentration of 0.1Mm

produced the earliest Ct values for amplified CHIKV culture and no amplification was observed in the negative control wells Table 2.3~~Table 2.3~~, Figure 2.7~~Figure 2.7~~. The melt curve displayed consistent amplification Figure 2.8~~Figure 2.8~~.

Table 2.3. Amplification sensitivity of CHIKV primers (by Lanciotti *et al.*) at different concentration of primers and positive control template

Concentration of Positive control	**Primer concentration**		
	0.1	**0.05**	**0.02**
10^{-4}	18.75	20.60	27.65
10^{-5}	21.66	23.77	32.28
10^{-6}	25.41	27.88	36.78
10^{-7}	29.32	32.83	-
10^{-8}	31.44	34.43	-
10^{-9}	33.18	-	-
10^{-10}	-	-	-
NTC	-	32.78	-

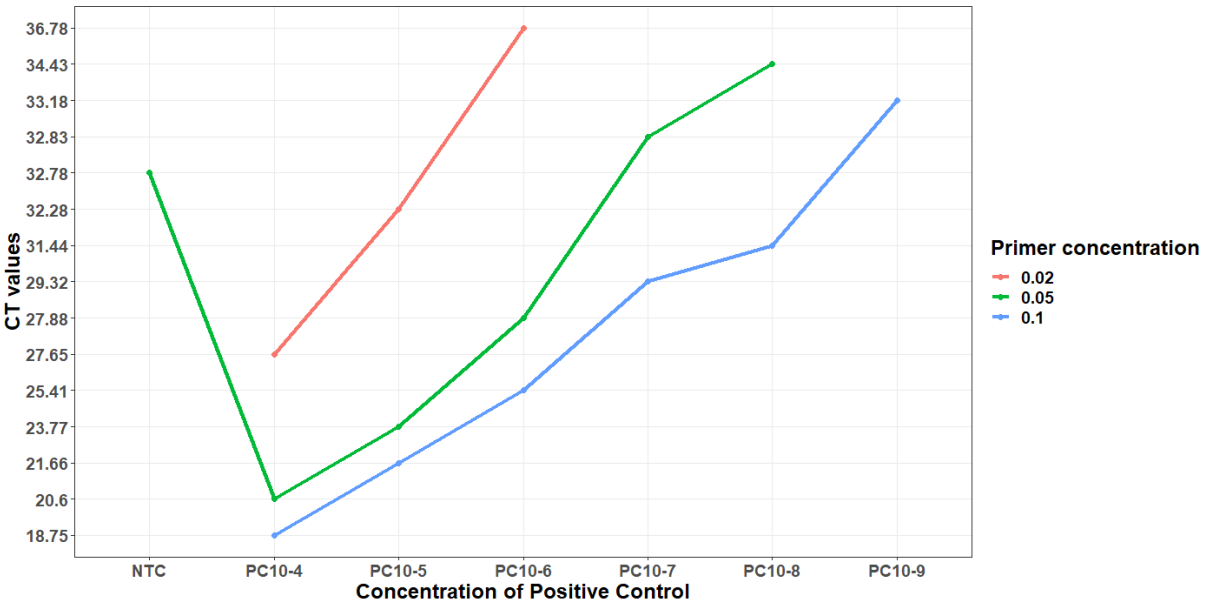

Figure 2.7. Amplification sensitivity of CHIKV primers (by Lanciotti *et al.*) at different concentration of primers and positive control template

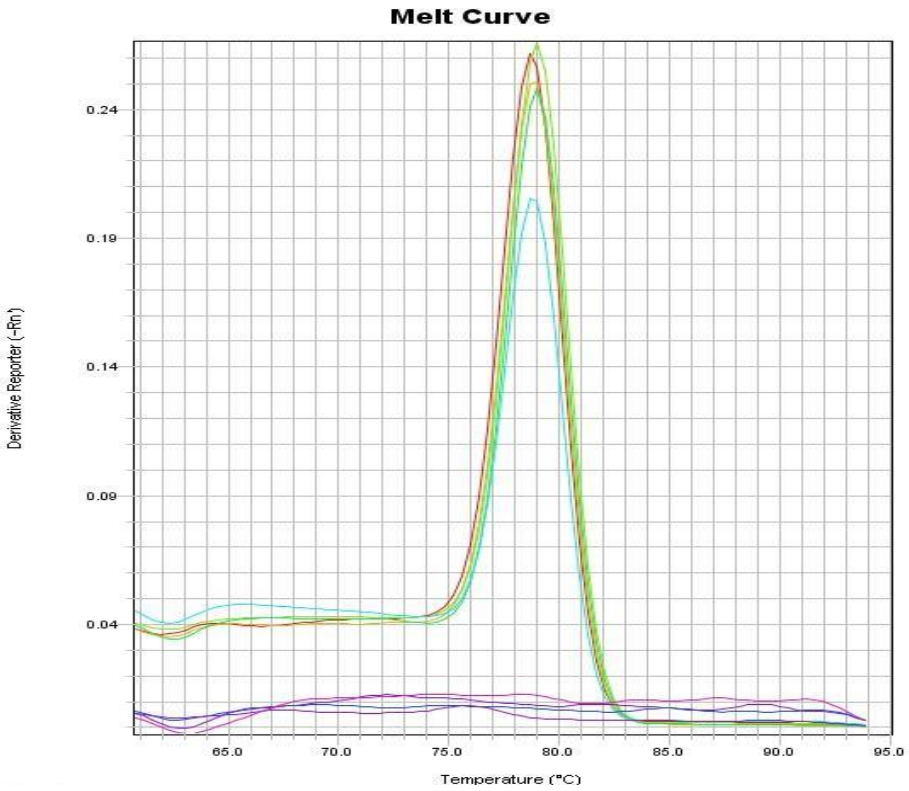

Figure 2.8. Melt curve derived from amplification of serially diluted positive control and negative controls using primer sets by Lanciotti *et al*. at 0.1 Mm concentration

II. Probe concentration

The initial probe working concentration was 0.05mM. Dilutions of 0.10, 0.06, 0.05, 0.04, 0.02, 0.01 were prepared and used in a qPCR with the optimized primer concentration of 0.1Mm on serially diluted positive control and no template control wells. The best amplification was observed at 0.02Mm concentration, without amplifications in the negative control wells **Table 2.4**~~**Table 2.4**~~.

Table 2.4. Observed Ct-values on qPCR assay using different concentrations of probe, serially diluted positive control and no-template controls

PCR Template	Probe concentration(Mm)					
	0.06	**0.05**	**0.04**	**0.02**	**0.01**	**0.1**
PC10^{-4}	25	25.4	26	17	28	24
PC10^{-5}	26	27	29	19	30	30
PC10^{-6}	31	32	33	22	33	31
PC10^{-7}	34	37	34	24	38	35
PC10^{-8}	-	36	-	29	-	-
NTC	-	-	-	-	37	36
NTC	-	-	-	-	-	-
NTC	-	-	-	-	17	-
NTC	-	-	-	-	-	-
NTC	-	36	-	-	-	36

Key; PC stands for positive control, NTC is a no template control

Using the obtained optimum conditions i.e. primer concentration = 0.1 Mm, probe concentration = 0.2Mm a standard curve was generated to determine the sensitivity of the assay. A 10-fold serial dilution of extracted RNA from cultured CHIKV with an initial absorbance of 30.3ng/ µl was used. **Figure 2.9**~~**Figure 2.9**~~

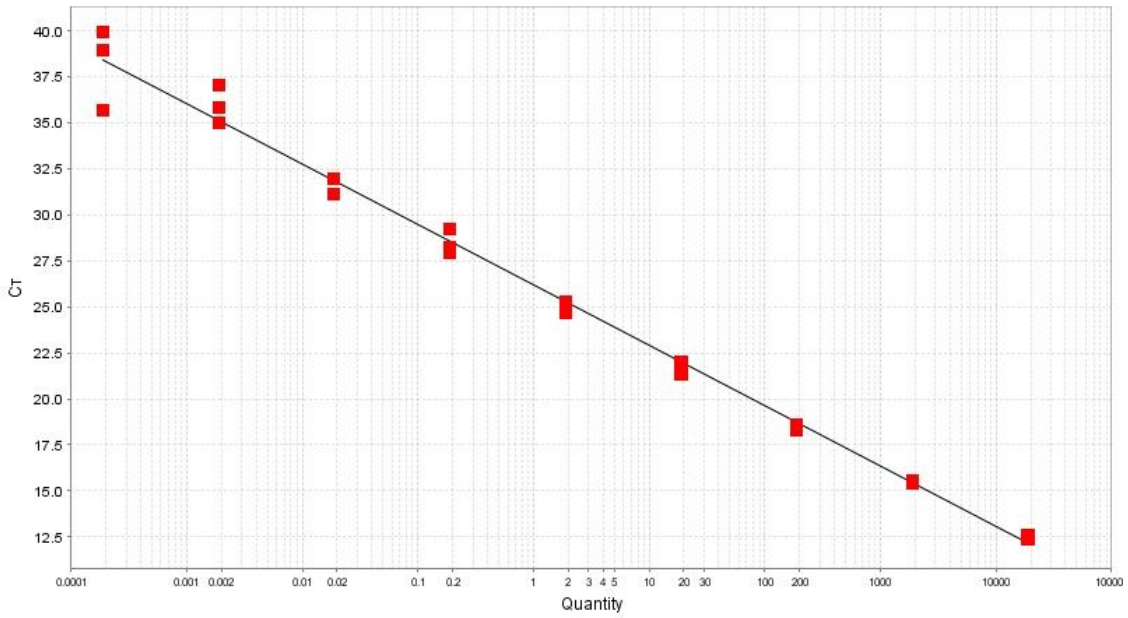

Figure 2.9. A standard curve produced using primers by Lanciotti et al and QuantiFast Multiplex kit; The assay can detect virus RNA from a CHIKV culture concentration of 4576.305 x 10^{-7} TCID50/ml.

2.4.2.1 RT-PCR kits and cycling conditions

The QuantiFast Multiplex RT-PCR Kit (Qiagen) kit was used for the community surveillance study and the Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix kit for clinical surveillance **Table 2.5**~~**Table 2.5**~~, **Table 2.6**~~**Table 2.6**~~. This was purely dependent on kit availability in the lab at the time of the study . There was no observed difference in sensitivity between the 2 kits as they produced similar standard curves using serially diluted RNA from a CHIKV cell culture.

Table 2.5. qPCR Master mix contents and cycling conditions using the QuantiFast multiplex kit

Description	**1 reaction (µl)**	**Cycling conditions**
2x buffer	12.5	50 °C for 20 min, 95 °C for 15 min, 45 cycles of 94 °C for 15 s and 60 °C for 1 min
5µm forward primer	0.5	
5µm reverse primer	0.5	
5 µm probe	0.1	
50x Rox	0.5	
RT mix	0.25	
RNase-free water	5.65	
Template	5	

Table 2.6. qPCR Master mix contents and cycling conditions using Fast Virus 1-step master mix kit

Description	**1 reaction (µL)**	**Cycling conditions**
Fast virus master mix	3	50 °C for 5 min, 95 °C for 20 seconds, 45 cycles of 95°C for 3s and 60 °C for 30 seconds
10mM Forward primer	0.8	
10mM Reverse primer	0.8	
5mM Probe	0.4	
Template (RNA)	5	

2.5 CHIKV genome sequencing

Some samples that positively amplified in RT-PCR for CHIKV were sequenced with the Nanopore MinION technology using methods described in the PrimalSeq approach [140]. CHIKV-specific multiplex primers were designed in the Primal Scheme software and used for pre-enrichment of CHIKV viral RNA in the samples using multiplex PCR. Barcodes and sequencing adapters were ligated into each sample, after which all samples were pooled into a single reaction tube and the library loaded on to a MinION sequencing device for sequencing. The sequences generated in this study were deposited in GenBank, accession numbers: MT526798-MT526807

2.6 Data Analysis

For the systematic review **(Chapter 3)**, global prevalence was estimated by pooling reported estimates in various studies and performing a meta-analysis by implementing random effects model. Sensitivity analysis was used to determine validity of effect size estimate. A meta-regression was used to determine the importance of predicting differences in effect sizes.

Data from the dispensaries (**Chapter 4**) were linked to the larger KHDSS data using unique person identifiers allowing estimation of disease incidence in the population, and to assess the influence of various demographic variables on CHIKF incidence. Incidence rate was computed as total number of CHIKF cases divided by total person years of observation (PYO) and was expressed per 100,000 pyo. Incidence rate ratios (IRRs) comparing incidence across various sociodemographic variables were computed using a univariate negative-binomial regression model.

In **Chapter 5**, identification of CHIKV positivity results was followed by descriptive analysis and significance testing using negative binomial regression. Demographic and clinical features were compared between CHIKV-positive and CHIKV-negative admissions using chi-squared

test for categorical variables and Mann–Whitney U test for continuous variables. Incidence of CHIKV infection in the KHDSS was calculated as the number of CHIKV-positive cases divided by total PYO using time-to-event analysis methods in a longitudinal dataset. Incidence rate ratios comparing rates between sociodemographic variables were estimated using negative binomial regression models. Univariate logistic regression models were used to estimate association between CHIKV positivity in cord blood and maternal or newborn variables in **Chapter 6**.

CHAPTER 3:

Chikungunya Disease among children: A systematic literature review

"This chapter forms the basis of a manuscript submitted to PLoS Global Public health for publication"

3.1 Introduction

Chikungunya fever (CHIKF) is an acute febrile illness caused by the mosquito-borne chikungunya virus (CHIKV) [89]. CHIKV is a positive sense single-stranded RNA virus of approximately 11 kilobases that is mainly transmitted to humans by *Aedes aegypti* and *Aedes albopictus* mosquitoes [50,51]. The disease is characterised by a sudden rise in body temperature and debilitating joint pain that could resolve within weeks or persist for months to years [3]. Other symptoms include: headache, maculo-papular rash, fatigue, myalgia, backache and tachycardia [8,9]. Severe complications like encephalitis, myocarditis, kidney dysfunction, hepatitis, oculitis, cardiovascular and respiratory disorders have been observed commonly among the elderly, infants and immunosuppressed individuals [1,3]. There are currently no specific antivirals or approved vaccines for CHIKV infection, though efforts towards vaccine development are underway [71,141–144].

First identified in Tanzania in 1952, CHIKF epidemics are now reported in tropical and subtropical regions within Africa, Asia, Europe, Pacific islands and the Americas [84]. This rapid expansion into new ecological niches follows adaptation of the virus to new vectors and their introduction into temperate regions [5,56] resulting in outbreaks associated with high morbidity and mortality [145]. However, accurate diagnosis of CHIKF remains a challenge due to non-specific symptoms that are similar to those of other common endemic illnesses such as malaria and dengue [109]. In addition, the general lack of awareness of the disease as well as lack of resources impedes efforts towards active surveillance and inclusion of CHIKF in routine screening of febrile illness. As a result, the burden of CHIKF remains underestimated with diminished attention on its public health impact [109].

There are few studies that have described the clinical manifestations of paediatric CHIKF which tends to be different from that described in adult infection [29,92,146,147]. For instance, the typical musculoskeletal symptoms reported among infected adults are rare in children [29].

However, haemorrhagic manifestations associated with thrombocytopenia and lymphopenia are commonly reported in paediatric CHIKF [126]. With delayed medical attention, infected neonates are at an increased risk of developing severe complications including status epilepticus and multiorgan failure due to sepsis and intracerebral haemorrhage [148]. Nevertheless, there remains limited descriptions of the clinical spectrum and epidemiology of paediatric CHIKF, particularly in Africa. I therefore set out to determine the epidemiology of CHIKF among children (<18 years) globally by describing its prevalence, geographical distribution, and clinical manifestations through a systematic literature review and meta-analysis. This work will help consolidate the understanding of CHIKF in children, identify critical knowledge gaps and areas of further research including on CHIKF diagnosis and vaccine development.

3.2 Methods

This systematic review protocol followed the Preferred Reporting Items of Systematic Review and Meta-Analyses protocols (PRISMA-P), and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42021238628.

3.2.1 Search strategy and selection criteria

I searched published literature in PUBMED, SCOPUS, EMBASE and Web of Science electronic databases for studies that have reported CHIKV infections in children using the search terms detailed in **Table 3.1**~~**Table 3.1**~~. The search and selection of articles was conducted without any restrictions on date or language of publication. Searches were done for individual groups using the Boolean operator 'OR' and a combined search done for groups A and B using the 'AND' operator. Citations of relevant articles were also screened to identify additional studies.

Table 3.1. Details of search strategy used to identify studies on epidemiology of chikungunya infections among children globally, from PubMed, Scopus, Embase and Web of Science databases

Group A: **Problem (#1)**	**Group B:** **Population (#2)**
Chikungunya OR "Chikungunya fever*" OR "Chikungunya virus" OR "Chikungunya infection" OR "Chikungunya fever virus" OR "Chikungunya virus infection*" OR "Chikungunya fever* virus infection" OR "CHIKV"	infant* OR infancy OR newborn* OR baby* OR babies OR neo nat* OR preterm* OR prematur* OR postmatur* OR child* OR schoolchild* OR "School age*" OR preschool* OR kid OR kids OR toddler* OR adoles* OR teen* OR boy* OR girl* OR minors* OR pubert* OR pube scen* OR prepubescen* OR paediatric* OR paediatric* OR peadi atric* OR "Nursery school*" OR kindergar* OR "Primary school*" OR "Secondary school*" OR "Elementary school*" OR "High school*" OR highschool*

The inclusion criteria for studies were: (i) The study was either a cross-sectional, longitudinal, surveillance, randomised and non-randomised clinical trial, before-and-after, interrupted time series or case-series; and (ii) reported on laboratory-confirmed CHIKV infection among children (<18 years of age). Studies were excluded if: (i) they reported on CHIKV infections among adults (≥18 years of age); (ii) they were experimental (i.e., studies using in vivo or in vitro analytical approaches), review, commentary, editorial, mathematical model or used previously published datasets; (iii) described clinical trials and laboratory techniques; or iv) provided insufficient information to investigate the question of interest.

3.2.2 Study selection

The study selection process began by screening titles and abstracts retrieved from different electronic databases by matching the search terms and the inclusion criteria using the Ryyan software (https://rayyan.ai). A collaborator (Jolynne Mokaya (JM)) independently screened the titles and abstract of the articles for comparison. We included articles that presented original data and that had undergone peer review. Disagreements in the study selection were discussed

and resolved by consensus or discussion with my DPhil supervisors. The full text of relevant articles was then considered for further screening of their content.

3.2.3 Data extraction

Following the selection of eligible studies, JM and I independently extracted relevant data using a data extraction in google spreadsheet**.** For each study, we extracted the following information: first author and year of publication, study site (country/ geographical location), study setting (hospital vs community), duration of study, whether study was conducted during epidemic or non-epidemic season, study design, sampling method, sample size, diagnostic technique, diagnostic tool sensitivity and specificity, case definition, type of study population, age of study participants, sex of study participants, patient symptoms, clinical/laboratory findings, treatment, disease outcome, detected viral strain, estimated prevalence/incidence and a statement on the key findings. Non-English-language studies were translated into English using Google Translate before data extraction.

3.2.4 Critical appraisal

To assess the quality of included studies, Joanna Briggs Institute Critical Appraisal tool checklist was employed in scoring case reports (joannabriggs.org). For assessment of quantitative studies and case series, the National Institute of Health quality assessment tool for Observational Cohort and Cross-Sectional Studies, and quality assessment tool for Case Series studies were used, respectively (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).

3.2.5 Data analysis

A summary of the clinical manifestations, laboratory diagnostics, treatment administration and disease outcome of CHIKV infection among children was done. To determine the global prevalence of CHIKV infection in children, all studies that screened participants for CHIKV

infection using reverse-transcriptase polymerase chain reaction (RT-PCR) to assess for CHIKV genomic RNA in body fluids and/or serologic tests for CHIKV specific immunoglobulin M (IgM) were pooled. A meta-analysis of reported CHIKV cases among children was performed using the *metaprop* package in R [149]. Overall estimates were calculated using random effects model to take account of between-study heterogeneity [150]. The restricted maximum likelihood estimator [151] was used to calculate the heterogeneity variance $\tau 2$ while the Knapp-Hartung adjustments [152] were used to calculate 95% confidence interval (CI) around the pooled effect. CHIKV prevalence data were pooled using logit-transformed proportions in a generalised linear mixed-effect model (GLMM). Test for heterogeneity was applied using the Cochran's Q, I^2, and H statistics, with an I^2 of more than 75% indicating substantial heterogeneity [153]. Sensitivity analysis was performed by checking for outlier and influential cases [154] to determine their impact on the validity and robustness of the effect size estimate. A meta-regression using multi-model inference was performed to obtain a comprehensive look at which predictors were more or less important for predicting differences in effect sizes. The test statistics and confidence intervals were computed using Knapp-Hartung adjustment. For model evaluation, corrected Akaike's information criterion (AICc) was applied. Interactions between included predictors were not considered. Existence of publication bias was assessed by using a funnel plot whose asymmetry was measured by Egger's linear regression test ($p<0.05$ levels were considered statistically significance for publication bias) [155].

3.3 Results

3.3.1 Search results and characteristics of the included studies

The initial search terms yielded 3175 articles, 1071 of which were removed as they were duplicates between different electronic databases **Figure 3.1**~~**Figure 3.1**~~. After screening titles of the remaining 2104 articles, 1281 articles were excluded because they did not fulfil the

inclusion criteria. These were articles that were not focused on describing any aspects of CHIKV infections among children. Following an abstract and full text review of 823 articles, a further 681 articles were excluded because they either described other arboviral infections, were inaccessible, provided inferences of CHIKV infection based on the general population, described previous infections by assessing exposure through detection of anti-CHIKV IgG antibodies, their diagnostic criteria was not clearly stated or absent, were conference abstracts or reviews. A total of 142 articles were eligible and included in the review.

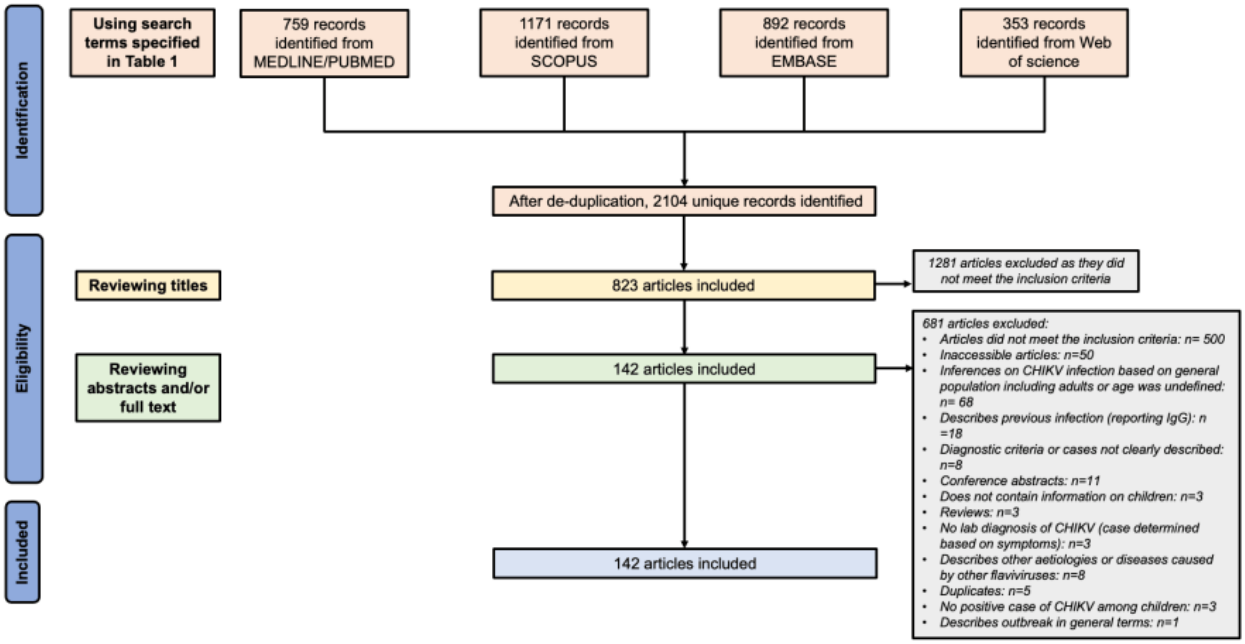

Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram illustrating identification and inclusion of studies for a systematic review of Chikungunya Disease among children**.**

All studies included in the systematic review were published ranging from the year 2006 to 2021, except for 1 study carried out in 1983. Most studies, approximately 70%, were conducted between 2016 and 2020 **Figure 3.2**~~**Figure 3.2**~~.

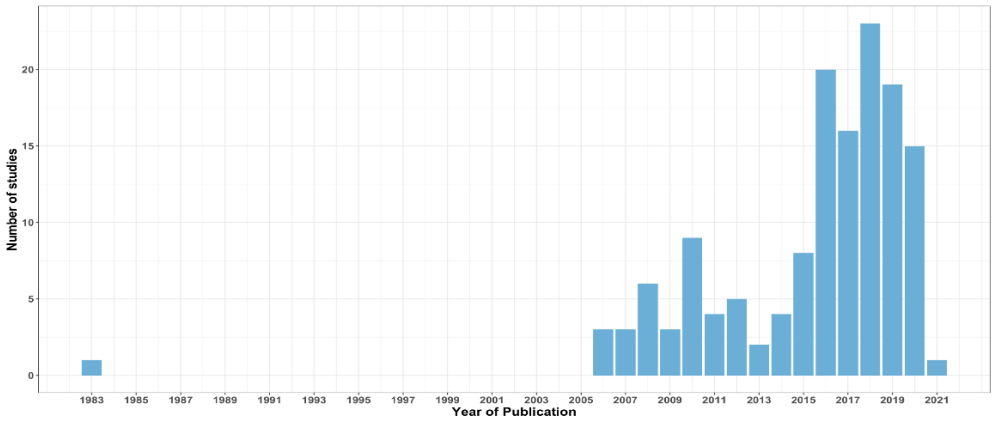

Figure 3.2. The distribution of studies included in the systematic review by their year of publication

Majority of the studies were from Asia (38%) and South America (22.5%)

Table 3.2

~~Table 3.2~~. Case reports and case series accounted for half of all studies reviewed. Hospital-based reports represented 78% of the included studies and majority of them (41.5%) were carried out during an epidemic period. A summary of the characteristics of the 142 articles that were included is provided in

Table 3.2

~~Table 3.2~~

Table 3.2. Summary of studies included in a systematic literature review to collate evidence for the global prevalence and clinical manifestation of chikungunya infection among children

Study design	**Number of studies (%)**
Case reports	55 (38.7)
Case series	18 (12.7)
Cross-sectional studies	36 (25.4)
Longitudinal studies	29 (20.4)
Surveillance	3 (2.1)
Unspecified	1 (0.7)
Regions represented	
Africa	24 (16.9)
Asia	54 (38.0)
Europe	5 (3.5)
North and Central America	19 (13.4)
South America	32 (22.5)

Oceania	1 (0.7)
Unspecified	7 (4.9)
Study Setting	
Hospital/health centre	111 (78.2)
Community	20 (14.1)
Unspecified	11 (7.7)
Season	
Epidemic	59 (41.5)
Non-epidemic	8 (5.6)
Epidemic/Non-epidemic	1 (0.7)
Unspecified	74 (52.1)
Sex	
Female only	11 (7.7)
Male only	25 (17.6)
Both female and male	49 (34.5)
Unspecified	57 (40.1)
Case definition	
IgM positive	36 (25.4)
CHIKV RNA positive	31 (21.8)
IgM positive and CHIKV RNA positive	9 (6.3)
IgM positive or CHIKV RNA positive	18 (12.7)
Unspecified	48 (33.8)
Age categories	
Infants (<1 year)	47 (33.1)
Children (1-12 years)	14 (9.9)
Teenagers/adolescents (13-18 years)	10 (7.0)
Mixed (0-18 years)	68 (47.9)
Unspecified	3 (2.1)
Viral strain	
Asian	1 (0.7)
West African	0 (0)
ECSA	11 (7.7)
Unspecified	130 (91.5)

3.3.2 Quality appraisal

Among the case reports, 18 studies were of good quality as there was a clear statement of the aims of research, clear description of participants' characteristics, clinical history, current clinical condition, diagnostic tests or assessment methods, interventions or treatment procedures, post-intervention clinical condition, and conclusion with key messages [92,156,165–172,157–164]**.** Among longitudinal and cross-sectional studies, seven studies [47,173–178] were good quality since their study populations were clearly described with participation rate of eligible persons being at least 50%, participants were selected from the same population, inclusion and exclusion criteria were clearly described, sample sizes were justified, analyses of the exposure of interest were measured prior to the outcome being measured, the outcome being measured were clearly defined, valid, reliable and consistently

applied across all participants, and key confounding variables were measured and adjusted statistically for their impact on the relationship between exposures and outcomes of interest. Only one case series [179] had good quality with the study objective clearly stated, study population clearly described, and cases consecutively described, subjects were comparable, the outcome being measured were clearly defined, valid, reliable and consistently applied across all participants, intervention was clearly described, length of follow-up was adequate, and results were clearly described**.**

3.3.3 Global distribution of CHIKV infections among children

The geographical distribution of studies that reported on children with CHIKV infection included in this review are summarised in **Figure 3.3**~~**Figure 3.3**~~. Most of the studies were from India (n=43) and Brazil (n=16). Only 8 countries in Africa were represented in this review. The bulk of studies from the Indian Ocean islands were from La Reunion Island (n=10) which is a constituent part of France that was most affected during the 2005 - 2006 CHIKV epidemic [180]. There were five studies from Europe, four of which were reports on traveller associated importation of CHIKV [181–184]. In North and South American continents, case series and case reports represented 37% and 72% of the studies, respectively.

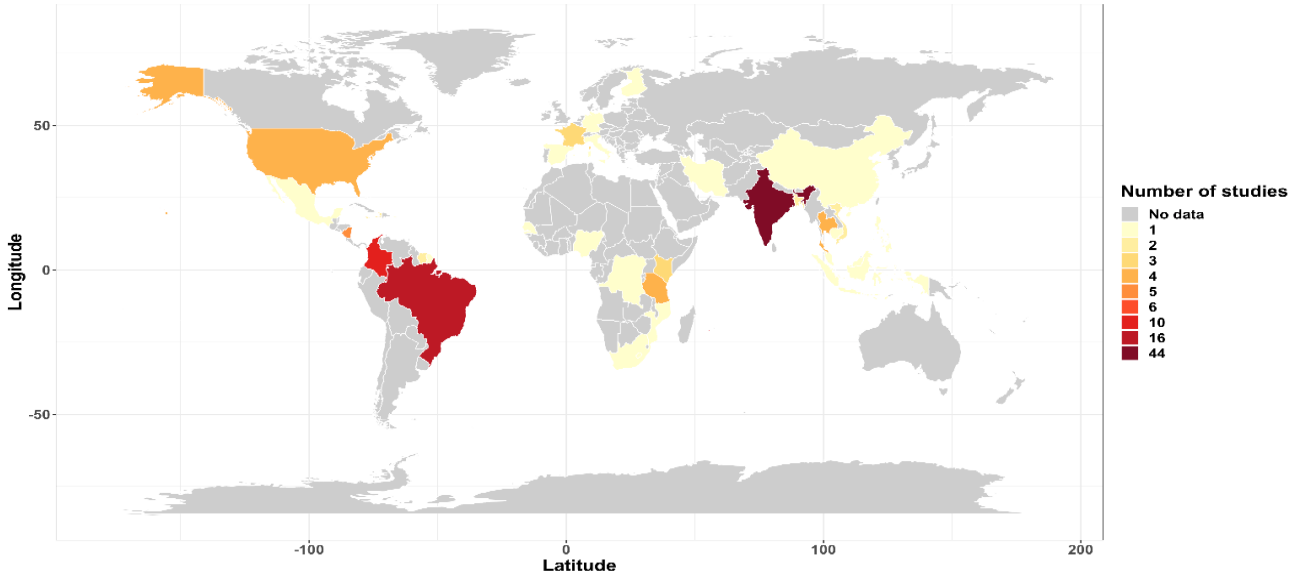

Figure 3.3. A global map showing the geographical distribution of study countries of the articles included in the review for CHIKV infection in children.

3.3.4 Global prevalence CHIKV infection among children

To determine the global prevalence of CHIKV infections, we focused on studies that had a defined numerator (number of CHIKV cases) and denominator (the total sample size). We excluded case reports, case series and observational studies that only reported on infected individuals – these studies were excluded as they were not representative of the target population. Prevalence of CHIKV among children in endemic regions ranged from 5% to 40% [109,110,185,186]. The highest rates were recorded during epidemic seasons

Table 3.3

~~Table 3.3~~ with highest positivity observed in the Carribbean islands.

Table 3.3. The proportion of CHIKV cases reported among children from various continents in different seasons

Continent	**N CHIKV cases per season**				**Total cases N (%)**
	Both	Epidemic	Non-epidemic	Unspecified	**Cases (%)**

Africa	32/383	516/4729	124/2011	43/749	715/7872 **(9.08)**
Asia	-	425/1954	31/1915	1087/14534	1543/18403 **(8.38)**
Central America	-	96/3377	-	-	96/3377 **(2.84)**
Caribbean	-	154/275	74/1390	-	228/1665 **(13.69)**
Indian Ocean Islands	-	224/2959	-	-	224/2959 **(7.57)**
Oceania	-	13/33	-	-	13/33 **(39.39)**

' - ' means there were no cases reported

3.3.4.1 Heterogeneity and sensitivity analyses

Significant heterogeneity of the included studies in the meta-analysis was observed, $I^2 = 98.61\%$, (95% CI 97.7–98.2). Sensitivity analysis using leave-one-out meta-analyses recalculated pooled effects, with one study omitted each time, revealed that I^2 remained constant at 98% **Figure 3.5**~~**Figure 3.5**~~. Plotting values of various influence measures including standardized residuals, difference in fits (DFFITS), the Cook's distance, covariance ratio, leave-one-out τ^2, Cochran's Q-value, Hat value and study weights showed that one study by Munoz et al., [187] was an influential case and was dropped from the analysis **Figure 3.4**~~**Figure 3.4**~~

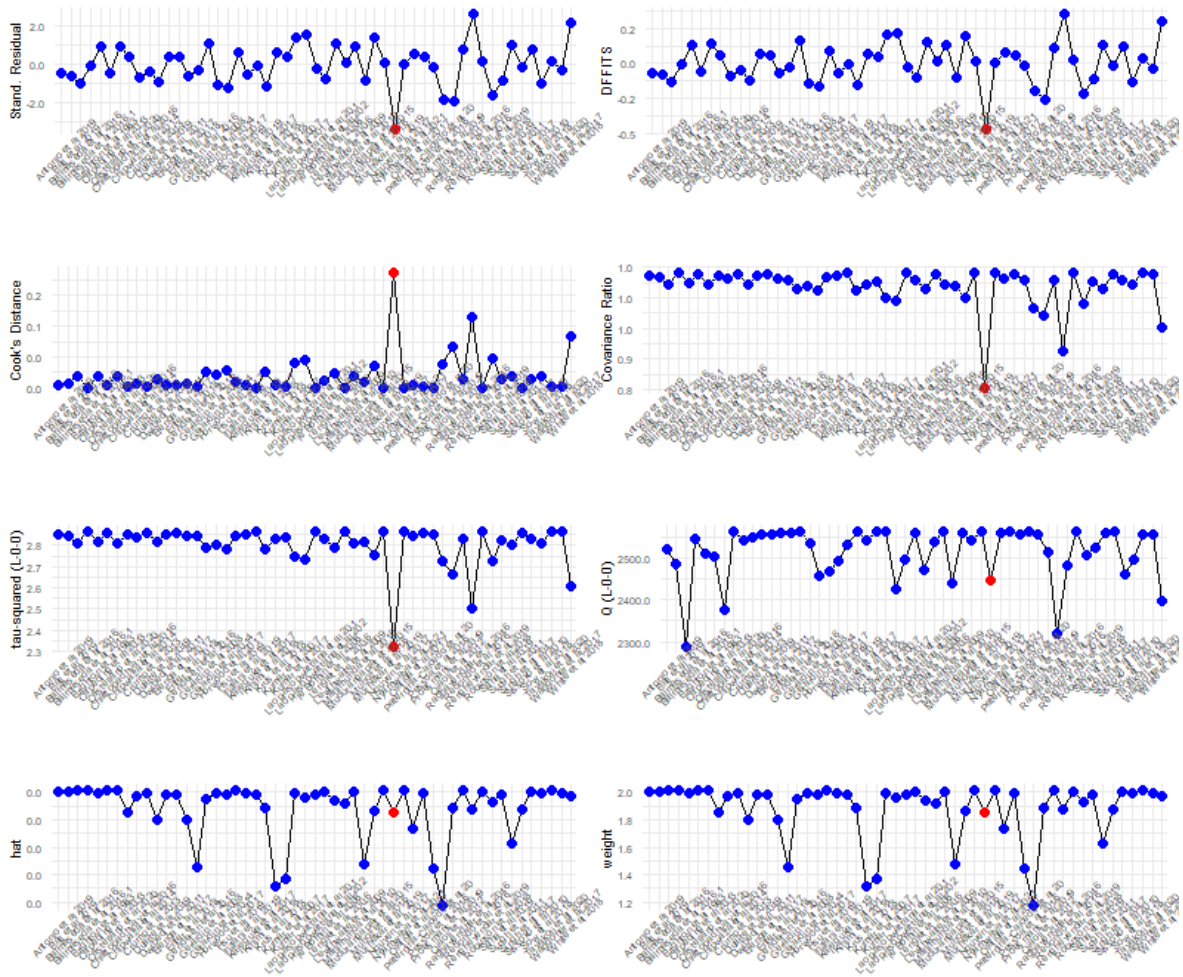

Figure 3.4: A plot of influential diagnostics of each of the included studies. Determined influential studies are displayed in red: Externally standardized residuals are indicative of the deviation of each observed effect size from the pooled effect size, a large residual show that the study is an influential case that does not "fit in". Difference in fits (DFFITS) value indicates how much the pooled effect changes when a study is removed; higher value indicates influential cases. The cook's distance value (D_i) directly summarizes how much the pooled effect size change when a study is deleted; large D_i indicates that the study strongly influences the fitted values. Covariance ratio measures influence on collinearity; values below 1 indicates that removal of the study would result in a more precise estimate of the pooled effect size. Leave-one-out τ^2 and Q-Values display estimated heterogeneity as measured by τ^2 and Cochran's Q after omitting a study; low value=low heterogeneity. Hat value and study weight are predictions made that summarize the potential contribution of the study to the estimated pooled effect size.

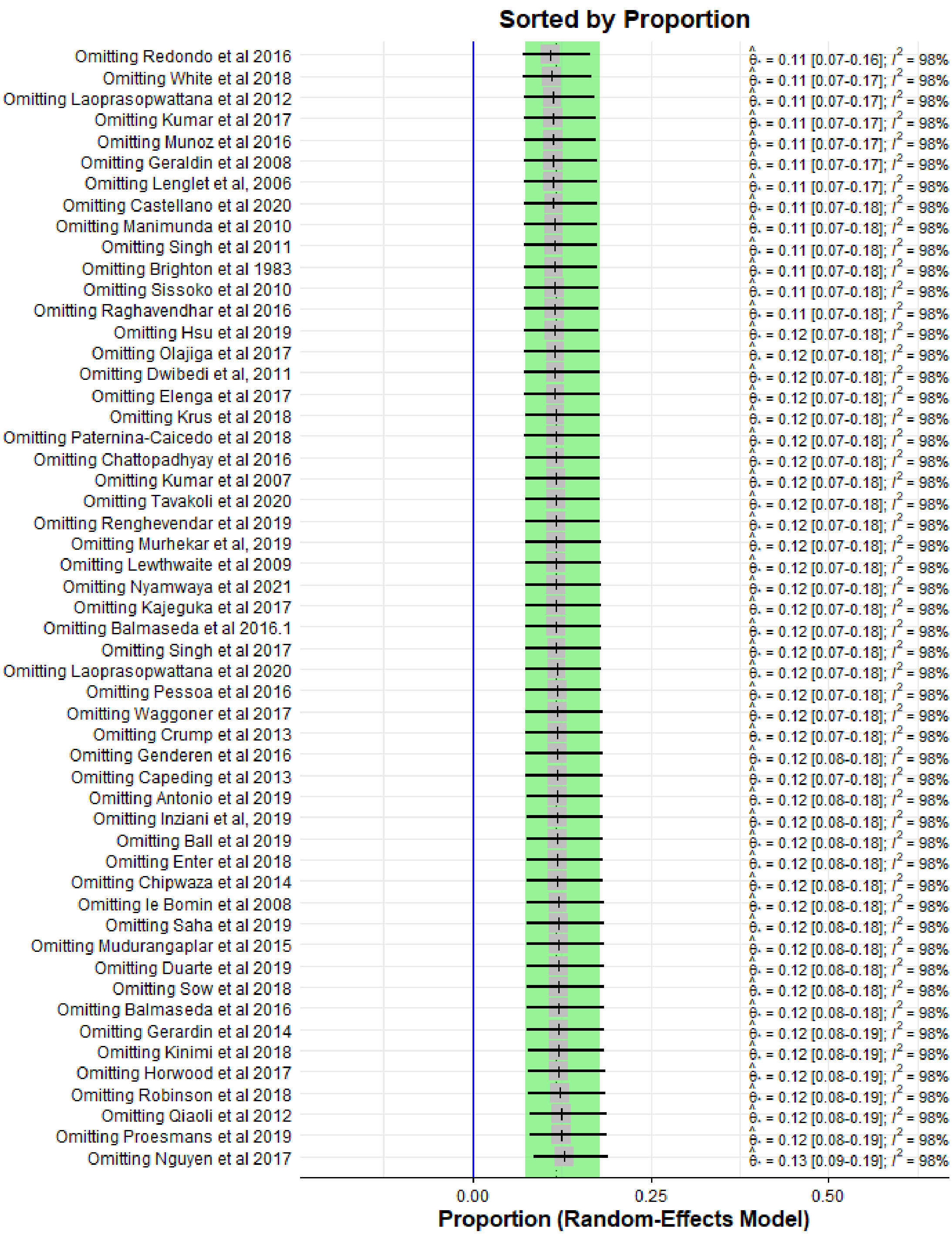

Figure 3.5. Forest plot of the overall proportions of meta-analyses conducted using leave-one-out method.

3.3.4.2 Meta-regression by multi-model inference

The multi-model regression fitted 256 models. The characteristics of the five best fitting models with their AICc are shown in **Table 3.4**~~**Table 3.4**~~. The best fit model (AICc = 10628.6) was close to other predictor combinations making it difficult to pinpoint the best model. However, all top 5 models contained "continent, diagnostic technique, season (epidemic/non-epidemic) and study design" suggesting these variables might be important. The study design used seemed to influence the effect size estimate, followed by reported method of laboratory diagnosis and the study setting either hospital or community. The season of sampling whether in or out of epidemics and the continent of the study have a moderate effect. Duration of study, year and sample size are observed to have the least effect. **Figure 3.6**~~**Figure 3.6**~~.

Table 3.4. Characteristics of the best five models fitted by the multi-model inference in meta-regression

Model	Intc	Continent	Diagnostic Technique	Study design	Season (Epidemic)	Sample size	Study duration	Year	DF	Loglik	AICc	Delta	Weight
256	+	+	+	+	+	2.53E-15	1.86E-13	-1.47E-12	34	-5279.83	10628.6	0	0.299
128	+	+	+	+	+	6.22E-19	-4.02E-16		33	-5281.3	10629.4	0.88	0.192
248	+	+	+	+	+		-3.52E-13	1.33E-09	33	-5281.3	10629.4	0.88	0.192
224	+	+	+	+	+	-2.70E-16		9.42E-13	33	-5281.3	10629.4	0.88	0.192
216	+	+	+	+	+			-4.04E-13	32	-5282.76	10630.3	1.76	0.124

*A number (weight) or + sign (for categorical predictors) indicates that a predictor/interaction term was used in the model, while empty cells indicate that the predictor was omitted

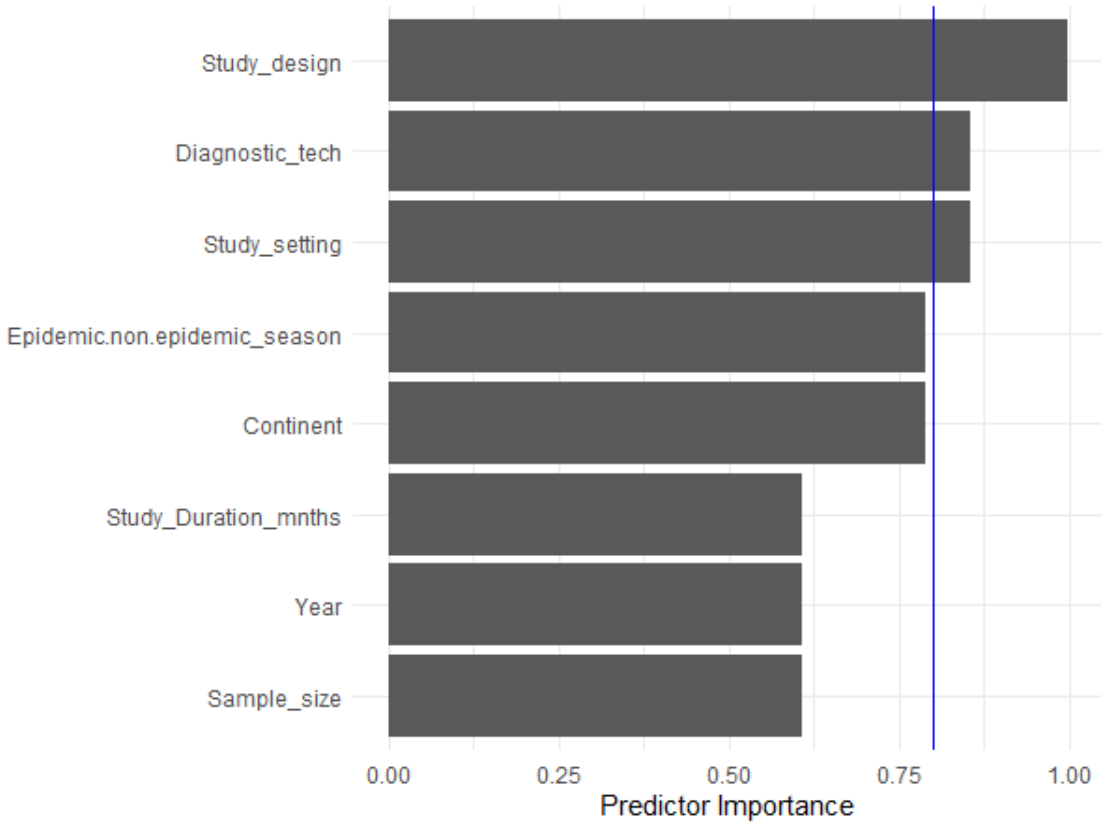

Figure 3.6. A plot of the averaged importance of each predictor (source of heterogeneity between studies reporting prevalence of CHIKV among children) across all models computed in the multimodel inference

3.3.4.2 Sub-group analyses

Most studies included in the meta-analysis (27/53 studies) were carried out during an epidemic season and they provided the highest prevalence estimate at 18% (95% CI; 10 - 30) when compared to those carried out during the non-epidemic seasons, **Table 3.5**~~**Table 3.5**~~. The observed prevalence estimate was highest in North America at 22% (95% CI; 4 - 64) and lowest in Africa at 8.9% (95% CI; 4 - 16). About 70% of the studies (n=37) were carried out in a health centre setting; these included a hospital, a school clinic or a general health centre, reporting a pooled prevalence of 13% (95% CI; 7 - 21). Detection of CHIKV RNA by RT-PCR was a frequently used diagnostic method (n=22/53 studies) followed by detection of anti-CHIKV IgM antibodies by ELISA (n=19 studies).

Table 3.5. Results of sub-group analysis for prevalence of CHIKV infection among children based on continent, study setting, diagnostic technique, study design and season

	Subgroup analyses		
	Number of studies	**Proportion in % (95% CI)**	**I^2, %**
Continent			
Africa	17	8.86 [4.42; 16.98]	97.2
Asia	20	8.78 [3.87; 18.70]	97.8
North America	6	22.20 [4.22; 64.89]	99.3
South America	10	22.83 [7.50; 51.88]	92.5
Study setting			
Hospital/health centre	37	13.02 [7.36; 21.99]	98.1
Community	11	10.11 [4.38; 21.64]	98.1
Unspecified	5	7.52 [0.68; 49.25]	91.3
Diagnostic technique			
Detection of anti-CHIKV IgM	19	11.09 [6.11; 19.31]	97.3
Detection of CHIKV RNA	22	14.64 [6.64; 29.27]	97.7
Detection of anti-CHIKV IgM and CHIKV RNA	12	8.56 [2.42; 26.12]	98.4
Study design			
Longitudinal	22	11.84 [5.14; 24.96]	98.5
Cross-sectional	21	8.75 [4.65;15.88]	97.4
Unspecified	10	21.08 [8.46; 43.55]	95.5
Season			
Epidemic	27	18.46 [10.52; 30.37]	98.4
Non-epidemic	6	3.86 [1.91; 7.62]	89.7
Epidemic/Non-epidemic	1	8.36 [5.97; 11.58]	NA
Unspecified	19	8.59 [3.36; 20.23]	96.7

3.3.4.2 Publication bias

The generated funnel plot was symmetrical **Figure 3.7**~~**Figure 3.7**~~ implicating absence of publication bias among included studies, as suggested by Egger's linear regression test (Intercept= -0.438 [95%CI; -3.33 - 2.45], t-value= -0.297, p-value= 0.767442).

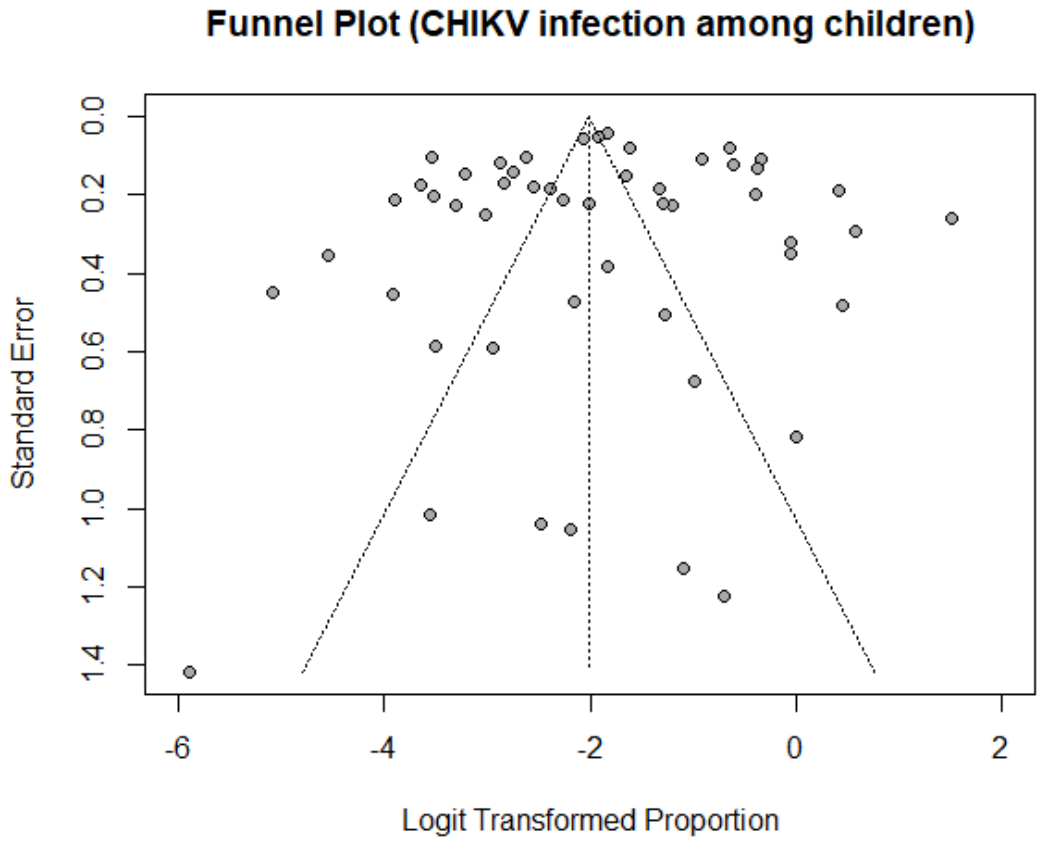

Figure 3.7. Funnel plot showing the precision of 53 reviewed studies included in the meta-analysis of CHIKV prevalence among children against their effect estimates. The funnel plot was used to examine whether the distribution of effect size estimates were less variable (i.e. whether higher number of studies were roughly symmetrical around the mean) and to detect whether the small-study effect was present. The Egger's regression was used to assess for publication bias by using precision to predict the standardized effect, a significant result suggests the possibility of publication bias. The Egger's regression test is a weighted linear regression test in which the standardized effect estimates (i.e. the quotient of effect size and its standard error) are regressed against precision (i.e., the inverse of the standard error).

3.3.5 Clinical manifestation of CHIKV infection among children

CHIKF among children can affect various body organs as shown in **Figure 3.8**~~**Figure 3.8**~~. Fever, rash and arthralgia were the most common symptoms reported in 80, 55 and 40 studies, respectively. The clinical presentation of paediatric CHIKF varies with geographical location and the circulating strain of CHIKV. In La Reunion island where epidemics were predominantly due to the Indian Ocean lineage, majority of infected neonates had thrombocytopenia, deranged coagulation, seizures , hemodynamic disorders and hemorrhagic syndromes [188]. In contrast, circulatory failure was common among CHIKV-infected infants in Kerala, India where the ECSA strain circulated [189]. The Asian genotype introduced to the Carribbean islands and central America induced weaker pro-inflammatory effect with a milder clinical course among infected neonates. It was not associated with thrombocytopaenia and clinical neurological sequelae, however the case fatality rate was comparably high [20,28]

The varied manifestations in different patients could be influenced by factors including viral / host genetics, prevailing environmental conditions, and the infective viral dose. The observed pathological difference resulting from different CHIKV strains indicate that infection by ECSA and IOL strains are more likely to result in a severe clinical course among children. The Asian genotype commonly leads to mild or subclinical disease. However, there were no paediatric CHIKV cases in the included studies that described disease progression due to West African genotype.

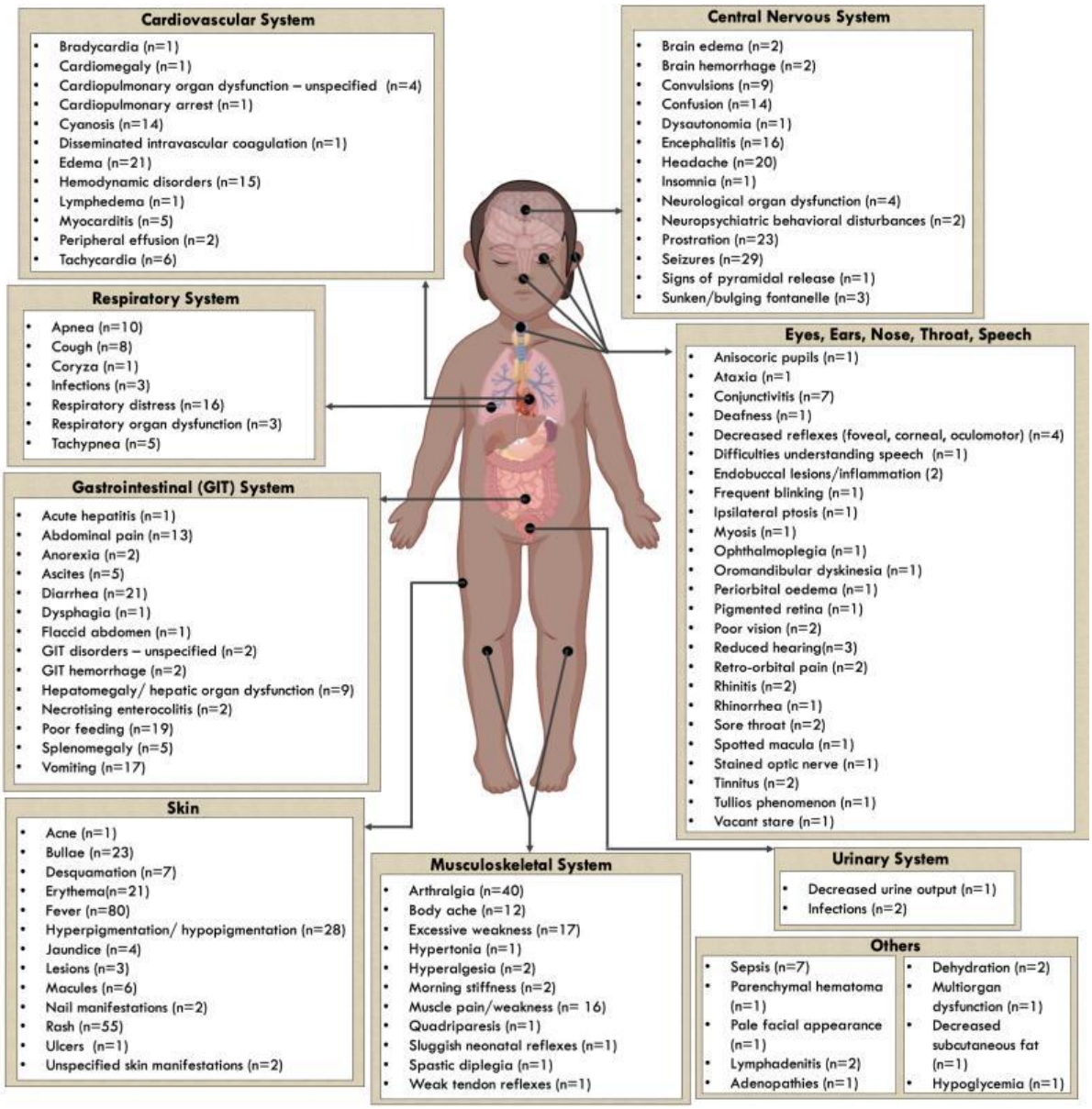

Figure 3.8. A summary of symptoms/complications of CHIKV infection in children. The number in brackets represents the number of studies that reported on this symptom/complication.

3.3.6 CHIKV diagnosis and treatment

Thrombocytopenia was the most commonly reported laboratory finding among children infected with CHIKV in 40 studies out of 59 that described laboratory investigation. Other laboratory findings also reported included: neutrophilia, leucopenia, lymphopenia, hypokalemia, hyponatremia, hypocalcemia, hyperbilirubinemia, anaemia, elevated C-reactive protein, transaminases (aspartate, alanine and oxaloacetic glutamic), elevated cellularity and neutrophil predominance, elevated creatinine kinase, reduced prothrombin concentration **Figure 3.9~~Figure 3.9~~.** CSF examination was reported in 9 studies [166,169,190–195] and showed pleocytosis (7 studies), increased protein levels (7 studies) and hypoglycorrhachia (3 studies).

Various imaging techniques including ultrasonography, magnetic resonance imaging (MRI), ultrasound, computed tomography and X-ray of CHIKV-infected children were reported in 14 studies [157,158,200–203,159,169,188,193,196–199]. They presented abnormal findings indicating diffuse brain lesions that were distributed around blood vessels, accentuation of cerebrospinal fluid spaces surrounding the carotid arteries, enlarged ventricles, loss of brain volume, cerebral oedema, cerebral atrophy and micro-calcifications, haemorrhagic leukoencephalopathy, vasculitis and soft tissue resorption

Detection of anti-CHIKV IgM antibodies in serum or cerebrospinal fluid samples by ELISA was used in 58 studies, whereas 46 studies used RT-PCR for diagnosis of CHIKV infection. Thirty-four studies used both molecular and serological techniques. Three studies diagnosed acute CHIKV infection based on stipulated clinical and epidemiological evidence. Cell isolation of the virus and indirect immunofluorescence test were each done in one study [190,204] .

Out of 142 included studies, 65 described treatment therapies for CHIKV infected children. Antibiotic prescription was predominantly given to children presenting with undifferentiated

fever before CHIKV infection was detected as reported in 35/65 studies. Treatment was supportive through management of aches using analgesics, volume resuscitation by intravenous hydration and mechanical ventilation in cases of respiratory distress. Neurological symptoms were managed using anticonvulsant therapy in 12 studies. Abnormal haematological values were corrected by transfusion of either platelets, red blood cells or plasma in 14 studies. Immunoglobulin therapy, antivirals, antimalarial and corticosteroidal treatment were also reported **Figure 3.9**~~**Figure 3.9**~~. There being no registered treatment regime for CHIKV, administration of some of these drugs without proper diagnosis can aggravate drug resistance in the patient. This would pose a challenge in subsequent treatment of other diseases in the patient. For instance, inappropriate use of antibiotics can lead to antimicrobial resistance that poses a significant clinical challenge in treatment of some dangerous bacterial diseases like tuberculosis.

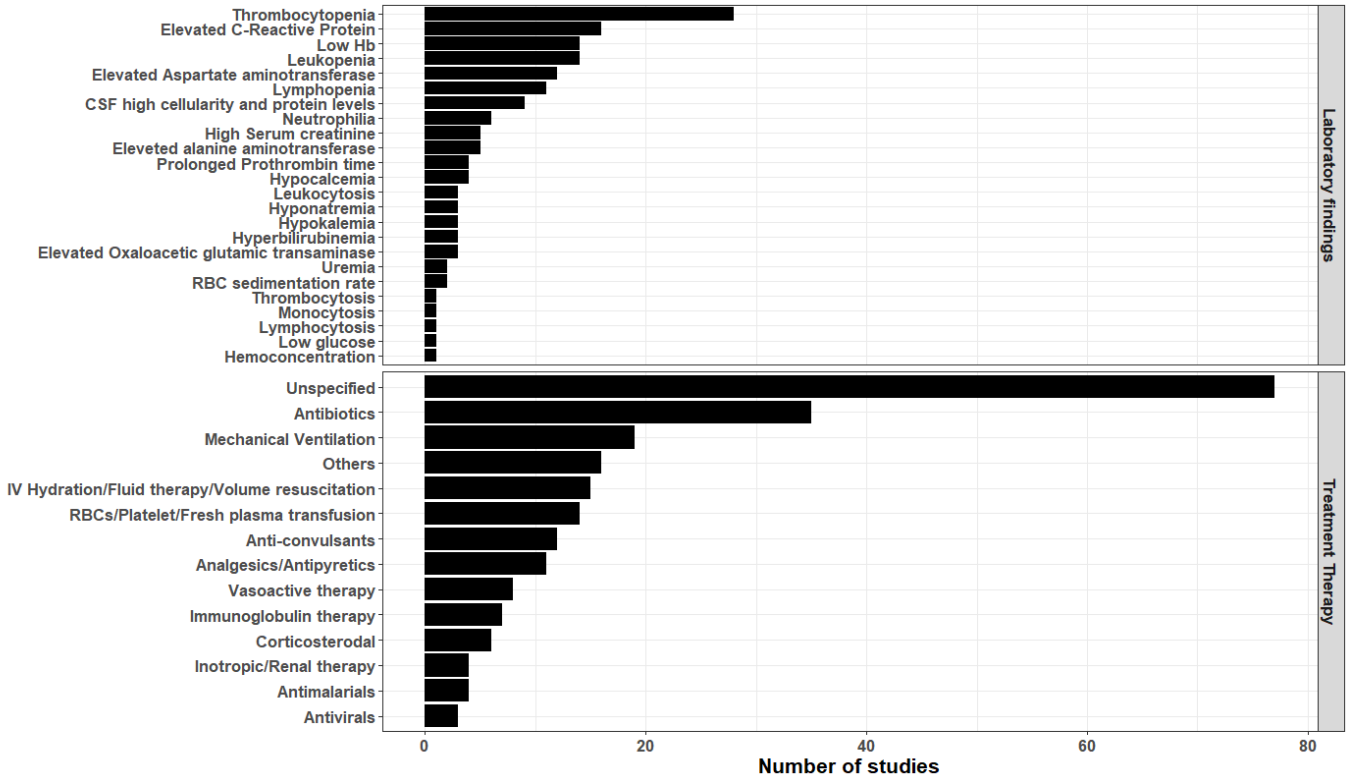

Figure 3.9. A summary of the treatments administered to CHIKV infected children and the reported laboratory investigation findings among studies included in this review

3.3.7 CHIKV Disease outcome

Most acute CHIKV infected children recovered except for a few that either developed a severe form of the disease or died. After pooling all the cases in the studies included in the review (n=3093), 34.5% (n=1068) underwent full recovery within an average of 11 days. A case fatality rate of 1% (n=33 cases) was observed. The causes of death included respiratory distress, cardiac arrest, intraventricular, gastrointestinal or cerebral haemorrhage, renal failure, cerebral edema, pleural and pericardial effusion, enterocolitis, coma and collapse of the circulatory system.

A total of 36 children (1.2%) progressed into a severe form of the disease that necessitated hospitalisation and special care. The severity included long-term neurological and dermatological sequelae, severe cerebral bleeding, neurocognitive delay, cerebral palsy with ataxia and blindness, ocular and behavioural or postural deficiencies (dysconjugate gaze), language delay, axial hypotonia, aphasia, tenosynovitis causing fixed flexion deformity of thumb, hypotonic cerebral palsy with mental retardation, deafness, persistent seizures, visual impairment, tone abnormalities, strabismus, persistent itchy rash, arthralgia, progressive sclerosis of digital skin with limited range of motion and further tapering of distal fingertips, postnatal microcephaly and retinopathy, ptosis and myosis, ataxia, apalestesis and hyperflexia in lower limbs associated with persistent clonus and urinary incontinence, bulging fontanelle, convulsion and dyspnea.

3.4 Discussion

In this study, I set out to understand the global epidemiology of CHIKF among children (aged <18 years) by describing its geographical distribution, prevalence among children presenting with fever, and clinical manifestations. CHIKF is common among children with febrile illness with a wide geographical range of transmission given that it has been reported in five continents. Several studies were from India during the CHIKF epidemic wave in 2005-6 [205–207], and a study from the Americas was published after 2013 when CHIKF was first reported in the region [8]. There were few studies published from Africa and Southeast Asia despite a high prevalence of CHIKV in these settings [109]. Most studies included in this review were from the Asian continent within the Indian territory. This was the region that was largely affected by the CHIKV epidemic wave in 2005-6 [205–207]. Children travellers from affected areas can contribute in the importation of CHIKV to new niches as indicated by the case reports from Europe [181–184]. Differential representation of available studies from endemic regions could be dependent on surveillance frameworks that depend on resource base to establish efficient diagnostic and reporting infrastructure.

We estimated the global test positive rate of CHIKF among children with acute illness to be 11%. However, this might be an underestimation of the actual disease burden due to lack of data from most CHIKF endemic regions. Underreporting of CHIKF cases could be due to exclusion of CHIKF from routine screening of undifferentiated febrile illness in many low- and middle-income countries, absence of affordable diagnostic infrastructure, low suspicion index for CHIKF among healthcare professionals and absence of an efficient surveillance system. Limited epidemiological data on CHIKV among children calls for more efforts towards control, prevention and management within the clinical and public health sector. On the other hand, we could have overestimated prevalence due to many studies being conducted in response to epidemics, and there was significant heterogeneity detected, indicating that the mean

proportion is very dependent on the spatio-temporal distribution of studies. Nevertheless, 11% figure indicates that there is a substantial correlation between CHIKF and fever among children.

There is no specific clinical sign that is discriminatory for CHIKF in children to aid in its diagnosis, and hence CHIKF may be missed in many countries that lack testing capacity. CHIKF manifests with a wide range of clinical symptoms affecting most parts of the body including musculoskeletal, nervous, cardio- respiratory, renal, cutaneous and gastrointestinal systems. Neonates may become infected during the peri-partum period if born by viraemic mothers, and in rare cases may develop long-term neuro-cognitive impairment or fatal pathological progression [126]. The rate of vertical transmission among CHIKV infected pregnant women was estimated at 48.5% during an epidemic [208].

Surveillance and detection of CHIKV infections among children is rare within community settings as most studies in this review were hospital-based reports. Community surveillance could inform transmission trends and enable timely detection of epidemics. Travellers from affected areas can contribute in the importation of CHIKV to new niches as indicated by the case reports from Europe [181–184]. This could be detrimental among CHIKV immunologically naïve populations. An effective surveillance system can detect transmission trends and help in prediction and identification of epidemic seasons.

I recognise some limitations in our review that calls for caution when interpreting our results. Differential representation of various geographical zones in terms of publications could lead to bias. Scarcity of published data from endemic regions may lead to an underestimate of the proportion of positive cases in this review, or a bias towards studies during epidemics may lead to an overestimate.

3.5 Conclusions

CHIKF is a significant unrecognised and underreported health problem among children globally and should be included in routine screening of febrile illnesses. Different isolates exhibit different degrees of pathogenicity in different geographical locations. Efforts towards treatment therapy and deployment of vaccines when available should target the vulnerable children <1 year of age who are at an increased risk of developing severe CHIKV-associated neurological complications and require adequate monitoring for potentially fatal outcomes.

CHAPTER 4:

The incidence of acute CHIKV infections among children visiting local dispensaries in Kilifi, Kenya

"This chapter forms the basis of a manuscript published in BMC infectious diseases;

Nyamwaya, D.K., Otiende, M., Omuoyo, D.O. *et al.* Endemic chikungunya fever in Kenyan children: a prospective cohort study. *BMC Infect Dis* **21,** 186 (2021). https://doi.org/10.1186/s12879-021-05875-5

4.1 Introduction

Community-based CHIKV surveillance can inform transmission trends and enable timely detection of epidemics. Availability of baseline epidemiological data including prevalence and incidence estimates enhance knowledge of the associated disease burden and could inform policy on management of CHIKV infections.

High seroconversion rates reported from coastal Kenya [129,209,210] indicate the presence of CHIKV activity that could suggest endemicity. However, the rate of occurrence over time remains unknown. Most studies reporting on CHIKF tend to be cross-sectional and carried out during an epidemic season.

Circulation and transmission of CHIKV in Kenya remains unknown outside declared epidemic seasons. Hospital records are not reliable in this setting as CHIKV is not included in the routine screening procedure for acute febrile illness. The cases therefore, remain unrecognized and unreported. This limits the attention given to CHIKV in terms of devising appropriate surveillance and monitoring systems. As transmission continues, the virus is prone to mutate and could result into more pathogenic variants that could cause severe disease [145].

In Kilifi, patients presenting with undifferentiated fever would first seek medical care in dispensaries that are near the villages. In cases of disease severity and need of better medical equipment, they are referred to the County hospitals for more intensive care. Due to the absence of diagnostic infrastructure for CHIKV, most cases are misdiagnosed for other endemic infections with similar clinical presentation like malaria. Hence, the public health burden due to CHIKV remains largely unknown in coastal Kenya. Additionally, information regarding inter-epidemic CHIKV exposure is scarce in the region.

Here I aimed to assess the epidemiological characteristics of CHIKV in Kilifi before, during and after a reported epidemic period (year 2016) [145].

4.2 Methods

I used stored EDTA blood samples collected during an ongoing passive surveillance project aimed at monitoring malaria cases in children presenting to local dispensaries in Ngerenya and Pingilikani, Kilifi. These dispensaries lie within KHDSS **Figure 2.1**~~**Figure 2.1**~~ where each inhabitant is described by their family relationships and their homestead of residence with geospatial coordinates and assigned a unique personal identifier (PID). Febrile children (axillary temperature ≥ 37.5°C or fever reported by parents) visiting these dispensaries were identified using their PID and finger-prick blood samples that had been collected and examined for malaria parasites.

To determine the prevalence, incidence and clinical characteristics of CHIKV infections, I surveyed febrile children visiting Ngerenya and Pingilikani dispensaries in Kilifi between March 2014 – March 2018, whose information is linked to the KHDSS as described in **section 2.1.2.1 (General methods).** Presence of CHIKV in their archived blood samples obtained from the KEMRI-Wellcome Trust Research Programme Kilifi biobank was determined through real-time PCR as indicated in **section 2.1.3**

Some of the CHIKV positive samples were sequenced as described in **section 2.5.** MUSCLE was used to align the obtained CHIKV genome sequences with those available in GenBank from other geographical locations. Maximum Likelihood phylogenies were reconstructed from the alignment using RAxML[211] using the GTR substitution model with 4 gamma categories (GTR + G4) and visualized in FigTree v1.4.4.

4.3 Results

4.3.1 Sampling

Between March 2014 and October 2018, a total of 29,819 children presented to the Ngerenya (n= 6,746) and Pingilikani (n=23,703) dispensaries for outpatient care and were all considered for inclusion in this study. From these, I excluded non-febrile children (n=16,123). Among those who had fever (n=13,696), 10,022 children had visited Pingilikani and 3,674 Ngerenya dispensaries. Some of the febrile children did not have PID numbers (N = 1,256). These could be children who were travellers, recent migrants into the area or those who had not been captured in KHDSS enumeration rounds despite being residents in the area, and were excluded. Some samples corresponding to the remaining records of febrile episodes (N=732) were not available in the biobank. After these exclusions, a total of 11708 records of febrile episodes from the two dispensaries [(Ngerenya (n=2,566) and Pingilikani (n=9,142)] were eligible for this study. Some children had 2 or more of these febrile episode records. The eligible visits represented records of 5,569 children, 1,897 children from Ngerenya and 3,772 from Pingilikani. Figure 4.1~~Figure 4.1~~. More than three-quarters of the samples were from children between 1 year and 10 years. Table 4.1~~Table 4.1~~

Table 4.1. Febrile episode case distribution over the various age groups

Age (years)	Frequency	%	Cumulative
<1	1,108	9.46	9.46
>=1 - <5	5,583	47.69	57.15
>=5 - <10	3,768	32.18	89.33
>=10	1,249	10.67	100
Total	11,708	100	

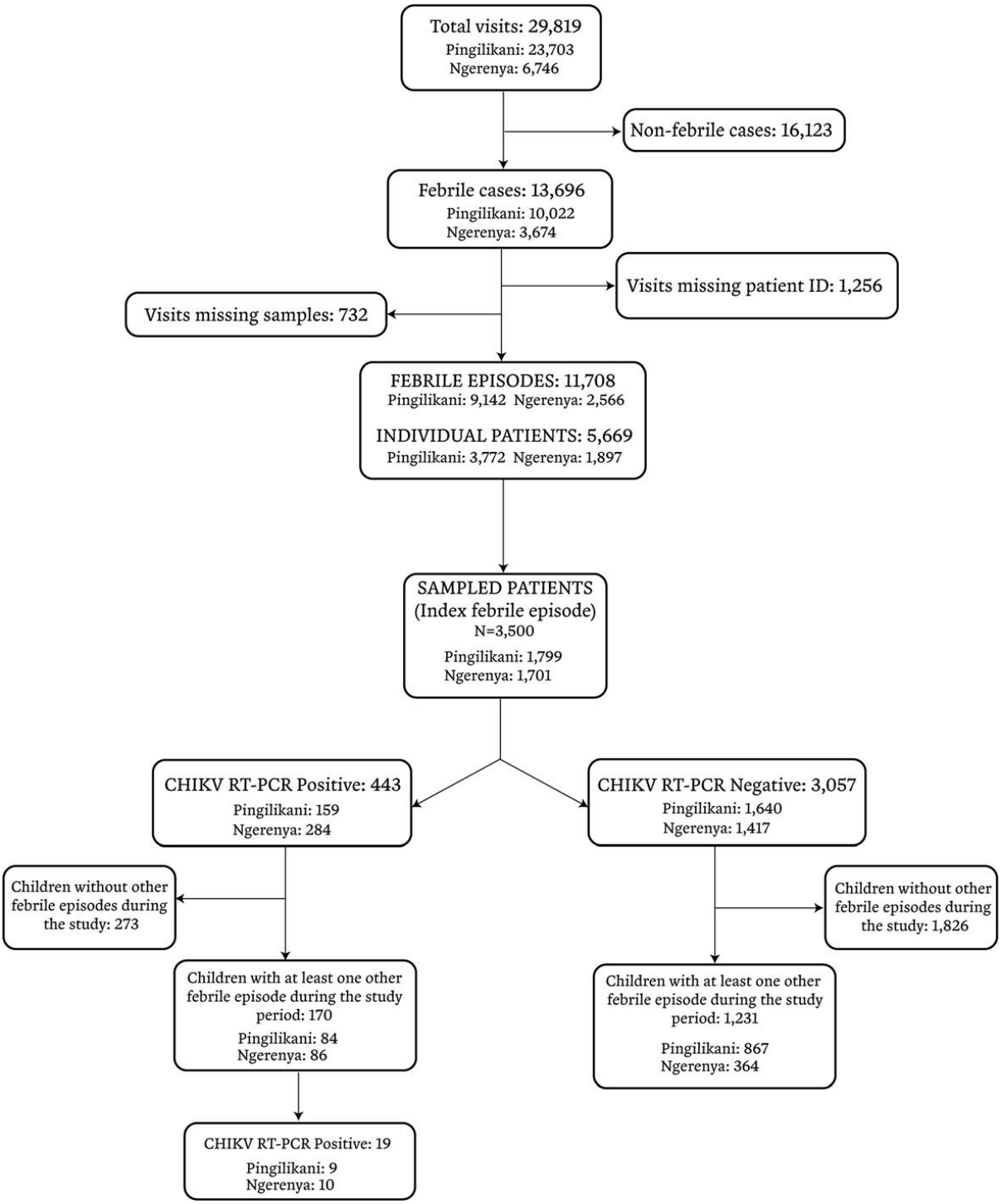

Figure 4.1. Consort diagram illustrating the sample selection criteria for CHIKV screening, the PCR output and recurrent infections

The 11708 febrile episodes were experienced by 5569 children, 1401 of whom recorded multiple febrile episodes (median 1 febrile visit per child during the study period, IQR 1 - 2). Only the first episode of the participants was considered for initial CHIKV RT-PCR analysis. A sample of 3500 febrile children out of the 5569 across the two dispensaries were randomly selected using the *runiform* () function in Stata™ version 15 and archived blood collected from their first febrile episode on record screened for CHIKV infection. The median age of the sampled children was 3.1 years (IQR 1.3 – 6.4), with 1701 being resident in Ngerenya and 1799 in Pingilikani.

Separately, samples collected from 435 children, who were residents within KHDSS were screened for presence of CHIKV. These children were afebrile and participated in a cross-sectional survey conducted in 2016 that aimed to monitor malaria transmission levels in the area.

4.3.2 Prevalence of symptomatic and asymptomatic CHIKV cases

Of the 3500 febrile children, blood samples from 443 children tested positive for CHIKV infection. This represents a prevalence of 12.7% [95% CI 11.60, 13.80]. The demographic characteristics of the detected CHIKV cases are summarized in **Table 4.2~~Table 4.2~~**. Positivity was twice as frequent in Ngerenya (16.7%, 95% CI 15.00, 18.54) than in Pingilikani (8.8%, 95% CI 7.61, 10.24). Most of the 443 CHIKF cases had a clinical diagnosis of upper respiratory tract infection but in no instance, was a diagnosis of CHIKF assigned. CHIKF case prevalence was highest in 2016 (23.1%), and was significantly lower in the other years where it ranged between 5% and 8%. Gender and age did not have any significant influence on the distribution of CHIKV cases. The frequency of detecting CHIKF cases was high in first quarter of the year (Jan – Mar) when compared to other months (**Table 4.2~~Table 4.2~~**).

Table 4.2. Demographic characteristics of 3500 febrile children screened for CHIKV infection

	Ngerenya dispensary (*N* = 1701)		**Pingilikani dispensary (*N* = 1799)**	
	CHIKF cases n/N (%)	***P* value (Chi^2 test)**	**CHIKF cases n/N (%)**	***P* value (Chi^2 test)**
Sex		0.55		0.07
Female	132/763 (17.3)		89/884 (10.1)	
Male	152/938 (16.2)		70/915 (7.6)	
Age (years)		0.27		0.67
< 1	54/260 (20.8)		25/341 (7.3)	
1 to < 5	151/926 (16.3)		69/774 (8.9)	
5 to < 10	66/430 (15.3)		43/469 (9.2)	
10 to 15	13/85 (15.3)		22/215 (10.2)	
Year		< 0.001		0.05
2014	No data		43/509 (8.4)	
2015	11/503 (2.2)		37/366 (10.1)	
2016	165/537 (30.7)		43/365 (11.8)	
2017	91/370 (24.6)		17/304 (5.6)	
2018	17/291 (5.8)		19/255 (7.4)	
Season		< 0.001		0.15
Jan – Mar	93/423(22.0)		49/432 (11.3)	
Apr – Jun	76/392 (19.4)		58/678 (8.5)	
Jul – Sep	66/496 (13.3)		31/443 (7.0)	
Oct – Dec	49/390 (12.6)		21/246 (8.5)	
Clinical diagnosis		0.58		0.06
Other[a]	26/160 (16.2)		35/506 (6.9)	
Malaria	13/94 (13.8)		70/591 (11.8)	
Pneumonia	9/58 (15.5)		18/242 (7.4)	
URTI	198/1197 (16.5)		20/231 (8.7)	
Gastroenteritis	13/83 (15.7)		1/12 (8.3)	
Undifferentiated fever	25/109 (22.9)		15/217 (6.9)	

a'Other' clinical diagnosis includes: helminthiasis, ear infections, and non-infectious conditions such as wounds, burns, and malnutrition

In contrast, the prevalence of asymptomatic CHIKV infections estimated in afebrile children was 3 out of 435 cases [0.7% (95% CI 0.22, 2.12)], yielding a clinical-to-asymptomatic CHIKV infection ratio of 18:1 during 2016 that was an epidemic year [145].

4.3.3 The incidence rate of CHIKF among children in Kilifi

The incidence of CHIKF in Kilifi was calculated over the 5 year study period, by restricting the analysis to children aged <16 years, whose residence was within the dispensary catchment area, defined as a radius of 5km from each dispensary. This represented a total of 136,509 person-years of observation (pyo) over the study period at both locations. For the numerator (CHIKF cases), the observed CHIKV RT-PCR positivity rate **Table 4.2~~Table 4.2~~** was applied to the total number of febrile children presenting to the respective dispensary on the assumption that the case positive rate among the randomly selected 3500 would hold true for the remaining 2069 samples. The CHIKV positivity rate was linked to the DSS data, **Figure 4.2~~Figure 4.2~~.** Individuals with a periodic follow-up whose CHIKV infection time was not precisely known were excluded (interval censoring).

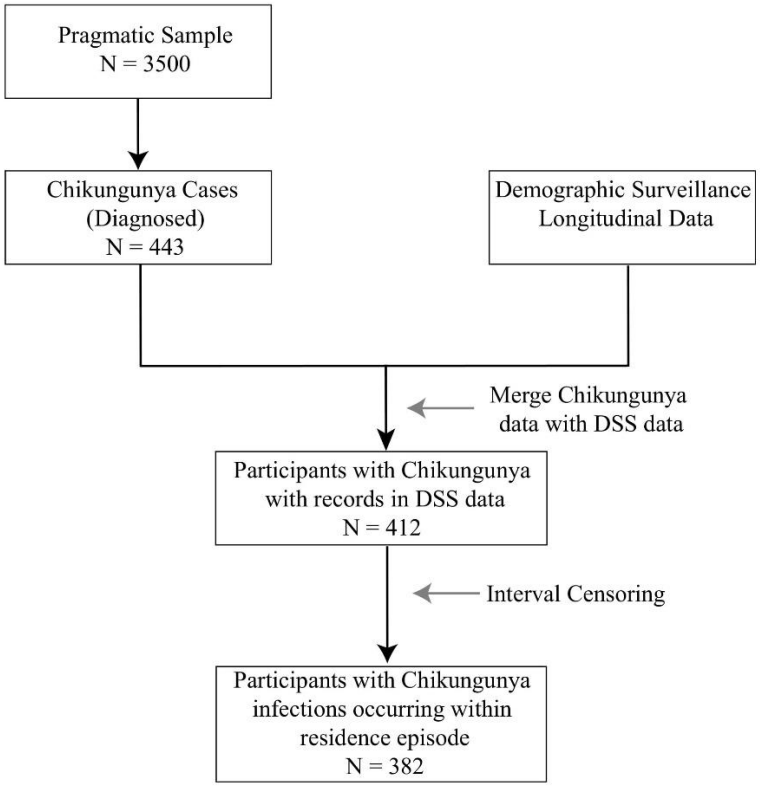

Figure 4.2: Consort diagram illustrating the CHIKV cases used as numerator for incidence calculation

The overall incidence of CHIKF during the study period was 314 cases/100000 pyo (95% CI 285, 345; Table 4.3~~Table 4.3~~). However, an inverse relationship was evident between CHIKF incidence and age (Table 4.3~~Table 4.3~~). CHIKF incidence was significantly higher in Ngerenya as compared to Pingilikani. While an increase in incidence was observed in Ngerenya during the 2016 CHIKF epidemic, this was not observed in Pingilikani where CHIKF incidence had been gradually declining since 2014. A negative correlation was observed between CHIKF incidence and residence distance from the dispensary at both locations **(**Table 4.3~~Table 4.3~~**).**

Table 4.3. Incidence of CHIKF among children < 16 years in Ngerenya and Pingilikani

	Ngerenya (N = 73,028 pyo)		**Pingilikani (N = 63,481 pyo)**	
	Incidence (95% CI)	**IRRs (95% CI)**	**Incidence (95% CI)**	**IRRs (95% CI)**
Overall incidence	336 (296,381)	–	288 (248,333)	–
Year				
2014	–	–	536 (418,676)	Ref
2015	79 (41,138)	Ref	348 (255,464)	0.65 (0.45, 0.94)
2016	920 (774,1086)	11.58 (6.42, 20.89)	274 (192,380)	0.51 (0.34, 0.77)
2017	514 (406,642)	6.47 (3.52, 11.88)	122 (69,198)	0.23 (0.13, 0.39)
2018	127 (72,207)	1.60 (0.76, 3.39)	129 (70,216)	0.24 (0.14, 0.43)
Sex				
Female	320 (265,384)	Ref	331 (270,401)	Ref
Male	352 (294,418)	1.10 (0.86, 1.41)	245 (194,306)	0.74 (0.55, 0.99)
Age (years)				
< 1	1363 (1030,1770)	Ref	979 (674,1375)	Ref

1 to < 5	658 (549,783)	0.48 (0.35, 0.66)	509 (406,629)	0.52 (0.35, 0.78)
5 to < 10	221 (166,288)	0.16 (0.11, 0.24)	208 (151,280)	0.21 (0.14, 0.33)
10 to 15	35 (16,67)	0.03 (0.01, 0.05)	93 (58,143)	0.10 (0.06, 0.17)
Distance from dispensary (Km)				
< 1	881 (599,1251)	Ref	1037 (677,1519)	Ref
1 to < 2	636 (483,822)	0.72 (0.47, 1.12)	855 (605,1173)	0.82 (0.50, 1.36)
2 to < 3	456 (353,580)	0.52 (0.34, 0.79)	883 (700,1099)	0.85 (0.55, 1.33)
3 to < 4	376 (277,498)	0.43 (0.27, 0.67)	207 (116,343)	0.20 (0.11, 0.38)
4 to < 5	181 (120,261)	0.21 (0.12, 0.34)	148 (81,249)	0.14 (0.07, 0.27)
5 to < 6	84 (47,139)	0.10 (0.05, 0.18)	32 (15,59)	0.03 (0.02, 0.06)
Season				
Jan - Mar	459 (368,566)	Ref	273 (199,365)	Ref
Apr - Jun	350 (271,445)	0.76 (0.55, 1.05)	433 (338,546)	1.59 (1.09, 2.30)
Jul - Sep	276 (206,362)	0.60 (0.43, 0.85)	281 (205,374)	1.03 (0.68, 1.55)
Oct - Dec	249 (179,338)	0.54 (0.37, 0.79)	147 (91,225)	0.54 (0.32, 0.91)

*Ref –comparator group

4.3.4 Reccurrent Chikungunya infections in Kilifi

Examination of 3500 samples collected from the first febrile episode of children participants during the study period showed presence of CHIKV in 443 children, of which 170 had more than one fever episode during the study period. CHIKV was detected in samples from recurrent febrile episodes of 19 of the 170 children. The timeframe between CHIKV infection episodes varied among individuals. More non-CHIKV repeat febrile episodes were observed in residents of Pingilikani than Ngerenya **Figure 4.3**~~**Figure 4.3**~~.

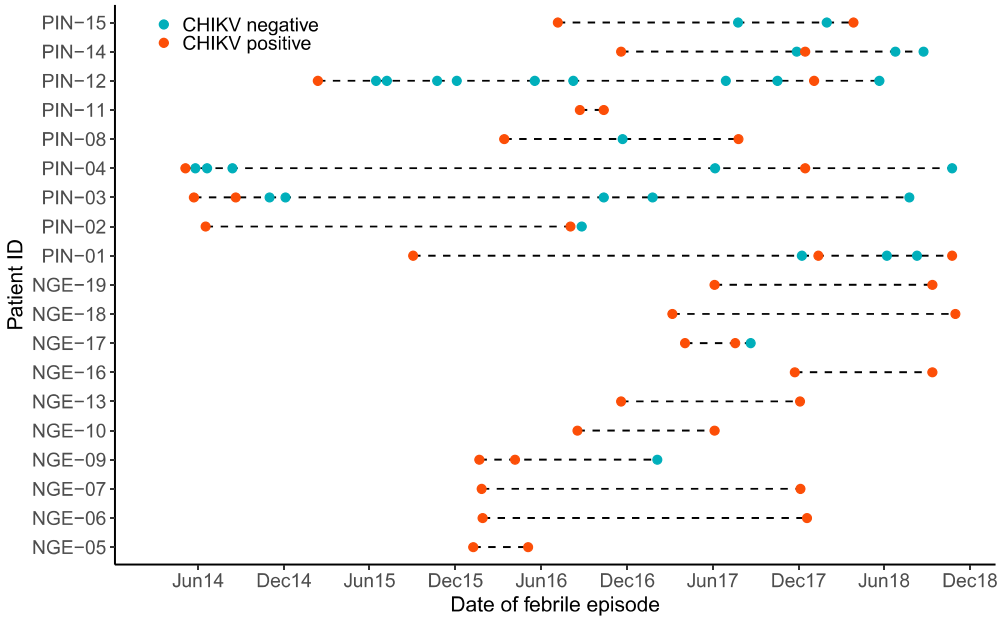

Figure 4.3. Recurrent CHIKF episodes. The distribution of febrile episodes for each of 19 children who had at least two CHIKV RT-PCR positive febrile episodes is shown. The timing of the CHIKV RT-PCR positive results is shown, with the index episode shown as the first data point in the follow up timeline. Children with prefix 'NGE' were resident in Ngerenya and those with 'PIN' from Pingilikani respectively

4.3.5 CHIKV phylogeny

All index and recurrent CHIKF episode samples from the 19 children **Figure 4.3**~~**Figure 4.3**~~ were sequenced. Complete and partial genome coding sequences were generated from 9 children. Reference-based assembly was done on the index and repeat cases whereby only 4 children had index-repeat genomes that covered > 80% of the reference genome from the Lamu outbreak of 2004 in coastal Kenya (HQ456255.1). The rest were partial genomes that mapped on to different protein coding segments of CHIKV genome including NSP2, NSP3, NSP4 and E1. BLASTn analysis of the near-complete genomes showed Kilifi sequences shared high nucleotide similarities with sequences from the 2017-2018 outbreak in Mombasa, China and India. All the obtained sequences mapped to the ECSA genotype, albeit distinct from the clade containing the IOL strains that emerged during the 2004 epidemic and the clade including

genomes from CHIKF patients sampled in Mandera, northern Kenya, during the 2016 epidemic. The E1 A226V mutation, associated with adaptation and increased transmissibility by Aedes albopictus [212] , was absent from all sequences sampled in Kenya [145]. Time-resolved phylogenies showed strong temporal clustering of CHIKV sequences from coastal Kenya that may suggest antigenic changes over time driven by acquisition of herd immunity **Figure 4.4**~~**Figure 4.4**~~. Index-recurrent CHIKF episode genome sequence pairs were available for four children with respective time intervals of 2.9, 3.5, 22.3 and 28.3 months between the index and recurrent episodes **Figure 4.4**~~**Figure 4.4**~~. The degree of relatedness of these index-recurrent episode sequence pairs was also time-dependent, with the sequence pair with the longest intervening time interval (28.3 months) being most divergent **Figure 4.4**~~**Figure 4.4**~~. The relatedness of CHIKV genomes from index-recurrent sequence pairs in the same child was similar to the relatedness of CHIKV genomes from different children **Figure 4.4**~~**Figure 4.4**~~ suggesting that these were reinfections rather than relapses.

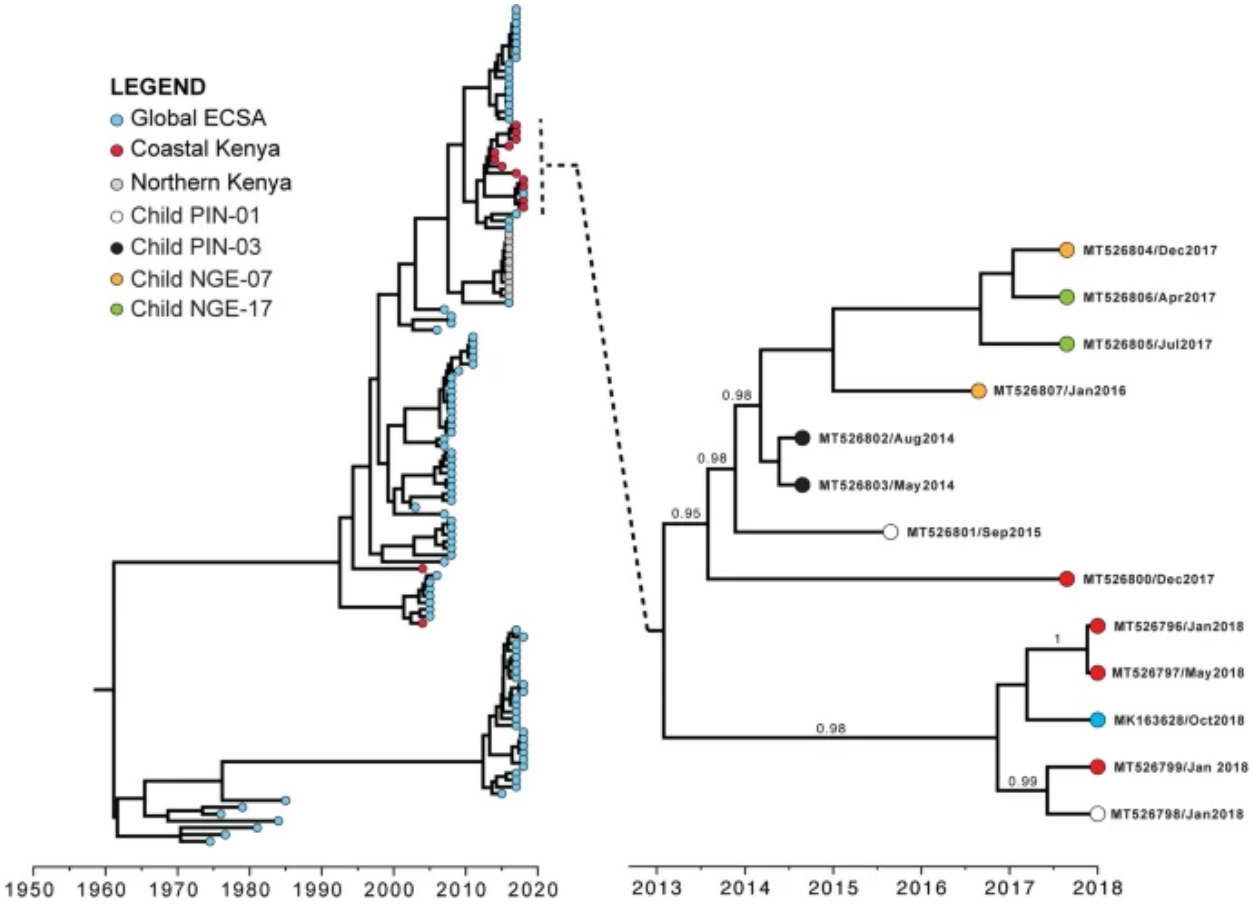

Figure 4.4 Phylogenetic analysis of CHIKV genomes from Kenya. BEAST MCC tree of 123 ECSA genomes collected across the world, including 24 from Kenya is shown in the left panel. On the right panel is an extraction highlighting the CHIKV sequences generated in this study. The tips are coloured according to geographic location or child identity (as in **Figure 4.3**~~**Figure 4.3**~~, for those with paired index-recurrent episode sequence pairs), with posterior probability support shown for select nodes with probability > 0.8. Sample MT526800 was from a child with a single CHIKF episode whose RT-PCR cycle threshold (Ct) value was low (Ct = 19)

4.4 Discussion

In this study I estimated the prevalence of acute CHIKV infections at approximately 12% of all childhood fevers in the local dispensaries in Kilifi, coastal Kenya. This corroborates documented findings from previous studies [96,110,213]. A systematic meta-analysis in Africa yielded a pooled prevalence estimate of acute CHIKV infections of 9.7% [110]. A study conducted in three sites of northern Tanzania with varying ecological and climatic conditions reported an average prevalence of 12.9% acute CHIKV infections (10.1%, 13.8% and 14.7%

respectively from the sites) [96]. In Kenya, 13 of 239 [5.4%] febrile children visiting health centres in coastal region had acute CHIKF [213].

Occurrence of new cases was evident throughout the study period (2014 – 2018) with varying infection intensities over time. Most cases were observed in 2016 when an outbreak was reported in northern Kenya [145] following the major global epidemic in 2004 [122]. The continuous circulation indicates that CHIKV is endemic in the study setting. Variation in climatic conditions can influence CHIKV infection rates. High temperatures are favourable for CHIKV transmission and more cases have been associated with drought seasons in coastal Kenya [120], and in La Reunion Island [31].

Heterogeneous distribution of acute CHIKF cases within the study region was observed in this study with Ngerenya reporting more cases than Pingilikani. During the epidemic year an increase in CHIKF incidence was only noted in Ngerenya while that in Pingilikani had been declining. Previous studies have described differences in malaria transmission intensities that is higher in Pingilikani than Ngerenya [132]. Kajeguka et al also noted that the prevalence of acute CHIKV infection was lower in a high malaria transmission zone compared to areas with low malaria incidences [214]. The ecological and biological factors underlying these geographic differences in risk of exposure to CHIKV and other infections need further investigation.

In this cohort CHIKF manifested with occurrence of fever in most of the cases although prevalence in asymptomatic residents was 0.7%. The proportion of subclinical CHIKV infections is reportedly highly variable ranging about 3% to 80% [215]. These differences could be attributed to the circulating CHIKV strain as the Asian genotype has been associated with high rate of inapparent infections when compared to ECSA that predominantly circulates in Kilifi [215]. A longitudinal cohort study in the Philippines in 2012 -13 evaluated the proportion of subclinical and symptomatic CHIKV infection in 853 subjects of different age

groups [215]. They established occurrence of subclinical CHIKV in 82% of the study population. This proportion was the highest reported compared with previous studies [3]. They attributed this finding to increased precision in detection of cases by the implemented study design which involved collection of baseline blood samples from a defined community-based cohort prior to infection, followed by the implementation of a sensitive active surveillance system [215]. The incidence of subclinical infection was age-dependent and was lowest among children below 5 years old [215]. Some cases present with other symptoms like joint pains without fever and can inappropriately be classified as asymptomatic [216].

The acute CHIKV case burden was observed to be inversely proportional with age in Kilifi. This could be due to accumulation of immunity over time hence the older children are more protected. Studies have shown that young infants below the age of 1year are more likely to develop a severe form of CHIKF that is associated with a high case fatality [127,170,217]. Most of these cases acquire the infection during birth from a viremic mother [188,218] however others could be exposed to an infected mosquito bite.

Consistent with previous reports of CHIKF in children in East Africa [98,109,118] , none of the patients in this study had a clinical diagnosis of CHIKF. Routine screening for undifferentiated acute febrile illnesses in health care centres does not include CHIKV hence the high probability of missing it. Investigation in Tanzania for the aetiology of febrile illness among children revealed that most CHIKV cases were diagnosed and managed as Malaria [109]. Lack of a differential diagnosis for CHIKV becomes an important factor in underreporting of CHIKV cases and subsequently underappreciation of the associated clinical burden. Co-infections do occur, but there is need to develop a system that could detect and record all cases to inform CHIKF prevention and clinical care.

Joint pathology that typifies CHIKF manifestations in adults was not described among the detected CHIKF cases in this study. Previous studies have indicated variation in CHIKV

clinical manifestation with cutaneous and neurological symptoms being common among children while musculoskeletal manifestations are frequent in adults [29,219]. The high frequency of URTI diagnosis in this study could imply presence of coinfections with other causative aetiologies of respiratory illness or a possible cognitive bias in diagnostic classification of undifferentiated fevers.

Reports have shown that exposure to CHIKV results in development of durable immunity that can be boosted by subclinical infections with any circulating strain of CHIKV [220,221]. However, the observation in this study revealed that recurrent CHIKV infections can occur in varying timeframes per individual. This could be linked to differences in immunological profiles that could be influenced by many factors including co-infections, genetic composition and age.

This study has unravelled a previously unrecognized burden of CHIKV infections among children in coastal Kenya. Further studies to determine the incidence of CHIKF in other geographical locations in the country will enable proper monitoring of transmission intensity and timely detection of epidemics. There is need to undertake a detailed characterization of clinical manifestation of the disease so as to establish a differential diagnostic criterion for CHIKF. It will be appropriate to undertake an assessment of the longevity and kinetics of anti-CHIKV neutralizing antibodies. Establishment of a reliable population-based surveillance framework could be informative on epidemic trends and identification of sub-clinical infections which can have significant public health implications on transmission dynamics and detection.

4.5 Conclusion

This study has confirmed that chikungunya is endemic and presents a substantial undetected health burden among children resident in Kilifi. Lack of a CHIKV surveillance framework within the health care system masks the actual disease burden in the community. There is need

to develop rapid diagnostic tools to augment CHIKF case detection, studies to inform clinical guidelines for CHIKF to increase the index of suspicion among healthcare workers.

Chapter 5 :

CHIKV-associated neurological disease among children in Kilifi County

“This chapter forms the basis of a manuscript published in PloS Medicine

Nyamwaya DK, Otiende M, Mwango L, Kariuki SM, Otieno B, Omuoyo DO, et al. (2022) Incidence of chikungunya virus infections among Kenyan children with neurological disease, 2014– 2018: A cohort study. PLoS Med 19(5): e1003994. https://doi.org/10.1371/journal.pmed.1003994”

5.1 Introduction

CHIKV continues to rapidly expand its geographical space causing outbreaks that are associated with high morbidity. Since its initial identification in humans, CHIKF has been described as a self-limiting illness that resolves within approximately 10 days [7]. Chronic cases involving relapse of joint pathology after months or years have been reported [12]. However, during the epidemic wave associated with re-emergence of CHIKV in 2004, severe clinical manifestations including; neurological, cutaneous, cardiological, renal, hepatic, respiratory and haemorrhagic defects were observed [6,222]. The case fatality rate has since been on the rise making CHIKF a disease of public health concern. Infants, the elderly and immunocompromised individuals are at a high risk of developing severe disease.

CHIKV associated neurological disease has been described since early 1960s [223,224] but descriptions of the prevalence and neurologic syndromes were only documented during the epidemic in 2004. Neurotropism of CHIKV results in a broad range of detrimental manifestations including simple and complex febrile seizures, meningeal syndrome, myelitis, choroiditis, acute encephalopathy and encephalitis [225]. It can lead to death in severe cases especially among neonates who are at a high risk of CHIKV associated encephalitis that could be attributed to intense viral replication and a weak immune system [127,225]. CHIKV targets leptomeninges, the choroid plexus and the ependymal wall while infecting neurons and glial cells inducing apoptosis [226].

Common causes of neurological illness among children in Africa include cerebral malaria and bacterial meningitis [227]. However, there is scarcity of data on viral neurological infections. Neurological complications due to CHIKV infection have been described in different parts of the world, with children being disproportionately affected [228,229]. However, there have been no studies describing CHIKV-associated neurological disease in Africa.

In this objective, I set out to estimate the incidence of CHIKV-associated neurological disease among children in coastal Kenya.

5.2 Methods

To determine occurrence of CHIKV-associated neurological disease in Kilifi, I used CSF samples of children admitted in Kilifi county hospital from 2014 to 2018, whose illness necessitated lumbar puncture and screened for presence of CHIKV genome. These samples were available from the KEMRI-Wellcome Trust Research Programme biobank that also integrates clinical and demographic data from sampled individuals. CHIKV detection was done by extracting viral RNA using TRIzol method then screening by real-time PCR as indicated in **section 2.1.3.** The obtained data on CHIKV positivity was linked to the information in the KHDSS records as described in **section 2.1.2.2** (General methods) to allow for incidence calculation.

5.3 Results

5.3.1 Sampled population

Kilifi County Hospital registered a total of 18,341 paediatric admissions during the study period (2014 – 2018). All of these admissions were eligible for inclusion into the study

Figure 5.1

~~**Figure 5.1**~~. These were children resident and non-resident within the KHDSS below 16 years of age admitted with meningism (n=136), coma (n=2780), impaired consciousness (n=6428), seizures (n=1524), prostration (n=131), fever (n=4292) and others (n=3050). Of these, 14,009 children were excluded from the study as they did not undergo lumbar puncture hence no CSF sample was obtained. Among the remaining 4,332 cases, CSF samples were available from

3,980 children after acute investigations for clinical care and these were all included in the study and screened for the presence of CHIKV. Among these, 2,145 were from KHDSS residents and 1,835 were non-residents

Figure 5.1

~~**Figure 5.1**~~.

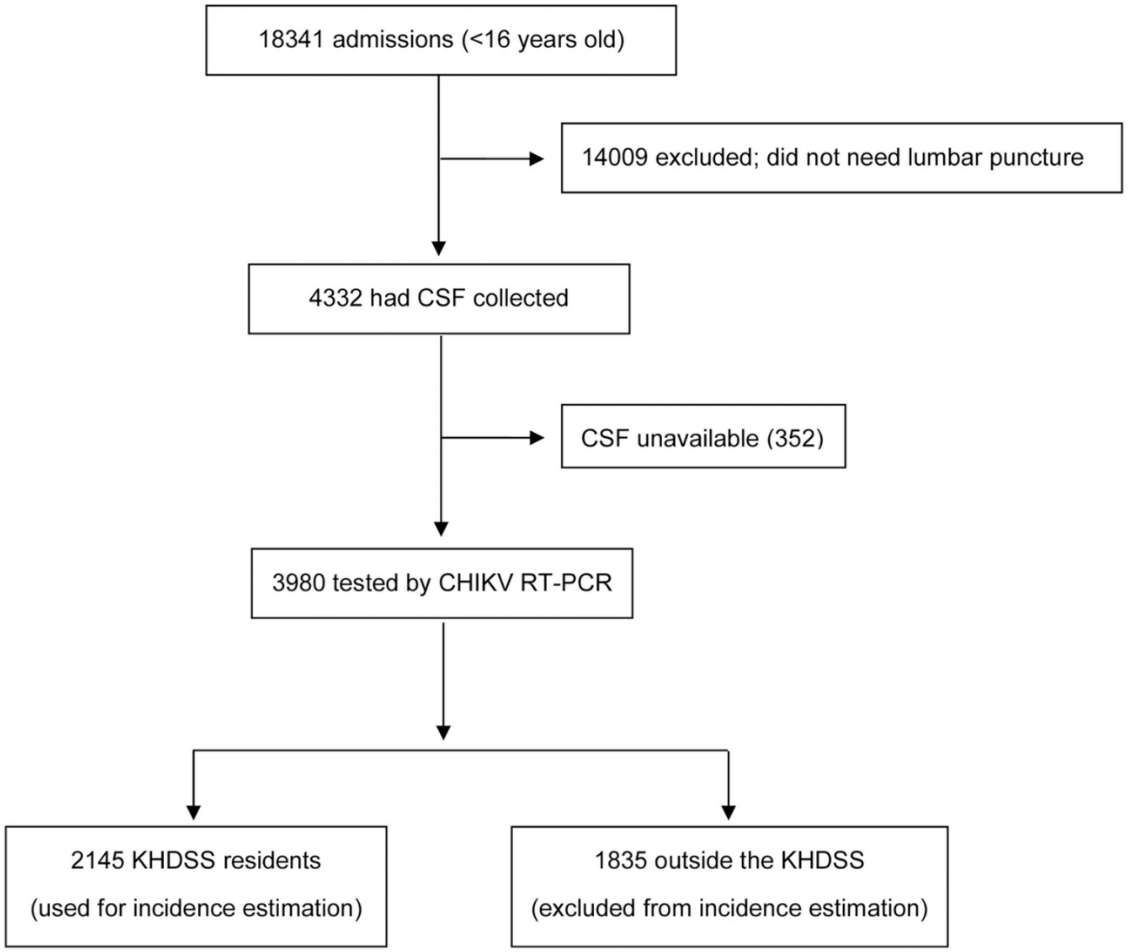

Figure 5.1. Consort diagram illustrating CSF samples available to study CHIKV-associated neurological disease and the resultant RT-PCR outcome

The most common clinical indications for CSF collection from the 4,332 cases who required lumbar puncture for routine clinical investigations included: meningism; coma (Blantyre Coma

Score [BCS] <3); impaired consciousness (BCS 3 or 4); seizures; prostration; fever; and other causes **Figure 5.2~~Figure 5.2~~**. Similar proportions of children with these lumbar puncture indications had CSF collected across the 5-year study period, suggesting a consistent pattern of clinical practice **Figure 5.3~~Figure 5.3~~**. During the study period, a total of 1,653 admitted children died representing a mortality rate of 9.0% (95% CI 8.6, 9.4) **Figure 5.4~~Figure 5.4~~**. Mortality among the 4332 admissions that had CSF collected during the study period was 3.2% (95% CI 2.7, 3.8; 139 deaths), compared with 10.8% (95% CI 10.3, 11.3; 1514 deaths) in those where CSF was not collected **Figure 5.4~~Figure 5.4~~**.

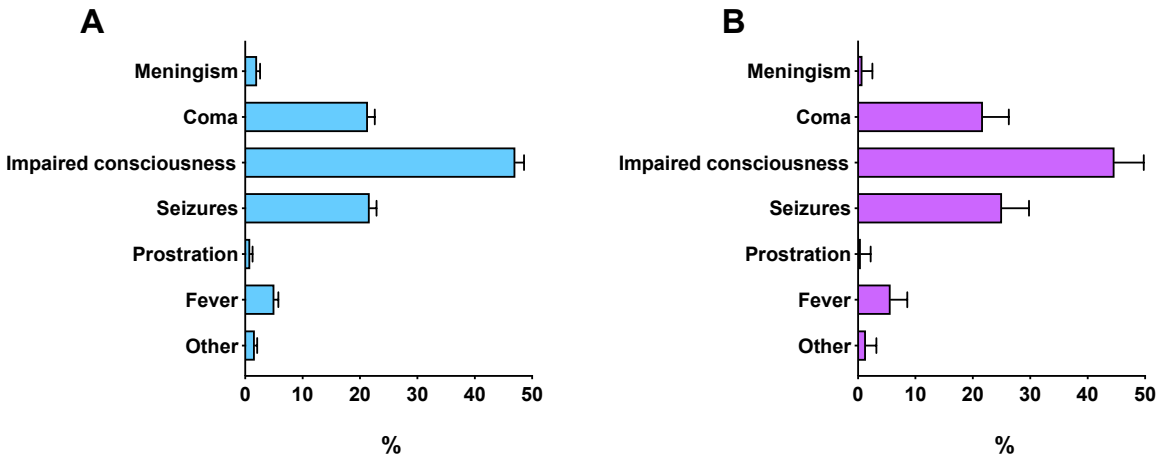

Figure 5.2. The distribution of clinical indications for lumbar puncture among all 4332 admitted patients that had CSF collected during the study period are shown in panel A. In panel B, the distribution of clinical indications for the 367 children whose CSF were CHIKV positive is shown for comparison. The data are shown as percentages of the respective denominator (4332 in A, and 367 in B). Error bars represent 95% confidence intervals

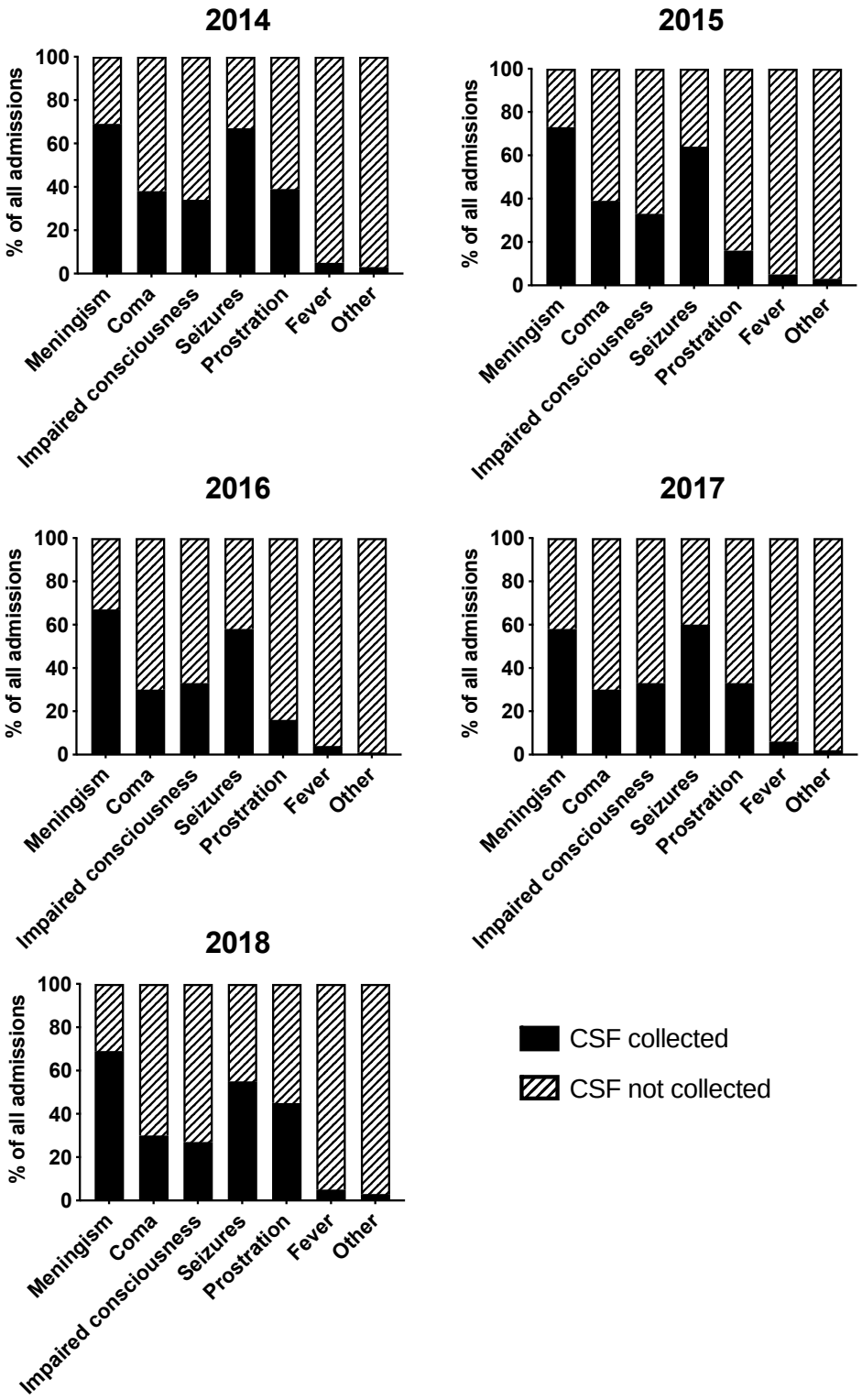

Figure 5.3. Annual distribution of admissions at Kilifi County Hospital stratified by clinical indication for CSF collection**.** The distribution of all 18341 children aged <16 years admitted at Kilifi County Hospital during the study period is shown, stratified by whether cerebrospinal fluid (CSF) was collected or not. The stacked bars for each clinical indication show the proportions whose CSF was collected or not collected and add up to 100% in each instance.

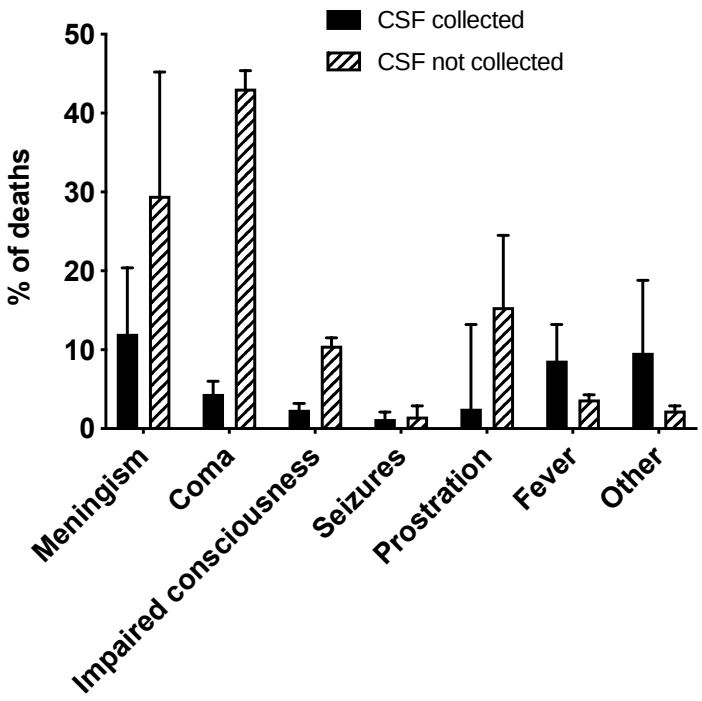

Figure 5.4. Distribution of deaths by clinical indication for CSF collection. There were 1653 (9.0%) deaths among all 18341 children aged <16 years admitted at Kilifi County Hospital during the study period. All deaths among children within each clinical indication are stratified by whether or not cerebrospinal fluid (CSF) was collected. The total number of deaths within each clinical indication are: meningism (n=24), coma (n=840), impaired consciousness (n=510), seizures (n=20), prostration (n=15), fever (n=169) and others (n=75). Error bars represent 95% confidence intervals.

5.3.2 CHIKV Positivity in CSF

All the 3,980 CSF samples from the cases included in this study were available from the KEMRI-Wellcome Trust biobank, Kilifi. All the samples were screened for presence of CHIKV

Figure 5.1

~~**Figure 5.1**~~. CHIKV PCR positivity was observed in 367 (9.2%, 95% CI 8.3, 10.2). Most of these CHIKV infections (308 of the 367; 84%) were in children aged under 5 years **Table 5.1**~~**Table 5.1**~~. Positivity rate was highest in 2016 (18%), and ranged between 4% to 9% in the other years **Table 5.1**~~**Table 5.1**~~. CHIKV infection showed no association with HIV, bacteraemia or malaria parasitaemia at the time of admission **Table 5.1**~~**Table 5.1**~~. Further, the

distribution of clinical history and symptoms recorded at admission and laboratory investigations undertaken to inform clinical care was similar for CHIKV positive and CHIKV negative children **Table 5.1**~~**Table 5.1**~~. Overall case fatality among CHIKV positive children was 1.4% (95% CI 0.4, 3.2; 5 deaths of 367 CHIKV positive children; two new-borns, two aged 2 years and one aged 8 years) and 3.2% (95% CI 2.6, 3.8; 115 deaths) among CHIKV negative children **Table 5.1**~~**Table 5.1**~~.

Table 5.1 Demographic and clinical features of patients screened for CHIKV infection**.**

	CHIKV positive # (%) (N=367)	**CHIKV negative # (%) (N=3613)**	**P value**
Sex			0.39
Female	161 (43.9)	1501 (41.5)	
Age group			0.39
<3 months	148 (40.3)	1641 (45.4)	
3 to <12 months	36 (9.8)	306 (8.5)	
1 to <5 years	124 (33.8)	1164 (32.2)	
5 to <10 years	48 (13.1)	399 (11.0)	
10 to 15 years	11 (3.0)	103 (2.8)	
Year of admission			<0.001
2014	68 (18.5)	918 (25.4)	
2015	91 (24.8)	895 (24.8)	
2016	144 (39.2)	639 (17.7)	
2017	33 (9.0)	457 (12.6)	
2018	31 (8.4)	704 (19.5)	
Season			0.32
Jan - Mar	92 (25.1)	986 (27.3)	
Apr - Jun	110 (30.0)	995 (27.5)	
Jul - Sep	90 (24.5)	792 (21.9)	
Oct - Dec	75 (20.4)	840 (23.2)	
Admission characteristics			
Duration (days) of hospitalization	3 (2, 6)	4 (2, 7)	0.26

Needed blood transfusion	22 (6.0)	185 (5.1)	0.46
General symptoms			
Fever	279 (76.0)	2683 (74.3)	0.46
Vomiting	57 (15.5)	548 (15.2)	0.85
Cough	60 (16.3)	629 (17.4)	0.61
Diarrhea	20 (5.4)	251 (6.9)	0.28
Jaundice	25 (6.8)	312 (8.6)	0.23
Wasting	15 (4.1)	150 (4.1)	0.95
Joint pain	2 (0.5)	10 (0.3)	0.37
Irritability	16 (4.4)	236 (6.5)	0.10
Rash	1 (0.3)	34 (0.9)	0.19
Deep breathing	29 (7.9)	356 (9.8)	0.23
Shock	2 (0.5)	33 (0.9)	0.47
Lymphadenopathy	3 (0.8)	27 (0.7)	0.88
Neurological symptoms			
History of seizures	56 (15.3)	469 (13.0)	0.22
Seizures during current illness*	179 (48.8)	1632 (45.2)	0.19
Headache	19 (5.2)	144 (4.0)	0.27
Bulging fontanelle	10 (2.7)	70 (1.9)	0.31
Neck stiffness	3 (0.8)	81 (2.2)	0.07
Agitation	27 (7.4)	239 (6.6)	0.59
Prostration	56 (15.3)	574 (15.9)	0.75
Impaired consciousness	165 (45.0)	1718 (47.5)	0.34
Coma	82 (22.3)	791 (21.9)	0.84
Laboratory investigations			
CSF-to-blood glucose ratio <0.67	63 (29.6)	681 (28.7)	0.80
CSF protein > 0.45g/L	154 (43.7)	1607 (46.5)	0.31
CSF Leukocyte count >5/µL	52 (14.7)	567 (16.2)	0.47
CSF turbidity	9 (2.7)	158 (4.8)	0.09
HIV positive	8 (2.8)	73 (2.6)	0.80
Bacteraemia	16 (4.4)	179 (5.0)	0.62
Malaria slide positive	90 (24.7)	815 (22.7)	0.39
Malaria parasite density (>2500/µL)	57 (15.6)	531 (14.8)	0.67
Impaired renal function (creatinine >80µmol/L)	89 (26.5)	894 (28.4)	0.45
Severe anemia (Hb <5g/dL)	13 (3.6)	108 (3.0)	0.56

Hypoglycemia (blood glucose <2.2mmol/l)	15 (6.8)	243 (9.9)	0.12
Thrombocytopenia (platelets <159 x10^3/µL)	81 (22.4)	767 (21.5)	0.71
Leukopenia (WBC count<3.9 x10^3/µL)	5 (1.4)	53 (1.5)	0.87
Lymphopenia (Lymphocyte count<1.7 x10^3/µL)	37 (10.2)	293 (8.2)	0.19

Key: *# Symptoms are not mutually exclusive**;** some patients had seizures and prostration, neck stiffness and agitation, and other overlaps in symptoms.* ***** *Refers to at least one seizure in the last 24 hours. The frequency of multiple seizures (>2 in the last 24 hours) was comparable between CHIKV-positive and CHIKV-negative patients (35.2% vs 30.2%, p=0.18); the bulk of seizures were generalized (84.2% and 79.3% for CHIKV negative and positive children, respectively).* † *Sample sizes for each variable do not always add up to the total number (N) for each group due to missing data. Analysis was only performed in those with data available. P values are from Chi2 test comparing variables, except duration of hospitalization for which a Mann-Whitney U test was used.* ***Abbreviations:*** *CHIKV – chikungunya virus; CSF – cerebrospinal fluid; IQR – interquartile range; HIV – human immunodeficiency virus; Hb – haemoglobin; WBC – white blood cell count.*

5.3.3 Incidence of CHIKV-Associated neurological disease in Kilifi

To estimate the incidence of CHIKV infection in the study area, the number of CHIKV positive cases were divided by total person years of observation (PYO) using time-to-event analysis methods. The denominator (PYO) was calculated as the duration of time from the latest date of birth or in-migration or study start date to the earliest of (i) CHIKV positive hospitalization; (ii) 16th birthday; (iii) date of last out-migration from the KHDSS; (iv) date of death; (v) end of the study (31 December 2018). Periods of absence from the KHDSS area, where an individual migrated out of the KHDSS and in-migrated back were excluded from the PYO. For comparison, incidence estimates for cerebral malaria and acute bacterial meningitis were also calculated similarly to the incidence of CHIKV infection. Total PYO in the three incidence

calculations would be slightly different because time-to-hospitalization with CHIKV, cerebral malaria or bacterial meningitis was not the same.

Of the 367 detected cases of CHIKV-associated neurological disease among the admitted children, 207 were resident in KHDSS region **Figure 5.5**~~**Figure 5.5**~~. The total risk time contributed during the 5-year study period by children aged <16 years within KHDSS was 691,588 person-years **Table 5.2**~~**Table 5.2**~~. The overall incidence of CHIKV-associated neurological disease within the KHDSS was 30 per 100,000 person-years (95% CI 26.1, 34.3) and showed no significant variation by sex, age or season **Table 5.2**~~**Table 5.2**~~. Disease incidence was highest during the 2016 epidemic but a substantial number of presentations with CHIKV infection were also detected in other years **Table 5.2**~~**Table 5.2**~~. CHIKV-associated neurological disease cases were distributed throughout the KHDSS area **Figure 5.5**~~**Figure 5.5**~~. A strong inverse relationship was observed between the incidence of CHIKV infection and age, estimated at 77 per 100,000 person-years in all children aged <5 years and 7 per 100,000 person-years among children aged ≥5 years **Table 5.2**~~**Table 5.2**~~. During the same period, the corresponding incidences of cerebral malaria and bacterial meningitis in children aged <5 years to be 20 per 100,000 and 7 per 100,000 person-years, respectively **Table 5.2**~~**Table 5.2**~~. The corresponding incidence of cerebral malaria and bacterial meningitis in children aged ≥5 years was 5 per 100,000 and 1 per 100,000 person-years, respectively **Table 5.2**~~**Table 5.2**~~.

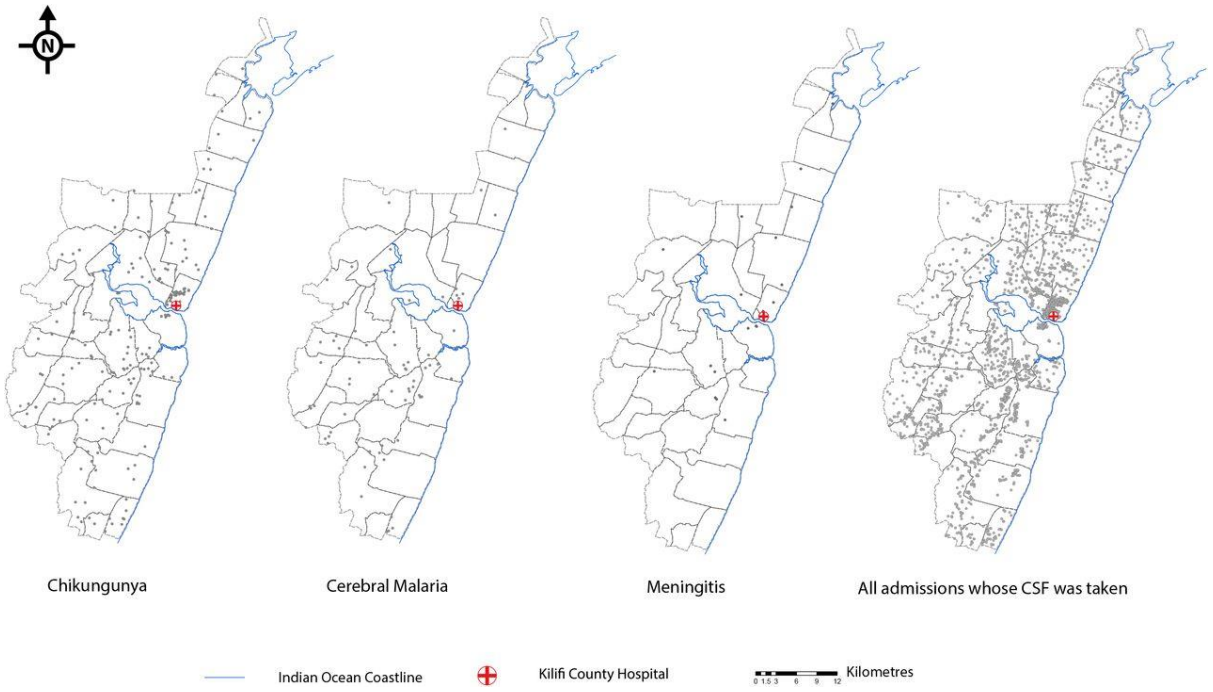

Figure 5.5. Distribution of children resident within the KHDSS that were admitted with neurological disease during the study period (2014-2018)**.** *Each point on the Kilifi Health and Demographic Surveillance System (KHDSS) map represents a child's residential coordinates. The distribution of CHIKV-associated neurological cases is shown in comparison with that for children with cerebral malaria, meningitis or for all paediatric admissions that had CSF available for CHIKV RT-PCR screening.*

Table 5.2. Incidence of CHIKV-associated neurological disease among children aged <16 years within the KHDSS during the study period (2014-2018)

		Chikungunya (N=207)			Cerebral Malaria (N=68)			Acute Bacterial Meningitis (N=22)		
Variable	**Categories**	**n / PYO**	**Incidence/100,000 (95% CI)**	**IRR (95% CI)**	**n / PYO**	**Incidence/100,000 (95% CI)**	**IRR (95% CI)**	**n / PYO**	**Incidence/100,000 (95% CI)**	**IRR (95% CI)**
Sex	**Female**	95/341753	27.8 (22.7-34)	1	33/341899	9.7 (6.9-13.6)	1	7/341753	2 (1-4.3)	1
	Male	112/349835	32 (26.6-38.5)	1.2 (0.9-1.5)	35/350019	10 (7.2-13.9)	1.0 (0.6-1.7)	15/349835	4.3 (2.6-7.1)	2.1 (0.8-5.1)
Age	**<3 months**	77/10837	710.5 (568.3-888.4)	1	0/10853	-	-	12/10851	110.6 (62.8-194.7)	1
	3 to <12 months	19/33700	56.4 (36-88.4)	0.1 (0-0.1)	2/33755	5.9 (1.5-23.7)	1	2/33750	5.9 (1.5-23.7)	0.1 (0.0-0.2)
	1 to <5 years	77/179432	42.9 (34.3-53.7)	0.1 (0-0.1)	42/179650	23.4 (17.3-31.6)	3.9 (1-16.3)	1/179697	0.6 (0.1-4)	0 (0-0)
	5 to <10 years	28/227125	12.3 (8.5-17.9)	0 (0-0)	20/227148	8.8 (5.7-13.6)	1.5 (0.3-6.4)	3/227227	1.3 (0.4-4.1)	0 (0-0)
	10 to 15 years	6/240495	2.5 (1.1-5.6)	0 (0-0)	4/240512	1.7 (0.6-4.4)	0.3 (0.1-1.5)	4/240519	1.7 (0.6-4.4)	0 (0-0)
Year	**2014**	41/138881	29.52 (21.7-40.1)	1	15/138891	10.8 (6.5-17.9)	1	4/138897	2.9 (1.1-7.7)	1
	2015	50/138008	36.2 (27.5-47.8)	1.2 (0.8-1.9)	24/138042	17.4 (11.7-25.9)	1.6 (0.8-3.1)	6/138062	4.3 (2-9.7)	1.5 (0.4-5.3)
	2016	79/136770	57.8 (46.3-72)	2.0 (1.3-2.9)	12/136847	8.8 (5-15.4)	0.8 (0.4-1.7)	5/136877	4.9 (1.5-8.8)	1.3 (0.3-4.7)
	2017	18/138861	13 (8.2-20.6)	0.4 (0.3-0.8)	7/138965	5 (2.4-10.6)	0.5 (0.2-1.2)	0/138998	-	-
	2018	19/139067	13.7 (8.7-21.4)	0.5 (0.3-0.8)	10/139172	7.2 (3.9-13.4)	0.7 (0.3-1.5)	7/139209	5 (2.4-10.5)	1.7 (0.5-6.0)
Season	**Jan - Mar**	56/172895	32.4 (24.9-42.1)	1	18/172967	10.4 (6.6-16.5)	1	5/172996	2.9 (1.2-6.9)	1
	Apr - Jun	54/173137	31.2 (23.9-40.7)	0.9 (0.3-2.3)	15/173216	8.7 (5.2-14.4)	0.8 (0.4-1.8)	9/173245	5.2 (2.7-10)	1.8 (0.6-5.4)
	Jul - Sep	53/172957	30.6 (23.4-40.1)	0.6 (0.2-1.6)	18/173043	10.4 (6.6-16.5)	1.0 (0.4-2.2)	3/173076	1.7 (0.6-5.4)	0.6 (0.1-2.5)
	Oct - Dec	44/172599	25.5 (19-34.3)	0.6 (0.2-1.6)	17/172692	9.8 (6.1-15.8)	1.0 (0.4-2.1)	5/172726	2.9 (1.2-7)	1 (0.3-3.5)

5.4 Discussion

This study provides the first documented report of CHIKV-associated neurological disease in Africa, despite the continued circulation and transmission within the continent. The result indicates that 9.2% of children admitted in Kilifi County hospital during the study period had a positive CHIKV PCR test on their CSF sample. None of them had a clinical diagnosis for CHIKV infection. Lack of knowledge on epidemiological and clinical aspects of CHIKF has significantly contributed to its exclusion from the routine screening list of common causes of neurological disease in resource poor settings for clinical management. As a result, the cases and burden of CHIKF has remained unrecognized and underappreciated albeit the consequential effects to the public health system.

An inverse relationship between CHIKF and age was observed in this study whereby young infants are at a high risk of infection as compared to the older children. This could be as result of acquisition of immunity with age. This age-related risk of CHIKV-associated neurological disease has previously been observed in a landmark study in La Reunion Island where disease incidence was highest in young infants and in adults aged >65 years [230]. CHIKV infections were commonly detected among newborns within the first week of life indicating possibility of maternal acquisition of the infection. This is in corroboration with observations described from other parts of the globe [188,231]. Infection during the perinatal period is reported to cause severe disease in approximately 50% of cases predominantly characterised by encephalopathy [126].

Temporal distribution of CHIKV infections during the study timeline indicated endemic CHIKV transmission with varying intensity over time. CHIKV-associated neurological disease was more common in 2016 that was an epidemic year, while other years had fewer cases keeping with findings from community-level surveillance in the same setting described in chapter 4 [185].

The estimated incidence of CHIKV-associated neurological disease in Kilifi was 77 CHIKV-positive admissions per 100,000 person-years among children aged <5 years resident within KHDSS. This incidence is higher than that reported for bacterial meningitis estimated at 7 per 100,000 person-years for invasive bacterial diseases, 37 per 100,000 for invasive salmonellosis or 3 per 100,000 for invasive pneumococcal disease [232,233] , and four times the incidence of cerebral malaria (20 per 100,000) in the same age group [234]. Because of incomplete case ascertainment due to children not presenting to hospital, or not having CSF collected, these are likely minimum estimates.

Following the reductions in severe malaria due to falling malaria transmission [234], and reductions in bacterial meningitis after vaccination [232], CHIKV may now be the most common cause of hospitalization with neurological disease among children aged <5 years in coastal Kenya.

The clinical indications for CSF sampling for routine clinical investigations was meningism, coma, seizures or mild reductions in Blantyre Coma Score that did not meet the threshold for coma. These accounted for 92% of the sampled children. Mortality among CHIKV positive children was low, despite coma in 22% and depressed levels of consciousness in 47%. The decision to collect CSF via lumbar puncture was based on clinical priorities rather than research criteria. Even in those without obvious indications for CSF collection, the illness must have appeared sufficiently significant to clinicians to justify hospital admission and lumbar puncture. Children with coma may not have CSF collected acutely if clinicians are concerned about raised intracranial pressure, and on clinical recovery clinicians or families may decide to forego lumbar puncture. Data in this study suggest that children in whom CSF is not collected are at high risk of death, and it is therefore possible that CHIKV is a cause of mortality among these children. Future studies using PCR on serum or IgM serology against CHIKV will help address this.

When comparing CHIKV-positive and CHIKV-negative children, none of the clinical symptoms or laboratory tests showed differences that were substantial enough for diagnostic use in clinical practice. Diagnostic uncertainty in the absence of CHIKV RT-PCR testing (or other laboratory tests such as CHIKV IgM serology) may be clinically challenging. For instance, treating children for possible culture-negative bacterial meningitis is costly and contributes to antimicrobial resistance in a hospital setting. Neuroimaging is difficult to access in resource poor settings and usually requires costs to be borne by parents. Capacity for definitive molecular or serological testing is therefore required for confirmatory diagnosis of a disease that appears to be a substantial cause of admission in young children in coastal Kenya. No specific anti-viral treatment is available for CHIKV infection. However, most children with neurological illness will receive prolonged antibiotics in the absence of a specific diagnosis. Diagnosis would therefore improve antibiotic stewardship and avoid treatment costs. Furthermore, given the good prognosis associated with CHIKF, a diagnosis may allow some reassurance to be given to parents regarding outcome. However, the sickest patients are likely to die before CSF can be collected as clinicians are less likely to collect CSF in the first 24 hours when a patient presents in acute coma. Post-discharge follow up of patients will help determine the impact of CHIKV infection on neurocognitive outcomes during childhood. Outcomes of CHIKV infection among children from other settings have tended to range from full recovery through to neurological deficits of varying severity (especially among perinatal infections) and death [198].

5.5 Conclusion

In summary, this study has uncovered a significant unrecognized burden of CHIKV-associated neurological illness in Kenyan children, with disease incidence being much higher than recent estimates for bacterial meningitis and cerebral malaria. This, together with previous studies, [126,230,235] support the importance of CHIKV neurotropism. Surveillance for CHIKV in CSF samples has not been undertaken systematically in Africa and should now be an urgent priority to describe this previously unidentified public health burden. Such work will provide the foundation for future research on preventive and therapeutic interventions.

Chapter 6: Mother -to -child transmission of CHIKV in Kilifi

6.1 Introduction

Mother to child transmission (MTCT) of viral pathogens like HIV, CHIKV, Zika, Hepatitis B, Herpes simplex and cytomegalovirus is associated with significant morbidity and mortality among children globally. MTCT can occur during pregnancy and or the postpartum period. Infection occurs by pathogens breaching the placental barrier though the mechanisms involved remain largely unknown [236] . Infected infants are predisposed to a wide array of birth defects and developmental disabilities.

Vertical transmission of CHIKV was first documented during the 2005/6 epidemic in La Re-union island [188,208] with subsequent reports from other regions [194,237]. However, cases of CHIKV MTCT have not been described in Africa. CHIKV infection can occur during pregnancy regardless of the gestational term with the mother presenting with typical CHIKF symptoms including hyperthermia, athralgia and rash [208,238]. However, a study by Foeller *et al*., showed that CHIKV infection during pregnancy may be protective against long-term joint pain sequelae that are often associated with acute CHIKV infection, but more muscle pain was reported by women in their first or third trimesters of pregnancy compared with those with CHIKV outside of pregnancy [239].

Studies in Reunion indicated that CHIKV infection during early gestation (before 16 weeks) can result in fetal deaths with no malformation [27,208,240]. Clinical investigation of 3 cases of intra-uterine deaths due to CHIKV in the first trimester, revealed presence of the virus in the amniotic fluid, placenta and the fetal brain [208] but absence in maternal blood [238]. Detection of CHIKV in the placenta indicates that CHIKV could not be cleared by maternal immune response [238]. CHIKV infection in the second and third trimester is thought to pose no risk of MTCT except when the mother is viremic during delivery [31,239]. Lenglet *et al.*, did not observe intrauterine fetal death nor find any difference in the reported rates of prematurity and

intrauterine growth retardation among mothers infected with CHIKV after 22 weeks of pregnancy compared to the general population of women who gave birth [208].

The risk of CHIKV MTCT is high during the perinatal period especially from mothers in the acute phase of the infection [31,194,197,241]. The neonate can get infected through placental breaches during labor [28,188,242]. Contamination during passage through the genital tract during delivery is possible, but no protective effect of cesarean section has been noted [242]. During the epidemic in La Reunion 2005/2006, a study indicated the rate of MTCT was approximately 50% for viremic women at the time of delivery, suggesting that this period is critical for transmission to the neonate [126].

Following peripartum MTCT of CHIKV, a severe clinical course is observed among most of the infected neonates [31,127,188,194,197,231]. This probably could be due to the maternal infection being at the acute phase hence protective maternal antibodies are not yet formed to be passed to the neonate. Also, the higher viral load observed and delayed clearance in this age group could be a factor [28,195,217,243]. In a survey of 33 newborns born to CHIKV viremic mothers at the time of delivery, 48.9% developed symptomatic neonatal disease [208]. Similary, Gerardin *et al.* reported that out of 61 neonates who were infected during delivery, 19 (31%) neonates developed symptomatic CHIKF and 52.6% of these were severe cases [31]. In a separate study, 61% of surveyed neonates developed symptomatic CHIKF that resolved within 2 weeks while 39% had complicated clinical disease [188]. In other studies where CHIKV infection from MTCT was reported, symptomatic neonatal disease was not observed [244,245] . A 50% risk of symptomatic neonatal disease was reported in a meta-analysis of 46 women with intrapartum symptomatic CHIKV infection [231]

Infected neonates present with a wide array of clinical manifestations in the first week of life [31,231,239,246]. Commonly reported symptoms in neonates are fever, poor feeding, irritability, pain, lethargy, recurrent apnoea, varied cutaneous manifestations including

erythematous maculopapular generalized rash, vesiculo-bullous dermatitis and diffuse hyperpigmentation [31,170,247,248]. Treatment is purely symptomatic support after which most cases recover within approximately 1 week [158,247]. The rash and the apnea usually subsides in 3-4 days while the hyperpigmentation persists for several weeks [170] . However, notable cases of complicated clinical presentation including intracerebral hemorrhages, status epilepticus, respiratory distress, sepsis, necrotizing enterocolitis, meningoencephalitis, myocarditis, pericarditis and multiorgan failure have been documented [29,247].

Thrombocytopaenia is a common physiological abnormality among neonates with CHIKF, that is associated with an elevated prothrombin time and disseminated intravascular coagulation [126,188,248]. During the 2005/6 CHIKV epidemic in La Reunion, 76% of infected neonates had thrombocytopaenia [188]. In a separate study of 19 neonatal CHIKF cases, most presented with thrombocytopaenia and this was associated with severe outcome [126]. In contrast, a study in El Salvador during the 2014 epidemic documented thrombocytopaenia in 1.2% of neonates with CHIKF [28]. Other reported biological abnormalities observed in neonatal CHIKF include lymphopenia, decreased prothrombin concentration, elevation of aspartate aminotransferase , and hypocalcemia [26,31,188].

Severe neonatal CHIKF can lead to death [247]. The reported case fatality rate (CFR) due to CHIKV MTCT varies in different studies. In Colombia, during the 2014/15 epidemic, a CFR of 37.5% was documented [247]. This was lower in La Reunion where in two separate studies a CFR of 0.8% and 2.6% was reported [188,249] and in Sri-lanka 6% [250]. However, in some studies no deaths were reported [170,171,251]. The reported causes of death were necrotizing enterocolitis with gastrointestinal hemorrhage and sepsis [188,247].

CHIKF is therefore a significant pathological consideration before, during and after pregnancy especially in CHIKV-endemic regions. Perinatal CHIKV transmission at birth has been associated with potential complications including congenital malformations, stillbirths,

intrauterine growth restriction and preterm delivery [231]. The neonatal clinical consequence can be fatal and necessitates timely diagnosis and appropriate clinical care. Scarcity of data on MTCT of CHIKV in endemic regions limits development of strategies for prevention, control and management of the associated clinical consequences.

Knowledge about CHIKV MTCT is drawn from studies outside Africa. The true prevalence of CHIKV during pregnancy and CHIKV neonatal illness remains unknown in the continent despite the high CHIKV sero-prevalence rates reported in different studies [122,252]. As described in some reports, misdiagnosis of neonatal CHIKF for other common endemic illnesses has contributed in underappreciation of the public health significance of CHIKV [109]. Neonatal fatality is reportedly high in LMICs accounting for almost half of all hospital admissions and two-thirds of the deaths among children below 13 years [253]. Some of these cases could be attributed to CHIKV MTCT.

In this chapter, I set out to determine the occurrence of CHIKV MTCT in coastal Kenya, including the prevalence and associated demographic and clinical characteristics.

6.2 Methods

To determine the occurrence of MTCT of CHIKV in Kilifi, I used RT-PCR to check for evidence of CHIKV in stored cord blood samples from the maternity ward in Kilifi County Hospital. I used samples collected in the year 2016 when an epidemic was reported in Kenya [145]. The samples were available and stored in the KWTRP biobank as described in **section 2.1.2.3** (General methods). Acute CHIKV infection was assessed by extracting RNA from cord blood samples using the QIAamp® Viral extraction kit as described in **section 2.1.3.1**, then subsequently screened using real-time PCR for presence of CHIKV genome as described in **section 2.1.3.2**. The resulting binary output indicating presence or absence of infection was

linked to available metadata as described in **section 2.1.2.2** (General methods) to allow assessment of the association of various demographic factors with MTCT in Kilifi.

6.3. Results

6.3.1 Sampled population

In 2016, KCH recorded 5,026 maternity admissions linked to 5,051 newborns **Figure 6.1**. Cord blood samples were collected for research purposes following consent from the mother. I excluded births whose cord blood samples were unavailable (n=2486). The remaining 2,565 cord blood samples were screened for presence of CHIKV of which 183 (7.13%) were CHIKV-positive **Figure 6.1**.

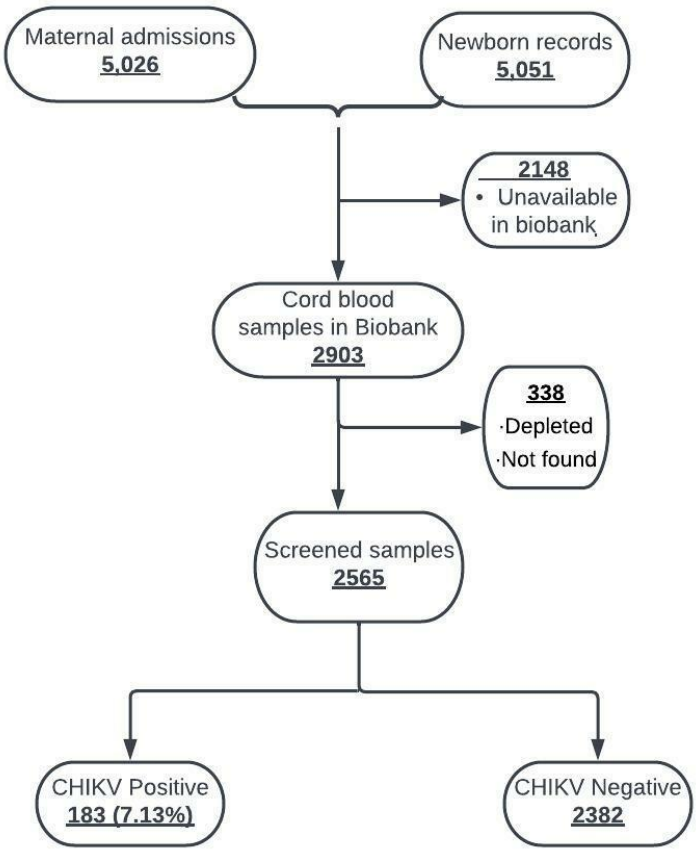

Figure 6.1. Consort diagram illustrating the data flow for cord blood sample availability and CHIKV screening

6.3.2 Monthly distribution of cord blood samples and CHIKV cases

The screened samples were collected between January and October 2016. The monthly sample size ranged between 18 and 363. The fewest samples were in May and June (n=18 and n = 73 respectively). There were 293 and 217 samples in January and February respectively. The remaining 6 months had more than 300 samples with most of them (n =363) collected in April **Figure 6.2~~Figure 6.2~~.**

CHIKV positivity was detected throughout the study months except in May. The highest positivity rate was observed in October at 12.54% and June at 12.33%. In January, February, July and September the CHIKV positivity rates were comparable at 8.19%, 8.76%, 8.62% and 8.44% respectively. The lowest rates were observed in August (2.46%) and March (2.49%). April recorded a positivity rate of 4.41% **Figure 6.2~~Figure 6.2~~**.

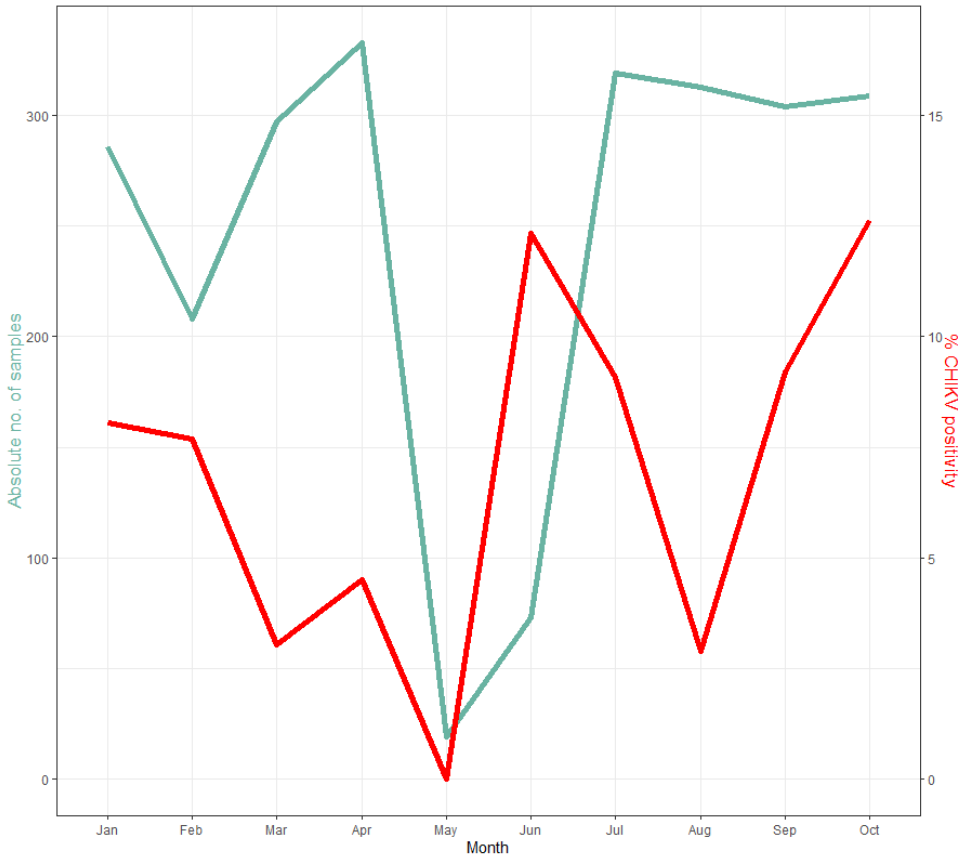

Figure 6.2. 2016 Monthly distribution of collected cord blood samples and CHIKV infection status

6.3.3 Association of demographic factors with CHIKV-MTCT

Crude odds ratios (OR), 95% confidence intervals (CI) and P values from univariable logistic regression models were used to estimate the association of maternal and newborn factors with CHIKV-MTCT. Positive detection of CHIKV in cord blood was assumed to be a case of CHIKV vertical transmission.

The odds of detection of CHIKV in cord blood were low in the months of March, April and August (OR 0.29 (95% CI 0.13-0.65), 0.52 (0.27-0.99) and 0.28 (0.13-0.64), respectively) **Table 6.1~~Table 6.1~~** .

Assessment of various maternal sociodemographic factors including, age, marital status, educational level, place of residence and the type of housing showed no significant association with CHIKV-MTCT **Table 6.1~~Table 6.1~~**. The maternal obstetric history including parity, clinic attendance for ante-natal care, taking folic supplements, vaccination against tetanus and malaria prophylaxis did not show statistical significance in association with CHIKV-MTCT **Table 6.1~~Table 6.1~~.** The observed maternal clinical symptoms including fever, convulsions and decreased foetal movement were also not associated with CHIKV-MTCT.

There was no association observed between CHIKV-MTCT and neonatal features i.e sex, gestational age, whether single or multifetal and status at birth either stillborn or alive.

Table 6.1. Association of demographic factors and CHIKV positivity in cord blood

Covariate	Available data (N=2883)	Categories	n/N cases (%)	Crude OR (95% CI)	P value
Month	2883	January	24/293	Ref	
		February	19/217	1.08 (0.57 - 2.02)	0.82
		March	8/321	0.29 (0.13-0.65)	0.00
		April	16/363	0.52 (0.27-0.99)	0.05
		May	0/18	1	
		June	9/73	1.58 (0.70-3.55)	0.27
		July	28/325	1.06 (0.60-1.87)	0.85
		August	8/325	0.28 (0.13-0.64)	0.00
		September	26/308	1.03 (0.58-1.84)	0.91
		October	43/343	1.61 (0.95-2.72)	0.08
Maternal factors					
Sociodemographic factors					
Maternal age	2883	<20 years	32/431	Ref	
		20 to 35 years	119/1830	0.87 (0.58 - 1.30)	0.49
		>35 years	28/289	1.34 (0.79 - 2.27)	0.28
Marital status	2868	Married	168/2332	Ref	
		Single	8/193	0.56 (0.27 - 1.15)	0.11
		Divorced	2/11	2.86 (0.61 - 13.35)	0.18
Education level	2833	None	19/305	Ref	
		Primary	115/1527	1.23 (0.74 - 2.02)	0.43
		Secondary	32/466	1.11 (0.62 - 2.00)	0.73
		Higher	11/206	0.85 (0.40 - 1.82)	0.68

Residence	1265	Kilifi South	19/278	Ref	
		Kilifi North	28/434	0.94 (0.51 - 1.72)	0.84
		Kilifi township	36/417	1.29 (0.72 - 2.30)	0.39
Type of house	1281	Stone wall	38/513	Ref	
		Mud wall	45/622	0.97 (0.62-1.53)	0.91
		Other	0/8		
Obstetric history					
Parity	2883	Primigravida	3/23	Ref	
		Multigravida	118/1622	0.52 (0.15 - 1.79)	0.3
		undefined	58/905	0.46 (0.13 - 1.58)	0.22
Antenatal care attendance	2825	None	2/25	Ref	
		1-3 visits	85/1086	0.98 (0.23 - 4.21)	1
		<3 visits	89/1388	0.79 (0.18-3.4)	0.75
Medication during pregnancy					
Folic acid supplements	2875	No	24/319	Ref	
		Yes	155/2224	0.92 (0.59 - 1.44)	0.72
Malaria prophylaxis	2806	None	17/228	Ref	
		1 to 2 doses	26/414	0.83 (0.44 - 1.57)	0.57
		3 to 4 doses	40/652	0.81 (0.45 - 1.46)	0.49
Tetanus vaccination	2767	None	24/365	Ref	
		1 dose	86/1110	1.19 (0.75 - 1.91)	0.46
		2 doses	64/974	1.00 (0.62 - 1.62)	0.1
Maternal co-morbidities and infections					
HIV status	2856	Positive	4/91	Ref	

		Negative	168/2339	1.68 (0.61 - 4.64)	0.31
		Not tested	5/96	1.20 (0.31 - 4.60)	0.8
Clinical symptoms					
Fever	2868	No	178/2525	Ref	
		Yes	1/11	1.32 (0.17 - 10.36)	0.79
Convulsions	2869	No	177/2518	Ref	
		Yes	2/19	1.56 (0.36-6.79)	0.56
Decreased foetal movement	2868	No	179/2501		
		Yes	0/35	1	
Newborn factors					
Sex	2586	Female	85/1253	Ref	
		Male	96/1333	0.07 (0.79 - 1.44)	0.68
Small gestational age (SGA)	2586	SGA	41/535	Ref	
		Normal	140/2,051	0.88 (0.61-1.27)	0.5
Type of birth	2580	Multifetal	12/174	Ref	
		Singleton	167/2406	1.01 (0.55 - 1.85)	0.98
Baby outcome	2554	Stillbirth	4/70	Ref	
		Livebirth	174/2,484	1.24 (0.45 - 3.45)	0.68

6.4 Discussion

In this chapter, I sought to determine the occurrence of CHIKV MTCT in coastal Kenya. There are no documented reports on the effects of CHIKF on pregnancy outcomes in Africa. Descriptions of CHIKV-MTCT elsewhere have pointed out possibility of fetal deaths following infection in the first trimester [208,238] and an approximately 50% probability of severe disease among neonates born to viremic mothers during the perinatal period [31,188]. Serosurveillance studies have indicated a high exposure rate of to CHIKV in coastal Kenya [129,131,222] but the associated clinical burden during and after pregnancy has not been documented. Here, I have estimated the prevalence of CHIKV MTCT cases by determining the presence of CHIKV genome in cord blood samples.

The assumption was that presence of CHIKV in cord blood was indicative of virus transfer to the neonate following a report that documented absence of histologic signs compatible with viral infection among 623/624 placentas examined from women found to be infected during pregnancy [249]. This was only observed in the placenta from one case of mother-to-child transmission [249] . I observed 7.13% CHIKV positivity among the screened 2,565 cord blood samples. This is a lower estimate compared to CHIKV MTCT rates reported during the 2014 epidemic in El Salvador due to the Asian CHIKV genotype (ranging 27.7% and 48.29%) [28]. In La Reunion however, prevalence of vertical transmission was estimated at 0.25% in a sample size of 7,504 deliveries during the 2005/6 epidemic caused by the Indian Ocean viral lineage [126]. These differences could be due to the pathogenicity of the circulating CHIKV strain or the immunological status of the study participants but this needs to be ascertained.

CHIKV was detected in the cord blood samples throughout the 10-month study period except in the month of May where the sample size was small thus reducing the probability of detection. This is indicative of continuous CHIKV transmission in coastal Kenya as indicated in previous studies [185,254]. The observed odds of CHIKV positivity were low in the months of March,

April and August demonstrating the seasonality in the dynamics of CHIKV transmission. Changes in climatic conditions can affect CHIKV transmission efficiency, for instance high temperature can enhance viral development in mosquito vectors [255,256]. A study in Kenya associated an increase of CHIKF cases with seasons of drought in Kenya [120]. Similar observations were made in La Reunion where CHIKV prevalence rate among parturient women was low during the cold season but peaked in the austral summer (hot and rainy) [126].

In this study, none of the analysed maternal and neonatal factors including demographics, obstetric history and co-morbidities were associated with MTCT. This is in agreement with the findings reported in Reunion, there was no difference in the pregnancy outcomes between CHIKV cases and mothers without CHIKF [249]. Maternal age has been positively associated with presence of anti-CHIKV IgG antibodies [257]. Older women in endemic regions are therefore expected to have immunological protection against CHIKV infection. However, in this study, maternal age was not associated with CHIKV positivity in cord blood.

The mode of delivery was not associated with CHIKV positivity in cord blood. This corroborates reports from other studies where caesarean section did not have a protective effect against MTCT [28,188]. However, postponement of delivery has been suggested for viremic mothers in the perinatal period in absence of fetal distress as a putative preventive measure against MTCT [126]. There was no association of maternal fever with CHIKV MTCT in this study. It is hypothesized that fever plays a direct role in intrauterine deaths [249] but in this study stillbirths were rarely observed among the infected foetus.

This study has established that CHIKV-MTCT occurs in coastal Kenya. CHIKV has remained neglected in the health sector portfolio with minimum attention in research. Considering the observed severe clinical consequences of the infection among neonates as documented in other regions [192,237] , there is need to devise effective ways of prevention, detection and management of CHIKV during pre- and post-partum. Due to its exclusion in routine screening

of neonatal illnesses, most CHIKV cases remain misdiagnosed or undetected and classified as illness of unknown cause [109]. Misdiagnosis for common endemic diseases with similar clinical manifestation like malaria has masked the burden of CHIKV among neonates and encouraged inappropriate clinical care like administration of antibiotics for suspected sepsis. Since there is no treatment or registered vaccine against CHIKF, there is need to establish a protocol to screen for CHIKV during the perinatal period. This will ensure that the viremic parturients and their newborns are closely monitored and provided with appropriate clinical support.

Studies to identify factors determining perinatal CHIKV MTCT could provide insights into prevention and control of transmission. Further follow up studies of the neonates could reveal the clinical burden associated with CHIKV-MTCT. There is need for more studies to clarify the transmission mechanism from the mother to the neonate to inform prevention strategies.

6.5 Conclusion

Maternal transmission of CHIKV occurs in coastal Kenya. In the absence of treatment or registered vaccine against CHIKF, there is need to establish a protocol to screen for CHIKV during the perinatal period. This will ensure that the viremic parturients and their newborns are closely monitored and provided with appropriate clinical support considering the associated clinical and socio-economic risks.

Chapter 7: Summarizing discussion

7.1 Introduction

Chikungunya is an important re-emerging epidemic-prone infectious disease of significant public health concern globally. It is considered a self-limiting infection that can sometimes be chronic affecting the musculo-skeletal system. However, CHIKF can sometimes present with severe clinical manifestations among children and immuno-compromised individuals. The infection can affect various organ systems including nervous, cutaneous, cardiovascular, respiratory, gastro-intestinal, optical and renal. The clinical presentation of CHIKF varies among individuals. This creates a challenge in defining a diagnostic criterion for general clinical observation as some of the symptoms are like those of other infections.

CHIKV was first identified in the African continent where it was confined within a sylvatic cycle involving primates and Aedes sp. Mosquitoes. Spillover into the human populations led to establishment of an urban cycle that has been associated with numerous epidemics in most parts of the globe. There has been limited research attention focusing on CHIKF especially in Africa. Most of what is known about CHIKF is derived from descriptions of the disease elsewhere.

CHIKF remains poorly characterized in Africa due to lack of an established surveillance infrastructure. This has contributed to the under-recognition of the associated disease burden. Consequently, there is diminished attention towards the need to formulate preventative and control measures. Considering the public health risk posed by CHIKF, in 2018, it was proposed to be included in WHO's Blueprint list of priority diseases for further research and development, including surveillance and diagnostics [258].

Routine surveillance is important in early detection of CHIKF outbreaks. This should be implemented at the community level to allow documentation of both symptomatic and asymptomatic cases. Lack of affordable diagnostic equipment and exclusion of CHIKV from the list of routine screening for acute febrile illnesses has greatly contributed to masking of the

associated clinical impact. Most CHIKF cases are grouped among those of unknown cause as described in **chapter 1**.

To guide decisions on CHIKF control and management in Africa, there is need to expand the available knowledge base about the disease. The few available sero-surveillance studies from Kenya have shown that there is significant exposure among residents in the coastal and western regions of the country.

In this thesis, I have explored the epidemiological status of CHIKF among children with an aim to summarise the transmission dynamics and clinical manifestations. This was achieved by first conducting a comprehensive systematic review to understand the global burden of CHIKF among children **Chapter 3**. I then conducted a population based observational study to determine occurrence of acute CHIKV infections in coastal Kenya **Chapter 4.** Whether CHIKV is associated with neurological disease in Kilifi is explored in **Chapter 5**. Lastly in **Chapter 6**, I investigated the probability of occurrence of CHIKV mother to child transmission in Kilifi.

7.2 Global assessment of CHIKV infections among children

CHIKF has been reported among children from most parts of the globe. Most of these records are available from Asia and the American continents **Chapter 3**. The few case reports from Europe were traveller associated CHIKV infections. Research activity and documentation of CHIKF among children is lacking from most parts of Africa. More attention is paid to the disease during epidemic seasons compared to non-epidemic period. Majority of the reviewed studies were case reports from a hospital setup.

The global assessment of CHIKV infections among children revealed lack of surveillance data from most of the areas where CHIKV transmission has been reported **Chapter 3**. Despite the estimated pooled CHIKV test positive rate of 11%, lack of data from endemic regions could

influence the accuracy of this proportion. The available studies included in CHIKV positivity estimate were mostly hospital-based rather than community-based descriptions. This limits the understanding of natural transmission pattern of CHIKV.

CHIKV can affect several organ systems. Those under 1year old are at high risk of developing severe CHIKF. The documented clinical descriptions vary among infected children. It would be therefore important to consider CHIKV diagnosis for ill children with unidentified aetiology. Timely detection of the infection can improve clinical management practices by ensuring administration of appropriate drugs and care.

7.3 Chikungunya community surveillance in coastal Kenya

The clinical impact of CHIKF among children can be better understood with intensified local community surveillance and documentation of cases. Sporadic CHIKF outbreaks have been reported in coastal Kenya **Chapter 1**. In **Chapter 4**, I have established that CHIKV transmission occurred throughout the 5-year study period (2014 – 2018) in coastal Kenya with varying magnitudes over time. I established that there were more CHIKV cases in 2016 when a CHIKV outbreak alert was raised in Kenya. Attention is paid to the infection during the outbreak season by devising short-term strategies like vector control measures in the community to reduce the observed clinical burden. There are no outlined long-term measures to prevent and control the infection. Some cases of CHIKF were classified as fevers of unknown cause. None of the detected cases was diagnosed and managed for CHIKF on visiting the community dispensary.

More CHIKF cases were observed in a low malaria transmission region (Ngerenya) as compared to Pingilikani; a region classified as high to moderate in malaria transmission. Geographically, Ngerenya is bordered by one of the largest natural forest in coastal Kenya (Arabuko Sokoke), that is home to a wide range of non-human primates. These could form a

rich natural reservoir for enzootic CHIKV that would be transmitted to the surrounding human population at a higher rate compared to people living away from the forest. There could also be an antagonistic effect between malaria and CHIKV vectors. A study to ascertain dynamics of the distribution of these vectors in this study area could further explain the inverse relationship of malaria and CHIKV cases

Asymptomatic infections create important reservoirs for CHIKV transmission cycle. Identification of these infections is difficult without an effective surveillance framework. It was observed that these infections are rare but they do occur in Kilifi.

Contrary to the dogma of long-term immunity acquisition after CHIKV infection, in **chapter 4,** I observed that some children had 2 or more episodes of CHIKF during the study period. The predisposing factors to recurrent CHIKV infections are not yet established but the genetic composition of the host which could influence the resultant immune profile, the infecting viral strain and the quantity of inoculated viral particles by the infecting mosquito could be playing a role.

Phylogenetic analysis showed temporal antigenic changes of circulating CHIKV in the study area that could be driven by acquisition of herd immunity. The viruses responsible for recurrent CHIKV infections in this study were genetically distinct ruling out the possibility of recrudescent infection.

7.3 CHIKV-Associated neurological disease in Coastal Kenya

Neurological complications due to the CHIKV infection have previously been shown in different parts of the world, with children being disproportionately affected. However, the burden of CHIKV associated with neurological disease in Africa was unknown. The lack of diagnostic tools in routine care could explain why CHIKV has escaped notice as a cause of encephalitis or other neurological illness. In **Chapter 5**, I aimed to estimate the incidence of

CHIKV-associated neurological illness in coastal Kenya where infections among children are relatively common.

Neurological diseases in children in developing countries cause significant illness and death. In Kenya about 65% of Paediatric hospital admissions with neurological manifestations have unknown causes. **Chapter 5** highlights CHIKV as an important pathogen resulting in neurological illness. It recognizes the need to consider CHIKV infections among children presenting with neurological symptoms and to establish diagnostic tools to allow detection and management of the infection. This will ensure proper clinical care and prevent use of inappropriate drugs like the commonly administered antibiotics.

It was observed that CHIKV infections are relatively more common than cerebral malaria and bacterial meningitis among children hospitalized with neurological disease in coastal Kenya. Following the reductions in severe malaria due to falling malaria transmission [234] , and reductions in bacterial meningitis after vaccination [232], CHIKV may now be one of the most common causes of hospitalization with neurological disease among children aged <5 years in coastal Kenya.

7.4 CHIKV MTCT in coastal Kenya

Neonatal age is the most critical and delicate phase of human life. With a poorly developed immune system, there is need to protect neonates from all kinds of pathogenic infections. Reports outside the African continent have indicated possibility of CHIKV MTCT. The infected neonates are predisposed to severe clinical manifestations involving various organ systems and neurodevelopmental delay. Owing to the absence of a registered treatment or prevention regime, it is important to consider CHIKV infection during antenatal and postnatal care.

In **Chapter 6**, I document occurrence of CHIKV MTCT in Kilifi during the 2016 epidemic. The risk of transmission was not associated with maternal factors including age, marital status, educational level, place of residence or type of house. The maternal obstetric history including number of antenatal clinic visits, parity, taking of folic supplements, malaria prophylaxis and tetanus vaccination was not statistically associated with MTCT. There was no association of MTCT and the neonatal factors including gender, gestational age, whether born alive or dead and if singletons or multifetal.

This study has revealed the unrecognized occurence of CHIKV infection during the perinatal period in coastal Kenya. It provides evidence for the need to advocate for inclusion of CHIKV screening in the obstetric protocols to allow timely detection and appropriate care for the infected and monitoring of neonates.

7.5 Study Limitations and future directions

7.5.1 Sampling

There are several limitations to the work presented in this thesis that warrant further research. First, my study relied on samples stored in the biobank initially collected for different research purposes. Freeze-thawing cycles could have reduced the viral-RNA recovery and consequently the detection sensitivity. For community surveillance **chapter 4**, only finger-prick whole-blood samples for RT-PCR analysis were available, thus limiting the possibility to do immunological investigations. Future longitudinal studies with serial blood sampling for immunological assays will help determine the natural course of infection, including duration of viraemia, and identify host and viral factors that may underlie the occurrence of recurrent CHIKF episodes in coastal Kenya. It could be useful to establish a standard sample collection and storage protocols for CHIKV epidemiological studies to enhance the accuracy of prevalence and incidence estimates.

7.5.2 Prevalence and incidence estimates

CHIKV cases were defined based on PCR detection of viral RNA. This is likely to underestimate the true burden of CHIKV due the short duration of CHIKV in blood during infection. Assaying for presence of anti-CHIKV IgM antibodies could improve the precision of incidence estimation for acute CHIKV infections.

In **Chapter 5**, severe CHIKV infections were likely to be underestimated since CSF is unlikely to be collected from severly ill children. For future studies, it could be useful to screen for a wider array of viruses that have been associated with neurological disease among children and assess comparability of clinical importance.

Some cases that did not present to the study health facilities could have been missed. This could be possible if the infection was self-limiting, the patient died at home or even sought clinical care from another health facility outside the study area. Future studies in other health facilities along the East African coast together with household surveillance will help determine the generalizability of the indicated incidence/prevalence estimates.

7.5.3 Geographical location

The incidence estimates were limited to a single geographical location on the Kenyan coast and were based on the KHDSS population as the denominator. Non-residents of KHDSS who visited Pingilikani and Ngerenya dispensaries (**chapter 4**) or KCH (**chapter 5**) were excluded from the incidence analysis as their denominator could not be established. To further understand the effect of geographical locations on the risk of CHIKV infection, sampling from different ecological zones will be useful.

7.5.4 Recommendations for future research

In this study, I have provided evidence that CHIKF is a substantial problem among children in coastal Kenya. Whether this finding can be generalized to other regions within the country or the African continent remains unknown. There is need to establish an intensive surveillance framework especially in endemic regions to allow assessment of the burden associated with CHIKV infections and generate a more elaborate understanding of the transmission dynamics in different areas. Mapping out the areas affected by CHIKV in the continent will enhance a more focused and targeted approach towards prevention and control of new infections. This could include vector control, community education and vaccine administration.

Owing to the co-circulation of CHIKV lineages, development of a specific treatment regime or vaccines can become a challenge. Information of circulating strains in Africa can be made available through well-structured longitudinal genomic studies. This will enable detection of genomic changes, the associated clinical manifestations and provide a platform for development of diagnostic equipment, vaccines and therapeutic drugs. Enhancement of CHIKV case recognition can alleviate challenges of misdiagnosis and consequently unrelated clinical care. There is need to work towards developing an affordable and readily available CHIKV diagnostic test. Development of an effective therapeutic regime for CHIKV patients is important but it would be better to generate a cross-reactive vaccine against circulating lineages and target endemic regions for mass vaccination to protect against infection.

In **Chapter 6**, I provide the first evidence for possibility of CHIKV MTCT in Africa. However, the risk factors associated with MTCT and the impact on the neonates' wellbeing need to be determined. Comprehensive analysis of maternal demographics, clinical history and socio-economic features of MTCT cases could reveal some of the predisposing factors to MTCT. A longitudinal follow up of neonates born to viremic mothers would provide a description of the

effect of MTCT. A genomic description of the virus isolated from the mother and that from the neonate would be important in determining management or treatment options.

7.6 Conclusion

In summary, this thesis reveals that CHIKF is a significant under-recognised and underreported health problem among children globally and should be included in routine screening of febrile illnesses. CHIKF may be a substantial public health burden in primary healthcare in coastal Kenya outside epidemic years. Recurrent CHIKV infections are common while asymptomatic cases are rare in coastal Kenya. CHIKV infections are relatively more common than cerebral malaria and bacterial meningitis among children hospitalized with neurological disease in coastal Kenya. Mother to child transmission of CHIKV occurs in coastal Kenya.

Studies to enhance knowledge on the geographical distribution of CHIKF in Africa are essential. Further studies on the associated clinical outcomes, including the incidence of severe ('atypical') manifestations of CHIKF, should help determine the wider public health significance of CHIKF in coastal Kenya. Given the wide distribution of CHIKV mosquito vectors, access to rapid diagnostics would be of value in early detection and control of transmission in the community. This would substantially reduce the burden on healthcare facilities. Efforts towards treatment and vaccine development should target young children who are at an increased risk of developing severe CHIKF-associated neurological complications and require adequate monitoring for potentially fatal outcomes. More attention is needed for the long-term impact of CHIKV on the health of newborns, and more efforts are required to clarify the mechanisms of pathogenesis to propose effective strategies to prevent neonatal CHIKV infection

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