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Epidemiological analysis of emerging and re-emerging virus infections in Mozambique: from arbovirus to SARS-CoV-2

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KEY WORDS

Emerging infectious diseases

Arbovirus

Dengue virus

Chikungunya virus

Zika virus

Malaria

Epidemiology

SARS-CoV-2

COVID-19

Mozambique

ABSTRACT

Background

Emerging and re-emerging viral infections are an increasing important concern for global public health. Previous studies conducted in Mozambique have shown occurrence of several emerging virus infections, including arbovirus. However, existing evidence on arbovirus is not recent and no data exists on co-occurrence of arbovirus and malaria. Besides, the ongoing COVID-19 pandemic hit Mozambique since March 2020, causing until mid-2021 three waves. There is a lack of an in-depth characterization of the epidemiologic profile of COVID-19 in Mozambique.

Methods

Samples from acute febrile patients selected retrospectively (2009 to 2015) and prospectively (2017 a 2018) were screened for Dengue, Chikungunya and Zika virus using commercially available ELISA. Additionally, we reviewed records of suspected and confirmed cases of COVID-19 collected in 11 provinces of Mozambique between March 2020 to September 2021. All of confirmed COVID-19 cases were subsequently mapped.

Results

From the 895 retrieved samples, the positive samples we found 54 (6.0%) were IgM anti-CHIKV, 160 (17.8%) positive for IgG anti-CHIKV, 16/577 (2.8%) for DENV-NS1. And IgM anti-ZIKV were also found in 42/850 (4.9%). For the prospective approach, of the 906 participants, the positive frequency was as follow 134 (14.8%) for IgM anti-CHIKV, 332 (36.6%) for IgG anti-CHIKV, 64 (7.1%) for IgM anti-DENV, 16 (1.8%) NS1-DENV and 83 (9.2%) for IgM anti-ZIKV. Malaria was diagnosed in 56 (6.2%) participants, 16 (1.1%) of whom were also IGM-positive for CHIKV, 3 (5.4%) for DENV-IgM and 10 (0.4%) for ZIKV. Regarding COVID-19, a total 778,926 individuals were screened for SARS-CoV-2 using Rt-PCR real time and Ag-RDT between 22 March 2020 and 30 September 2021, of whom (17.8%; 138,468/778,926) returned positive. The number of cases was increased by more than 60,000 from the first to the third wave of COVID-19 pandemicand the Chi-square test revealed significant differences between the three waves ($p<0.01$).

Conclusion

This study represents the largest serological study of arbovirus in febrile patients conducted in Mozambique. The results from this study indicate that first: for several years CHIKV, DENV and ZIKV have silently circulated and the Mozambicans across all provinces. And second, that co-occurrence between malaria and CHIKV, DENV and ZIKV among febrile patients is more common than previously thought. In addition, our analysis also describes the three waves of COVID-19 in Mozambique. The findings raise the need for increased awareness of arboviral infection as another cause of acute febrile illness and recommend active surveillance of viral emerging diseases to improve human public health.

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LIST OF ABBREVIATIONS

Abbreviation	**Explanation**
Ag-RDT	Antigen detection rapid diagnostic test
AU	African Union
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya Virus
CIBS-INS	*Comité Institucional de Bioética para Saúde do INS*
CI	Confidence Interval
CIF	Case investigation form
CNBS	*Comité Nacional de Bioética para Saúde*
COVID-19	Coronavirus diseases 2019
CT value	Cycle threshold
DENV	Dengue Virus
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
EID	Emerging infectious disease
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HF	Health Facilities
HIV	Human immunodeficiency virus
INS	*Instituto Nacional de Saúde*
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LV	*Laboratório de Virologia*
MERS	Middle East respiratory syndrome
MISAU	Ministry of Health of Mozambique
NS1	Nonstructural protein 1
O.D.	Optical density
RDT	Rapid diagnostic test
rRT-PCR	real-time reverse transcriptase polymerase chain reaction
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

SSA	Sub-Saharan Africa
UGD	Unidade de gestão de dados
WHO	World Health Organization
WNV	West Nile Virus
YFV	Yellow fever
ZIKV	Zika Virus

1. INTRODUCTION

1.1 Emerging and Re-emerging Viral Diseases

Emerging and re-emerging viral diseases are part of human history and have led to inestimable misery and death because of huge impact on health and economic [1]. Although the term “emerging diseases” become wide recognized after HIV/AIDS and genital herpes outbreaks recorded between 1970s-1980s [2, 3], these infections only caught the attention of the scientific community in the last decades with the report of large outbreaks, such as Zika [4, 5], Ebola [6] and many others.

The definitions of emerging and reemerging infectious diseases (EID) can vary or be adapted to the context. Joshua Lederberg, Robert B. Shope, and Mary Wilson formally presented the term emerging and reemerging diseases in 1987 [7]. The World Health Organization (WHO) identified “Emerging infectious diseases” as those caused by pathogen which for the first time have occurred in a population, or those that have taken place in the past but are increasing in prevalence or geographically. For example, Influenza (1918), Ebola (1976), HIV/AIDS (1981), SARS (2002) and MERS (2012). The term “re-emerging infectious diseases “are frequently used to classify those diseases caused by old or well-established pathogen that may have been concern in the past, that reappeared in a new geographical area and increased incidence [8].

Morens and Fauci have categorized the emerging diseases into 4 groups: “newly emerging”, “re-emerging or deliberately emerging” and “accidentally emerging”. For the group of “deliberately emerging” they linked with bioterrorism. The “accidentally emerging” were considered to be that diseases created by humans, such as those deriving from mutations of live virus vaccines. In their assessment, they considered these four categories as distinct, although they are likewise correlated: for example, newly emerging diseases can persist in a limited region and later re-emerge in new geographical region and can turn out to be agents of deliberate or accidental release (Figure 1) [9-11].

Pathogens of animal origin, also called zoonotic diseases, cause majority of EIDs [12, 13], and among them coronaviruses (SARS and MERS-CoV) [14], influenza A (H5N1[15] and H7N9 [16]), and Ebola hemorrhagic disease (1976-2020) [17]. Another

important group of EIDs are the diseases transmitted by vectors, also known as “vector-borne diseases”. As example, Chikungunya, dengue, zika and many others. The Vector-borne diseases account annually for more than 700,000 deaths, with higher incidence in tropical and subtropical regions [18]. Among the vectors of major concerns, such as ticks, and fleas, mosquitoes are the most globally distributed and can transmit a variety of pathogens. Some authors suggest that, about 25% of the EID events that pose a significant public health threats were related to vector-borne diseases [13, 19]. Additionally, Jones *et al* (2008) after analyzing biological, temporal and spatial data of the origins of more than 330 EID indicate that emerging disease outbreaks caused by zoonotic and vector-borne pathogens are the more frequent and those seems to be more concentrated in developing countries [14].

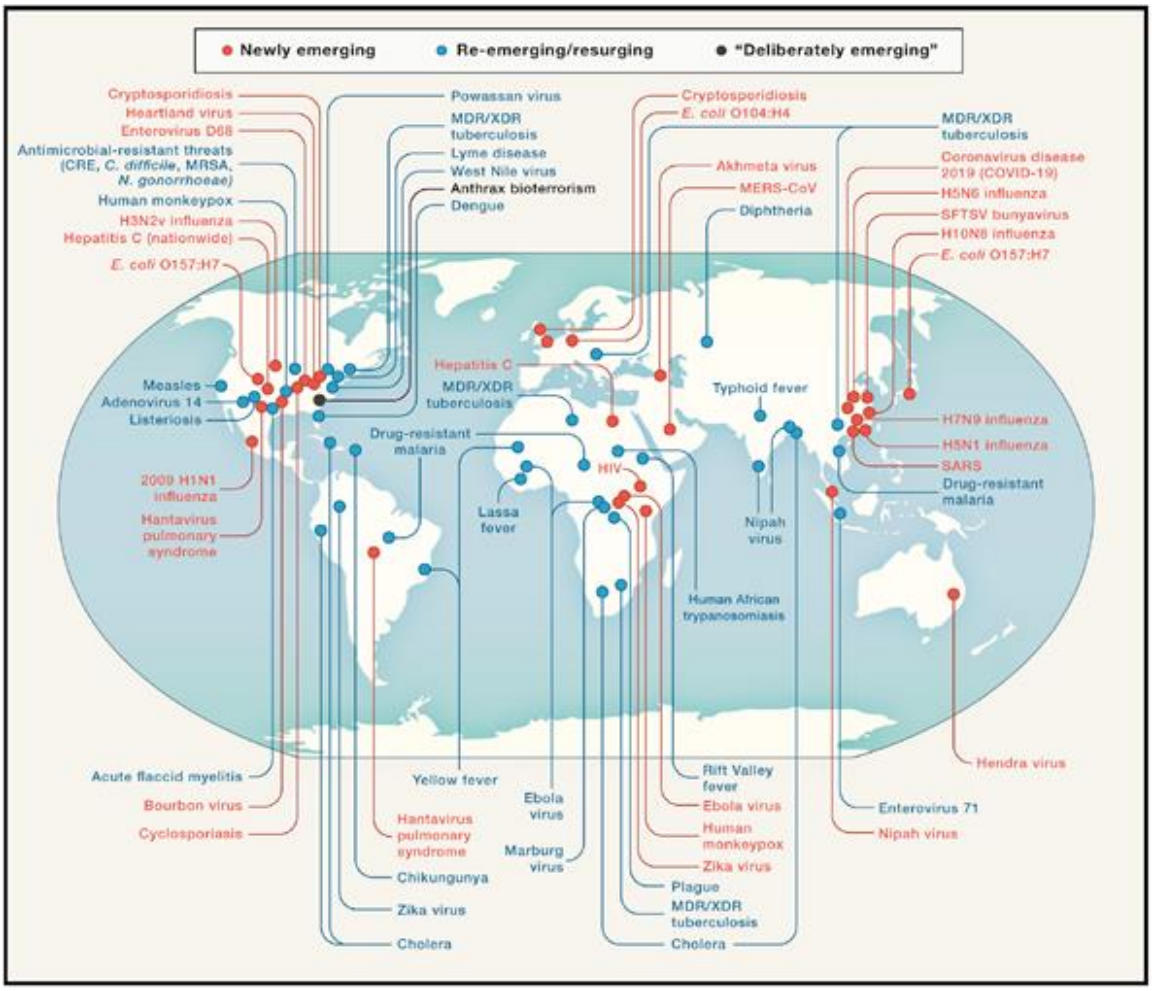

Figure 1.1 : The global extent of the 3 categories of emerging diseases: newly emerging, re-emerging, and “deliberately emerging” infectious disease from 1981 to 2020.

Source *:* Morens D and Fauci A (2020) [20]

The notable increase in reports of EID outbreaks between 1980 and 2017, may reflects in part to the advances in abilities to detect and diagnose infections. Although, other aspects such as socio-economic, environmental, ecological factors, and weakness of healthcare systems could have also contribute to the increase in EIDs [12, 13].

After they reviewed the process of pathogen emergence over both ecological and evolutionary time scales, Woolhouse and Gaunt (2007), noted four characteristics that they believe describe the most EIDs: they are caused by an RNA virus, a pathogen with non-human (animal) reservoir, pathogens with a broad host range and a great potential for human-to-human transmission [21].

The Mechanisms and determinants that lead to the emergence of infectious diseases have been heavily discussed in the last decades. Laperche (2011), suggest that the emergence of infectious diseases in humans and animals is result of a combination of two mechanisms. Initially, an unpredicted contact with the environment, can be through arthropod bites, ingestion of contaminated food, or unintentional inhalation, followed by adaptation of pathogen to new host, human or animals. Besides, several spatial and temporal components have been implicated in diseases emergence, such as animals traffic, host aspects, genetic evolution , environmental and social changes and mobility [16].

However, the viral pathogens are responsible for about two-thirds of infectious diseases burden in humans, and represent a great proportion of EID threat, given example of filoviruses, Ebola and Marburg which are part of the most devastating infectious disease in the history [17, 22].

Several strategies have been used by the governments and public health coordinators to properly control EIDs. In general, those strategies ranges from prediction, early and rapid detection, and surveillance. Nevertheless, predict which pathogen as potential to become a public health problem, can be challenging due to high pathogen diversity [19]. Laboratory has the responsible to identify a specific etiology of the disease, and reporting new pathogens, but in case of new and viral EIDs this is particularly challenging due to limited availability quality diagnostic tools. [23]. Thus, serological studies have been considered as an important component of public health preparedness, by monitoring the prevalence of antibodies which indicate previous exposure. Thought to provide useful

knowledge, particularly about the rate of spillover events into humans and the potential for person-to-person transmission [38].

a) The pandemic potential of Emerging and Re-emerging infectious diseases

Humanity have already been affected by devastating pandemics and epidemics caused by EIDs such as plague (*the plague of Justinian, the Black Death and the third plague*), influenza, Ebola, cholera, severe acute respiratory syndrome coronavirus (SARS-CoV) and many others [24].

Through the history, few aspects of the epidemiology of EID have called the attention of Schwartz (2021), one includes the fact that among all the groups of emerging infectious diseases, the viral pathogens comprise the most severe risk to global populations. Other aspect is that in the last decades viral pathogens, such as HIV, Ebola virus, chikungunya virus, dengue virus, Zika virus and at present severe acute respiratory syndrome virus 2 (SARS-CoV-2) have related to substantial morbidity and mortality globally. Analysing the origin of the pathogen that cause, around 75% are zoonotic pathogens, also from those the great significance are to vector borne diseases, including chikungunya, dengue, Rift Valley fever, Zika, yellow fever, and others [25].

So more and more the scientist a focused in clearly understand the determinants of disease emergence and persistence, that seems to involve a triad of the infectious agents, host, and the environment (Figure 1.2). The major factors include genetic, biological, climate change, social, political, and economic factors [9, 11, 26-28]. Semenza and colleagues (2016), identified several drivers in Europe, and grouped them into 3 categories: "globalization and environment, sociodemographic, and public health systems" [29]. The categories globalization and environment were founded to contributes to 61% of threat events [29], nonetheless for establishment of endemicity or pandemicity, emergence infectious pathogens, has to adapt to person-to person transmission [20].

The biology of the emerging pathogens has been thoroughly studied. And through the history viral agent with single-strands of RNA are the common pathogen involved int he pandemics that imposed high impact on human survival. As example, Dengue, Ebola, Chikungunya, Influenza, Lassa, and all of them have RNA genomes. A particularly aspect of these virus the ability to easily adapts from reservoir hosts to humans and as

humans develop immunity to the initial infection, and successfully spread the disease [22, 30].

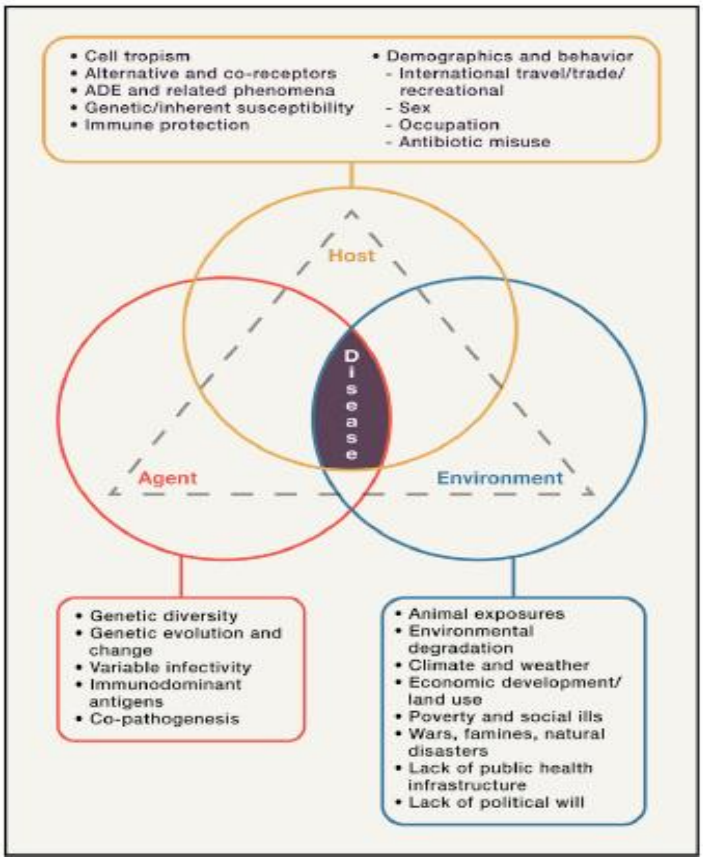

Figure 1.2: The interactions between the determinants of disease emergence and persistence.

Source : *Morens D and Fauci A* (2020) [20]

1.2 Arbovirus

a) Global burden of arboviruses

Arthropod-borne viruses, known as causative agent of arbovirus diseases, represent a group of zoonotic diseases transmitted to humans by arthropods [31, 32]. Although they have been neglected for many years, recent data suggest that they are common causes of disabling fever syndromes worldwide [33]. The majority of the arboviruses that cause diseases in human or animal belong to, the alphaviruses (e.g. Chikungunya), the flaviviruses (e.g., Dengue and Zika), nairoviruses (e.g., Crimean-Congo hemorrhagic fe-

ver), the orbiviruses (e.g., Epizootic hemorrhagic disease virus and African horse sickness virus)and phleboviruses (e.g. Rift Valley fever) [34-36].

From the public heath perspective, among the arboviral vectors, the Aedes mosquitoes represent the main concern. The clinically most important arbovirus, for example chikungunya (CHIKV), dengue (DENV), and yellow fever (YFV), are transmitted mainly by two species, *Ae. aegypti* and *Ae. albopictus* [36]. Other forms of transmission of arbovirus has been described, including vertical transmission [37-39], by transfusion [40, 41], sexual transmission [40, 42], and in nosocomial settings [43].

Dengue virus (DENV) infections is considered one of the most predominant and rapidly spreading mosquito transited virus worldwide, causing per year between 100 to 400 million infections. It is also estimated that around 50% of the global population may be at risk to dengue infection [47]. A recent meta-analysis conducted by Eltom and Colleagues (2021), reviewed published articles of DENV prevalence of in Sub-Saharan African countries between 2010 to 2020, revealed high prevalence of dengue infection in the region [44]. DENV belongs to the family Flaviviridae, and it has four distinct serotypes that causes in most of the cases a self-limiting disease ranging from mild febrile illness characterized to death. The mild form of the diseases can be presented with headache, fever, skin rash, and myalgia. In case of secondary DENV infections, an antibody-dependent enhancement can worsen the disease prognostic inducing to severe and or fatal clinical manifestations of the diseases, which are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [48]. In the early phase of diseases can be difficult to predict the prognostic, there are no particularly characteristics to help predict the prognostic [45].

Regarding CHIKV, through the years the global public health concern has significantly increased, since the CHIKV has been reported in areas not previously affected, including in some European countries such as Italy characterized by moderated temperatures [15, 51]. Although it is reported that the disease mostly occurs in Africa, Asia, and the Indian subcontinent with low public health impact [52], there is no real estimate for the number of people affected by the disease globally on an annual basis. The period of incubation is typically 3–7 days, but may range from 1 to 12 days [46]. The disease is characterized by fever, headaches, rash, myalgia, and severe joint pain, that can persist

for months [53]. Although CHIKV cause-specific mortality and morbidity represent considerable health deficits, these are usually not frequently part of the discussions of disease control priorities [54].

Zika virus, was first isolated in 1947 in Uganda [56], since then it has been poorly investigated in sub-Saharan. Considering the wide distribution of the vector (Aedes mosquitoes) and its efficiency in transmitting several arboviruses on the African continent, all the countries in the African Region were at risk of Zika virus transmission. The first largest outbreak of ZIKV occurred in others continent different from Asia and Africa was reported in 2007 [57].

Although about 20% of the overall diseases burden is attributed to vector-borne viruses, effective vaccines to help protect and prevent the spread the diseases have still accessed. [36, 37]. According to the US Food and Drug Administration (FDA) and WHO updates, there are few vaccines available against DENV, YFV, tick-borne encephalitis virus (TBEV) and JEV. Few others are still under research, such as vaccines against CHIKV, WNV (human) and ZIKV [38-40]. The discovery of “insect-specific viruses (ISVs)”, can offers an great opportunity to booster the vaccine development [37].

Public health importance of these viruses has increased due to rapid spread to previous unaffected regions and countries [26, 47]. They are the forefront and top list of emergence and resurgence mostly in the Africa region, but also in North America, Europe and the Arabian Peninsula [26, 47, 48]. In fact, for the past 20 years it has observed a remarkable resurgence of epidemic arboviral diseases [49-60].

Clinical suspicions and diagnostic of arbovirus is difficult because of absence of specific symptoms, together with similarity of its symptoms with malaria [61]. Frequent co-infection of arboviruses and malaria among febrile patients were described elsewhere where both pathogens co-occur [62]. Arboviruses are often only considered by clinicians if samples testing turn negative for malaria. Thus, arboviral diseases are often underdiagnosed and underreported [63].

Malaria represent one of the main contributes to morbidity and mortality on sub-Saharan Africa, despite the massive efforts to reduce Malaria transmission [64] In addition, other arboviral diseases, including dengue, yellow fever, chikungunya, and Zika are known to

circulates frequently in humans, wildlife, and livestock in sub-Saharan Africa, although their burden are not well described [65-67].

Several authors have discussed the factors responsible for the dramatic emergency and reemergence of arboviral diseases as a public health threat, and include: a) climate change; b) intense traffic of people between different geographical areas, c) increased human contact with wildlife; d) dissemination and adaptation of arthropods vectors to other geographical areas; e) deforestation ; f) altered farming practices, such as irrigation projects; g) urbanization [26]. Between these, the highest risk of arboviral emergence seems to appears from urbanization and the wide spread of mosquitoes responsible for the virus transmission [33]. It is important to highlight that, in sub-Saharan Africa, most of the public efforts are focused in malaria control, creating an great chance to dengue, chikungunya, and other arboviruses emerge as public health threats [68].

b) Preliminary epidemiological evidence of Arbovirus in Mozambique

The burden and epidemiology of arbovirus in Mozambique is scarce. Mozambique is localized in the sub-Saharan Africa, characterized by a tropical climate. Over the last decades dengue outbreaks have been reported in Mozambique [69], and also in other African countries, such as Tanzania [70]. Likewise, vector mapping in Mozambique showed the presence of *Ae. Aegypti*, *Ae. Albopictus* across all country [71] and *Ae. luteocephalus* [72] vector species of CHIKV, DENV, YFV and ZIKV. Those collectively suggest that flavivirus, could be endemic in Mozambique.

In Mozambique, the first reported epidemic of dengue were identified in Pemba, Cabo Delgado province, in 1984-85 [73], and it was associated with dengue-3. Since then no further cases have been reported in Mozambique, until March 2014, when an outbreak Dengue-2 was confirmed after a report of an increase in the frequency of patients with non-malarial febrile illness in Pemba and Nampula cities, situated in northern Mozambique [69, 74]. In addition, subsequent serologic studies conducted in the suburban area of Maputo city also demonstrate that Dengue virus circulates among febrile patient in southern Mozambique [75]. Gudo and colleagues, made a brief summary earlier published and unpublished work on chikungunya in Mozambique [76]. Among those works Gudo and colleagues cited a work conducted by *Kokernot et al.,* in 1957 cited by, con-

sidered the oldest serological studies on arbovirus in Mozambique. In his study, blood samples collected in 29 localities were serological tested for arboviruses. Results showed presence of antibodies against CHIKV in 191/871(21.0%), with higher frequency registered among adults 175/467 (37.5%). Those results leads the authors to conclude that arbovirus circulates in the entire extension of the country [76].

In 1971-1973 [77] and 1987 [78] a smaller serological studies also provided evidences of CHIKV circulation in Mozambique. This virus remains unnoticed up to 2013, when it was reported for the first time that CHIKV is a common cause of fever in southern Mozambique. In their study, of the 209 paired samples, 55/208 (26.4%) presented IgG antibodies against CHIKV in the convalescent sample. Following studies conducted in northern Mozambique in 2014, describe a severe case of CHIKV infection [79], and in southern Mozambique among febrile patients suggesting the inclusion of CHIKV as a part of the algorithmic of differential diagnosis of acute febrile illness [80]. In 2016, an outbreak of CHIKV was confirmed in Quelimane City, after investigation carried on among a febrile patient with arthralgia [81].

Mozambique was listed among the countries with high risk of zika outbreak [40]. Zika antibodies were identified in Mozambique for the first time in 1957 [82]. A significant portion (up to 80%) of patients infected with ZIKV are estimated to be asymptomatic and symptoms patients in general will experience a mild and self-limited illness [83, 84]. Beside, ZIKV are not only transmitted by Aedes mosquitoes, but other modes of transmission have been reported, including sexual encounter [85], and blood transfusion [84, 86].

Mozambique has a tropical climate, and characterized by rapid and poorly planned urbanization, and spread *Ae. Aegypi* recently identified, this all combined supports the hypothesis that Mozambique could be an arbovirus disease endemic country [71, 72]. As outlined above, there are few reports on arboviral infection in Mozambique. However, the data available on arbovirus infections is still limited, does not clear describe the burden and risk of these infections for public health.

1.3. Emerging pandemic diseases: COVID-19

a) The global spread of COVID-19: epidemiology perspective

Humans Coronavirus (HCoVs) are mostly originating from bats, mice, or domestic animals, and they can be categorized into two groups, alpha-CoVs and beta-CoVs [87]. Before 2003, two human CoVs (HCoVs), HCoV-229E and HCoV-OC43, has been described to cause cold mild respiratory diseases and frequently transmitted during the winter and most predominantly occurred in temperate countries [59].

In 2013, was reported the first pandemic in human involving a HcoV, epidemic of severe acute respiratory syndrome (SARS), resulted in over 8,096 cases and 774 deaths, and affected over 26 countries [88, 89]. In 2012, another previously unknown coronavirus emerged identified as Middle east respiratory syndrome (MERS) [90]. Since then, at least 27 countries have reported more than over 2500 confirmed cases were recorded. Most of these cases have been limited to the Middle East, Saudi Arabia with a case–fatality rate of 37.2% [91]. Several years later, a new type of coronavirus appeared in China, and it has named severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) because of his genomics similarity with SARS-CoV [92].

As of August 15, 2021, cumulative number of cases reported globally was over 206 million and the cumulative number of deaths almost 4.4 million. The America region has the highest cumulative number of cases reported, 80,121,215 (39%). By individual country, the United States of America (37,085,214), India (32,358,829) and Brazil (20,457,897) reported the highest numbers of cumulative cases. The European region account for the total of 62 474 616 confirmed cases and 1 242 204 deaths, followed by south-east Asia region with 39 908 781 confirmed cases and 610 389 deaths. The eastern Mediterranean account for 6,65% (13,813,261) of the global cases. Western Pacific and Africa region with 5,526,878 and 5,360,584 respectively [93]

The transmission mode of COVID-19 is mainly person to person, by aerosols and/or droplets that are released in cough or sneeze. However other sources of transmission have also been described, including airborne viral particles and environmental contamination through droplets accumulation on surfaces [94]. The viability of SARS-CoV-2 seems to one of the direct responsible for high transmissibility of the virus. It is known

that in the air, the SARS-CoV-2 aerosols particles can persist viable for several hours and contagious to infect the human host for about one hour [95].

The clinical manifestation presentation of COVID-19 can vary from mild (55% of cases) to very severe disease. The most frequent reobserved manifestation are fever (98-77%), cough (82-46%), fatigue, anorexia and myalgias [96]. In 10% of COVID-19, nausea and diarrhea followed the respiratory symptoms [97]. Others, like loss of sense of smell and taste are all so reported to be a COVID-19 symptoms COVID-19 [98]. As the current pandemic progresses, several risk factors for severe disease were described, such as age above 65 years, chronical diseases such as cardiovascular disease, chronic lung disease, diabetes, hypertension, and immunosuppression. Others clinical and immunological indicators have been listed as prognostic of severity, including lymphopenia, thrombocytopenia, and elevated inflammatory markers (IL-6, ferritin) [99]. In terms of risk infection, the gender also seems men are more commonly affected with SARS-CoV-2 infection, and even has higher risk to hospitalization [100, 101]. In terms of transmissibility, SARS-CoV-2 seems to be less lethal but more transmissible when compared to MERS-CoV or SARS-CoV. Taking in account the spread of the dieases infectious disease, SARS-CoV-2 appears to be more contagious, presenting the higher basic reproduction number 2.5 (range 1·8–3·6) compared with 2.0–3.0 for SARS-CoV, 0.9 for MERS-CoV, and 1.5 for the 2009 influenza pandemic [102].

Up to date, more than year have passed since COVID-19 has been declared a pandemic [103], and despite the all efforts is not yet controlled, and many countries have seen two or more waves of reported cases [104-109]. Some European countries have experiment higher proportion of confirmed cases in the second wave compared with the first wave, while the proportion of deaths apparently lower. These tendency corroborate with the hypothesis that the second have reported in Europe was characterized of higher infectivity and lower virulence SARS-CoV-2 [110].

A descriptive analysis of the reported COVID-19 epidemiology data from the AU Member States allowed to compare the COVID-19 waves registered up to the end of 2020. And the result of these study indicates that African countries have experienced more severe second wave, which were characterized by any increase of 30% on weekly incidence and the mean daily new cases the of the COVID-19 pandemic when compared

with the numbers reported during the peak of the first wave. [109]. The observational study performed by Zhang and colleagues 2021, listed several aspects that have corroborate for the early onsets of COVID-19 epidemics in Africa. In part they indicates that international mobility may have contributed for the high first wave mortality rate, while for the second wave the predictors of high the mortality rate seems to be related to urbanization, others infectious diseases highly prevalent in the region [111].

Another particular aspect of the SARS-CoV-2, is the emergence of different variants [112, 113]. To better analysed and focus the efforts, WHO instituted the term “SARS-CoV-2 variant of concern (VOC)”, which is considered as a variant that presents changes in the RNA genome that is suspected to affect virus transmissibility, virulence, and even the influence the sensibility and specificity of the available diagnostics, vaccines, and treatments [114]. In September 2020, the first major variant of Concern was documented in the United Kingdom (UK), called the “variant 202012/01 (alpha variant)”, [115]. The scientists observed mutation in the position N501Y of the genome of this these variant that was described as a increasing concern due to the ability of virus strongly adhere to the receptor in the humans cells [115, 116]. So as the pandemic progress a second major variant, these presented “501Y.V2 mutation” and was detected first detected in South Africa, the genomic analysis of these variants revealed a similar profile to the N501Y in the UK variant [115, 116]. The south African variant (501Y.V2 variant) was characterized by presence of mutations in the S protein, and additional presences of residues in the “RBD—K417N, E484K, and N501Y” [117]. In a case of COVID-19 reported in Denmark was also discovered other variant with several mutations in Spike protein called the “Cluster 5”, characterized by to be immune to neutralizing antibodies [118]. Most recently, India registered an devastating wave of COVID-19 cases, and followed it new variant of concern was identified, named “SARS-CoV-2 variant B.1.617.2 (delta Variant)” [119], which was consider to have a higher rate of transmission than other variants [120]. The appearance of different variants has highlighted the importance of molecular epidemiology, that could allow the early identification of potential for higher infectivity, transmissibility of the pathogen [121, 122].

The approached used for screening and diagnostic testing of COVID-19 a based in three types of assays. The Molecular diagnostic-RT-PCR, that detect genomic material

of a specific pathogen in the sample were considered to be the definitive diagnosis, and these presents sensitivity of 75%. The positivity of the test can be affected by the type and quality of specimen, stage and severity of diseases and the characteristics of specific test kit [123]. Additionally, it was established other types of diagnostics such as, the rapid diagnostic tests, that detects the presence of a viral antigen and antibodies, and the last one more used in the sero-epidemiology studies. And as part of the genomic surveillance it is also performed, whole genome sequencing to analyses the SARS-CoV-2 genomic sequence in a sample and allow identification of possible variants [124-126].

There are different proposed strategies for treat and prevent COVID-19, currently under investigation and some authors classified them as pharmacological and non-pharmacological [124, 125]. Scarabel and Collegues (2021) have extensively reviewed the current status of the pharmacological strategies for primary prevention, and have divided the drugs into four groups: “vaccines, antiviral drugs, immune-based drugs” [125]. Vaccination seems to represent the greatest strategies for primary prevention and control, but also posed a great challenge due the time and machinery required to development. As described by Scarabel et al, the scientific community have come with new technologies, the next-generation vaccine platforms, that allowed the production of two RNA-based vaccines, “Moderna (mRNA-1273)” and “Pfizer BioNTech (BNT162b2)” [125]. Regard the antiviral drugs, the current drug approved is Remdesivir, although there is scare evidence of the benefits. [125, 126]. In addition favipiravir has been rolled for management of COVID-19 cases, but they effectiveness is still debatable [127].

Other relevant approach and theme of various debates are “non-pharmacological interventions” being used to control and prevent the spread the current COVID-19 reviewed by Pereira and colleagues (2021). For this group the authors listed the following measures: “social distance, washing hands, use of masks, use of disinfectants, restrictive mobility, many others”. In addition, the authors also mentioned about the “non-pharmacological treatments” been used, which they described as the measures related to supportive treatments, such as oxygenation, although there is no clear evidence of the benefits or harms to the patients of these measures [124].

b) COVID-19 in Africa.

The first case of COVDI-19 in Africa was announced in Egypt 14 days after WHO declared outbreak of COVID-19 a “public health emergency”, on 30 January 2021 [128, 129]. Within a few months, there was of reports of COVID-19 cases in roughly all African countries, suggesting a rapidly spread of the diseases [93]. But a broad analysis of the pandemic progression across the continent indicates that the COVID-19 pandemic had a slower rate of progression compared with other continents [93, 130, 131].

As of August 3, 2021, a 55 African Union (AU) Member States have reported a cumulative of 6,780,837 COVID-19 cases and 171,752 deaths. This total contributes for 3% of all cases and 4% of all deaths reported globally. There are differences in the contributions of the five AU regions to the cumulative cases of COVID-19 and deaths. The major contribution are from the southern region with 48% cases and 52% of deaths, followed by eastern region with 12% of cases and 10% of deaths, the Northern region with, 29% of cases and 32% of deaths, the Central region with 3% of cases and 2% of deaths, and the minor contribution from Western region with 7% of cases and 4% of deaths[132].

About 44% of the AU members states have reported higher case fatality rates when compared with the global figure. In terms of pandemic progression, since the beginning of the pandemic in Africa up to the end of august almost 98% of the AU members states have experienced at least a two wave, and around 58% experiencing the third wave. And Alegria, Tunisia and Kenya had report to be experiencing the fourth wave of COVID-19 cases.

A modeling study performed in the beginning of the pandemic assessed the risk of importation of cases of COVID-19 to Africa from affected provinces in China, has revealed that most countries from SSA region were at risk of COVID-19 outbreak, but present a low level of preparedness to contain due to low capacity building and lack of resources [133].

In general, the risk factors of severe COVID-19 diseases and worsen prognostic include older age groups (>65 years old) and presence of comorbidities, such as diabetes, cardiovascular disease, immunocompromised status, obesity, chronic respiratory disease,

and cancer [14, 134]. Additional risk factor heavily study is the gender. For one side is seems that there is no significant differences between male and females infections rates, but when look to the odds to hospitalization male patients had three times higher odds to admission intensive care unit and also higher risk of death compared to females[135]. However, differences in demographic profile in African population may raise debates around the risk factors of severe COVID-19 disease and death. Some authors have listed different factors such as high population density, disproportional urbanization, higher mobility and limited or poor healthcare system [136].

Nigeria announced the first confirmed case of COVID-19 on February 27, 2020, being the first country to report a confirmed case in sub-Saharan Africa (SSA) [14]. Within the first 2 months of the first introduction into the region, disperses imported events have been registered across SSA; nevertheless, the level of preparation to respond, such as capacity for detection, reporting, and control varies from country to country [133]. Since the introduction of the COVID-19 in SSA region, South Africa and Rwanda were among the countries reporting highest numbers of confirmed cases, and these situation was in part attributed to their heir level of preparedness and also their strong health systems [137]. From the total number is cases reported in Africa, the Sub-Saharan countries accounted for about half of the cases. Although it has been predicted a high risk for COVID-19 deaths in Africa continent [138], as the pandemic progress a different scenario has been observed when compared with other continents such as Asia.

The low burden of COVID and low case fatality rates are subjects of different type of analysis, such as synergy between the infectious agent, the host's characteristics, the environment, socioeconomic conditions [136]. Particularly attention a given to the mitigation measures, such as time for better response preparation, since the reported later introduction of SARS-CoV-2 in the SSA region [130, 133]. But in other hand it might have registered an underreporting due to limited testing capacity [137]. In terms of sociodemographic aspects, it seems a great advantage to Africa and specially SSA countries is due to young age structure of the population, under 20 years old (52.7%) [139]. Several other explanations have been hypothesized, including early mobility restrictions, underreport due to limited testing capacity leading to a possible selecting bias testing

only seek persons, and cross-immunity from other coronaviruses other parasitic diseases [140-143].

1.4. Rationale

Globally, the public health importance of arbovirus has increased in recent years. In Mozambique, these viruses were heavily neglected for several decades. Importance of arbovirus in Mozambique has increased because of recent outbreaks of Dengue and Chikungunya in northern and central Mozambique. Since then, few epidemiological and entomological investigations were conducted to well determine the occurrence and epidemiological characteristics of arbovirus. However, previous studies are smaller in sample size and cover a smaller period. In this study, we intend to include a larger number of participants and during a longer period using both retrospective and prospective approach of sampling.

Another challenge related to arbovirus is the fact that clinical suspicions and diagnostic of arbovirus among acute febrile patients is difficult because of absence of specific symptoms, together with similarity of its symptoms with malaria [57, 59, 146]. Besides this, a study conducted among acute febrile patients in 22 health facilities in the country demonstrated that almost half of patients who receive anti-malarial drugs have their malaria result negative indicating a major problem in management of acute febrile illness. However, information on the co-occurrent of malaria and arbovirus is scare [147]. This study aims to provide more insights into this.

In this context, this study was conducted with the following purpose:

· Conduct a large retrospective serological investigation of CHIKV, DENV and ZIKV in blood samples from febrile patients between 2009 and 2015.

· Conduct a prospective investigation of the seroprevalence of arbovirus and its co-occurrence with malaria among febrile patients in 6 selected health facilities over 24 months, to provide data on the co-occurrence of malaria and arboviruses which are relevant to guide improvement of case management for febrile illness in Mozambique.

Emergence of SARS COV-2 is a reminder to the entire world that emerging virus represents and will continue to represent a major threat to public health. Understanding the epidemiological profile of the pandemic at country level is relevant to better prepare the country for further SARS COV-2 waves. Until November 2021, Mozambique has suffered the impact of three SARS COV-2 waves. However, no data with full pictures of these waves has been so far characterized. This study aims to characterize these three first waves of SARS COV-2 viruses.

The first case of COVID 2019 in Mozambique was confirmed on the 22 of March in Maputo city. Since then, it has spread throughout all 11 provinces of the country [148], causing three waves until November 2021. In this context, the purpose of this study was to better understand the COVID epidemic, as well as its temporal and geographical trend. This is of importance to guaranty a better preparation for future waves.

2. OBJECTIVES

2.3. Primary Objective

I. Determine the epidemiological characteristics of arbovirus infections in Mozambique from 2009 – 2016 and 2017 to 2018

II. Describe the epidemiology of Coronavirus diseases (COVID-19) epidemic in Mozambique from 2020 to 2021

2.4. Secondary Objectives

a) Investigate the presence of CHIKV, DENV and ZIKV antibodies using samples collect from febrile patients from 2009 to 2015.

b) Describe the demographic and socioeconomic characteristics of arboviral infection in Mozambique from 2017 to 2018.

c) Determine the co-occurrence of DENV, CHIKV, ZIKV and malaria.

d) Determine the frequency of COVID-19 in Mozambique from March 2020 to September 2021.

e) Describe the sociodemographic characteristics of coronavirus disease 2019 (COVID-19) epidemic.

f) Describe the main sociodemographic characteristics of three waves of COVID-19 cases in Mozambique.

3. METHODS

3.1. Study settings

Mozambique territory covers a total 801,590 km² and over 2,500 km of coastline. The country is located at the southeaster coast of Africa. The country is administratively divided into 11 provinces and 152 districts. The climate in Mozambique is tropical characterized for high relate humidity that could ranges between 70 and 80%. There are two different seasons, the raining season (October – March) and dry season (April – September). The average annual precipitation is estimated to vary between 500 and 900 mm. The country's population was estimated at 28.9 million, of which approximately 70% live in rural area. The main sources of income are familiar farming, livestock activity and artisanal fishing [144, 145].

3.2. Output I: Sero-epidemiology of arboviruses (CHIKV, DENV and ZIKV)

The sero-epidemiology of arboviruses was investigated using two different approaches:

1) It was retrospectively retrieved samples from the biobank of measles surveillance in Mozambique from 2009 to 2015.
2) It was conducted a cross-sectional study from 2017 to 2018, based on samples collection from acute febrile patients attending 6 health facilities.

3.2.1. Retrospective approach: serum samples from a biobank (2009-2015)

I. Study design

For the retrospective study it was selected samples in the biobank of national measles surveillance with the aim to investigate the occurrence and geographical spread of antibodies against arboviruses in Mozambique. It was selected and screened serum samples from the national case-based surveillance for measles The serum samples selected corresponded for the period of 2009 and 2015 and were stored in the biobank of the National Institute of Health in Mozambique.

A Blood sample were collected from suspected measles cases, who had to fulfill at least one of the following criteria, defined as "patient with a fever and one of the following

symptoms: rash and cough, coryza or conjunctivitis" [146]. This biobank was selected because the frequent sings of arbovirus infection are fever and rash [147]. The sample size was determined by the number of available stored sera.

II. Eligibility criteria

For this investigation, it was selected samples collected up to seven day of onset of fever, that presented negative results for measles and rubella for the period of 2009 to 2015. Samples with volume less than 500ul, with unsuitable labelling, samples which had deteriorated and without any sociodemographic information in the database, were excluded.

III. Demographic information

The demographic characteristics, such as age, gender, district, province, and year was obtained from electronic database system (DISA, lab informatics system set in INS, Laboratory system Technologies, cape Town, south Africa), available at the Laboratory of serology of National Institute of Health.

IV. Laboratory testing

The serum samples were examined using commercial kits for Dengue by antibody-capture enzyme-linked immunosorbent assay (ELISA) for non-structural protein 1 (NS1) (Panbio Abbott, Australia). CHIKV, and Zika virus IgG and IgM antibodies detection were performed (Euroimmune Lübeck, Germany) at Virology Laboratory (LV), in Maputo, Mozambique following the manufacturer's instructions. The interpretation of the test results and respect sensitivity and sensibility of the tested used are listed in table 3.1.

Table 3.1: Results interpretation and sensitivity and sensibility of the ELISA kits used.

	Ratio of optical density (OD)				
Kit _ELISA	**Positive**	**borderline**	**negative**	**Sensitivity**	**specificity**
Panbio Dengue IgM Capture	≥ 1	≥ 0.9 to < 1.1	< 1	1ª infection: 94.7% 2ª infection: 55.7%	100%
Panbio Dengue Early	≥ 1	≥ 0.9 to < 1.1	< 1	76.0%	98.4%
Euroimmun Anti-CHIKV IgM	≥ 1.1	≥ 0.8 to < 1.1	< 0.8	98.1%	98.9%
Euroimmun Anti-CHIKV IgG	≥ 1.1	≥ 0.8 to < 1.1	< 0.8	96.8%	98.0%
Euroimmun Anti-ZIKV IgM	≥ 1.1	≥ 0.8 to < 1.1	< 0.8	56%	97%

Source: Product catalogues [148, 149]

3.2.2. Prospective approach: facility-based study (2017-2018)

I. Study design

A descriptive cross-sectional study was performed between January 2017 and December 2018 in 6 provinces in Mozambique. An urban or suburban districts within each provincial capital were selected. At each facility, the study clinician recruited eligible patients that fulfill the inclusion criteria. Each patient was requested to sign the consent form prior to recruitment.

Samples were collected for 24 months. Under the assumption that arbovirus seropositivity would be at 10.0 %, a sample size of 360 per site would have resulted in a 95% confidence interval (CI) of 6.9 – 13.1%, which was deemed sufficiently precise for comparisons between settings and between other variables of potential.

II. Eligibility criteria

a) Inclusion criteria

- Acute elevated body temperature (axillary temperature > 37.5°C) for less than 5 days;
- Age > 5 years;

b) Exclusion criteria

- Pregnant woman;
- Individuals with mental health disorders;
- Individuals with an clear identifiable focus of infection, such as arthritis, cellulites, otitis media, pharyngitis, sinusitis, urinary infection, pneumonia or pelvic inflammatory disease;
- Unlikely to return for the convalescence sampling.
- Those that refuse to participate.

III. Questionnaire

The demographic and socioeconomic information, date of onset of fever and contact with animals was collected using a questioner specially developed for this (See annex 1). The study participants were observed for clinical manifestation suggestive of fever and related to diseases conditions. Axillary body temperature was recorded using a digital thermometer.

IV. Laboratory testing

A Venous blood sample (20 ml) was drawn from each participant using standard precautions into Vacutainer tube with K3EDTA (10 mL) and without anticoagulant (10 mL) on the day of first medical appointment. The blood was centrifugated at 3000 rpm for 10 min to allow serum separation. Aliquots with unique identifications were transported to the reference laboratory where they were freeze (-80°C) until analyzed. Serum samples were examined for Dengue by antibody-capture enzyme-linked immunosorbent assay (ELISA) for non-structural protein 1 (NS1) antigen and human immunoglobulin M (IgM) antibodies using commercial kits (Panbio Abbott, Australia). CHIKV, and Zika virus IgG and IgM antibodies detection were performed using commercial ELISA kit (Euroimmune Lübeck, Germany) at Virology Laboratory (LV), in Maputo, Mozambique. The procedures were carefully executed following the manufacturer's instructions. The interpretation of the test results and respect sensitivity and sensibility of the tested used are described in table 3.1.

V. Ethical considerations

a) Retrospective study: this study was approved by national Ethics (CNBS) in Mozambique for the secondary analysis of samples stored at biobank, collected as a part of measles surveillance in Mozambique.
b) Prospective approach: For the cross-sectional study, the Mozambique's National Review Board approved the ARBOMAP study protocol and informed consent for the cross-sectional study (letter of reference 487/CNBS/2017) and the Ethics Commission of Ludwig-Maximilians Universität, Munich also reviewed. At the study clinic, each participant received written information about the study and

provides consent on a form that was signed and dated by member of staff administering the consent and by the patient. All patient information was treated as confidential and personal data were anonymized by unique study ID number. The laboratory and clinical data were recorded daily in the case report form designed for the study. Case report forms were checked for completeness. All files were stored at a secure, locked place to which only allowed study staff had access. Paper case report forms were entered into a secured clinical data management system. In term of risk, we didn't anticipate any risk due to participation in this study besides a mild discomfort during the phlebotomy. Study patients were not paid for their participation in the study.

VI. Data collection and analysis

a) Retrospective study: Sociodemographic information were obtained from the measles surveillance electronic database available at the Serology laboratory of the National Institute of Health. For this study, the variables collected were age, gender, district, province (considered region), and year of case notifications. It was used Statistical Package for the Social Sciences" – SPSS version 22.0 (SPSS Inc, Chicago, USA) to analyze the data. Medians and interquartile ranges (IQR) were used to describe the continuous variables, and it was calculated frequencies (%) and proportions to present the categorical variables. The uncertainty around proportions were reported using binomial exact 95% confidence intervals (CI).

b) Prospective study: Data entering, and matching were performed using Data compare tool of EPI Info software package. Double data entry was done by two different individuals. Data from this study was analyzed "Statistical Package for the Social Sciences" – SPSS version 22.0 (SPSS Inc, Chicago, USA). Comparison between frequencies was done using The Chi-square test. Test were considered statistically significant at type I error level of $p < 0.05$.

VII. PhD candidate role on the project

For this project (arbovirus prospective and retrospective approach), the PhD candidate was responsible for general study coordination, direct examinations of all procedures, data acquisitions, samples processing, analysis, and interpretation.

3.3. Output II: coronavirus disease 2019 (COVID-19): dynamics of SARS-CoV-2 in Mozambique throughout the course of the three waves.

I. Study design and study population

Descriptive epidemiological research was performed to analyze secondary data of all screened patients for COVID-19 in Mozambique. Using a convenient sampling to capture the data of confirmed cases of SASR-CoV-2 on INS database during the research period, from March 2020 to September 2021.

a) <u>The methodology used by the surveillance system to collect information of the confirmed cases of COVID-19</u>

The healthcare workers investigated the suspected cases and completed the case investigation form (CIF) (see annex 2) and from each of these individuals it was collected at least one nasopharyngeal and/or oropharyngeal swab. Under controlled temperatures conditions (2–4 °C), the swab was transported in a viral media transport to the reference laboratory. Samples were collected from suspect COVID-19 cases in order with the Ministry of health (MoH) case definitions (which were derived from WHO guidelines [150]). Initially the testing was performed only at the Instituto Nacional de Saúde - Marracuene, but gradually the laboratory capacity was expanded.

Following the WHO interim protocols, the laboratory testing of COVID-19 suspected cases was conducted by molecular diagnostics, Nucleic acid amplification tests (NAAT), [14]. Due of the shortage of reagent available, variety of methods or kits were used, such as LightMix sabercoV E-gene (TIB Biomol, Berlim, Germany), DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit (SolGent Co., Ltd, Korean), Da an gene (da an gene Co., Ltd. Sun yat-sen university, Guangzhou, China), TaqPath COVI-19 test (Life Technologies Ltd, UK) and many others. Further platforms and methodologies were also implemented, with the objective to respond the clinicians demand for rapid results

for the hospitalized patients, those tests include antigen rapid diagnostic tests (Ag-RDTs) and GenXpert using Xpert Xpress SARS-CoV-2 cartridges (Cepheid, Sunnyvale, CA, USA).

The sociodemographic information of patients, signs and symptoms in the 14 days before diagnosis, laboratory results, and clinical outcome and all other detailed collected in the CIF was entered in an electronic system (DISA, lab informatics system set in INS, Laboratory system Technologies, cape Town, south Africa). All confirmed COVID-19 cases were managed according to the Ministry of health case management protocol. Some of the case definition and strategies used in Mozambique were based in the guidelines from WHO and were also adopted and heavily described by other groups.

b) Definition

The case definitions in this study were as follows:

Suspected case: individual presenting with fever and at least one sign or symptom of respiratory infection and:

- Primarily, suspected COVID-19 case with history of travel to country or region with active transmission, including the Asian countries that reported cases active cases of COVID-19, such as China, Cambodia, Japan, North Korea, Singapore, South Korea, Thailand, and Vietnam. Gradually, other countries were included based on the development of the list of most affected countries from WHO. After community transmission were started to be verified across the country, hospitalized patients with severe respiratory symptoms, were also tested.
- Contact with a confirmed or suspected COVID-19 case was characterized as direct contact with individuals with COVID-19 confirmed infection, including individual that declared direct contact with a confirmed case during the symptomatic period.
- Patients that narrate travel history to an affected country in the prior 14 days were considered imported cases. A local transmission was attributed for the cases that not meeting the imported criterion.

Confirmed case: persons with a laboratory result confirming of SARS-CoV-2 infection that presents or not signs and symptoms.

II. Eligibility

a) Inclusion criteria

Information on COVID-19 suspected and confirmed cases of COVID-19 that present a result of PCR test or Ag-RDT for SARS-CoV-2 existing in the UGD database and used to produce daily COVID-19 bulletins in the country.

b) Exclusion criteria

Suspected and Confirmed case of COVID-19 that does not have a result for PCR test and Ag-RDT result for SARS-CoV-2.

III. Ethical considerations

A review board from Instituto Nacional de Saúde and national ethics committee for Mozambique approved the Epidemiological study of COVID-19 epidemic (Ref. No. 137/CIBS/2021). Full medical confidentiality was preserved. The study was conducted according to the Declaration of Helsinki (Version 2013). No new protocol or experiment were performed. All data were collected from Mozambique MoH and INS repository. The *Unidade de gestão de dados* (UGD) at INS codified and omitted the name and personal identification (name, address, cellphone number) of all confirmed cases from March 2020 to March 2021. Databases were password protected. No personal identifying information such as names, contact information, biometric identifiers (such as voice and fingerprints) and postal address information other than town, cities, provinces, districts, were collected from the data sources. Instead, random IDs were created to identify patients in the dataset used for analyses.

IV. Data collection and analysis

We described patient's social-demographic and clinical characteristic, including geographical distribution, and characterization by age and gender. We also compared characteristic according to state of survival, using the statistical methods

described in detail below. Data analysis for this study results was performed with “Statistical Packages for Social Sciences (SPSS)” version 22.0 (SPSS Inc., Chicago, USA). Frequencies and percentages (%) were used for binary or categorical variables. For normally distributed data, it was used means and standard deviations (S.D.) and medians and interquartile ranges (IQR) for the non-normally distributed variables. To evaluate possible associations of sociodemographic features between confirmed COVID-19 and negative cases the Pearson $\chi 2$ test as used. Logistic regression analysis was employed for multivariate analysis. The test was considered of statistical significance if p value < 0.05.

V. PhD candidate role on the project

For this project (epidemiology of COVID-19), the PhD candidate lead the national testing Lab, samples processing, analysis, and interpretation.

4. RESULTS

4.1. Output I: seroprevalence of arboviruses: Retrospective approach.

I. Demographic Characteristics of participants

In total 895 serum samples were retrieved from the biobank at serology Laboratory at National Institute of Health in Mozambique. From these 895 serum samples were screened for anti-CHIKV IgM and IgG, 850 screened for anti-ZIKV IgM, and 577 screened for DENV-NS1 antigen (see Figure 4.1). In general, serum samples tested for each antibody were mostly from 2015, followed by 2013 and 2010. In terms of median age distributions among participants screened for antibodies against CHIKV and NS1-DENV was similar, where the most frequent category was 1-4 years old (463/895; 51.7% and 284/577; 49.2% respectively). For the group of participants screened for ZIKV; antibodies, category 0-1 years 318/850 (37.4%) old was most frequent, followed by age category of 2 - 4 years old 240/850 (28.2%).

Serum samples tested screened for antibodies against CHIKV and ZIKV were most from 2015 (218/895; 24.2% and 275/850; 32.4% respectively) and for the serum samples tested for DENV-NS1 antigen most were from 2013 135/577 (23.4%). Serum samples were collected from throughout the country as illustrated in Figure 4.2 and 4.3. The geographical distribution of participants screened for antibodies against CHIKV and ZIKV from central region was 339/895 (37.9%) and 356/850 (41.9%), followed by participants from north (295/895; 33% and 301/850; 35.4%). Participants screened for DENV-NS1 antigen had a different geographical distribution, 245/577 (42.5%) from north region, followed by participants from central region 192/577 (33.3%) (Table 4.1 – 4.3).

II. Frequency of CHIKV, DENV and ZIKV

The prevalence of antibodies/dengue NS-1 protein is summarized in Figure 4.1, according to sex, age, and geographical regions. The overall prevalence of antibodies to each target was as follows: 54/895 (6%) for anti-CHIKV IgM, 160/895 (17.8%) for anti-CHIKV IgG, 42/850 (4,9%) for anti-ZIKV IgM, and 16/577 (2.8%) for DENV-NS1 antigen.

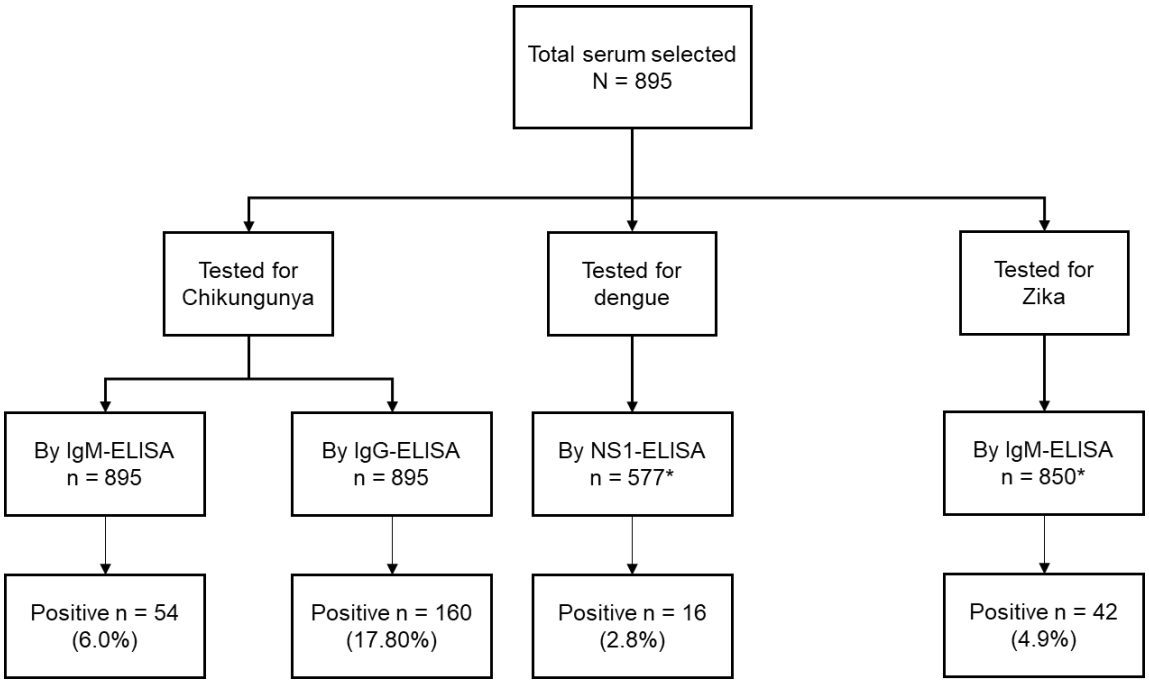

Figure 4.1: Diagram of samples selection and performed tests for the retrospective study.

Antibodies against CHIKV

From the the 895 screened samples, 6% (64/895) were IgM anti-CHIKV positive (Table 4.1). For the category gender, the frequency of IgM against CHIKV positive cases was higher in males (7.0%) than in females (4.8%) although the not statistically different. Focusing on age, in childhood age (<1 years old) groups there was no difference the frequency of IgM anti-CIKV while the older age group >15 showed a higher proportion (3.26; 95% CI; 1.12-9.42) (see table 4.1).

In terms of geographical distribution, IgM anti-CHIKV were observed in 66.1% (84/127) of the districts studied and in all provinces. The Maps presented in Figure 4.2 also show different patterns around the study area, indicating in the central region it was founded slightly higher frequency of IgM anti-CHIKV compared to the other regions, but these were not statistically significant (p=0.511). It was not observed a specific temporal pattern or trend for the distribution of IgM antibodies between 2009 and 2015 (Figure 4.2).

Table 4.1: Demographic characteristics of positive cases for IgM anti-CHIKV

	Suspected cases reported, n (%)	CHIKV IgM+, n (% of all positives)	CHIKV IgM+ (95% CI)*	Proportion Ratio (95% CI)	p-value†
Total	895 (100)	54 (100)	6.0 (4.6 - 7.8)		
Sex					0.171
Male	498 (55.8)	35 (64.8)	7.0 (4.9 - 9.6)	1.00	
Female	395 (44.2)	19 (35.2)	4.8 (2.9 - 7.4)	0.68 (0.40 - 1.18)	
Age category					0.131
Median age (IQR)	2.0 (1.0 – 5.0)	2.0 (1.0 – 4.3)	-	-	
< 1	198 (22.1)	8 (14.8)	4.0 (1.8 - 7.8)	1.00	
1 – 4	463 (51.7)	33 (61.1)	7.1(5.0 - 9.9)	1.76 (0.83 - 3.75)	
5 – 9	149 (16.6)	7 13.0)	4.7 (1.9 - 9.4)	1.16 (0.43 - 3.14)	
10 – 14	47 (5.3)	1 (1.9)	2.1 (0.1 - 11.3)	0.53 (0.07 - 4.11)	
≥ 15	38 (4.2)	5 (9.3)	13.2 (4.4 - 28.1)	3.26 (1.13 - 9.42)	
Regions					0.511
North	295 (33.0)	19 (35.2)	6.4 (3.9 - 9.9)	1.00	
Central	339 (37.9)	23 (42.6)	6.8 (4.3 - 10.0)	1.05 (0.59 - 1.90)	
South	261 (29.2)	12 (22.2)	4.6 (2.4 - 7.9)	0.71 (0.35 - 1.44)	
Year of onset					0.179
2009	67 (7.5)	5 (9.3)	7.5 (2.5 - 16.6)	1.63 (0.58 - 4.59)	
2010	129 (14.4)	11 (20.4)	8.5 (4.3 - 14.7)	1.86 (0.81 - 4.26)	
2011	91 (10.2)	3 (5.6)	3.3 (0.7 - 9.3)	0.72 (0.20 - 2.55)	
2012	98 (10.9)	5 (9.3)	5.1 (1.7 - 11.5)	1.11 (0.39 - 3.17)	
2013	123 (13.7)	13 (24.1)	10.6 (5.7 - 17.4)	2.30 (1.04 - 5.10)	
2014	169 (18.9)	7 (13.0)	4.1 (1.7 - 8.3)	0.90 (0.35 - 2.32)	
2015	218 (24.4)	10 (18.5)	4.6 (2.2 - 8.3)	1.00	

*Percentage of positives in stratum: n of positives out of N in stratum
†p value result from the comparison between proportion ratio of the variables of interest.
CI – confidence interval
Source: Adapted Antonio, Amade, Muianga, Ali *et al.* Rectrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control.

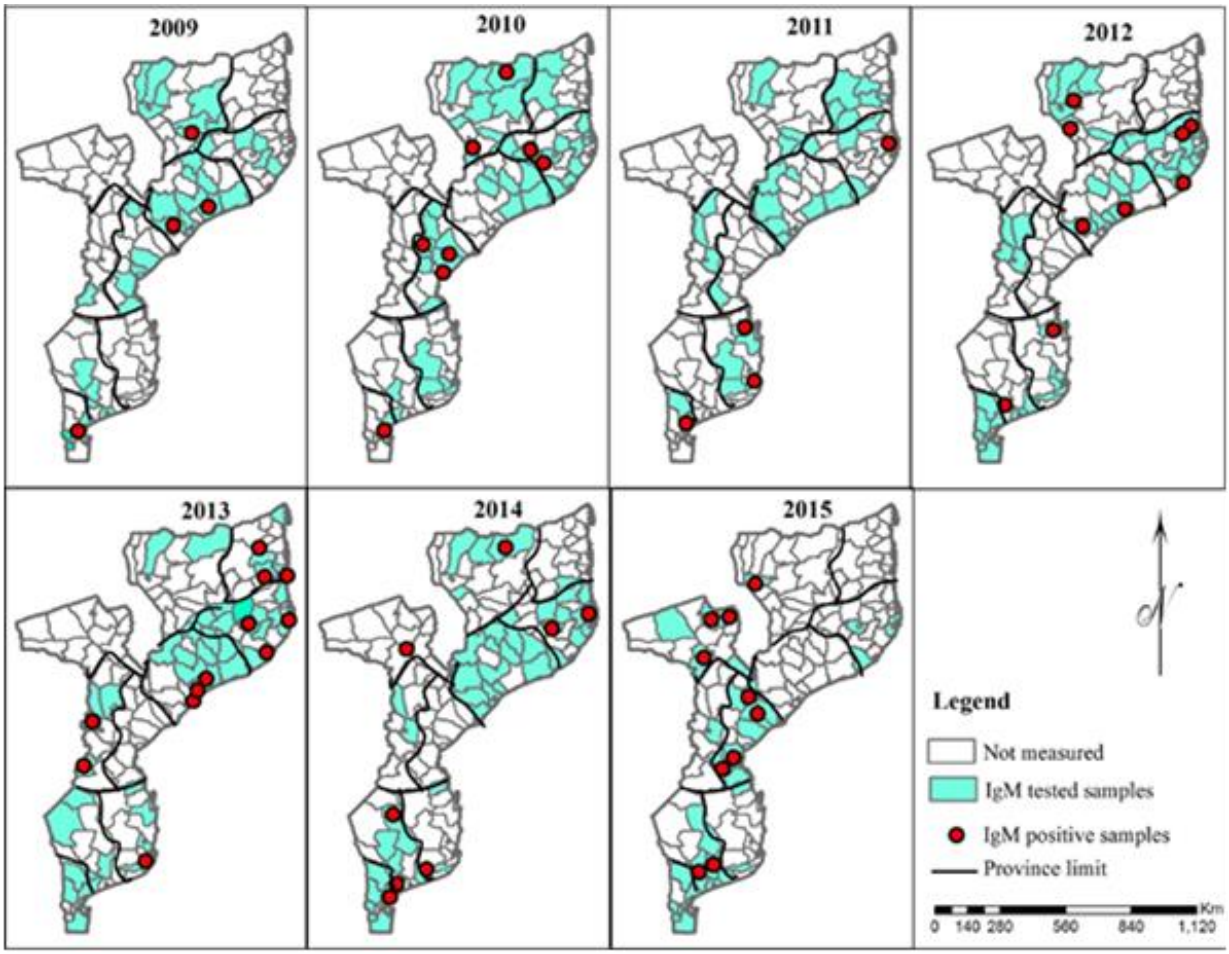

Figure 4.2: Geographical distribution of retrieved samples and IgM anti-CHIKV antibody detected.

Source: Adapted from Antonio, Amade, Muianga, Ali *et al.* Rectrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control.

The proportion of anti-CHIKV IgG positive samples was 17.9% (160/895) and the median age of the positive cases was 2 years (IQR: 1-4 years). There was not found a significantly different when compared the frequencies of IgG anti-CHIKV in males and females (p=0.571). Regarding the geographical spreading, in the central region of the country it was found a higher frequency of IgG against CHIKV (21.8%: 95% CI: 17.5 - 26.6) when compared to Northern region (16.3%; 95% CI; 12.2 -21.0) followed by the southern region (14.6%; 95% CI; 10.5 - 19/4) of the country.

Table 4.2: Demographic characteristics of positive cases for IgG anti-CHIKV

Characteristics	Suspected cases reported, n (%)	CHIKV IgG+, n (% of all positives)	CHIKV IgG+ (95% CI), %*	Proportion Ratio (95% CI)	†p-value
Total	895 (100)	160	17.9 (15.4 - 20.5)		
Sex					0.571
Male	498 (55.8)	86 (53.8)	17.3 (14.1-20.9)	1.00	
Female	395 (44.2)	74 (46.3)	18.7 (15-22.9)	1.08 (0.82-1.44)	
Age category					0.911
Median age (IQR)	2.0 (1.0 – 5.0)	2.0 (1.0 – 4.0)	-	-	
< 1	198 (22.1)	34 (21.3)	17.2 (12.2 - 23.2)	1.00	
1 – 4	463 (51.7)	87 (54.40	18.8 (15.3 - 22.7)	1.09 (0.76 - 1.57)	
5 – 9	149 (16.6)	26 (16.3)	17.4 (11.7 - 24.5)	1.02 (0.64 - 1.62)	
10 – 14	47 (5.3)	8 (5.0)	17.0 (7.6 - 30.8)	0.99 (0.49 - 2.00)	
≥ 15	38 (4.2)	5 (3.1)	13.2 (4.4 - 28.1)	0.77 (0.32 - 1.83)	
Regions					0.050
North	295 (33.0)	48 (30.0)	16.3 (12.2 - 21.0)	1.00	
Central	339 (37.9)	74 (46.3)	21.8 (17.5 - 26.6)	1.34 (0.97 - 1.86)	
South	261 (29.2)	38 (23.8)	14.6 (10.5 - 19.4)	0.89 (0.60 - 1.32)	
Year of onset					< 0.001
2009	67 (7.5)	14 (8.8)	20.9 (11.9 - 32.6)	0.69 (0.42 - 1.15)	
2010	129 (14.4)	21 (13.1)	16.3 (10.4 - 23.8)	0.54 (0.35 - 0.84)	
2011	91 (10.2)	13 (8.1)	14.3 (7.8 - 23.2)	0.47 (0.27 - 0.81)	
2012	98 (10.9)	16 (10.0)	16.3 (9.6 - 25.2)	0.54 (0.33 - 0.88)	
2013	123 (13.7)	15 (9.4)	12.2 (7.0 - 19.3)	0.40 (0.24 - 0.67)	
2014	169 (18.9)	17 (9.4)	8.9 (5.1 - 14.2)	0.29 (0.17 - 0.49)	
2015	218 (24.4)	66 (41.3)	30.3 (24.3 - 36.8)	1.00	

*Percentage of positives in stratum: n of positives out of N in stratum
†p value result from the comparison between proportion ratio of the variables of interest.
CI – confidence interval
Source: Adapted Antonio, Amade, Muianga, Ali *et al.* Rectrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control.

The Central region (1.50) also had a higher seroprevalence when compared to the Souther region (reference group) (p=0.05). In terms of temporal trends, in 2015 it was observed a significantly highest frequency of IgG against CHIKV (30.3%; 95% CI; 24.3%-36.8%) than the other years (p<0.001). The figure 4.3. ilutrates the study districts, the distribution of IgG anti-CHIKV positive samples stratified by years.

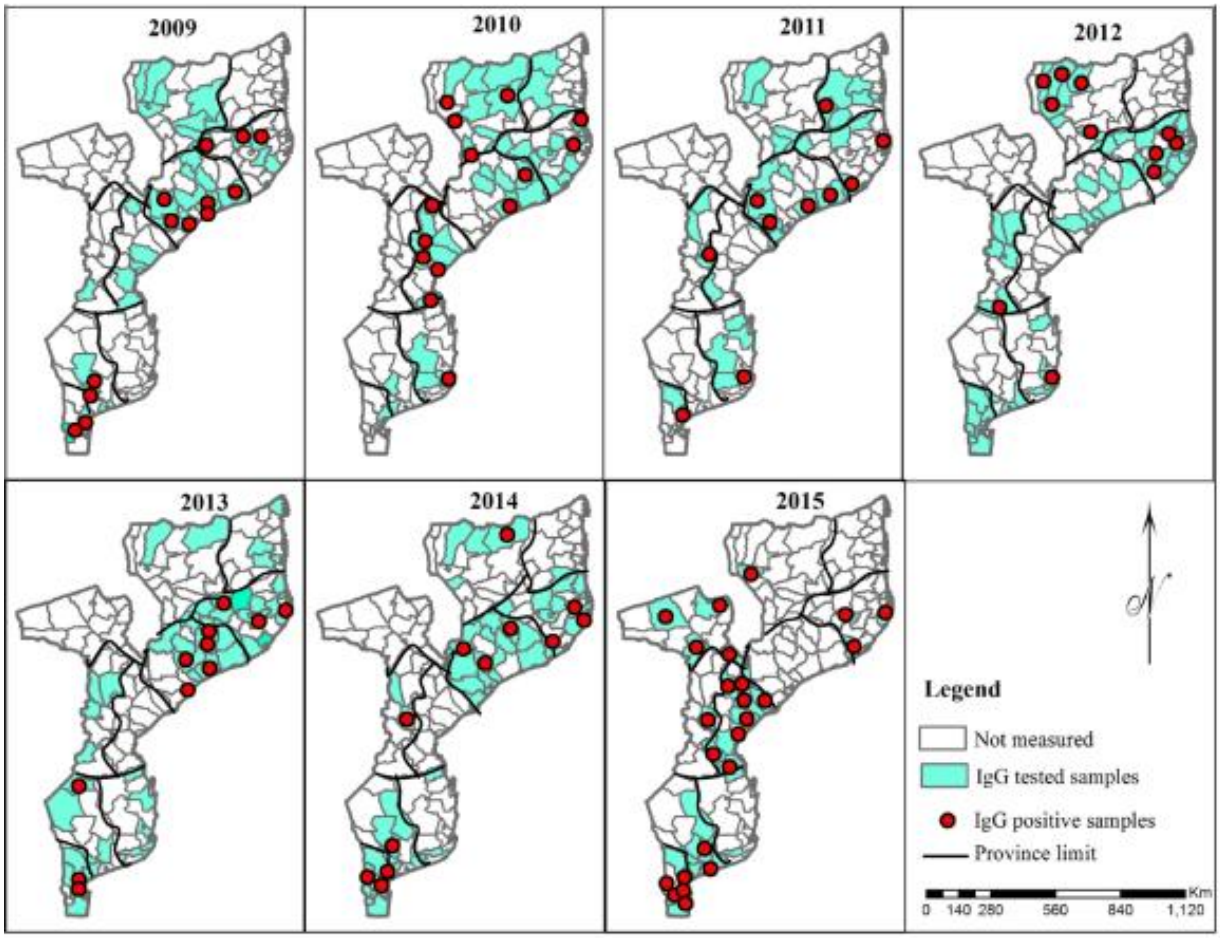

Figure 4.3: Geographical distribution of retrieved samples and IgG anti-CHIKV antibody detected.

Source: Adapted from Antonio, Amade, Muianga, Ali *et al.* Rectrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control.

Dengue

Laboratory results showed a positive finding for NS1 among 16/577 (2.8%) NS1 tested samples. Frequency of NS-1 positives was higher in participants 1-4 years old 8/284 (2.8%). In terms of geographical distribution data showed in table 4.1 indicates that the frequency of NS1 positive samples was higher in the Northern region 15/245 (6.1%). No NS-1 positive samples were found in participants from Central region. And it was not noted any trends cross the years (see Table 4.2).

Table 4.3: Demographics characteristics of positive cases for NS-1 DENV.

	DENV	
Characteristics	**Suspected cases, n (%)**	**NS1+, n (%)**
Total	577	16 (2.8)
Sex		
Male	314 (54.4)	6 (1.9)
Female	263 (45.6)	10 (3.8)
Age category		
< 1	124 (21.5)	0
1 – 4	284 (49.2)	8 (2.8)
5 – 9	107 (18.5)	2 (1.9)
10 – 14	33 (5.7)	0
≥ 15	29 (5.0)	2 (6.9)
Region		
North	245 (42.5)	15 (6.1)
Central	192 (33.3)	0
South	140 (24.3)	1 (0.7)
Year of onset		
2009	62 (10.7)	0
2010	122 (21.1)	1 (0.8)
2011	86 (14.9)	3 (3.5)
2012	87 (15.1)	8 (9.2)
2013	135 (23.4)	2 (1.5)
2014	85 (14.7)	2 (2.4)
2015	0	0

Antibodies against ZIKV

Of 850 samples, 4.9% (42) were IgM anti-ZIKV positive for. From those participants positive for IgM positive, most of them were male 27/42 (64.3%) and the median age was 3.0 years (IQR: 1.0-5.0 years). A higher frequency of IgM anti-ZIKV was founded in the participants that belong to the aged categories 5 -9 years old (7.5; 95% CI:4.2-12.3) (see Table 4.3). For the variable region, in 9 out 11 provinces screened was detected IgM anti-ZIKV. Although it was not detected positive cases from Cabo Delgado, the higher frequency of positive cases for IgM anti-ZIKV were founded in north region (see Figure 4.4). No temporal trends were observed, but the in 2011 it was registered the highest frequency of anti-ZIKV IgM antibodies. (See Table 4.2).

Table 4.4: Demographic characteristics of positive cases for anti-ZKV IgM

Characteristics	Suspected cases reported, n (%)	Zika IgM+ n (% of all positives)	ZIKV IgM+ (95% CI), %*	Proportion Ratio (95% CI)	†P-value
Total	850	42	4.9 (3.5. - 6.6)		
Sex					0.299
Male	480	27 (64.3)	5.6 (3.7 - 7.9)	1	
Female	369	15 (35.7)	4.1 (2.1 - 6.2)	3.1 (2.6 - 3.7)	
Age (years)					0.521
Median age (IQR)	3.0 (1.0 – 6.0)	3.0 (1.0 – 5.0)	-	-	
0-1	318	7 (16.7)	2.2(0.8 - 4.4)	1	
2—4	240	14 (33.3)	5.8 (3.2 - 9.6)	1.45 (0.25 - 8.16)	
5—9	186	14 (33.3)	7.5 (4.2 - 12.3)	0.71 (0.15 - 3.28)	
10—14	67	3 (7.1)	4.5 (0.9- 12.5)	0.59 (0.12 - 2.74)	
≥ 15	39	4 (9.5)	10.3 (2.8 - 24.2)	0.54(0. 11 - 2.55)	
Regions					0.087
North	301	20 (47.6)	6.6 (4.1 - 10.1)	1	
Central	356	17 (40.5)	4.7 (2.8 - 7.5)	0.69(0.81 - 1.32)	
South	193	5 (11.9)	2.5 (0.8 - 5.9)	1.89 (0.61 - 4.32)	
Year of onset					0.356
2009	62	2 (4.8)	3.22 (0.3- 11.1)	1.61 (0.35-7.29)	
2010	121	8 (19.0)	6.6 (2.9 - 12.6)	0.76(0.31 - 1.86)	
2011	84	7 (16.7)	8.3 (3.4- 16.4)	0.59 (0.23 - 1.52)	
2012	88	6 (14.3)	6.8 (2.5- 14.3)	0.73 (0.27- 1.97)	
2013	142	4 (9.5)	2.81 (0.7 - 7.1)	1.85 (0.6 - 5.75)	
2014	78	1 (2.4)	1.2 (0.03 - 6.9)	4.14(0.53 - 32.0)	
2015	275	14 (33.3)	5.0 (2.8 - 8.3)	1	

*Percentage of positives in stratum: n of positives out of N in stratum
†p value result from the comparison between proportion ratio of the variables of interest.
CI – confidence interval
Source: Adapted Chelene & Ali *et al.* Retrospective investigation IgM antibodies against Zika virus in serum from febrile patients in Mozambique, 2009-2015.

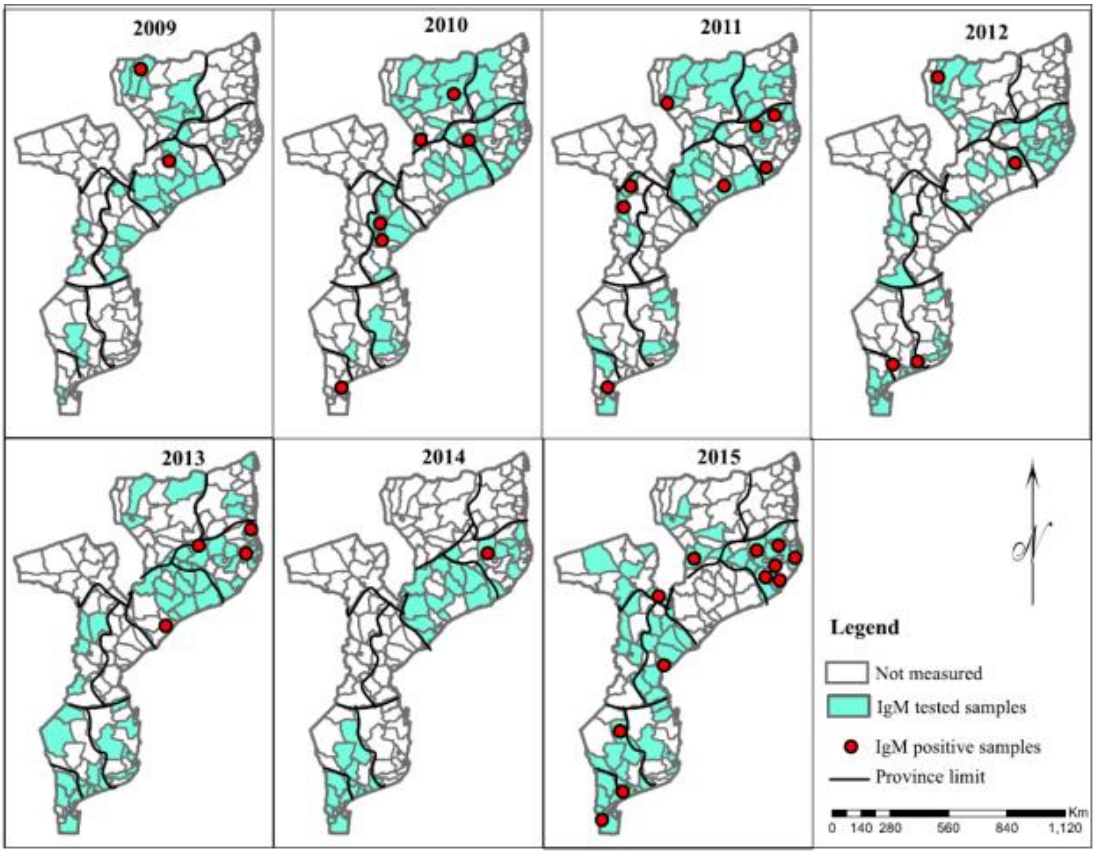

Figure 4.4: Geographical distribution of retrieved samples and IgM anti-ZIKV antibody detected.

Source: Adapted from Chelene & Ali *et al.* Retrospective investigation IgM antibodies against Zika virus in serum from febrile patients in Mozambique, 2009-2015.

4.2. Output I: seroprevalence of arboviruses: Prospective approach.

I. Demographic Characteristics of participants

A total of 906 febrile patients were recruited between January 2017 through December 2018 from the following six Health facilities (HF): Polana Canico – Maputo City (15.1%), Massingir, Gaza Province (12.9%), Caia – Sofala province (13.2%), Eduardo Mondlane – Chimoio Province (27.2%), Coalane – Zambezia Province (23.3%) and Natite- Cabo Delgado Province (8.3%). Out of 906, 62.5% were female. The cases had a median age of 25 years old (IQR: 18–36 years). Most of the participants were aged between 18 and 24 years 28.8% (258/906) (Table 4.4).

II. Frequency of CHIKV, DENV and ZIKV antibodies and Malaria among participants

Of the 906 participants, IgM specific antibodies against CHIKV were detected in 134 (14.8%; 95%CI: 12.5-17.1), IgG anti-CHIKV in 332 (36.6%; 95%CI: 33.5-39.8), IgM anti-DENV in 64 (7.2%; 95%CI: 5.5-8.9) and IgM anti-ZIKV in 93 (9.2%; 95%CI: 7.4-11.2) of participants, respectively. Frequency of serum samples positive for DENV-NS1 antigen was 16/906 (1.8%; 95%CI: 0.9 – 2.6). The frequency of malaria among participants were 56/906 (6.2%; 95%CI: 4.7-8.0) (Figure 4.5). In general, it was registered differences between malaria, DENV and ZIKV positivity rate. Statistical differences were found in frequencies against arbovirus and malaria in different geographical areas ($p < 0,05$). While no statistical differences were found in the proportion of CHIKV, DENV, ZIKV and malaria between the age groups ($p > 0.05$) (Table 4.5 – 4.7).

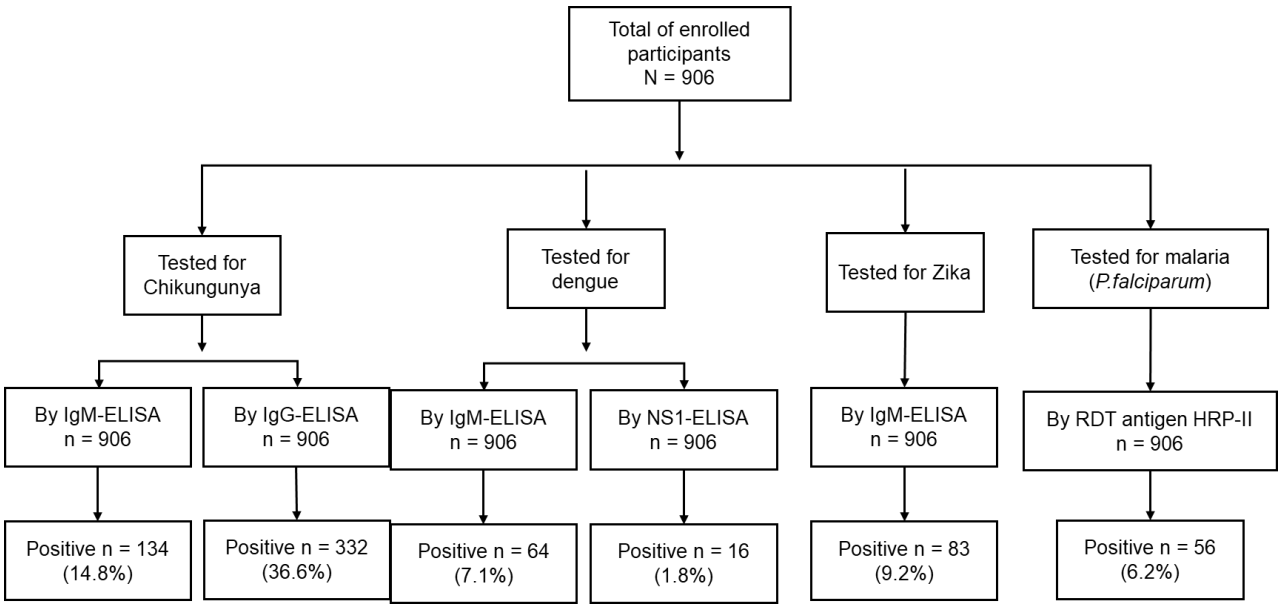

Figure 4.5: Diagram of enrolment of study participants and performed tests – Cross sectional study (prospective approach)

Antibodies against CHIKV

The frequency for IgM anti-CHIKV was 134 (14.8%; 95%CI: 12.5-17.1), with median age of 25 (IQR: 18 – 36.5), being patients with more than 54-year-old, who registered the highest frequency 10/57 (17.5%). Analysing the frequencies among the age categories, patients with 18-24 years old 40/258 (15.5%) had the higher frequency of IgM anti-

bodies against CHIKV. It was verified high frequency among female participants 80/560 (14.3%). In terms of geographical distribution, we observed statistical differences between CHIKV infection and Health facilities (p <0.000), and Coalane was the HF that showed higher frequency of anti-CHIKV IgM antibodies as compared to other study HF (Table 4.5).

In Table 4.4. are also summarized the demographic characteristics for the participants positive for IgG anti-CHIKV. A total of 332 (36.6%; 95%CI: 33.5 – 39.8) was observed in IgM anti-CHIKV antibodies, with median age of 27.5 (20 – 37.5), being patients aged 35 – 44 years old, who registered the highest frequency 59/116 (50.9%) (p=0.00). It was verified slightly high frequency among female participants 210/560 (37.5%). In terms of geographical distribution, we observed statistical differences between CHIKV infection and Health facilities (p <0.000), and Coalane was the HF that showed higher frequency 112/211(53.1%) of anti-CHIKV IgM antibodies as compared to other study HF.

Table 4.5: Demographic characteristics of positive cases for IgM and IgG anti-CHIKV

		CHIKV IgM+		CHIKV IgG+	
Characteristics	**Subjects n (%)**	**Positive cases n (%)**	**p-value**	**Positive cases n (%)**	**p-value**
Total sample	906 (100)	134 [14.8 (12.5-17.1)]		332 [36.6 (33.5 – 39.8)	
Gender			0,396		0.738
Male	336 (37.5)	54(16.1)		119 (35.4)	
Female	560 (62.5)	80(14.3)		210 (37.5)	
Age-group			0,993		< 0.001
Age, Median (IQR)	25 (18 - 36)	25 (18 - 36.5)		27.5 (20 – 37.5)	
5 – 17	168 (18.8)	25 (14.9)		38 (22.6)	
18 – 24	258 (28.8)	40 (15.5)		105 (40.7)	
25 – 34	205 (22.9)	28 (13.7)		73 (35.7)	
35 – 44	116 (12.9)	19 (16.4)		59 (50.9)	
45 – 54	87 (9.7)	11 (12.6)		34 (39.1)	
≥ 55	57 (6.4)	10 (17.5)		18 (31.6)	
Health facilities			< 0,001		< 0.001
Polana Canico	137 (15.1)	23 (16.8)		42 (30.1)	
Massingir	117(12.9)	20 (17.1)		38 (32.5)	
Caia	120(13.2)	15 (12.5)		52 (43.3)	
Eduardo Mondlane	246(27.2)	17 (6.9)		50 (20.3)	
Coalane	211(23.3)	46 (21.8)		112 (53.1)	
Natite	75(8.3)	13 (17.3)		38 (50.1)	

IgM anti-DENV antibodies and DENV NS1

A total of 64/906 (7.1%; 5.5-8.9) participants tested positive for DENV, with a median age of 25 (IQR: 18 – 34.25). Older participants (>55 years old) registered higher frequency 8/57 (14%) compared to others (p=0.19). There was verified a slightly higher tendency of higher frequency among females 44/560 (7.8%). The frequency of IgM anti-DENV positive cases between males and females (p = 0.558), and between age categories (p =0,19) were not statistically significant different. When compared frequency of IgM anti-DENV, data shows differences between HF, where higher frequency was verified in Polana caniço HF (18; 13.1%), followed by Caia HF (p = 0.000).

DENV NS1 was the arbovirus that registered the lowest frequency 16/906 (1.8%), with a median age of 22 (IQR:17.25 – 36), being 25 – 34 the age group that shows higher frequency compared to others. Higher frequency was also found in Polana Caniço HF (5.1%), followed Caia (4.2%) and Coalane (1.4%). No positive cases were found in Massingir and Natite HF (Table 4.6).

Table 4.6: Demographic characteristics of positive cases for IgM anti-DENV and NS1

		DENV IgM+		DENV NS1+
Characteristics	**Subjects n (%)**	**Positive cases n (%)**	**p-value**	**Positive cases n (%)**
Total sample	906 (100)	64 [7.1 (5.5-8.9)]		16 [1.8 (0.9-2.6)]
Gender			0.558	
Male	336 (37.5)	20 (5.9)		4 (1.2)
Female	560 (62.5)	44 (7.8)		12 (2.1)
Age-group			0,19	
Age, Median (IQR)	25 (18 - 36)	25 (18 - 34.25)		22 (17.25-36)
5 – 17	168 (18.8)	11 (6.5)		4 (2.4)
18 – 24	258 (28.8)	19 (7.4)		6 (2.3)
25 – 34	205 (22.9)	17 (8.3)		5 (2.4)
35 – 44	116 (12.9)	3 (2.5)		0
45 – 54	87 (9.7)	4 (4.6)		0
≥ 55	57 (6.4)	8 (14.0)		1 (1.8)
Health facilities			<0,001	
Polana Canico	137 (15.1)	18 (13.1)		7 (5.1)
Massingir	117(12.9)	5 (4.3)		0
Caia	120(13.2)	14 (11.7)		5 (4.2)
Eduardo Mondlane	246(27.2)	11 (4.5)		1 (0.4)
Coalane	211(23.3)	12 (5.6)		3 (1.4)
Natite	75(8.3)	4 (5.3)		-

IgM anti-ZIKV antibodies

Regarding IgM anti-ZIKV, a total of 83 (9.2%) positive samples were registered, with a median age of 28.5 (IQR: 19 – 41), with higher frequency reported in patients aged 45-54 years old 15/87 (17,2%), and males the gender that registered higher frequency 57/606 (10.2%) compared to females 25/336 (7.4%). When compared frequency of IgM anti-ZIKV, data shows differences between HF(p=0.002), where observed higher frequency among samples from Natite HF 16/75 (21.3%) followed by Eduardo Mondlane HF (see Table 4.7).

Malaria

Among enrolled patients, 56/906 (6.2%) were malaria confirmed cases, with a median age of 28 (IQR: 20 – 48), being 45 – 54 the age group with frequency 8/87 (17,2%). Males registered slightly higher frequency 23/336 (6.8%) (see Table 4.7). It was founded a significant association between health facilities and malaria infection (p<0.001), being Polana caniço the HF that verified a higher cases frequency compared to other health facilities, 26/137 (19%).

Table 4.7: Demographic characteristics among positive cases for IgM anti-ZIKV and Malaria.

		ZIKV IgM+		RDT Malaria+	
Characteristics	**Subjects n (%)**	**Positive cases n (%)**	**Pvalue**	**Positive Cases n (%)**	**Pvalue**
Total sample	906 (100)	83 [9.2 (7.4-11.2)]		56 [6.2 (4.7–8.0)]	
Gender			0.139		0.569
Male	336 (37.5)	25 (7.4)		23 (6.8)	
Female	560 (62.5)	57 (10.2)		33 (5.9)	
Age-group			0,504		0.059
Age, Median (IQR)	25 (18 - 36)	28.5 (19 - 41)		28 (20 - 48)	
5 – 17	168 (18.8)	12 (7.1)		5 (2.9)	
18 – 24	258 (28.8)	23 (8.9)		18 (7)	
25 – 34	205 (22.9)	17 (8.3)		9 (4.4)	
35 – 44	116 (12.9)	11 (9.5)		8 (6.9)	
45 – 54	87 (9.7)	15 (17.2)		8 (17.2)	
≥ 55	57 (6.4)	4 (70.1)		8 (14.0)	
Health facilities			0,002		0.000
Polana Canico	137 (15.1)	13 (9.5)		26 (19)	
Massingir	117(12.9)	0		3 (2.6)	
Caia	120(13.2)	10 (8.3)		17 (14.2)	
Eduardo Mondlane	246(27.2)	27 (11)		2 (0.8)	

Coalane	211(23.3)	17 (8.1)	5 (2.4)
Natite	75(8.3)	16 (21.3)	3 (4)

We performed an assessment of the monthly distribution of positivity to IgM anti-CHIKV, DENV and ZIKV detected over the 12-month period of study. During the period of the study, it was noted that the number of febrile patients increased and so did the number of cases with CHIKV, DENV, ZIKV and malaria (Figure 4.6).

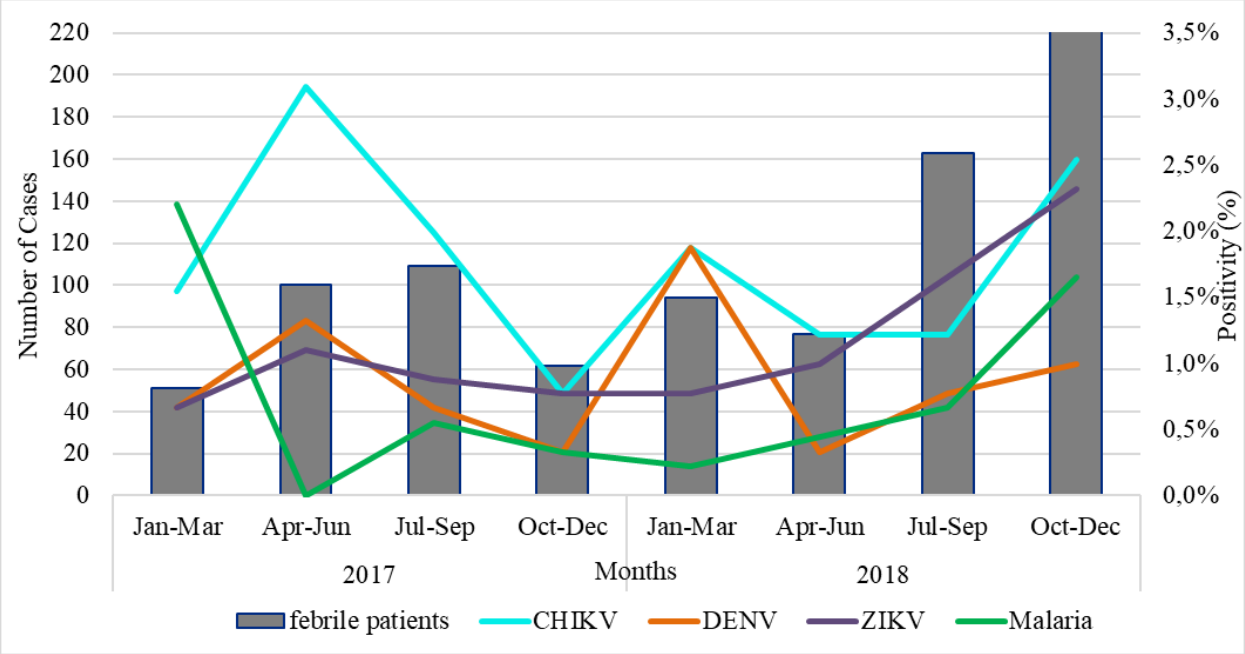

Figure 4.6: Monthly trend in the frequency of participants with positive IgM against CHIKV, DENV, ZIKV and of malaria infections among acute febrile individuals

III. Co-occurrence of IgM antibodies against CHIKV, DENV, ZIKV and Malaria

In Table 4.7 is described the distribution of co-occurrence of CHIKV, DENV, ZIKV and malaria among the febrile participants of the study. From a total of 134 CHIKV positive cases, 107(79.9%) registered CHIKV unique infection, and 16 (11.9%,95%CI: 6.98 – 18.7) were CHIKV and Malaria.

Among DENV positive cases, 44/64 (68.7%, 95%CI:55.9– 79.8) were only DENV positive cases, and 3/64 (4.7%, 95%CI: 0.98 – 13.1) were DENV and Malaria co-infection. Among DENV NS1 positive cases, 7/16 (43.8%, 95%CI19.8 – 70.1) registered DENV

NS1 unique infection and 1/16 (6.25 %, 95%CI:0.16 – 30.2) was DENV NS1 and malaria co-infection.

A majority of IgM anti-ZIKV positive cases, were unique IgM anti-ZIKV infection, 64/83 (77.1%, 95%CI:66.6 – 85.6) and 10/83 (12.0%, 95%CI: 5.93 – 21.0) were IgM anti-ZIKV and Malaria co-infection. (See Table 4.8).

Table 4.8: **Distribution of co-occurrence of CHIKV, DENV, ZIKV and Malaria.**

	Infection	Positive cases/ (n)	% (95% CI)
Unique Infection	Malaria	32/56	57.1 (43.2 – 70.3)
	CHIKV	107/134	79.8 (72.1 - 86.3)
	DENV	44/64	68.7 (55.9– 79.8)
	DENV-NS1	7/16	43.8 (19.8 – 70.1)
	ZIKV	64/83	77.1 (66.6 – 85.6)
Malaria co-infection	Malaria +CHIKV	16/134	11.9 (6.98 – 18.7)
	Malaria +DENV	3/64	4.7 (0.98 – 13.1)
	Malaria +DENV-NS1	1/16	6.25 (0.16 – 30.2)
	Malaria +ZIKV	10/83	12.0 (5.93 – 21.0)

* It was considered the positive cases for IgM antibodies.

4.3. Output II: Epidemiologic characterization COVID-19. (Examine the dynamics of SARS-CoV-2 prevalence in Mozambique throughout the course of the three-epidemic wave)

I. Overview of the COVID-19 pandemic

In March 2022, Mozambique announced the first laboratory confirmed case of imported SARS-CoV-2. Following, several imported cases were detected. And on about two months later, on 25 May 2020, it was registered the first death. Since middle of May 2020 the number of daily cases reported started to increase drastically (Figure 4.7). Since the begin of COVID-19 epidemic up to September 2021, three different waves were registered. The first wave occurred from 06 September to November 2020. The second wave occurred from January to 02 April 2021 and the third wave occurred from 13 June to 13 September 2021 (Figure 4.7).

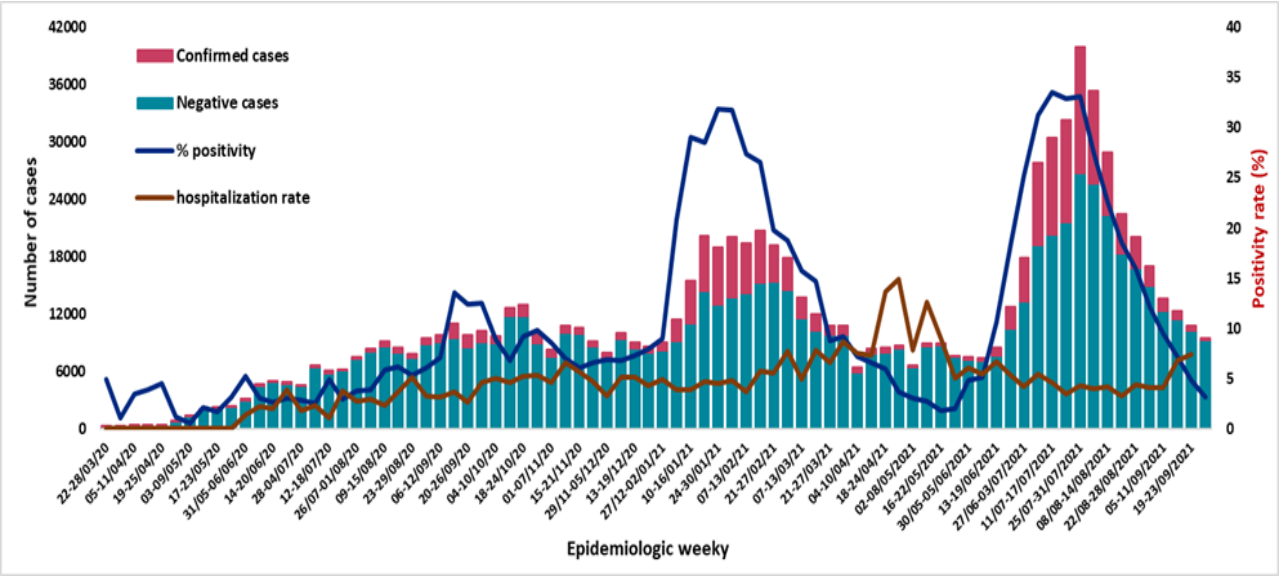

Figure 4.7: Epidemic Curve of the COVID-19 epidemic in Mozambique 2020 to 2021.

II. Demographics characteristics of subjects

From March 2020 to September 2021, approximately 772,250 individuals were screened for COVID-19 in Mozambique. Demographics data of the persons screened are shown in Table 4.9. Of all subjects, 412,701/772,250 (53.4%) were males. The highest samples were from the 15 - 29 years 226,409/758,055 (29.9%) group. Most of the individuals were from Maputo City 259,348/778,749 (33.3%). Table 4.9 gives demographic characteristics of screened and confirmed cases. A total of 149,142 cases were confirmed by the end of September 2021. Their median age was 33 years and 75,744/359,549 (21.8%) were female. Th high positivity was reported among the older 65 +years old 7,794/305,502 (25.6%) In terms of geographical distribution, the most prevalent geographic origins were Maputo province 21,140/87,189 (24.2%) and Maputo city 57,800/259,348 (22.3%). Regarding city/rural dichotomy, most of the study population live in urban area 100,623/469,602 (21.4%).

Table 4.9: Demographic Characteristics of screened patients

	Total		Confirmed cases		Negative cases	
	n	%	n	%		
Gender	**772,250**		**149,142**		**623,108**	
Female	359,549	46.6%	75,744	21.1%	283,805	78.9%
Male	412,701	53.4%	73,398	17.8%	339,303	82.2%
Age groups	**758,055**		**146,067**		**611,988**	
0 – 14	103,885	13.7%	14,473	13.9%	89,412	86.1%
15 – 29	226,409	29.9%	43,420	19.2%	182,989	80.8%
30- 44	254,504	33.6%	50,799	20.0%	203,705	80.0%
45 – 59	118,682	15.7%	24,128	20.3%	94,554	79.7%
60 – 64	24,073	3.2%	5,453	22.7%	18,620	77.3%
65+	30,502	4.0%	7,794	25.6%	22,708	74.4%
Province	**778,749**		**150,079**		**628,670**	
Niassa	36,911	4.7%	7,378	20.0%	29,533	80.0%
Cabo Delgado	39,668	5.1%	4,501	11.3%	35,167	88.7%
Nampula	60,628	7.8%	7,015	11.6%	53,613	88.4%
Zambezia	49,247	6.3%	8,257	16.8%	40,990	83.2%
Tete	45,013	5.8%	8,681	19.3%	36,332	80.7%
Manica	38,428	4.9%	6,443	16.8%	31,985	83.2%
Sofala	56,879	7.3%	9,399	16.5%	47,480	83.5%
Inhambane	63,780	8.2%	10,564	16.6%	53,216	83.4%
Gaza	41,658	5.3%	8,901	21.4%	32,757	78.6%
Maputo Province	87,189	11.2%	21,140	24.2%	66,049	75.8%
Maputo City	259,348	33.3%	57,800	22.3%	201,548	77.7%
Residence	**596,708**		**121,210**		**475,498**	
Rural	127,106	21.3%	20,587	16.2%	106,519	83.8%
Urban	469,602	78.7%	100,623	21.4%	368,979	78.6%

III. Different waves during the COVID-19 Pandemic

Table 4.10 summarizes the demographic and case data across the three waves. The 12,897 cases during the first wave involved slightly more females (54.1%) than males (45.9%), and most of them were aged 30 - 44 years 4515 (35.7%). In terms of clinical presentation, 1,279/12,897 (10.1%) were symptomatic patients from whom males presented slightly higher frequency 641 (50.1%). Most cases were from Maputo City 6605/13028 (50.7%).

The 48,058 cases during the second wave comprised slightly more males 24,675 (51.3%) than females (48.7%), and most of them were aged 30 – 44 18,120 (38.6%) years. The proportion of symptomatic patients were 11,665 (24.8%), from whom males presented slightly higher proportion 6037 (51.8%).

The 77,513 cases during the third wave comprised slightly more males (53.7%) than females (46.3%), and most patients were aged 30 – 44 years 26,598 (35.0%). During these waves 32,549 (42.8%) presented symptoms, from whom 17,991 (55.3%) were females.

The proportion of confirmed cases in the different epidemic waves was 12,897 (11.2 %; 95%CI: 11.1 – 11.4) with mean daily number of new cases was 143 in the first wave, 48058 (28.8%; 95%CI: 27.8 -28.2) in the second wave the total cases 77513 (25.1%; 95%CI: 24.9 – 25.2) and the mean daily number of new cases was 536, and in the third wave, total 77,513 (25.1%: 95%CI: 24.9 – 25.2) and mean daily number of new cases was 844 cases (Table 4.10). Thus, the number of cases has increased by more than 60,000 from the first to the third wave of the COVID-19 pandemic. The gender distribution across the waves, during the first and third wave females registered slightly higher proportion of confirmed cases, 54.1% and 53.7% respectively, and in contrast during the second wave males registered the higher proportion (51.3%). In general males were the gender with slightly higher proportion of symptomatic cases, except for the third wave were females had higher proportion (55.3%). During the three waves most of the patients were from Maputo City, corresponding to 50.7% in the 1st wave, 35.8% in the 2nd wave and 38.5% in the 3rd wave. Ir was founded a significant differences between the waves ($p < 0.01$).

Table 4.10: Sociodemographic characteristics of positive COVID-19 cases during the three different waves during COVID-19 pandemic in Mozambique

	All	First wave	Second wave	Third wave	p value*
Time periods of COVID-19 Pandemic		06 September - 14 November 2020	January 2021 – 02 April 2021	13 June - 13 September 2021	
Total		12,897 [11.2 (11.1 – 11.4)]	48,058 [28.8 (27.8 - 28.2)]	77,513 [25.1 (24.9 – 25.2)]	**<0,01**
Gender, n	**138,468**	**12,634**	**46,921**	**75,987**	**<0,01**
Female	71,995 (52.0%)	6,974 (54.1%)	23,383 (48,7%)	41,638 (53.7%)	
Male	66,473 (48.0%)	5,923 (45.9%)	24,675 (51,3%)	35,875 (46.3%)	
Age, years — median	**135,547 (34.9)**	**12,634 (33.0)**	**46,921 (36)**	**75,992 (34.46)**	**<0,01**
0 – 15	13,433 (9.9%)	1,474 (11.7%)	3,977 (8.5%)	7,982 (10.5%)	
15 – 29	40,403 (29.8%)	4,075 (32.3%)	12,637 (26.9%)	23,691 (31.2%)	
30- 44	49,233 (36.3%)	4,515 (35.7%)	18,120 (38.6%)	26,598 (35.0%)	
45- 59	20,183 (14.9%)	1,730 (13.7%)	7,782 (16.6%)	10,671 (14.0%)	
60- 64	5,089 (3.8%)	379 (3.0%)	1,895 (4.0%)	2,815 (3.7%)	
65+	7,206 (5.3%)	461 (3.6%)	2,510 (5.3%)	4,235 (5.6%)	
Clinical Characteristics					**<0,01**
Symptomatic		**1,279 (10.1%)**	**11,665 (24.8%)**	**32,549 (42.8%)**	
Female	71,995 (50.8%)	638 (49.9%)	5628 (48.2%)	17991 (55.3%)	
Male	73,398 (49.2%)	641 (50.1%)	6037 (51.8%)	14558 (44.7%)	
Hospitalization	6,298 (4.9%)	408 (3.2%)	2,438 (5.2%)	3,452 (4.5%)	
Deaths	1,918 (1.4%)	82 (0.6%)	612 (1.3%)	1016 (1.3%)	
Geography (Provinces)	**139,298**	**13,028**	**48,472**	**77,798**	**<0,01**
Niassa	6,671 (4.8%)	258 (2.0%)	1,906 (3.9%)	4,507 (5.8%)	
Cabo Delgado	3,785 (2.7%)	396 (3.0%)	2,141 (4.4%)	1,248 (1.6%)	
Nampula	5,751 (4.1%)	242 (1.9%)	2,248 (4.6%)	3,261(4.2%)	
Zambezia	7,446 (5.3%)	1,042 (8.0%)	3,066 (6.3%)	3,338 (4.3%)	
Tete	8,169 (5.9%)	430 (3.3%)	1,874 (3.9%)	5,865 (7.5%)	
Manica	6,193 (4.4%)	196 (1.5%)	1,904 (3.9%)	4,093 (5.3%)	
Sofala	8,934 (6.4%)	610 (4.7%)	4,768 (9.8%)	3,556 (4.6%)	
Inhambane	10,132 (7.3%)	319 (2.4%)	3,424 (7.1%)	6,389 (8.2%)	
Gaza	8,901 (5.9%)	524 (4.0%)	2,960 (6.1%)	5,043 (6.5%)	
Maputo Province	21,140 (14.1%)	2,406 (18.5%)	6,825 (14.1%)	10,541 (13.5%)	
Maputo City	57,800 (38.5%)	6,605 (50.7%)	17,356 (35.8%)	29,957 (38.5%)	
Residence	**112,420**	**13,027**	**48,469**	**50,924**	**<0,01**
Rural	19,068 (17%)	1,374 (10.5%)	6,942 (14.3%)	10,752 (14.3%)	
Urban	93,352 (83%)	11,653 (89.5%)	41,527 (85.7%)	40,172 (85.7%)	

* chi-square test was carried out and p values < 0.05 were considered significant

The incidence of cases per wave and stratified by age and gender are represented in the Figure 4.8. is clear observed an increase of incidence across the waves. During the first wave the absolute incidence of confirmed cases was around 200 cases per 100.000 inhabitants, and this increased approximately for 1000 cases per 100.000 inhabitants in the got higher as 1400 cases per 100.000 habitants second and in the third wave.

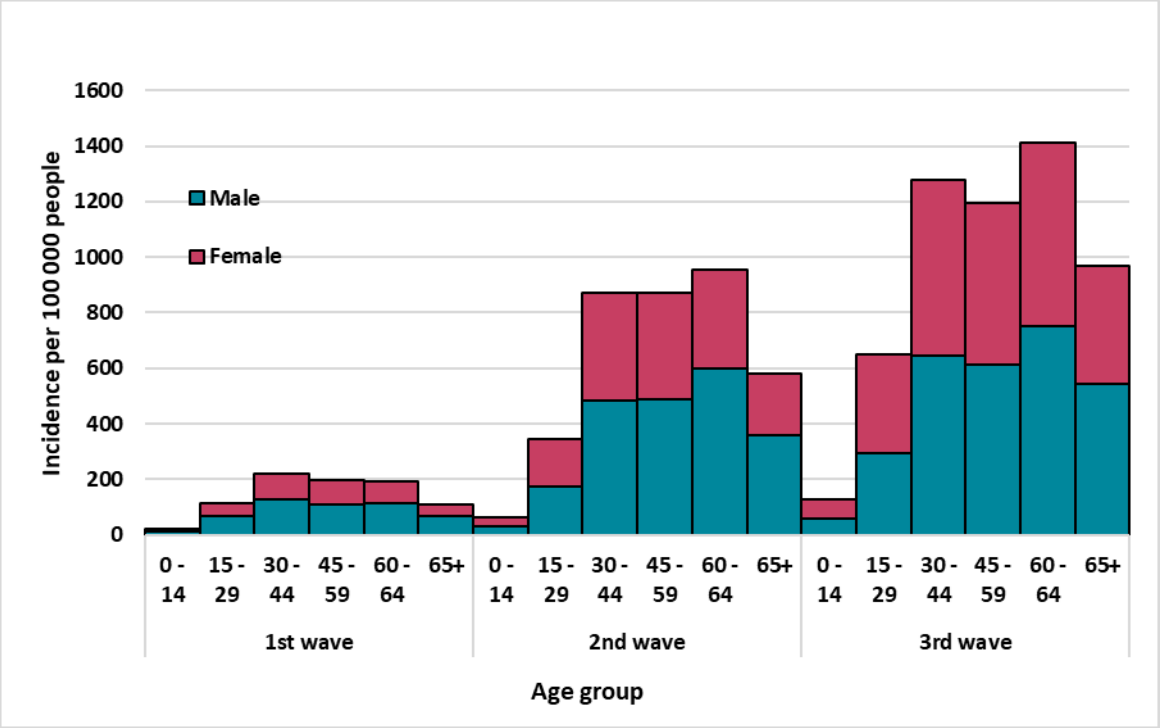

Figure 4.8: Cumulative incidence of COVID-19 per 100,000 inhabitants stratified by age group and sex for each wave.

In Figure 4.9 it is showed the geographic distribution of COVID-19 confirmed cases and deaths registered during the each COVID-19 wave. Through the three waves the south region reported the higher incidence of cases per 100,000 habitants and Maputo City and Maputo province accounted for more than 200 cases por 100 00 habitants followed by the central region. The incidence of death was slightly higher in Maputo City and Maputo province and there was a tendency of increase through the waves. In terms Niassa and Manica province has no deaths register during the three waves. Although

both Niassa and Manica province has registered slightly higher incidence in the second and third wave compared with the first wave.

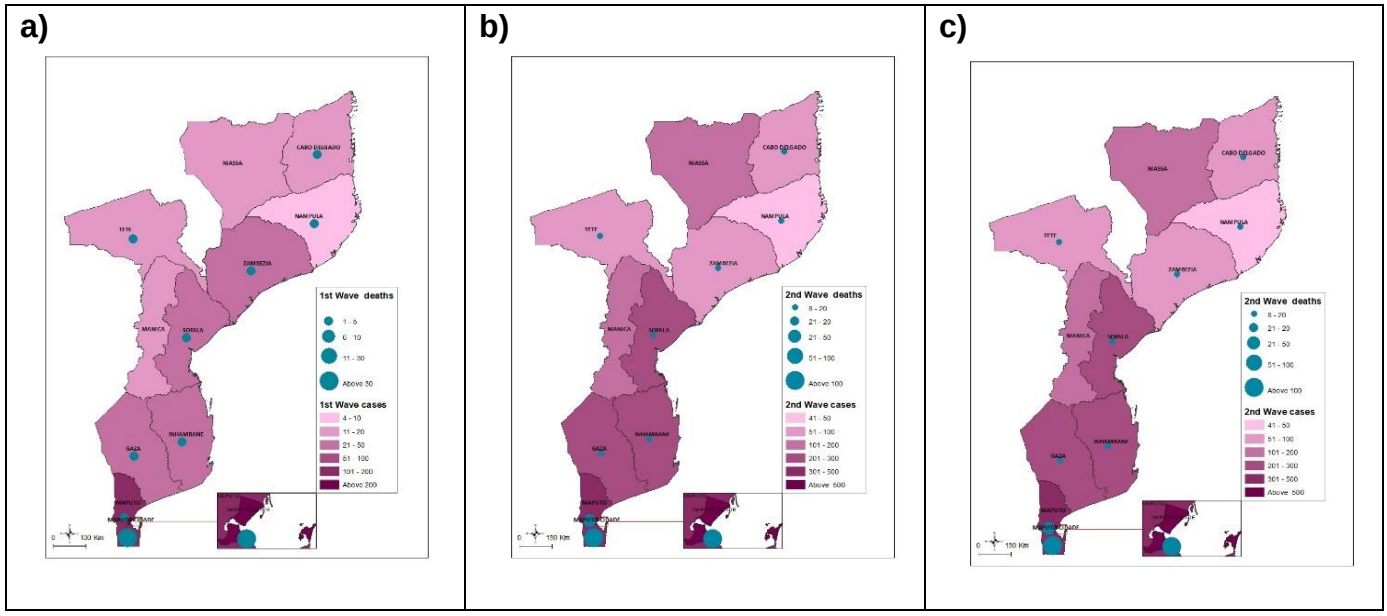

Figure 4.9: Distribution of SARS-CoV-2 Incidence in Mozambique. a) first wave, b) second wave and c) third wave.

5. DISCUSSION

This study represents the largest and most recent investigations of emerging and re-emerging virus in Mozambique. First, particular attention was directed to investigate past exposure to arbovirus and secondly to assess current exposure to arbovirus and co-occurrence of arbovirus and malaria in serum from febrile patients in Mozambique. Thirdly, we performed a descriptive analysis of epidemiological data of the three waves of COVID-19 in Mozambique to allow deeply understanding of the SARS COViD-10 epidemic in the country.

5.1. Output I: Seroprevalence of arboviruses

The seroprevalence of arbovirus was investigated using two different approaches. A retrospective study using a serum sample stored in biobank, that were collected from febrile patients in the context of national measles surveillance from 2009 to 2015. These were screened for antibodies against CHIKV, DENV and ZIKV. The measles and rubella surveillance were mainly focused on children, limiting the samples participants for children between <1 – 15 years old. The second approach was a cross-sectional serological investigation for antibodies against CHIKV, DENV and ZIKV, and for NS1 for DENV, in serum from febrile patients in 6 selected health facilities in Mozambique. This cross-sectional study also investigated co-occurrence of arbovirus and malaria. Participants of this study were people older than 5 years.

a) Chikungunya

In general, seropositivity for IgM (6.0%; 95%CI: 4.6 - 7.8) and IgG (17.9%; 95% CI:15.4 - 20.5) against CHIKV reported in the retrospective study which involved younger children was lower than the frequencies reported in the more recent prospective study, conducted among adults which found a frequency of IgM anti CHIKV of 14.8% (95%CI: 12.5-17.1) and IgG anti CHIKV of 36.6% (95%CI: 33.5-39.8), respectively. This corroborates with the finding from previews studies conducted by Manimunda and collegueas [151] and others group of research [146, 152], that founded that antibodies against chikungunya were more frequent in adults as compared to children. A potential reason for Chikungunya being less frequent in young children, could be the fact that in both

retrospective and prospective studies, persons with fever were enrolled. So, younger children have more frequent episodes of febrile illness per year than adults, which leads to a more dilution of positivity rare among younger febrile patients [153]. However, these findings are different of those reported by *Kajeguka, et al* and *Sissoko et al* that reported similar frequency of antibodies against CHIKV in both children and adults [154, 155].

Our data from the retrospective assessment did not find a difference in the seropositivity of IgM anti-CHIKV between 2009 and 2015 but found substantial increase of IgG seropositivity from 2009 through 2015. This can be indicative of an undetected outbreak, causing an increase in seroprevalence. The fact that Antibodies anti-CHIKV were detected in all provinces suggest that the virus is wider distributed throughout the country as also suggested in one of the oldest serologic studies conducted in Mozambique by Kokernot and colleagues in 1957 [16].

In terms of geographic spread, it was noted that the frequency of antibodies anti-CHIKV reported both in retrospective and prospective studies indicates an increased trend in the central region. This trend can also be supported by the recent investigation that reported a high frequency of antibodies against CHIKV in Quelimane city, located in the central region of the country [156]. Of note, in the latest Malaria survey, it was reported higher prevalence of Malaria in the central region of the country [157], suggesting that in this region of the country, suitability to mosquito transmissible infections is higher in the country.

Note that, it is not clear whether CHIKV has been circulating in Mozambique since 50`s or if this represents a more recent introduction of the virus. However, previous evidence of CHIKV circulation from smaller serologic study conducted in 1970s [77] and later 1980s [78] reported 65%-81% and 12,1% (24/199) of antibodies anti-CHIKV respectively. Knowledge about prior occurrence, distribution, and trends of CHIKV in the country is relevant for better understanding the epidemiology of the virus, as well for the definition of appropriate interventions for its control and prevention.

Few studies investigated the frequency of IgM and IgG antibodies against CHIKV in the Mozambican population. In this study, we found a higher frequency of antibodies anti-

CHIKV as compared to the reported in previous studies conducted by Muianga and colleagues [158]. The increased frequency of IgM anti-CHIKV antibodies when compared with previous studies might suggest that CHIKV is becoming more prevalent in the country. However, more studies are needed to ascertain this hypothesis. This also indicates that establishment of sentinel surveillance for monitoring the trend of CHIKV is urgently needed.

b) Dengue

Data from the retrospective study indicated that 2.8% of samples were positive for NS1, suggesting active infection with DENV among acute febrile patients with measles negative diagnostic. This raise awareness that dengue is an important pathogen to consider in the differential diagnostic of exanthematous illness. In contrast analysis from the prospective study indicated a slight lower seroprevalence of 1,8% (95%CI: 0.9-2.6) of DENV-NS1 in Cabo Delgado. The frequency of NS1 were slightly lower in the northern region, these results contrast with the previews description of circulation and outbreaks in 2014 occurred in north of Mozambique [69]. Our prospective surveillance showed that 2 years after the most recent report of Dengue outbreak in northern Mozambique, there is an ongoing circulation of DENV-2 [74].

IgM anti-DENV antibodies were positive in 7.1% (5%CI: 55-8.9) of the prospective study. which is relatively lower than that reported after a pooled frequency from 76 studies across Africa (80,977 participants; 24 countries across Africa) [65]. The positivity for IgM anti-DENV was higher than the positivity for DENV-NS1. These results were expected and corroborate findings that NS1 protein is positive for a shorter period than the persistence of IgM.

c) Zika

Few studies have investigated circulation of ZIKV in the country. The positivity for IgM anti ZIKV found in the retrospective study was 4.9% (95%CI: 3.5-6.6), and in the prospective study was 9.2% (95%CI:7.4-11.2). This relative increase in the frequency of antibodies against ZIKV in Mozambique is an additional argument demanding the urgency for the establishment of sentinel surveillance for arbovirus in the country. Regarding geographical distribution, we found higher frequency of IgM against CHIKV in north-

ern region anti-ZKV. Recent studies also reported the occurrence of antibodies against DENV and CHIKV in north, and center of the country [156, 158]. Although in 1957, researchers reported a frequency of antibodies against ZIKV of 4.0% (10/249) in Mozambique [159], the country were not included in the list of countries affected by ZIKV. This could have led errors in the estimation of the burden of de Zika in Mozambique and in the region.

d) Co-occurrence of IgM antibodies against CHIKV, DENV, ZIKV and Malaria

In the prospective study it was also investigated the co-occurrence of arbovirus and malaria. The frequency of malaria infection in this study was 6.2% (95%CI: 4.7-8.0). This finding is concurrent with a study conducted in Ethiopia, that found the frequency of malaria among febrile patients of 7,3% [160]. But malaria prevalence found in this study were lower as compared with the results found in the neighboring country. For instance in Tanzania, malaria prevalence was 28.75% (116/400) [62]. Increase in arbovirus burden in increasing globally because of climate change and urbanization that affects vector spread and activity, [68, 161].

Malaria is known as the major public health concern in Mozambique, representing the one of the main causes of morbidity and mortality in the country [162]. Co-occurrence of malaria and IgM antibodies against CHIKV, ZIKV or DENV found in this study may suggest that arbovirus was silently circulating among febrile patients misdiagnosed as malaria. This reinforces the need to establish arbovirus screening as a part of malaria differential diagnostic [163]. In this study, co-occurrence of malaria and IgM antibodies against CHIKV (17.9%) was higher than co-occurrence of malaria with either IgM antibodies against DENV (5.2%) or IgM antibodies against ZIKV (1.8%). In Nigeria, it has been reported are similar results by Ayorinde and collegueas [164]. However a lower frequency of Malaria and CHIKV co-infection (7.4%) was reported in Tanzania during a study among 400 febrile patients [62].

Arboviral infection and malaria have a similar clinical presentation, thus leads to difficulties to clearly identify the responsible pathogen for the clinical signs and symptoms in case of the simultaneous infections. As shown here for Mozambique arboviral infections are prevalent and should be part of the differential diagnostics for febrile diseases.

those are not considered by healthcare workers. In this study only 6.1% of the patients had a positive result for malaria and of those, 30/56 (53,5%) were co-infected with arbo-viral diseases.

4.3. Output II: Epidemiologic characterization COVID-19

This study is the first most comprehensive descriptive analysis of COVID-19 epidemiology, including a brief comparison of the three waves that occurred in Mozambique. By the end of September 2021, a total of 149,142 confirmed cases has been reported Mozambique and around 1,778 deaths recorded. Mozambique was listed among the twenty most affected countries in Africa [165].

During the first wave (06 September - 14 November 2020), it was experienced a low testing capacity, marked by insufficiency of diagnostic tests forcing for established a restricted criteria for a sample testing, only more severe patients were tested, and the laboratory facilities were only available in the Maputo City which might have influenced in the availability of diagnostic. During the second wave, Mozambique expanded the laboratory capacity throughout the country, and RDT-antigen was incorporated as a tool for surveillance and clinical management. The third wave hit Mozambique in July 2021.

The positivity rate in the different epidemic waves were 11.2% (12,897/114,775) in the first wave, 28.8% (48,058/171,560) in the second wave, and 25.1% (77,513/ 308,091) in the third wave. Thus, the number of confirmed cases increased by more than 60,000 from the first wave to the third wave of the COVID-19 cases. These results could indicate that there a higher rate of virus transmission. However, the increase in testing capacity, which from 600 tests per day, to 5.172 tests per day by April 2021 in the public sector may all so could lead for the rise of confirmed cases between the three waves [166]. Other factors that contributed was the increased surveillance activities and different control strategies implemented by the public and private sector. Across Africa, others country have reported a similar evolution pattern [167], such as South Africa [168] and Nigeria [169].

Although, Maputo City registered the higher number of COVID-19 confirmed cases and this was the epicenter of the local epidemic, adherence to the COVID-19 preventive

measures were considered relatively high in this province [170]. Further analyses may be needed to understand other aspects that promote the spread of virus in the region.

Our results indicates that the younger age group (30 - 64 years) showed the highest cumulative incidence, suggesting that this is the age group responsible for the spread of the virus. In contrast, the higher risk for severe disease were observed among the older individuals (>65 years of age) and those with medical conditions, such as cardiovascular disease obesity, chronic respiratory disease, hypertension, and obesity [126, 127].

The country registered an exponential increase of COVID cases and deaths in January and February 2021, with the average number of cases daily about four times greater than that seen in the first wave. The second wave in Mozambique was characterized by the circulation of the Beta variant.

The third wave occurred from July to September 2021 and has been considered the most severe wave than the previous. Many aspects have contributed for that scenario, such as the occurrence of SARS-CoV-2 Delta variant which was considered the more transmissible among the circulating variants. Delta variant had a shorter incubation period and more severe clinical disease than other variants [110]. Some of the neighboring countries of Mozambique, such as Zimbabwe as experienced even harsher third wave of the COVID-19 pandemic, reporting an increase in the cumulative number of cases from approximately 38,000 to 120,000 in just two months.

6. LIMITATIONS

The arbovirus investigation in this study was performed centred on serologic commercial kit, ELISA. No molecular tests or neutralizing assays were performed. Results from this study suggest that follow up studies should consider molecular or neutralization assays for better ascertainment of acute arbovirus infection. This fact can be considered a limitation of the study. However, we used commercial kits that are described as sensitive and specific [148, 149]. We also recognize that our sampling methodology can represent a limitation. First, in the retrospective study it was used pre-existing data collected in the context of surveillance for measles which may underestimate the seroprevalence of the arbovirus. Second, case definition of the arbovirus may not full overlap the definition for measles. Third, in the retrospective study the participants were chidrens

and in the prospective serology investigation the participants were alder than 5 years old, main are young adults.

Other important limitation of this study was our incapacity to test other respiratory virus among the participants with acute respiratory infection. This is important because SARS-CoV-2 negative results does not necessarily rule out possibility of co-infection and role in severity of the COVD-19 cases.

7. CONCLUSIONS AND RECOMENDATIONS

Data from this study:

- represents the first largest investigation on antibodies against arbovirus in febrile patients performed in Mozambique. The results these indicates that: for several years the population across all provinces have been exposed to CHIKV, DENV and ZIKV.
- Suggest that co-occurrence of malaria and CHIKV, DENV and ZIKV among febrile patients are more common than previously thought. Highlighting the need for the establishment of surveillance for arbovirus.
- Indicates that there are epidemiological and demographic differences between the three waves of COVID-19 pandemic in Mozambique. The urban areas and the southern region of the country were the most affected during the pandemic.

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9. APPENDIX

9.1. STATEMENT ON PRE-RELEASE AND CONTRIBUTION

a) **Title: Retrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control**

Submission: published March 2019 **Journal:** PLoS One

Aim: to retrospectively investigate the occurrence and geographical distribution of anti-CHIKV antibodies between 2009 and 2015.

Role of PhD candidate: Study coordinator, Data acquisition, samples processing, interpretation of data, analysis and interpretation, revision of manuscript,

b) Title: Retrospective investigation of IgM antibodies against Zika virus in serum from febrile patients in Mozambique, 2009-2015.

Submission: published July 2019 **Journal:** BMC Res Notes

Aim: to investigate the occurrence and geographical distribution of IgM antibodies against ZIKV between 2009 to 2015 in Mozambique

Role of PhD candidate: study coordinator, data acquisition, samples processing, interpretation of data, analysis and interpretation, revision of manuscript,

c) **Title: Investigations of antibodies against Chikungunya, Dengue, and Zika virus in serum samples from febrile patients and its co-occurrence with malaria in five districts highly endemic for malaria in Mozambique from 2017 – 2018**

Submission: yes (under editorial review) **Journal:** Viruses

Aim: to determine the co-occurrence of DENV, CHIKV and ZIKV and malaria in five districts highly endemic for malaria in Mozambique from 2017 to 2018

Role of PhD candidate: Study coordinator, conceptualization, data acquisition, samples processing, interpretation of data, analysis and interpretation, revision of manuscript,

d) Title: The descriptive epidemiological analysis of the three different waves occurred during COVID-19 epidemic in Mozambique.

Submission: No (under internal review) **Journal:** To be determined

Aim: aims to describe epidemiology of Covid-19 in Mozambique, with special attention to geographic and social demographics characteristics of different waves during COVID-19 pandemic.

Role of PhD candidate: lead of national testing Laboratory, data acquisition, samples processing, interpretation of data, analysis and interpretation, revision of manuscript,

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9.3. LIST OF PUBLICATIONS

a) Antonio VS, Amade NA, Muianga AF, **Ali S**, Monteiro V, Mula F, Chelene I, Oludele J, Chongo I, José A, Augusto O, Gudo ES. Retrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control. PLoS One. 2019 Mar 21;14(3):e0213941. doi: 10.1371/journal.pone.0213941. PMID: 30897135; PMCID: PMC6428254.

b) Chelene IR, **Ali S**, Mula FI, Muianga AF, Monteiro VO, Oludele J, Chongo IS, José A, Amade NA, António VS, Gudo ES. Retrospective investigation of IgM antibodies against Zika virus in serum from febrile patients in Mozambique, 2009-2015. BMC Res Notes. 2019 Jul 31;12(1):469. doi: 10.1186/s13104-019-4511-x. PMID: 31366379; PMCID: PMC6670129.

c) Mugabe VA, **Ali S**, Chelene I, Monteiro VO, Guiliche O, Muianga AF, Mula F, António V, Chongo I, Oludele J, Kerstin F, Paploski IA, Reis MG, Kitron U, Kümmerer BM, Ribeiro G S, Gudo ES. Evidence for chikungunya and dengue transmission in Quelimane, Mozambique: Results from an investigation of a potential outbreak of chikungunya virus. Plos one. 2018 ;13(2):e0192110. DOI: 10.1371/journal.pone.0192110. PMID: 29415070; PMCID: PMC5802900.

d) Gudo ES, Falk KI, **Ali S**, Muianga AF, Monteiro V, Cliff J. A Historic Report of Zika in Mozambique: Implications for Assessing Current Risk. Plos Neglected Tropical Diseases. 2016 Dec;10(12):e0005052. DOI: 10.1371/journal.pntd.0005052. PMID: 27930650; PMCID: PMC5145135.

9.4. ANNEX 1: ARBOMAP QUESTIONNAIRE

EPIDEMIOLOGICAL AND ENTOMOLOGICAL INVESTIGATION OF DENGUE AND OTHER ARBOVIRUS IN 11 DISTRICTS IN MOZAMBIQUE

A. Demographic Data

1. Name: _____ **2. Admission date**:____/_____/_____

3. Patient Code: _____

3. Residence: District_____ Neighborhood _____
Q_____ Street/Avenue _____ House no._____

4. Service Location:
_____

5. Date of birth: ____/____/____ **5. Age**: _____ **6. Gender**: Male❑ Female❑

6. Race: Black❑ Caucasian❑ Asian❑ Mixed race ❑ Other: _____

7. Occupation:

Tradesman □ Administrative □ Unemployed □ Driver □ Cleaning Agents □ Forest Guard □ Farm / Animal Husbandry □ Fisherman □ Health Professional □ Lumberjack Veterinarian □ Gold Miner □Other: _____

8. Schooling: None□ Primary□ Secondary□ Technical□ University□

9. Nationality: Mozambican□ Other: _____

B. Epidemiological and risk factor data (last 30 days)

13. Recent trip: Yes□ No□

If yes: Date of travel ____ / ____ / ____ to ____ / ____ / ____ Location _____

14. Contact with similar cases of acute fever: Yes□ No□

15. Exposure to dengue transmission area/mosquito presence: Yes□ No□

If yes: Location _____

16. Previous Dengue History: Yes□ No□ Year_____

17. Yellow Fever Vaccination (<10years): Yes□ No□

18. Contact with animals: Yes□ No□

If Yes Specify: Mouse□ Birds□ Monkey□ Bat□ Dog□ Cat□

Cattle: Sheep□ Caprine□ Swine□ Poultry□ Other_____

19. Contact with insects: Yes□ No□

If yes specify: Mosquito□ Flea□ Tick □ Other_____

20. Recent contact with poor sanitation site: Yes□ No□

If yes: Drainage ditches□ Dumpster□ Sewers / Ditches□ Other: _____

21. Recent Flood Exposure: Yes□ No□

If yes: Location_____ Date _____

22. Blood transfusion: Yes□ No□

23. Water Supply: Tap Water□ Well / Spring□ Other_____

24. Drinking Water Treatment: No□ Staining / Filtration / Boiling□ Other_____

25. Ingestion of unpasteurized milk: Yes □ No□

26. Insect Bite Prevention Method: Mosquito Netting□ Repellent □ Intra-Home Insecticide extra Extra-Home Insecticide□ Other _____

C. Clinical condition

27. Temperature in ° C: _____ **28. Fever Duration (number of days):** _____

28. Symptomatology

General

Fever ❑	Headache ❑	Nausea ❑	Vomiting ❑	Diarrhea ❑
Chills ❑	Myalgia ❑	Arthralgia ❑	Rash ❑	Retroorbital Pain ❑
Dehydration ❑	Photophobia ❑	Jaundice ❑	Oropharyngeal Pain ❑	Abdominal Pain ❑

Haemorrhages

Epistaxis ❑	Hematemesis ❑	Melena ❑	Hemoptysis ❑
Gingivorrhagia ❑	Bruises ❑	Petechias ❑	Major Bleeding ❑

Shock Signs ❑ Agitation ❑ Positive Loop Proof ❑

D. Exams Requested at the local Health Units

29. RDT malaria: Yes□ No□ **If yes:** Positive□ Negative□

30. RDT Dengue: Yes□ No□ **If yes:** Positive□ Negative□

31. CBC: Yes□ No□

32. Biochemistry: Yes□ No Others : _____

E. Therapeutic Conduct (Sentry Post)

33. Drug treatment:

Antibiotic ❑ Analgesic ❑ Antipyretic ❑ Anti-Inflammatory ❑
Corticoids ❑ Oral Hydration / Ev❑ None ❑

Other_____

F. Clinical Conduct (Sentry Post)

34. Outcome:

Hospital Discharge □ Transfer □ Hospitalization □ Urgency □

Clinician Signature: _____ Date: _____ / _____ / _____

Checked By: _____ Date: _____ / _____ / _____

9.5. ANNEX 2: COVID-19 INVESTIGATION FORM

REPÚBLICA DE MOÇAMBIQUE
MINISTÉRIO DA SAÚDE
DIRECÇÃO NACIONAL DE SAÚDE PÚBLICA

FICHA DE INVESTIGAÇÃO/NOTIFICAÇÃO DE CASO DE COVID-2019

Nº_____

Data de notificação: ___/___/___

CASO SUSPEITO DE COVID-19: uma pessoa que cumpre os critérios clínicos (A ou B) e epidemiológicos (C - descritos no verso)
A. Um individuo com início agudo de febre **E** de tosse; **OU**
B. Um individuo com início agudo febre E de tosse e MAIS UM dos seguintes sinais ou sintomas: fraqueza/fadiga geral, dor de cabeça, mialgia, dor de garganta, coriza, dispneia, alteração do estado mental.

PARTE 1: INFORMAÇÃO SOBRE O CASO PROVÍNCIA DE NOTIFICAÇÃO (_____)

Dados individuais

Sexo: Masculino ( ) Feminino ( ) Idade: _____ anos, Idade se for <1 ano I__I__I meses ou se for <1 mês I__I__I dias

NOME DO CASO (*preenchido pelo clínico*) EPINUMBER - *PROVÍNCIA/DISTRITO/NÚMERO DE CASO/ANO* – (*preenchido pelo RVE*)

_____/_____

Profissão:_____ Nacionalidade: _____

Caso tenha viajado:

Proveniência:_____Data de entrada no país: _____Ponto de entrada_____ Meio de transporte_____ Nº_____ Telefone: _____Telefone alternativo: _____ Pessoa de referência: _____ Telefone da pessoa de referência: _____

Relação com pessoa referência_____

Detectado no ponto de entrada: Sim ( ) Não ( )

Classificação: Caso Suspeito ( ) Caso Confirmado ( ) Caso Provável ( ) Contacto com Caso Positivo ( )

Vindo de área de alta transmissibilidade ( ) Indicar a área_____ Outro_____

INFORMAÇÃO DA RESIDÊNCIA DO CASO

Província de residência _____ Distrito de residência _____Detalhes do local de residência_____

Em isolamento: Sim ( ) Não ( ) Província de isolamento _____ Distrito de isolamento _____

Local de isolamento: Casa ( ) Hospital ( ) Outro ( ) Indique_____

PARTE 2: INFORMAÇÃO CLÍNICA DO CASO/CONTACTO *(coloque (X)*

Dados Clínicos

Sintomas: **Não ( ) Sim ( ) Data início de sintomas: I__I__I/I__I__I/20I__I__IDuração dos sintomas:** ____ (em dias)

Vacina BCG: (verificar cicatriz no braço) sim ( ) não ( ) não sabe( )

Estado clínico: (listar todos os presentes) Febre referida ( )

Febre	( )	Dor muscular	( )	Dor de garganta	( )	Dor de cabeça	( )
Náuseas	( )	Vómitos	( )	Diarreia	( )	Coriza	( )
Fraqueza geral	( )	Falta de ar	( )	Tosse	( )	Dores nas articulações	( )

Alteração do estado mental ( ) Perda de paladar ( ) Outros sintomas_____ Portador de doenças crónicas? sim ( ) não ( ) não sabe( ). Se sim, quais? _____ Contacto com um doente de COVID/caso suspeito nos últimos 14 dias antes do início dos sintomas? sim ( ) não ( ) não sabe( ), Se sim; nome do caso _____; local de contacto_____

Viagem nos últimos 14 dias antes de início dos sintomas? sim ( ) não ( ), Se sim qual o país ou província_____

PARTE 3: DETALHES LABORATORIAIS

Dados da Testagem

Motivo de testagem: Viajante com sintomas ( ) Contacto ( ) IRA ( ) Vigilância das IRAs ( ) Testagem de TS com IVRS ( )

1ºControle de Cura ( ) 2º Controle de Cura ( ) Controle de positividade pós cura( ) Outro:_____

Prioridade para a testagem: Baixa ( ) Média ( ) Alta ( ) Tipo de amostra (assinale todas as opções válidas):

Zaragatoa nasofaringea ( ) Zaragatoa orofaringea ( ) Escarro ( ) Lavado bronco-alveolar ( )

Aspirado endo-traqueal ( ) Outro ( )_____. Data da colheita ___/___/_____ Hora: ___:___

Resultado laboratorial: SARS-COV_2 ( ) (1-positivo, 2-negativo, 3 indeterminado, 4 Não testado)

Influenza (H1N1 ou outro) ( ) (1-positivo, 2-negativo, 3 indeterminado, 4 Não testado)

RSV ( ) (1-positivo, 2-negativo, 3 indeterminado, 4 Não testado)

Desfecho do caso: **Recuperado** ( ) Óbito ( ) **Desconhecido** ( ) Data do desfecho: I__I__I/I__I__I/20I__I__I

Nome da Unidade Sanitária: _____ Sector: _____ Cama Nr_____

Nome do clínico_____ Contacto do clinico _____

Data da requisição _____/_____/_____Hora: ____:_____ Comunicado ao MISAU ao _____/_____/__

C. Critérios epidemiológicos:

- Residir ou trabalhar em uma área com alto risco de transmissão do vírus: por exemplo, ambientes residenciais fechados e ambientes humanitários, como campos e ambientes semelhantes a campos para pessoas deslocadas, a qualquer momento nos 14 dias anteriores ao início dos sintomas;
    - (Exemplos: **Centros de acolhimento de idosos; quarteis; serviços penitenciários; instituições de caridade; acampamentos de refugiados de guerra, calamidades naturais e de trabalho; lares e internatos; profissionais afectos a enfermarias de internamento prolongado; creches e infantários; Cidades, Bairros, Aldeais e Povoados de grandes aglomerados populacionais)**
- Residir ou viajar para uma área com transmissão comunitária a qualquer momento nos 14 dias anteriores ao início dos sintomas;
- **Trabalhar em locais de risco de transmissão (unidades sanitárias; mercados incluindo informais; residências particulares; estabelecimentos comerciais; instituições bancárias e de atendimento directo ao público, etc) a qualquer momento nos 14 dias anteriores ao início dos sintomas.**