AIX-MARSEILLE UNIVERSITY

Faculty of Medicine Marseille

Graduate School of Health and Life Sciences Studies

(ED62)

A Doctoral Thesis On

Re-emerging Human Viral Pathogens of the Republic of Djibouti (Africa), Reporting on Pandemic Influenza A/H1N1/2009 and Arboviruses Epidemiology

A Thesis Submitted to the Faculty of Medicine Marseille
In Partial Fulfillment of the Requirement for the Degree Doctor of Philosophy in Human Pathology - Infectious Diseases Specialty (Virology) of the University of the Aix Marseille

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I. SPECIAL MENTION

This thesis was prepared under the Public Health Doctoral Network of the EHESP French School of Public Health

«Thèse préparée dans le cadre du Réseau doctoral en santé publique animé par l'EHESP «

II. COLLABORATING AND FUNDING INSTITUTIONS

IRD, Marseille France

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EHESP French School of Public Health, Rennes France

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III. ACKNOWLEDGMENT

Four years ago when I begun this doctoral project, I had no idea how this work would evolve to reach this level. It all begun with a proposition, gaining admission and a grant, followed by planning and execution of the field work, conducting bench work, statistical analyses, manuscripts preparation and ends today with this report. Along this treacherous and glorious journey as i strived towards the goal, i experienced both wonderful moments and others, not so well. Besides the science of epidemiology and virology, I ventured in new but interesting non-scientific areas, of project co-ordination. Initially, i felt were untenable, but i am grateful i did. These unique areas were in *project management*, *planning and budgeting*, *program liaison*, *database management*, *negotiations* and *human resource management*.

The experience of implementing the project, not only did it bolster my confidence, but also remained as a treasure for my future professional assignments. During this time of doctoral pursuit, i faced challenges and my spirit was kept alive, strong and motivated by many special people who i came to know and work with closely. They are the ones who made this program a success; i wish to celebrate them by acknowledging their contributions:

Prof Xavier de-Lamballeries: A BIG THANK YOU for accepting to be my thesis director, for hosting and sponsoring the project laboratory phase, for providing a wise counsel during field work, for taking interest in my work with keenness for details, for gently and patiently guiding me through difficulties in bench work, analysis and manuscript preparation without complaining. Sometimes you went out of you way to ensure my understanding and grasp of intuitive concepts. For these and others aspects, not mentioned, i wish to let you know, forever will be grateful for you contribution.

Prof Antoine Flahault: Special Thanks for identifying the potential in me, for by accepting to be my thesis director, for the Copanflu PhD grant award. You always inspired us/me as a Professor while i was an MPH student. As the first Dean of EHESP you reminded and championed us to go forth and change the world with the skills we gained. I am glad i met you, worked with you and learnt a lot from you. Merci beaucoup!

Dr Ammar Ahmed Abdo, *Dr. Alexia Kieffer*, *Imane Elhamdi* and *Dr. Regina Ducos* : Thanks to you all , for your support and guidance during my tenure as a co-ordinator, Copanflu Djibouti program. *Dr Pascal Crepey*, *Dr Jocelyn Raude*, *Prof Michel Setbon*, *Prof.Ernest Gould* and *Dr. Alexia Kieffer* : A special thanks , for you all for your

various help with my work; of note is in statistical analysis and data management (PC & AK), on insights in socio-epidemiological concepts (JR &MS) and in native English language peer review (EG). *Ms. Sarah Kitar*, *Ms. Maud Subtil*, *Ms. Ilana Levin*, *Ms. Imane Elhamdi*, *Ms. Regina Ducos*: I wish to appreciate for your administrative support (from EHESP Rennes France) during my field work operation in Djibouti (IL, IE & RD), and for updating us through the wonderful concept of EHESP doctoral network program that invests, coordinates and connects PHD students (SK & MS). *Dr. Ammar Abdo*, *Maoulid Barkhat*, *Basra Munera Ibrahim*, *Fatiah Ahmed*, *Abdo Garad*, and the entire Djibouti COPANFLU Field Research Team: I am very grateful for accepting to work with us, for your endurance and cooperation even under very difficult conditions.

MY colleagues at the host research laboratory (EPV/UMR/IRD 190), including PhD students, technicians, engineers and secretaries; I am so grateful for your company, willingness to assist, and to some of you, thanks for a lot of *soirees* together. It was good practicing my broken French with you, these includes the good people like *Fabien*, *Gael*, *Cidgem*, *Therese*, *Mimi*, *Loriane*, *laurance*, *Julie*, *Ines*, *Antoine*, *Jamal*, *Gregory*, *Christophe*, *Salez*, *Marie Theresa*, *Nathalie Blanc*, *Roland*, *Magali*, *Morgan*, *Morgane*, *Boris*, *Souand*, *Cecile*, *Abdenoor*, *Kathy and Klittling etc*. My doctoral Colleagues from other doctoral network and other establishment: You formed my extended family wing, such persons as *Anne Laure Cailet Brilliat*, *Mano*, *Niyaz*, *Projarkta*,, *Cecil Dupin*, *Sophie*, *Stephanie*, *Ndei*, *Yousef*, *Regis* etc. Thanks for your company and for being comrades at arm. To my spiritual and divine comrade *Saja Eren Cecil*, lots of appreciation

I wish to thank my family for their prayers, encouragement and for being a source of comfort and inspiration. *I want to let you know i will always love and treasure you. You are a special gift to my life*: my wonderful Parents dad *Shem Outo. Andayi* (deceased) and *Mom Anne TM Webeka*, my siblings; *Jayne Opiri* , *Andrew Warren*, *Joseph Kizito*, *Kennedy Otiende*, *Erika Were and George Manasseh*. Last but not least, to my two dear and lovely girl friends, Tracie and Lauren, and the two spiritual moms Ernestine and Gezelle in Paris, you made it possible to have a home far away from home, *asanteni sana*, *na Mungu wa nehema awabariki na kuwajalia kwa kazi za mikono zenu.*

In all things i have learnt to give thanks to you God of heavens, for with You all things are possible!

IV. DEDICATION

I dedicate this work to my beloved parents Mwalimu ANNE TERESA MULLAH WEBEKA and Papa SHEM OUTO ANDAYI (deceased), despite your limitations you strived and gave us what was precious-most; the education, love and the spiritual guidance. You set the bar and made us believe in our potential from an early age. I Thank God for you two! *Murio Muno Khandi*

V. THESIS ABSTRACT

(a). English

Influenza and influenza like illness (ILI) and vector borne diseases, forms the largest proportion of viral disease burden for many low income countries of the world, but largely remains undocumented. The republic of Djibouti is one such example. The purpose of this thesis was to provide an inventory of these two groups of infection in the country, focusing on the *general ILI*, the recent pandemic Influenza A/H1N1/2009 (H1N1p) and arboviruses events in the winter of 2010.

We begun by reporting the findings from the ILI syndromic surveillance, based on the data from the major healthcare facilities, diagnostic laboratories, pharmacovigilance, and weather pattern. It was followed by the documentation of the findings from an observation study, in the cohort of pandemic influenza (COPANFLU) study, which investigated the prevalence and the associated risks of infection to individuals at household level. The last part of the work reported on the arboviral epidemiology, a study based on the copanflu study protocol and specimens.

On influenza; this work showed that the local surveillance was capable of detecting ILI peaking during the first pandemic wave, whose profile was consistent with H1N1p. In the subsequent copanflu investigation, which occurred during the second pandemic wave, the study confirmed a high (30%) prevalence of H1N1p, and showed that the young and the residents from a district (District 4) with highest social inequalities bore the greatest burden. This demonstrated that the future ILI control would require a tailed approach to reach specific and vulnerable individuals. In general, the lack of robust ILI data from surveillance in southern countries could be responsible for the underestimation of the epidemiological burden, even when the illness profile resembled those of developed countries.

On arboviruses; this work confirmed a high prevalence of Aedes borne (Chikungunya-2.6% and Dengue-21.8%) and sandlfy borne (Toscana-3.7%) viruses cases, with most of them concentrating in the city centre (District 1). Limited number of Culex borne (rift valley fever) and Tick borne (tick borne encephalitis or Alkhumra related) viruses were noted, but deserves

further investigations to identify the viruses and vectors. Overall, most of arboviral cases predictors were statistically best described by individuals' housing space and neighbourhood environmental characteristics, which correlated with the ecological actors of their respective transmission vectors' survival in the local niche. This study demonstrated autochthonous arboviral circulations in the republic of Djibouti and provided an epidemiological inventory, with useful findings for risk mapping and future prevention and control programs.

Of general interest was that the cases for arboviruses were most prevalent in city centre (District 1), but cases declined towards the periphery of the suburb (District 4). The inverse was true for pandemic and ILI cases. This work, therefore has demonstrated that a tailored solutions suiting local area health needs, would guarantee an optimal return on allocated resources.

* * *

KEYWORDS: arbovirus, vector-borne, epidemiology, Djibouti, horn of Africa, H1N1, influenza, Eastern Mediterranean, prevalence, pandemic influenza, public health

(b). French

Ré-émergents virales pathogènes humains de la République de Djibouti (Afrique) ; Rapport sur la pandémie de grippe A/H1N1/2009 et Arbovirus épidémiologie

Résumé de la thèse

La grippe, les maladies pseudo grippales (ILI) ainsi que les maladies à transmission vectorielle constituent la majeure partie des infections virales qui touchent les pays émergents. A ce jour, peu d'informations sont disponibles sur ce sujet. C'est notamment le cas de la République de Djibouti. Le but de cette thèse a été de réaliser un inventaire des infections liées à ces deux groupes de pathogènes (grippe et ILI d'une part, maladies à transmission vectorielles d'autre part) à l'occasion de la pandémie de grippe A/H1N1/2009 (H1N1p) et des infections à arbovirus qui ont eu lieu au cours de l'hiver 2010.

Nous avons commencé par faire un bilan sur les données concernant la surveillance des syndromes ILI. Nous nous sommes basés sur les éléments fournis par les établissements de santé et les laboratoires de diagnostique, les informations issues de la pharmacovigilance et les données climatiques. Ces résultats ont été confrontés à ceux obtenus par une étude d'observation réalisée sur une cohorte en contexte de pandémie grippale (COPANFLU). Cette étude examinait les possibles liens entre la prévalence et les facteurs de risques associés à l'infection au niveau domestique. La dernière partie du travail portait sur l'épidémiologie des arboviroses. Elle a été réalisée sur les mêmes individus et en suivant un protocole identique à celui de l'étude COPANFLU.

En ce qui concerne la grippe, ce travail montre que la surveillance locale a été à même de détecter le pic d'ILI lors de la première vague pandémique, pic correspondant bien à l'infection par H1N1p. L'étude copanflu (réalisée durant la seconde vague pandémique) a confirmé une forte prévalence (30%) de H1N1p. Elle met également en évidence le fait que la population la plus fortement touchée correspond à des individus jeunes ainsi qu'à des populations résidant dans le quartier (District 4) où les inégalités sociales sont les plus fortes. Ces données indiquent que le contrôle

des ILI doit se faire (dans le futur) par une approche ciblant les individus vulnérables ainsi que certaines populations spécifiques. Sur le plan général, le manque de données de surveillance robustes sur les ILI est certainement une des causes de la sous estimation du poids de la maladie au niveau épidémiologique dans les pays en voie de développement, et ce malgré un profil de la maladie similaire à celui observé au sein des pays développés.

Sur le sujet des arbovirus, ce travail confirme la forte prévalence des infections par des virus transmis par les moustiques de type *Aedes* (Chikungunya 2.6% et Dengue 21.8%) et par les phlébotomes (Toscana 3.7%). Les cas d'infection sont concentrés dans le centre de la ville (District1). Peu d'infections par des virus transmis par les moustiques de type *Culex* (Rift Valley Fever) et les tiques (Tick Borne Encephalitis et Alkhumra Hemorrhagic Fever) ont été rapportées. Ce dernier sujet demande à être approfondi. En général, les prédicteurs d'infections à arbovirus les plus puissants au niveau statistique sont les caractéristiques liées au type de logement et l'environnement dans lequel il se trouve. Ces caractéristiques sont corrélées aux facteurs écologiques qui influent sur la survie des vecteurs au sein de la niche locale. Cette étude démontre une circulation autochtone des arbovirus au sein de la République de Djibouti et fournit un bilan épidémiologique détaillé qui pourra être utilisé dans des programmes de contrôle et de prévention futurs.

Un point fort de cette étude est que les cas d'arboviroses sont plus fortement prévalents au centre de la ville (District 1) et qu'on observe une décroissance du nombre de cas avec l'éloignement vers la périphérie (District 4). Le phénomène inverse est observé pour les cas d'ILI et de grippe pandémique. Ces deux observations illustrent bien l'importance de la mise en place de solutions ciblées et adaptées à la zone concernée afin d'optimiser le coût des programmes de santé.

* * *

MOTS-CLES: arbovirus , vecteurs ,'épidémiologie , de Djibouti ,corne de l'Afrique , H1N1 , grippe , Méditerranée orientale , prévalence , pandémie de grippe , santé publique.

VI. SCIENTIFIC PRODUCTION

(a). Articles

1. **Surveillance and monitoring of pandemic flu in resource limited environment – a case of Djibouti and a WHO-Copanflu international study preliminary report**. *Influenza Other Respiratory Viruses 2011, 5:159-194.*

2. **Determinants' of individuals risk to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents- a CopanFlu cross-sectional study**. *Virology Journal 2014, 11:13 doi:10.1186/1743-422X-11-13*

3. **Pandemic (H1N1) 2009 influenza vaccination intention; the determinants and implications on future vaccines uptake in Djibouti** *(submitted to BMC Public Health)*

4. ***Arboviruses and hemorrhagic fevers epidemiology in Djibouti, horn of Africa*** *(submitted to PlosOne Neglected Tropical Diseases)*

(b). Conference presentation and posters

1. In the Control options IV influenza conference held in Hong Kong SAR, China from 3rd to 7th September 2010. Presented a poster on ***Surveillance and Monitoring of pandemic flu in resource limited environment: a case of Djibouti and WHO-Copanflu International study preliminary report***
2. The *Joint Conference on emerging and re-emerging Epidemics affection Global Health* held in Orvieto Italy from 19th to 23rd September 2012. Presented a poster entitled ***Risk factors and prevalence of flaviviruses in Djibouti*** and an oral presentation on ***integrated sero-surveillance of arboviruses and hemorrhagic fevers in WHO-EMRO Region***
3. The 19th annual symposium of the Graduate school of health and life sciences (ED62) held in Luminy Campus, Marseille France from 23rd to 24th June 2011. Presented a poster entitled ***Influenza and respiratory infections; the risk determinants and epidemiology in Djibouti population***
4. The 1st (May 2010), 2nd (May 2011) and 3rd (May 2012) EHESP French School of Public Health Annual Doctoral Network Symposiums held in Hotel Dieu Notre dame Paris France. Presented on ***Determinants of risk of individuals infection to H1N1p 2009 virus at household level in Djibouti , a copanflu cohort program***

VII. PREFACE

The preface provides a brief introduction and summary of each chapter in the thesis, with emphasis on four manuscripts derived from the work. This thesis has two main sections, on Influenza A viruses, and that of the arbovirusal fevers.

In The first section begin with a short review of the influenza A viruses, in **Chapter 1**, is a description of the general influenza virus characteristics, including the ecology, transmission cycle and the global epidemiology of both seasonal and pandemic influenzas. The brief review is followed by two articles manuscript reporting the significant finding of influenza events in the republic of Djibouti. The first paper in the thesis "*Surveillance and monitoring of pandemic flu in resource limited environment – a case of Djibouti and a WHO-Copanflu international study preliminary report*" is reported in **Chapter 2**. It looked at how the clinical incidences, over-counter ILI drug sales, laboratory diagnosis, and health promotion reports were used in surveillance of ILI. The second paper in the thesis "*Determinants' of individuals risk to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents- a CopanFlu cross-sectional study*" is documented in **Chapter 3**. This article entails a report on the pandemic influenza sero-prevalence and it risk predictors of infection in individuals at household level.

The second section of this thesis begins in **Chapter 4. It** is a literature review on the three major families of arboviruses, namely the Bunyaviruses, Togaviruses and Flaviviruses, which are responsible for most arboviral fevers outbreaks in the region. Here, the details on specific family, genus and arbovirus characteristics, ecology, transmission cycle and the global epidemiology are discussed. The third paper in the thesis "*A sero-epidemiological study of arboviral fevers in Djibouti, horn of Africa*" is reported in **Chapter 5**. This paper reports on the prevalence and risk determinants of infection to the eight arboviral fevers among Djibouti city residents.

In last chapter, **Chapter 6**, it is a brief overview of this work. Giving a general discussion on the main findings, conclusion and providing perceptive of the subject matter. This includes epidemiological and policy contextualization and extrapolation of our finding at the country, regional and global level. It also gives an honest assessment, proposition and recommendation for future scientific work on the subject. This part forms the end of the thesis. Supplemental study on social epidemiology is given in the appendix section. This fourth paper, entitled "*Pandemic (H1N1) 2009 influenza vaccination intention; the determinants and implications on future vaccines uptake in Djibouti*" *is* provided. The article reports the application of protective motivation theory in determination of the health behavior of H1N1p vaccination intention among Djibouti city residents.

VIII. TABLE OF CONTENTS

IX. LIST OF ABBREVIATION

IHA	Indirect Heamagglutination Assay
VNT	Viral Microneutralisation Assay
CDC	Centre for Diseases Control and Prevention USA
HA	Hemagglutin
NA	Neuraminidase
qRT-PCR	Quantitative real time polymerase chain reaction
WHO	World Health Organisation
RNA	Ribonucleic Acid
EHESP	EHESP French School of Public Health
DEIS	Department of Epidemiology and Health Information Djibouti
UMR	Multidisciplinary Research Unit
IRD	Institute of Research for Development
AMU	University of Aix Marseille II
CHU	Public Hospital Unit (Timone)
GMT	Geometric Mean titer
OR	Odd ratio
aOR	adjusted odd ratio
H1N1s	Seasonal influenza H1N1
H1N1p	Pandemic influenza H1N1
BSL3	Biosafety Level 3 Containment Laboratory Facility
IFT	Indirect ImmunoFluorescent Test

CHAPTER 1

INFLUENZA

Human Influenza is a highly contagious, acute, febrile respiratory illness caused by influenza viruses. It can vary from mild to severe illness, leading to hospitalization or death, especially among the elderly, the younger and those with underlying health conditions. Use of annual vaccination remains the best prevention option. Morphologically, the Influenza virus subtypes are generally identical under electron microscope, as illustrated in the **Figure 1** below

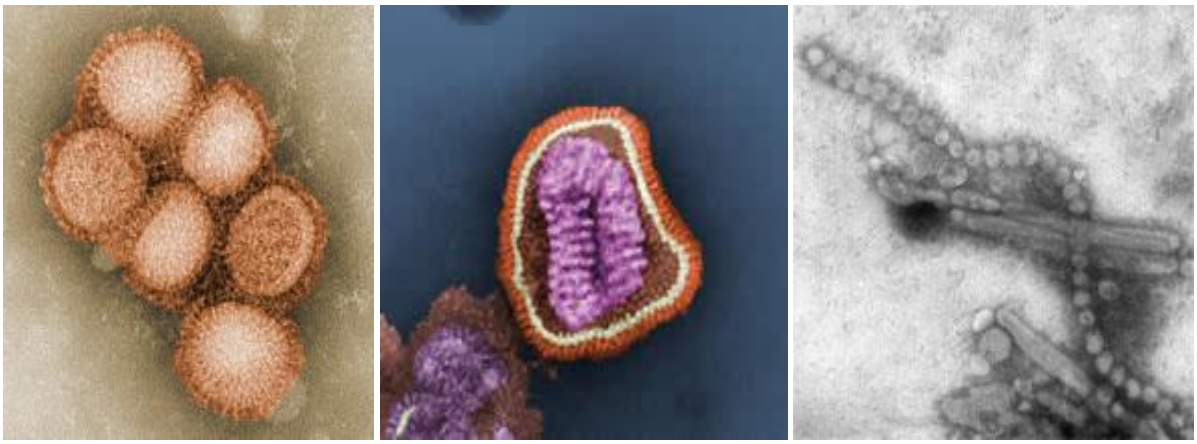

Figure 1: Influenza A prototype virus (Image courtesy of CDC Media 2013)

First Image shows a negative stain EM image of the 2009 H1N1 Influenza A/CA/4/09. Second image shows a negative-stained transmission electron micrograph (TEM) that depicts the ultrastructural details of an influenza virus particle (virion). The last Image shows the Influenza A H7N9 as viewed through an electron microscope.

1.1 Influenza virus characteristics

Influenza virus belongs to the *Orthomyxoviruses* family and has three genera of A, B and C. For the purpose of this work, emphasis was on type A. The relationship of the three influenza genera A, B and C, to the other members of the family is illustrated below in **Figure 2,** through a phylogenic tree based on the amino acid genetic characteristics (McCauley, Hongo et al., 2012).

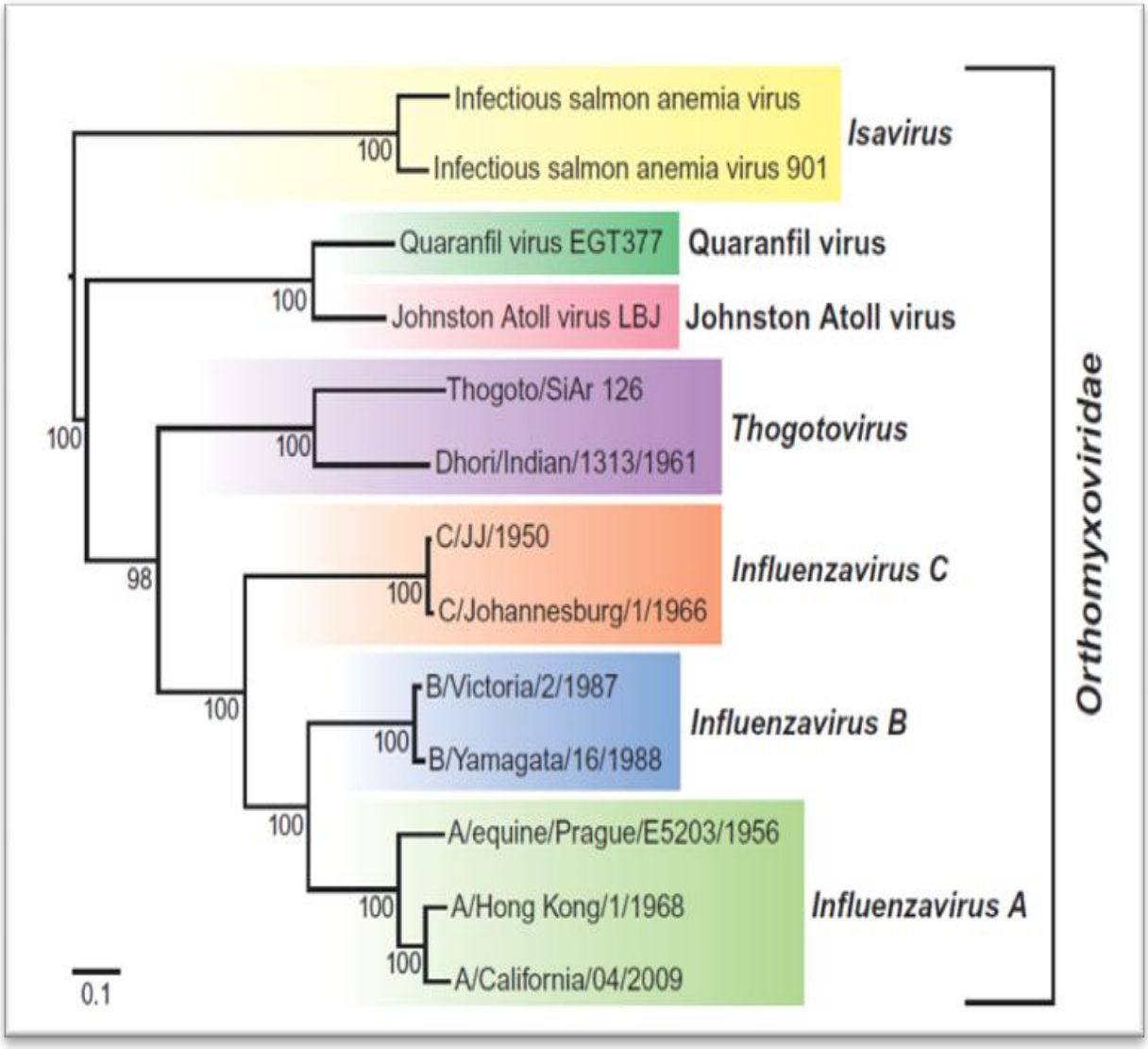

Figure 2: Phylogenic tree of Orthomyxoviruses

Phylogenetic tree showing the relationships between different genera in the family Orthomyxoviridae. This tree was constructed by alignment of amino acids (aa) of the gene encoding the polymerase (McCauley, Hongo et al., 2012).

A mature influenza virus particle is enveloped and has a negative sense, single stranded RNA genome, which is segmented eight times. The genome encodes 11 functional genes, namely; the hemagglutinin (HA), neuraminidase (NA), matrix 1 (M1), matrix 2 (M2), nucleoprotein (NP), non-structural protein 1 (NSP1), non-structural protein 2 (NS2), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase basic protein (PB1-F2). Individual gene function,

relative size and location within the genome are graphically represented in **Figure 3** below (Hoffmann 2007).

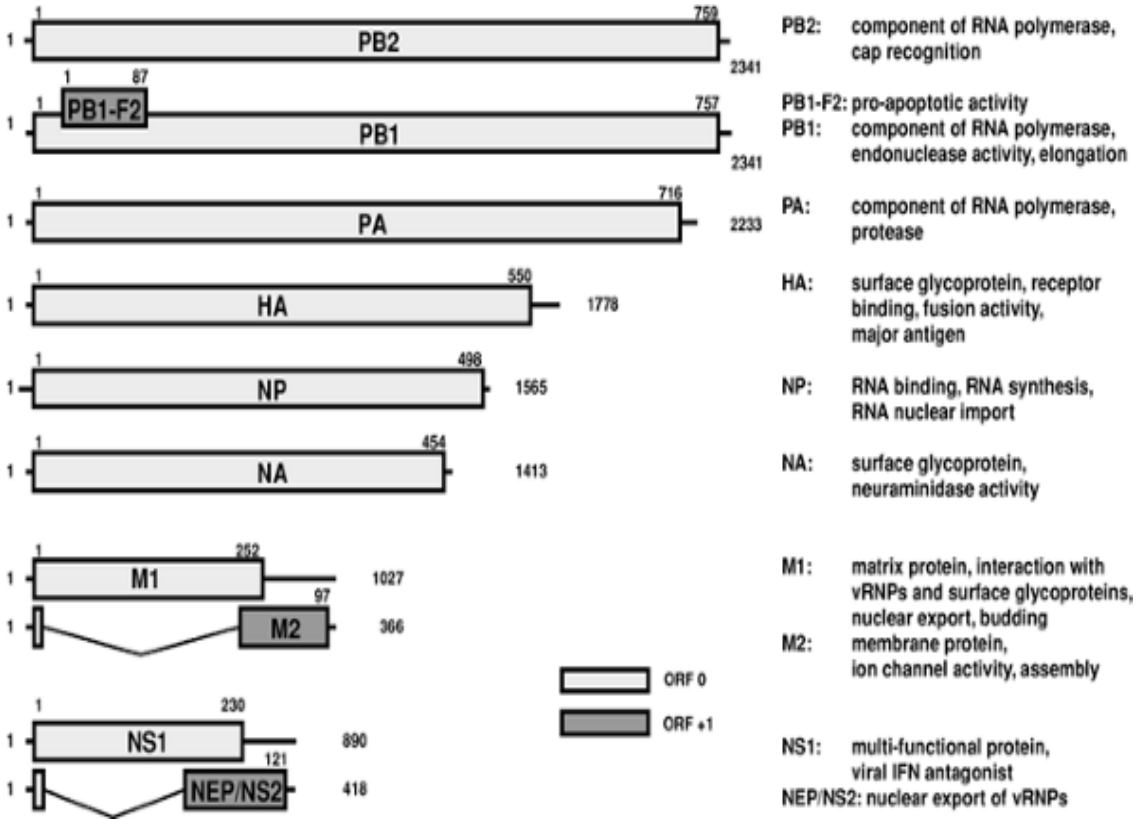

Figure 3: Influenza virus A genomic Structure.

The RNA segments (denoted nucleotide size) are presented in the positive direction and the encoded proteins ORF (size indicated in amino acid) are indicated by rectangles. The line terminal 5 'region and 3' represent the noncoding regions. The PB1 segment contains a second ORF in the +1 reading frames which encodes a protein PB1-F2. And M2 proteins are encoded by NEP/NS2 a messenger RNA (mRNA) spliced (introns are indicated by lines V-shaped) (Hoffmann 2007).

1.2 Influenza A replication cycle

Influenza virus has a unique replication process that enables a successful invasion and infection of susceptible hosts. This process is illustrated in Figure **4** below. It begins with the virus attachment to the surface of susceptible host cell, before initiating entry process via receptor-mediated endocytosis. The HA binds to a Sialic acid receptor on the surface membrane of the infected cell and is then encapsulated into the acidic endosome. Here, there is a conformational change on the HA protein due to the low pH form

and the hydrophobic fusion peptide exposed. After the entry, the clathrin coat is removed and vesicles fuse with the endosome, leading to the virus exposition inside the cytoplasm.

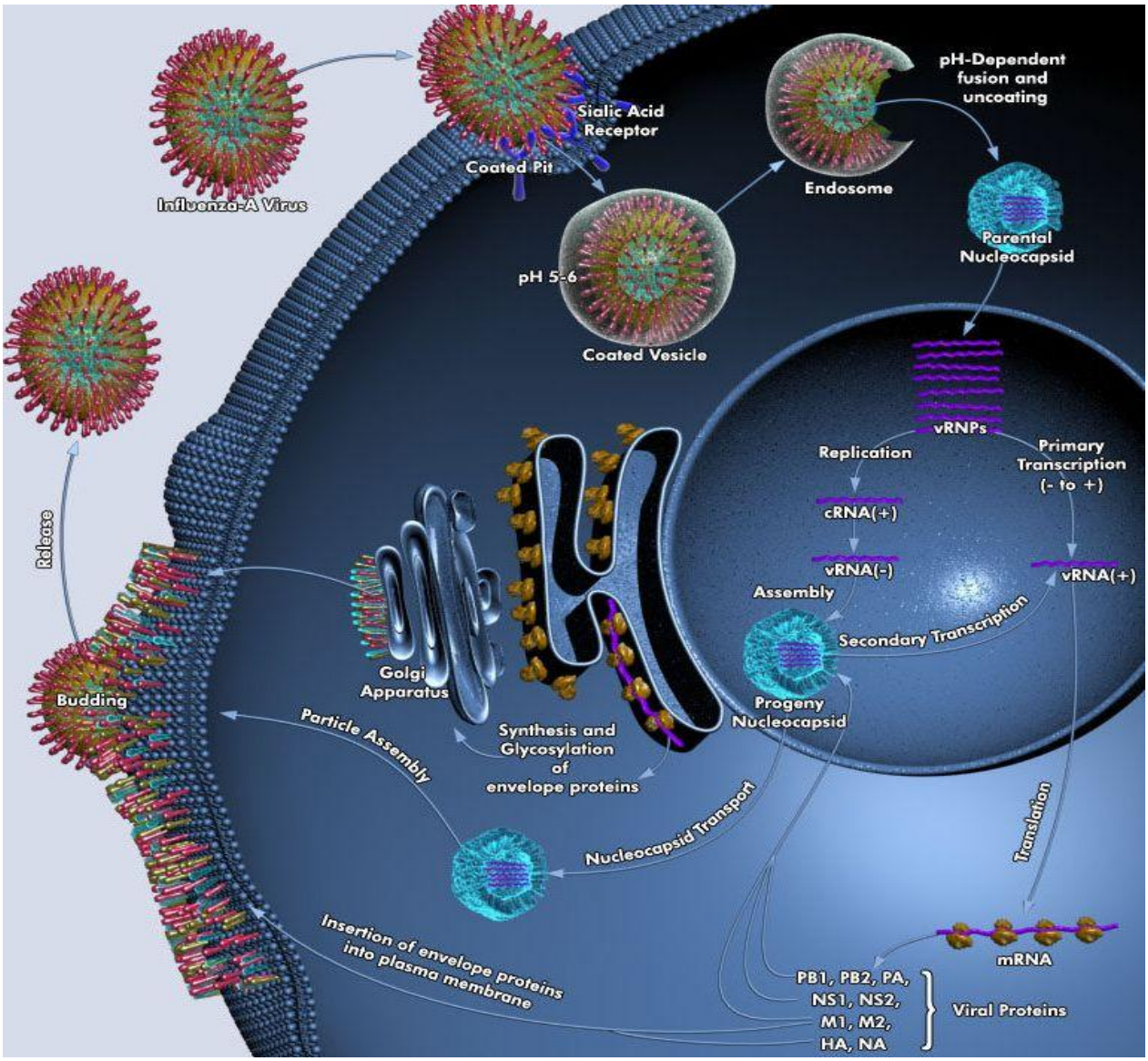

Figure 4: Influenza A virus replication cycle

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Consequently, the vRNPs (viral Ribonucleoprotein Complex) are released and then transported into the nucleus (Kelly et al 2003), where it (vRNPs) acts as the templates for the production of two positive-senses RNAs, the viral mRNA (messenger RNA) and the cRNA (complementary RNA). The mRNA synthesis is catalyzed by the viral RNA-dependent RNA polymerase, made of PA, PB1 and PB2, and part of the vRNP complex. This process is similar to other eukaryotic mRNAs synthesis. It entails; the methyl capping (addition of methylated 5 guanosine residue) and polyadenylation (addition of sequence of polyadenylic acid at their 3 end), and relocation from the nucleus to cytoplasmic ribosomes for translation. This relocation process employs the host cell machinery, but controlled by the viral non-structural protein NS1 (Deng et al 2005). Several other viral proteins; NP, M1, NS2 and the polymerases, are then assembled in the nucleus for the last phases of replication and for vRNP production. The viral cRNA does not undergo capping and polyadenylation, but is the exact copy of the template. Therefore the cRNAs continue to form the template for synthesis of further negative-sense genomic vRNA segments for amplification of mRNA synthesis and packaging into progeny virions. Both cRNA and vRNA molecules contain a 5' triphosphate group. Progeny virions are assembled at the apical surface of the plasma membrane and, therefore, newly synthesized RNPs are exported from the nucleus and directed to the plasma membrane to allow their inclusion into budding virions, and new virion released (Kelly et al 2003, http://www.qiagen.com/products/genes%20and%20pathways/pathway%20details.aspx?pwid=247) .

1.3 Influenza A Virus Evolution

(a) Influenza A nomenclature

Influenza A viruses are one of the most studied human viral pathogens in the world, because of the huge disease burden caused by seasonal and pandemic influenza events. This group virus has a unique genetic dynamism that allows for frequent generation of new variants, with subsequent infection of wide host range, including humans (**Figures 5, 6 & 7**). Due to high genotype (serotype/subtype) diversity, in 1979, the International convention on influenza viruses naming, adopted 6 attribute approach, for naming these viruses, namely; (i) the ***antigenic type*** (e.g., A, B, C), (ii) the ***host of origin*** (e.g., swine, equine, chicken, etc For human-origin viruses, no host of origin designation is given.), (iii) ***Geographical origin*** (e.g., Denver, Taiwan, etc.), (iv) ***Strain number*** (e.g., 15, 7, etc.), (v) ***Year of isolation*** (e.g., 57, 2009, etc.), (vi) For influenza A viruses, the ***hemagglutinin*** (HA) and ***neuraminidase*** (NA) antigen description in parentheses (e.g., (H1N1), (H5N1). For example; A/duck/Alberta/35/76 (H1N1) is influenza A virus, of H1N1 genotype, from a duck of Alberta Canada in 1976 (CDC 2013).

(b) Formation of Influenza subtypes (*antigenic drift* vs *antigenic shift*)

There 16HAs and 9NAs genes identified as representative of all the known influenza A virus subtypes variants. These genes are topical; expressed on the virus envelope as surface antigen, and are used to determine the subtype identity and pathogenicity profile. The process of subtype's formation heavily depends on the forces of antigenic shift and antigenic drift. In ***antigenic shift*****,** the HA and or NA genes are replaced to form a new variant or the ancient one that circulated many years ago. Often, the formed strain encounters naïve herd immunity, resulting in large outbreaks that may circulate across the globe (pandemic). Such occurrences are rare, and they are estimated to happen at least three times in a century. Of which, in the last 95years, more than three pandemics have been observed, involving the following HA genes A(H1) in 1918, A(H2) in 1957, A(H3) in 1968, A(H1) in 2009 (Miller, Viboud, Simonsen, Olson, & Russell, 2009). It's now thought that the formation of a pandemic subtype happens when there is a **co-***circulation of various subtypes in a flu season or co-infection of several subtypes into one host, such as pigs or birds*(F Carrat & Flahault, 2007). Unlike the Antigenic shift, that has long time interval; the ***antigenic drift*** *occurs between 2-8years, due human immune selective pressure that gradually initiates an inconspicuous point mutation during viral replication at the HA and or NA proteins epitopes* (F Carrat & Flahault, 2007). The cumulative effect is the generation of immune escape mutant subtype, capable of circumventing the human immunity and cause illness in general population, also referred to as common cold or seasonal flu. Recent studies(Miller et al., 2009), have confirmed that pandemic subtypes often spread rapidly and replaced the seasonal influenza in circulation at that time, and could persist for a while before receding. Of interest is that exposure to an influenza sub-type conferred a lifelong immunity, and a stronger protection with years, a postulate that is now referred to as "original antigenic sin" (Amesh A. & Henderson, 2010). For example a review of antigenic and Genetic Characteristics of Swine-Origin 2009 A(H1N1) Influenza Viruses Circulating in Humans using phylogenic tree relationship (**Figure 5**), suggests that the lack of similarity between the 2009 A(H1N1) virus and its nearest relatives indicates that its gene segments have been circulating undetected for an extended period (*Garten et al 2009*). Its low genetic diversity suggests that the introduction into humans was a single event or multiple events of similar viruses (*Garten et al 2009*).

(c) Pathogenicity and virulence subtypes

Pathogenicity(level severity) and virulence (rate of spread) in Influenza A virus are determined in part by specific host pathogen interaction parameters, at the receptor site. There are two types of host receptors to influenza A virus identified, the ***human alpha 2, 6 receptor***, and ***birds alpha 2, 3 receptor***. The pig has both receptors, implying that only pigs can be infected by either of the subtypes. It's common therefore for co-infection of both avian and human subtypes to happen inside the Pig, and when it does, the re-

assortment might occur leading into a new strain. Such strains are capable of infecting human and avian and pigs, causing big outbreaks (Pandemic). For this reasons pig have been labeled a "mixing vessel", and plays a significant role in epidemiology of influenza A viruses(Itoh et al., 2009; Neumann et al., 2009). For example, in late 1990's, a re-assortment happened between human H3N2, North American avian, and classical swine viruses, which resulted in a triple re-assortant H3N2 and H1N2 swine viruses, which have since circulated in North American pig populations. Most recently, in 2009, a triple re-assortant of swine virus, were also re-assorted with a Eurasian avian-like swine virus, resulting in the swine pandemic influenza of 2009 (Neumann et al., 2009). See the illustration in the **Figure 5 and Figure 6**

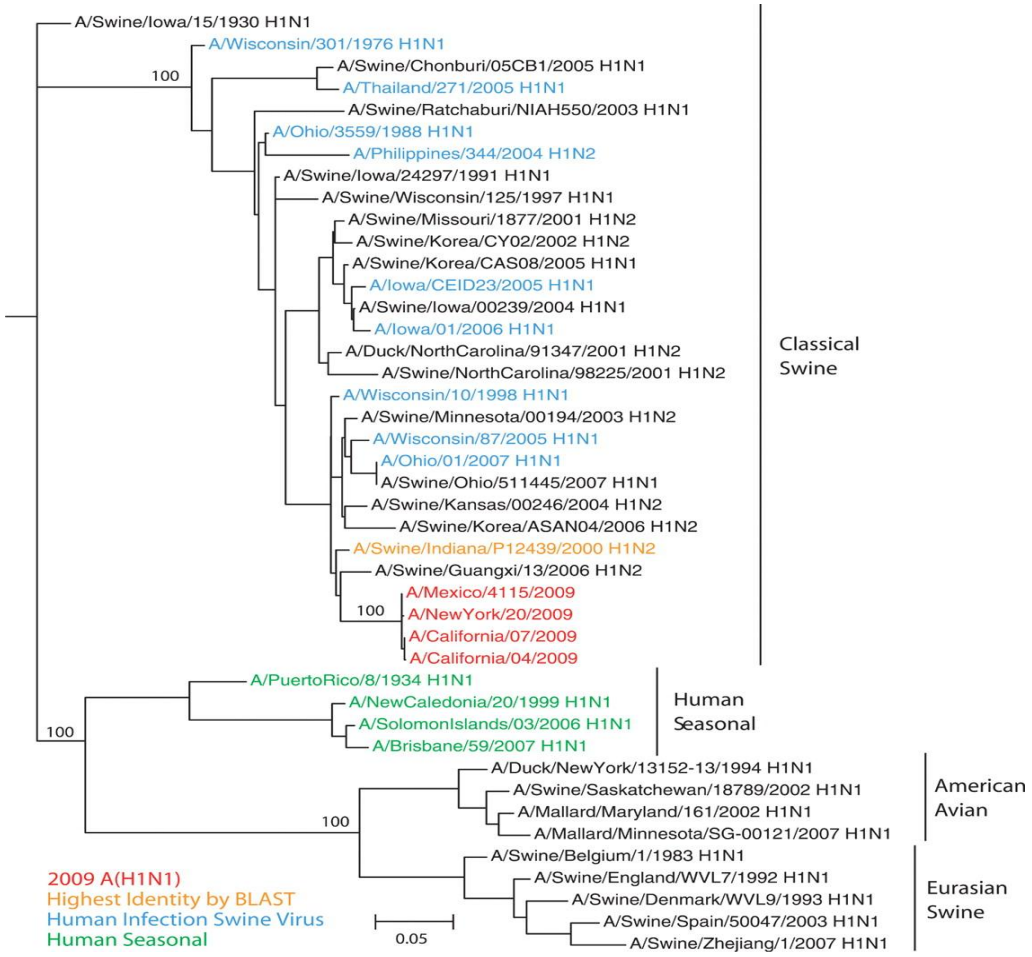

Figure 5: A maximum likelihood phylogenetic tree of influenza virus.

A phylogenetic tree for nucleotide sequences of the HA gene of influenza H1N1 (or H1N2) viruses selected to be representative of HA gene segments in relevant hosts (Garten et al 2009).

The influenza A α 2-3 and α 2-6 receptors are located in the upper and lower respiratory tract of humans, respectively. This distribution is significant in the eventual manifestation of influenza A virus pathogenesis. In fact, it explains why the bird adapted subtypes infections such as H5N1 were rare in man, but more often resulted with pneumonia.

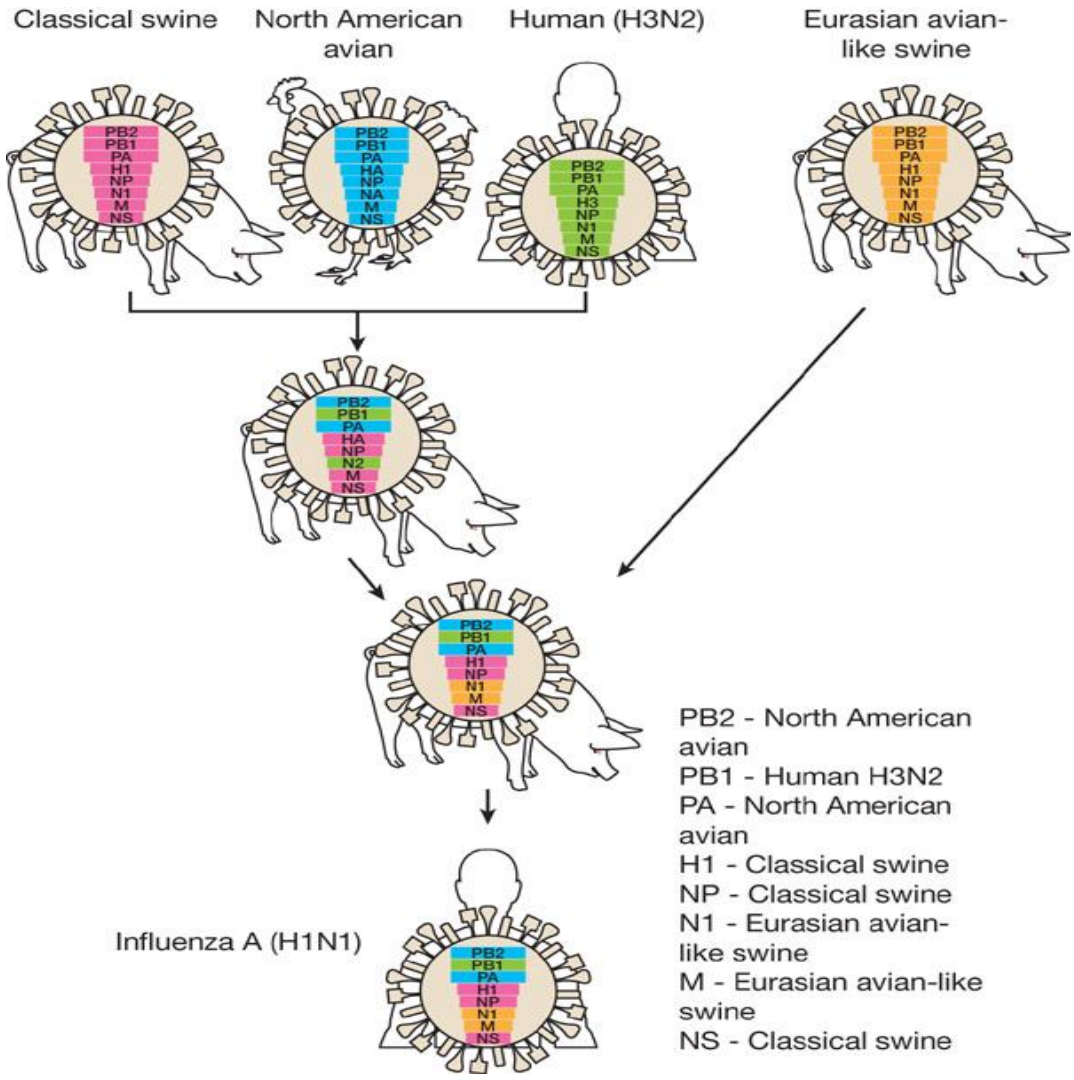

Figure 6: Genesis of swine-origin H1N1 influenza viruses

In the late 1990s, reassortment between human H3N2, North American avian, and classical swine viruses resulted in triple reassortant H3N2 and H1N2 swine viruses that have since circulated in North American pig populations. A triple reassortant swine virus reassorted with a Eurasian avian-like swine virus, resulting in the S-OIVs that are now circulating in humans (Neumann et al. 2009).

(d) Transmission cycle

At the centre of influenza As' ecology and evolution is the water fowl, which is the natural reservoir of the virus. The pathogen replicates in these birds species, but rarely results in lesion. However, it takes the swine as intermediate host for the involved subtypes to gain competence to efficiently adapt and infect humans. This process is thought to be due to accumulative mutations or by genetic re-assortment, as mentioned above. The rapid evolution of influenza viruses following interspecies transmission often results from the selection of genetic variants that favor optimal interactions between viral proteins and cellular factors. This in turn leads to increased multiplication potential and escape to the host antiviral response. Whereas influenza A viruses usually cause asymptomatic infections in water fowl, they may be highly pathogenic in other species. In **Figure 7**, is a summary of illustration of the potential evolutionary path of isolated subtypes and the interspecies transmissions.

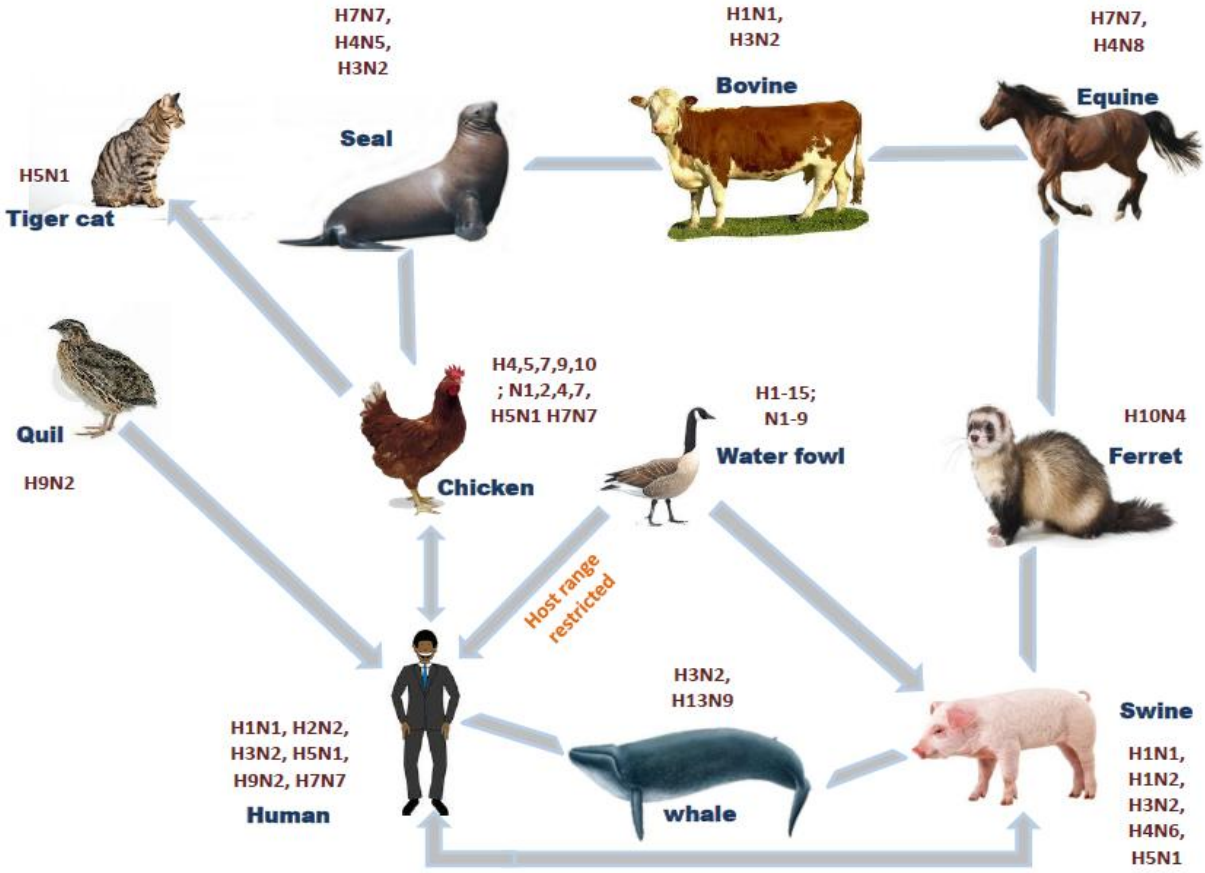

Figure 7: schematic representation of influenza A subtypes circulation in human and other animals

1.4 Seasonal Influenza A (flu)

Seasonal influenza occurs around the winter period. In the Northern hemisphere, with a temperate climate, outbreaks occur once a year ie in the winter months (around January), from ***November through April***. While in the southern hemisphere outbreaks occurs (around July), from ***May through September*** (Simonsen, 1999). In the tropical and subtropical climate countries the influenza epidemics episodes are less distinct, with influenza viruses' isolation occurring year round. In some countries, a biannual pattern may occur, but mainly in spring (April) and autumn (October), and that somewhat coincides with the peak influenza epidemics seasons in temperate zones (Simonsen, 1999).

Every time a new variant (subtype) capable of infecting humans is produced (via antigentic drift), it comes with public health implications. This could be in form of lack of herd immunity, ineffective formulated vaccines, and associated economic burden. The large proportion of seasonal influenza morbidities observed undeniably causes untold human suffering. It is now estimated that seasonal influenza epidemic affects about 5-15% of global population, 3-5million of them experience severe forms and up to a half a million of them loss their lives. The very young, below 5 years and the very old, above 65years are the ones at the greatest risk. The USA estimates each seasonal influenza epidemic season cost them between US$ 12-14billions (F Carrat & Flahault, 2007). A recent meta-analysis study revealed a considerable difference in seasonal influenza disease burden between the developed and the developing nations (Nair et al., 2011). The developing countries suffer three times more fatalities (case fatality rate - CFR 2.96%, 111,500 deaths) than the developed nations (CFR 0.17%, 28,000 deaths) for the under five children subpopulation.

1.5 Pandemic Influenza A

A pandemic outbreak is occasioned by the continual antigenic shift among the circulating subtypes. The process is very unpredictable but once it happens, the subtype circulates across the globe with devastating impact. As mentioned before, up to 3 pandemics have been experienced in the last century. However, Many medical historians, asserts that pandemic had been happening for the past 300 years(Cox & Subbarao, 2000; Potter, 2001). Information of influenza earlier pandemic before 1900 remains sketchy compared to those reported to occur afterwards, with exception of the one of 1889-1890. Subtypes responsible for the pandemic had varied spatial spread, severity and composition of the HA an NA genes **(See Table 1 below)**.

The 1889-1890 pandemic was reported to have caused up to 1 million deaths, and is thought to have been caused by the subtypes H3N8 or H2N2 (Valleron et al., 2010). The 1918 `Spanish flu A(H1N1) pandemic was the most severe, causing 20-100 million deaths world-wide (Mills, Robins, & Lipsitch, 2004;

Taubenberger & Morens, 2006). While the more recent pandemics, A(H2N2) `Asian flu in 1957 and A(H3N2) `Hong Kong flu in 1968, were associated with moderately severity and mortality (Oswald, Shooter, & Curwen, 1958; Simonsen, 1999). Since 1968, the strains of influenza A(H1N1), A(H3N2) and B viruses have co-circulated and caused discrete or overlapping epidemics each season (Simonsen, 1999).

In 2009, swine-origin H1N1 influenza viruses (H1N1pdm09) emerged from Mexico and spread rapidly across the global resulting over 20,000 death and unaccounted for incidence cases. Persons born before 1940 were found to be less vulnerable to scourge compared to others, like the young who were most severely, (Hancock et al 2009) due to naïve immunity (See **Figure 8 below**). The mortality rate associated with H1N1pdm09 infections was estimated to be comparable to that of seasonal influenza virus outbreaks, but it's possible it could have been higher (Neumann et al., 2009). According to mathematical modeling studies reported by Tricco et al (Tricco, Lillie, Soobiah, Perrier, & Straus, 2012); the last pandemic had high disease burden associated with hospitalizations, severe illness, absenteeism and intervention cost. For example, In the United States (US), there were 1.8 million to 5.7 million cases reported, which included 9,000 to 21,000 hospitalizations.

Name of pandemic	Date	Deaths	Case fatality rate	Subtypes	Severity index*	Reference
Asiatic or Russian flu	1889–1890	1 million	0.15%	H3N8 or H2N2	2	(Simonsen, 1999; Valleron et al., 2010)
Spanish flu	1918–1920	20 to 100 million	2%	H1N1	5	(Mills et al., 2004; Simonsen, 1999; Taubenberger & Morens, 2006)
Asian Flu	1957–1958	1 to 1.5 million	0.13%	H2N2	2	(Oswald et al., 1958; Simonsen, 1999)
Hong Kong Flu	1968–1969	0.75 to 1 million	<0.1%	H3N2	2	(Cox & Subbarao, 2000; Potter, 2001; Simonsen, 1999)
Russian flu	1977–1978	Inaccurate count	N/A	H1N1	1-3	(Potter, 2001; Simonsen, 1999)
Swine flu	2009–2010	18000	0.03-0.1%	H1N1/09	1	(Mishra, Chadha, Choudhary, & Potdar, 2010; Shrestha et al., 2011)

Table 1: Characteristics of Pandemic influenzas experienced in the last century

**Pandemic severity index (PSI) is a proposed classification scale of 1-5 for reporting the severity (CFR) of influenza pandemics in the United States. PSI 1 corresponds to 0.1% CFR and PSI 5 to the ≥2% CFR.*

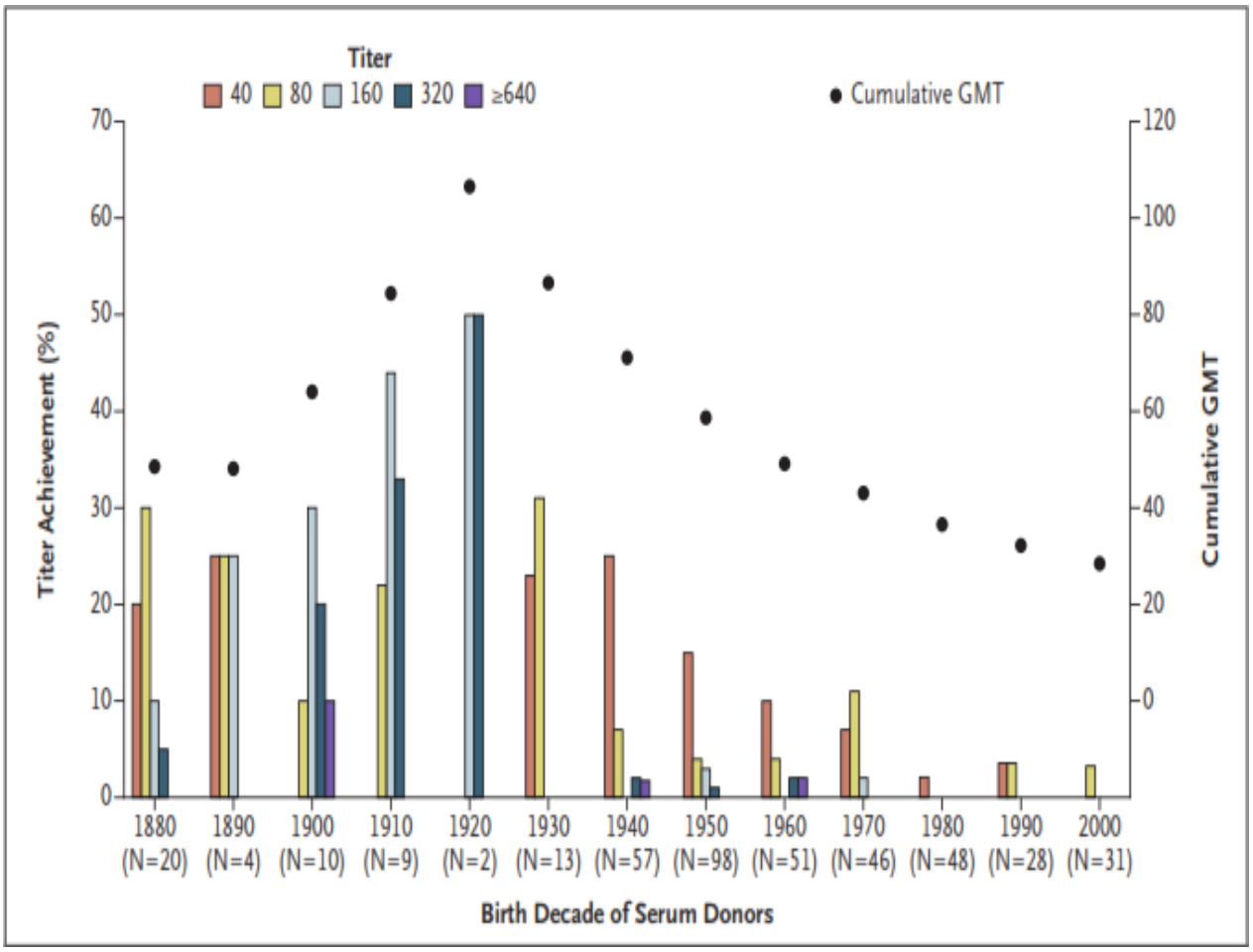

Figure 8: Neutralizing Antibody Titers against the 2009 Pandemic H1N1 Virus among Serum Donors, According to Birth Decade (1880–2000) (Hancock et al 2009)

1.6 Global influenza Disease Burden

On 10th august 2010, the WHO Director General Dr. Chan declared the pandemic was over after running its full course. At that time over 214 countries had reported the incidences and or mortalities. The exact burden was yet to be estimated. However, the WHO cartographic illustration released in 8th August the same year, two days before declaration of pandemic end, as shown in **Figure 9** below, indicates that the temperate countries had higher incidences, probably due to better case identification and reporting mechanism compared to the tropical and subtropical countries.

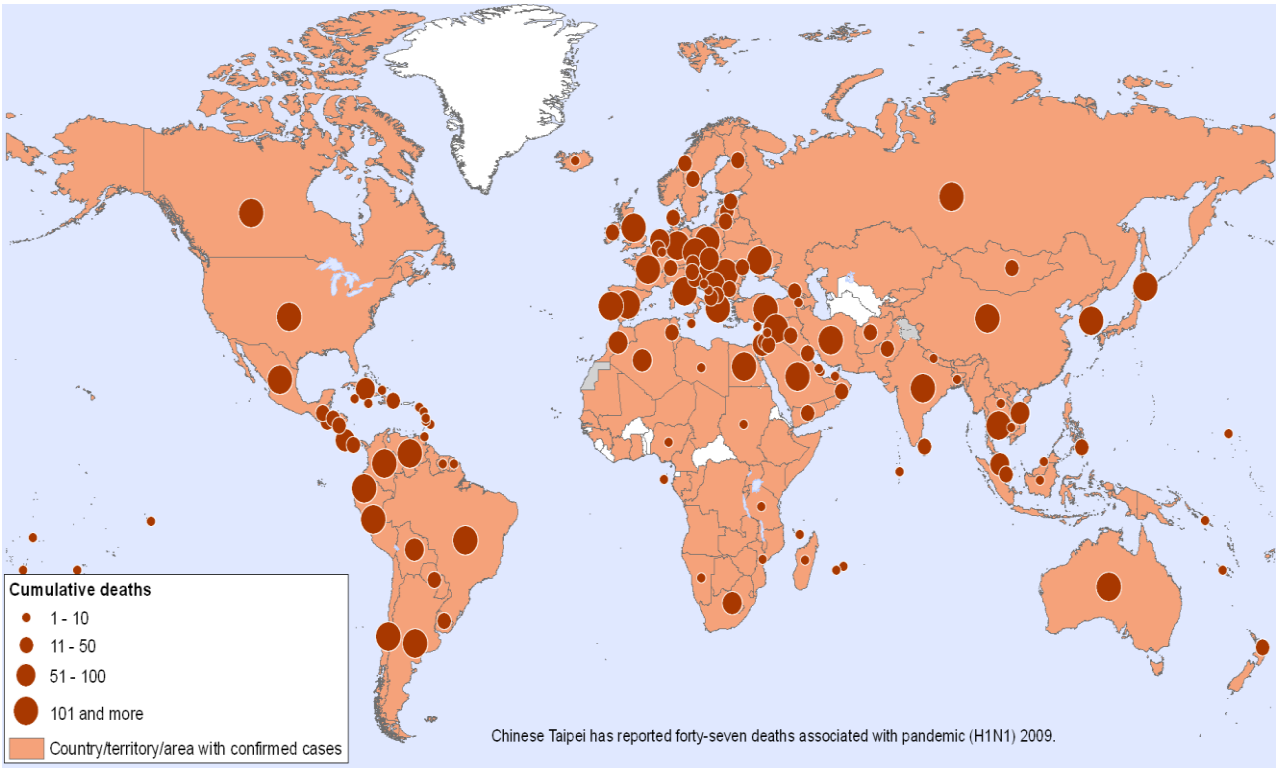

Figure 9: Pandemic (H1N1)2009 countries, territories and areas with laboratory confirmed cases and number of death on 8th August 2010, when WHO declared end of pandemic period (WHO 2010).

This burden as mentioned earlier was associated with hospitalizations, severe illness, absenteeism, and cost. In a recent study, it was estimated that during the pandemic period, from 12 April 2009 to 10 April 2010, there were approximately 60.8 million cases (range: 43.3–89.3 million), 274 304 hospitalizations (195 086–402 719), and 12 469 deaths (8868–18 306) that occurred in the United States (Shrestha et al., 2011). Tricco et al (Tricco et al., 2012) study, which focused on Italy, Spain, Australia and USA, equally confirmed a worldwide high disease burden. For example, in Spain, 9 to 30.5 days was the average work absenteeism due to the 2009 H1N1 pandemic and associated loss of about €144,773,577 (range 13,753,043-383,467,535). In Italy, they estimated economic burden due to laboratory-confirmed H1N1 2009 pandemic to range from €1.3 to €2.3 billion. In Australia, the economic burden of treating H1N1-admitted patients at the intensive care unit (ICU) was over AU $65,000,000.

In another study (Dawood et al., 2012), the severity of pandemic cases was found to be elevated among individuals with underlying respiratory and cardiovascular associated illness, which was about 15 times higher than the general population, and with the young less than 65 years old accounting for more than

80% of the cases(Dawood et al., 2012). In this study, 201,200(range 105,700–395,600) deaths were due to respiratory and 83,300 (46000–179900) were due to cardiovascular associated cause. Interestingly, the overall distribution of incidences confirmed that most cases, about 60%, were from Southeast Asia and Africa. This observations by Dawood et al (Dawood et al., 2012), are consistent with other risk assessment studies of the pandemic infection in the region (M. a Katz et al., 2012).

In general, a review of the published data, indicates that pandemic influenza 2009 cases were overrepresented among the healthy young to the middle-aged adults followed by children and adolescents. The severity and hospitalization was common among the elderly, obese individuals, pregnant women, or those with co morbidity. The greatest burden in many studies was associated with poverty and accessibility to preventive healthcare (Falagas et al., 2011).

1.7 Influenza Prevention and Control

Seasonal and pandemic influenza are shown to spread in a population in three main ways; (a) directly when an infected person sneezes mucus directly into the eyes, nose or mouth of another person; (b) the airborne route, when someone inhales the aerosols produced by an infected person coughing, sneezing or spitting; and (c) through hand-to-eye, hand-to-nose, or hand-to-mouth transmission, either from contaminated surfaces or from direct personal contact such as a hand-shake (Hall, 2007). These three modes therefore determine the prevention and control approach to be used.

Broadly, they're two approaches recommended by the WHO to limit the transmission; the *Non-pharmaceutical intervention* (NPI) and the *pharmaceutical intervention* (PI). The NPIs are actions, apart from getting vaccination and taking medicine that slows the person - to - person, and community' transmission of influenza. These actions can generally be referred to as community mitigation strategies (WHO, 2006b). To slow person - to - person transmission at individual level includes; covering coughs and sneezes, washing hands often, staying home when sick, and cleaning surfaces and objects routinely. Whereas, at community level, the main principle is social distancing, and this may include closing schools temporarily, making sick leave policies more flexible, offering telework or remote-meeting options and postponing or canceling mass gatherings (WHO, 2006b). The second option was that of pharmaceutical intervention. This is the administration of pharmaceutical agents to an individual by health care providers to treat, prevent and or ameriolate the progression of influenza A infection. There are several options, but the approved ones include the vaccines, antivirals and antiobiotics for treatment of secondary bacterial infection (WHO, 2010b).

1.8 Epidemiology of Influenza in Republic of Djibouti

On 12 May 2006, the Ministry of Health of Djibouti confirmed the country's first case of human infection with the H5N1 avian influenza virus. The patient was a 2-year-old girl from a small rural village in Arta province. The symptoms had begun on 23rd April and lasted for a week before recovery. Three tests were conducted on 10th May by the Cairo-based US Naval Medical Research Unit 3 (NAMRU-3) and confirmed the child's infection to be H5N1 virus. It was the first case of human infection reported in the Horn of Africa (WHO, 2006a). Later, several cases of poultry deaths were reported and found to be positive to the same pathogen.

Three year later, there was a pandemic swine flu (H1N1p) that had laid claim to more than 16,455 human lives and millions of infections worldwide (WHO, 2010a), since the first zoonotic reportage in late February 2009 in Pigs' farm in Mexico. The pandemic later rapidly spread to the USA, Asia, Europe, Canada and to other parts of the world. The fear of magnitude, based on the then pace of spread, was suggestive evidence of human-human transmission. This fact prompted WHO to raise a highest alert level of phase 6.

In the WHO Eastern Mediterranean region (WHO-EMRO), to which the republic of Djibouti belongs, the invasion was not spared by this new pathogen. As of that time, 3rd March 2009; at least 1018 deaths had been reported in the region. According to the unconfirmed data, from online flucount website, as of 7th December 2009 estimates for cases and deaths for selected four countries were as follows; Djibouti [9 cases 0 deaths], Ethiopia [6 cases 0 deaths], Egypt [15,738 cases 267 deaths] and Yemen [5,038 cases 28 deaths] (http://www.flucount.org/ accessed on 3rd March 2010). Although Djibouti remained without a reported death, this could presumably be a factor of inadequacy on the part of health department to conduct active aetiological surveillance of the influenza and influenza like illness.

It is very unlikely that the reported incidences in the region were autochthonous, but a recent introduction. For the republic of Djibouti, its strategic position puts it in a vulnerable position for easy introduction of the virulent pandemic H1N1 swine flu from Asia, America and Europe. This is because currently, the country has over 4,000 foreign military troops and their families, from the USA, Japan, France, Germany, Koreans and others (Verjee, 2013). At the time of study, most of these countries had reported pandemic flu incidences and or deaths cases within their territory. In addition, the frequent flights to and fro these nations and great social networks existing in Djibouti city between the natives and the foreigners was a potential 'mixing point' of introduction. This risk predisposition warranted a watchful eye to check the circulation or the new arrival of the pandemic flu so as to protect the troops or expatriates, their families and the native population from H1N1 infection.

The DJIBOUTI COPANFLU STUDY, as part of WHO-EHESP COPANFLU INTERNATIONAL CONSORTIUM, was therefore set up to investigate the determinants of individual risks to pandemic influenza infection at household level, in the republic of Djibouti.

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CHAPTER 2

METHODOLOGY

In this chapter, is a brief description of the study; *objective*, methodology *(design, population and area)* and *field work operation experience*

2.1 General Objective

The main objective of this study was to report on the epidemiology of re-emerging human viral pathogens of Djibouti, focusing on *influenza* (influenza and influenza like illness (ILI), and 2009 pandemic influenza viruses -H1N1p) and *arbovirus fevers*. The data was derived from ILI surveillance data and the household Cohort of Pandemic Influenza (CoPanFlu) study that was established in Republic of Djibouti's, four administrative districts of Djibouti city.

(a) Specific Objective

1. To assess the influenza and influenza like illness (ILI) surveillance system, and evaluate its potential to predict the evolution and seasonality of respiratory illness such as the H1N1p
2. To assess the prevalence, distribution and the risk determinants of H1N1p infection at household level among the Djibouti city residents.
3. To assess the population influenza knowledge, risk perception, and health behavior towards the H1N1p. Of interest being, how these factors might influence individuals vaccination intention (see **appendix**).
4. To assess the prevalence, distribution and the risk determinants of infection to arbovirus fevers among Djibouti city residents. Focusing on specific families of Bunyaviruses, Togavirus and Flavivirus families, and or their mode of transmission ie Culex borne viruses (rift valley fever-RVF, chikungunya-CHIKV), Aedes borne (dengue-DENV, west nile virus-WNV, and yellow fever-YFV), Sandfly borne viruses (Toscana-TOSV) and Tick borne viruses (tick borne encephalitis-TBEV and alkhurma-AHFV).

2.2 Project Design

(a) Cohort of Pandemic Influenza (COPANFLU) Study Program

The urge to setup a multicenter international cohort project, involving as many countries as possible was palpable, in the context of emerging pandemic influenza of 2009, with objective of assessing health and social impact of the pandemic, and allow for an international comparison. Of interest at that time was to know; (a) *How the dynamic of the pandemic evolves within households, in each country? And How many waves, when and of what amplitude?*; (b) *What was the attack rates in each country?;* (c) *What was the use of antivirals, antibiotics, vaccines and other drugs?* ;(d) *What was the use of protective masks, hand wash?* ;(e) *What was the role of co-infections with other infectious agents?*; (f) *What was the risk perception level and its trends with time in each country sample?;* (g) *What was the level of knowledge, attitudes and behaviors and their trends with time?;* (h) *What was the economic burden of the pandemic on households, in terms of direct (drugs, vaccines, hospitalization,...) and indirect costs of the disease, (days of sickness leaves, loss of income,...)?*; and (i) *What was the impact of seasons and climate variability on the epidemic dynamics?*.

In response to these unknowns and potential threat by H1N1p, several international institutions and governments invested in the developing surveillance and monitoring capacity among the low income nations(M. A. Katz, Schoub, Heraud, Breiman, Njenga, Widdowson, et al., 2012). The assumption as of that time was that the H1N1p could be absent in Africa and thus least of burdened, presumably based on recent works (Tamerius et al 2011, Lipsitch and Viboud 2009, Viboud et al 2006), that influenza in tropics and subtropics is mild and less synchronised compared to temperate climate.

To test for this hypothesis and others, the *World Health Organisation*, the *French school of Public Health (EHESP) Rennes France*, the *university of Aix Marseilles II IRD UMR 190 Lab Unit Marseille France* and *Ministries of Health* from several countries, initiated an *WHO-EHESP Cohorts for Pandemic Influenza (CoPanFlu) International Consortium* to study the global events of H1N1p(Lapidus et al., 2012). The overall objective was to advance knowledge on five aspects of H1N1p transmission and severity, namely the *virology*, *epidemiology*, *immunology*, *sociology*, and *genetics*; and to allow for international comparisons. This consortium developed a multicentre longitudinal prospective household cohort's study core protocol, with three phases and a two-year follow-up in six countries from five continents. In Phase I: it was the creation of cohort and blood sampling 30days prior to flu (winter) season and respond to questionnaires. In Phase II: it was in flu (winter) season, to collect virological specimen for viral isolation and respond to questionnaires to establish transmission pattern via socio-contact network, and study clinical profile of ILI. In Phase III: it was the final blood sampling 30 days after flu season. The three phases were to be repeated for at least 2 flu seasons(Lapidus et al., 2012).

The participating centres (nations) were required to co-sponsor and adapt the core protocol to the local context of the host country. Six centres which finally took part in the study, were namely; France, Laos, Djibouti, Mali, Bolivia, and Ré-union Island in the Indian Ocean(Lapidus et al., 2012). The number of households and subjects enrolled were determined as per the Copanflu international Core protocol as described by Carrat and colleagues(Fabrice Carrat et al., 2002). A household was defined to be made of two or more persons staying in the same house, sharing meals and living room space, with or without familial relationship. Only households with all members consenting participation (be blood sampled and give respond to questionnaires) were enrolled. All enrolled households had freedom to withdraw their participation at any point of the program. Households were to be geo-coded to allow for spatial analysis. The Djibouti Program received study approval from the WHO-EHESP CoPanFlu International Consortium, based at the French School of Public Health (EHESP) in Rennes France, and the Ethical Review Committee of the Djiboutian Ministry of Health's *National Institute of Public Health* (INSP). A written informed consent was obtained from all study participants or their parents/guardians for minors below 16 years to allow their enrolment.

After receiving authorization from relevant government departments, The Djibouti program took place between 11th November 2010 and 15th February 2011, and enrolled 1045 subjects from 324 households. These households were derived from the initially 1,835 household heads who were recruited from two sources: 1,335 were from the 2009 Hajj Pilgrim database and 500 from the community of health workers (CHW) cognizance list (**Figure 2**). Hajji Database was an annual document constituted by the Djibouti Ministry of Religious Affairs and Immigration for participants to Muslim pilgrimage to Mecca, Saudi Arabia. The CHW database was a document constituted by the Djibouti Ministry of Health and that includes a list of vulnerable households earmarked for emergency government support in case of natural disaster or disease outbreaks.

Information was given to all households' members and recruitment was performed when all members could be included and participants or their legal representatives given informed consent (that also included the use of specimen in other studies). Capillary blood samples (~100-500µL) were collected and assisted response to standardized French questionnaire was collected, using the local dialect to translate questionnaire whenever necessary. Information on *subject profile, health record, Socio-demographic Information, housing environment, risk perception and health behaviour on H1N1p* were obtained.

2.3 Material and Methods

(a). Influenza study

i. Syndromic Surveillance system evaluation

The purpose of this section inclusion was a preliminary report and to assess the potential of the local ILI monitoring system to detect H1N1p. Therefore, a secondary raw data was obtained from the *Department of Epidemiology and Health Information* (DISED) of Djibouti's Ministry of Health. This data was from 2007 to September 2010, constituting of summary of clinical consultations reports, over counter and prescription ILI drugs sales report, laboratory diagnostic report, and health promotion report. This data was summarised into appropriate variables and categories for a descriptive analysis in excel. The purpose was to evaluate the correlation of these parameters and seasonality of the influenza in Djibouti. Additional weather information for the relevant same period was obtained to argument the study. A legal consent was obtained from the ministry for the use of the data in the study.

ii. A pandemic Influenza epidemiological Study

The Copanflu Djibouti program goal was customised to investigate two aspects of sero-epidemiology (prevalence and determinants of individuals' risks to infection) and the socio-epidemiology (the knowledge, risk perception and the health behaviour), of the swine flu pandemic. These findings were to confirm the preliminary report, and to lay down the future platform for reinforcement of surveillance system of respiratory diseases in the general population, for Djibouti.

Laboratory detection of antibodies to A(H1N1)pdm09 virus was performed according to CoPanFlu standardized HI protocols, as previously reported[13,14,](Lapidus et al., 2013). This entailed twofold automated dilution 10^{-1} to 10^{-7} of test samples and control (positive and negative) sera, performed in the presence of a serum non-specific agglutination inhibitor. A highly specific cut off of HI titre at ≥80 was used to identify positive samples. For the detection of sero-neutralisation antibodies, we performed analysis on the HI positives (≥80) using a standard microneutralisation (VNT) assay protocol(Delangue et al., 2012). It entailed an automated twofold serial dilution 10^{-1} to $10^{-7}$of test samples and control sera in flat bottomed 96-well cell culture microplates (Nunc™). A 50µL sample of titrated virus at 100$TCID_{50}$ was then added to an equal volume of serum and incubated at 37°C in a CO2 incubator for 60 minutes. Afterwards, a 50µL aliquot of freshly prepared MDCK cell culture suspension at 2x10^5cells/µl was added, and then incubated at 37°C in a CO2 incubator until the cytopathic effect (CPE) formed in the control, which was usually about 3-5days. Absence of CPE was considered to reflect complete neutralisation

(positive reaction). A serum with standard VNT titre at ≥10 was considered to be positive(Fan et al., 2012; Meijer et al., 2006).

(b). Arbovirus fevers Study

This part of work was expanded from the initial copanflu project, and was informed by the need for additional information in these groups of pathogens in the country and the region. The study was motivated by four factors on Republic of Djibouti: (a) It is located in the greater rift valley and the neighbourhoods nations are endemic to YFV, RVF, WNV Ebola and AHFV(Malik et al., 2013); (b) Majority of DENV cases reported in France originate from Djibouti(De Laval, Plumet, Simon, Deparis, & Leparc-Goffart, 2012), courtesy of military foreign mission, (c) Suspected CHIKV outbreak occurred (in 2011) soon after this CoPanFlu sampling (Dr Ammar Abdo Personal communication MOH Djibouti), and there was need to know if the virus was circulation before the outbreak.

First intention detection of antibodies (IgG) against various pathogens was determined by two different enzyme-linked immunosorbent assay (ELISA) protocols. In the first protocol, in-house kits (in which antigen derived from whole-virion particles in non-inactivated cell culture supernatants) were used to test for YFV, TOSV, RVFV and CHIKV antibodies. In the second protocol, commercial kits were used for detection of DENV (PanBio®, Brisbane, Australia), WNV and TBEV (EuroImmun®, Lübeck, Germany) antibodies. Positive and negative control sera were provided by the *French National Reference for Arboviruses* or by the kits' manufacturers. Additional sero-neutralisation experiments were conducted in which wild-type laboratory-adapted viral strains were used, with exception of the YFV, in which the D17 vaccine strain was used. Appropriate cell culture lines and reagents were used in accordance to the established Standard Operating procedures (SOP) and Good Laboratory Practice (GLP) of the laboratory. All experiments were conducted in *Biosafety level 3* laboratory containment facilities, at the EPV UMR_D 190 research laboratory, or at the French National Reference Centre for Arboviruses, Marseille France.

2.4 The Republic of Djibouti

The republic of Djibouti is located at the horn of Africa, along the Gulf of Eden's Red Sea, bordering the Eritrea, Ethiopia and Somalia. It lies between latitudes 10° and 13°N, and longitudes 41° and 44°E, covering a total area of 23,200 square kilometres, see **Figure 9**. (United Nations- cartographic section, 2010) Djibouti city is the capital city, and one of the six administrative provinces of the republic. The others provinces are Arta, Ali Sabieh, Dikhil, Tadjourah and Obock. This nation was found in 1977 as a former French Territory of the Afars and the Issas, hence the long standing ties to the republic of France. At the moment, the nation hosts several foreign troops from France, Germany, Spain, Japan, Korea, china and the USA. These troops are involved in counter terrorism and anti-piracy operations in the horn of Africa,

which in recent times; the Somali militia pirates have posed a threat to international trade along the gulf of Eden (https://www.cia.gov/library/publications/the-world-factbook/geos/dj.html accessed 10/102013).

Figure 9: Map showing the geographical location of the study area, the republic of Djibouti (United Nations- cartographic section, 2010)

The climate ranges from the arid in the north eastern coastal regions to semi arid in the central, northern, western and southern parts of the country. The coolest areas in the country are in the forests of the Day National Park in the northern region, at 10 °C (50 °F) and the rest of the country is hot. In summer, the

temperatures are extreme and may exceed 40 °C (104 °F). The relative humidity may ranges from 40% during day time to a maximum of 85% in the night. Rainfall is generally low and occurring mostly in the winter, and ranges from 5 inches (130 mm) to 15 inches (380 mm). In a year, there are two seasons, the short winter and prolonged summer. Winter occurs from October to February with heavy rainfall intensity that causes flash floods often from January to February (see Figure 4), the rest of the year is hot summer (http://en.wikipedia.org/wiki/Djibouti accessed 10/102013).

Along the varied terrains of Djibouti, there exist diversity of fauna and flora. Over 534 species of floras and 399 species of fauna are documented endemic in Djibouti (Encyclopaedia Britannica 2006). Most of these species are concentrated in the cool mountainous ranges of the north. However, in semi-arid, presence of some grassland, shrub land and succulent scrub is evident. Along the coastal area, they are desert ranges and some parts close to sea host mangrove forest. A variety of plant species forms part of the evergreen forests in the mountains ranges of the Mount Goda, the Day National Park near Tadjoura and Mabla Mountains. The distribution of animals also seem to follow that of the plants, with most of animals preferring to reside where more diversity of plants are. Some of the reported species present are the reptiles, rodents, wildlife ruminants, low primates and birds-including passerine species(Magin, 2013).

The Djibouti population is concentrated mostly in urban areas. According to the 2009 national census(DISED, 2012), it has more than 820,000 people, 85% of them living in urban, of which 440,066(53.8%) are in Djibouti city. Among the general population, 378,093 (46.2%) were women. Distribution by age groups showed that those aged 0-14years were 32.5%, those 15-24years were 22.2%, those 25-54years were 39.4%, those 55-64years were 3.9% and those >65years were 2.1%. This is a multiethnic nation, with Somalis and Afars as the majority tribe, followed by the Arab, Ethiopians and Oromos. Other inhabitants are the foreigners from all over the world, and the refugees.

Economic wise, Djibouti's is dependant heavily on service industry that surrounds the presence of foreign mission, and on Ethiopian railway-Port harbor cargo handling. Crop production is untenable due to harsh climate, but livestock keeping is maintained informally courtesy of nomadic pastoralists' (FAO, 2005). Therefore, almost all the basic commodities are imported from outside. The United Nations rated the country 164th out of 184 in 2013, at 0.445 in terms of the Human Development Index (UNDP, 2013). 10% of sedentary households were classified as extremely poor, living below the extreme poverty line, which was set at USD 566 (FDJ 100623) per adult, per year (see **Figure 10**). This indicator rises to 60% of the population, if the nomadic and homeless persons are counted(African Development Bank, 2002).

The primary health care system of Djibouti is far from the best. There some challenges in acquisition of clinical services, preventive care and proper nutrition, this in part has contributed to persistence of

infectious and parasitic diseases, such as tuberculosis, HIV, acute respiratory diseases, and diarrheal diseases; and malnutrition in the population(African Development Bank, 2002; WHO, 2008, 2013). Under-five morbidity and mortality still remain high and yet to be contained. Most of the mortality cases are due to diarrheal diseases, acute respiratory diseases, protein-calorie malnutrition, pneumonia, and the childhood vaccine preventable infection. Disease Surveillance and monitoring programs are work in progress, thanks to partnership with international health agencies such as CDC, WHO and military foreign missions, who are involved in risk mapping and prevention programs

2.5 Djiboutian as Research Subjects

Republic of Djibouti is a multiethnic country, composed of both the locals and foreigners. Among the locals are the Afar, Somalis; Ethiopian, Arabs and Oromo's. Among the foreigners are the European, Americans, Asians and Africans (see **Figure 12** and **Table 2**). In addition, there are refugees who flock into Djibouti from the neighbouring countries experiencing civil unrest. Majority of the population live in the urban centres and have adopted a mixture of culture, which blends the lifestyle from Arab-Muslim, European-French and that of natives-Somalis or immigrant-Ethiopians.

This culture adoption has influenced the social life, feeding and dressing behaviour. For example, among the practicing Muslims, women commonly dress in "Hijab", a long dress covering all body except for the eyes, and men dress in long dress too "kanzu" in accordance to the Islamic provisions. Some of them annually travel to Mecca Jeddah for Hajji Pilgrim, as part of Islamic ritual rites. In this Hajji period, the Islam community enjoy a lot of celebration which demands many animal sacrifices, sharing meals and performing art. Apart from Hajji, many of the Djiboutian with disposable income do travel to Ethiopia annually, in summer (June to October) to escape from the extreme temperatures and humidity. Some may also relocate temporarily to their rural villages, closer to the cooler mountain, in the countryside of DJIBOUTI.

At family level, most domestic chores are performed by women and children at home, men are often heads of the household, but disparagingly involved. When free, men may have privileges to entertain their colleagues over drinks, shisha (Ethiopian traditional flavoured smoking pot) and bunch of Khat leaves (*Catha edulis*). This entertainment begins late in the afternoon and may run through into the night. Of concern in recent times has been the abuse of Khat among the youth of Djibouti, especially among the unemployed and students. Usually, most of the youth are in school, but when free, they pass their time

swimming in the ocean or at the beach, because most of the time it is hot and dry, and the natural parks or gardens are absent, limited or inaccessible.

For the working class, majority are in public service sector, hospitality service industry, and harbour port of Djibouti or in employment associated to foreign military mission. The large number of youth who are not in school, are jobless, contributing to the over 45% of unemployment in the entire population(UNDP, 2013; WHO, 2006c). The situation is exacerbated by the harsh ASAL eco-climatic condition, which impedes any crop production and limits livestock production to nomadic. Therefore, most of basic foods are imported from outside (USA, EU, ASIA, Middle East and neighbouring African countries). The imports are then merchandised in large food markets, vegetable markets and meat markets, which forms epic of Djibouti urban lifestyle.

The refugees and other illegal immigrants form a significant proportion of the entire population. Majority of whom, are in high deprivation, marked by congestion and malnutrition (see **Figure 10**). A large number of them resort to destitution (pegging in the street) for a living. These conditions, in part, contribute to the poor health status and associated to perennial outbreaks of hygiene related gastroenteritis (ie cholera and typhoid) in their camps. It is at the behest of the international relief agencies such as World Food Program, UNHCR, CARE International, World Vision etc that this section of the population are provided with primary health care, portable water and fortified prefabricated nutrition supplement for the under five children(WHO, 2006c, 2008, 2011b, 2012b, 2013).

The nomadic pastoralists who live in rural areas are also the beneficiaries of these agencies services, since, they too faces the same challenges. The nomads, mostly the *Afars* or *Somalis* live in large colonies (10 to <50) members. Their daily chores evolve around herding livestock (camels, cows, goats and sheep) from one terrain to another, foraging wild foods and maintenance of built temporary makeshift. Once the water and pasture is consumed they relocate to another and so on so forth. Although, they depend on their livestock for food (milk, blood and meat) and transport, they are vulnerable to extreme weather patterns. For example, in winter when it rains and floods, they enjoy lush pasture and abundant nutrition, but also suffer most from the vector born diseases that comes with the high insect population during this time. And they (nomads) could also be a good source of re-introducing infections to the urban centres where they throng in when its extreme weather conditions high flooding (see **Figure 11**) or extreme hot and dry, seeking for water and food for their animals.

2.6 Data Collection and Manipulation

Data collection in this study was bicameral, at subject and at household level. This choice was informed of due to relevancy to the local situation and potential influence on infectious disease dynamics. Households in Djibouti are generally large, closely knit and with high frequency of social physical contacts. This has a potential of either enhancing the spread of respiratory infections like influenza and other contagious illness, and in none severe cases lead to herd immunity. By investigating at household level, it provided a clearer picture of epidemiological risk situation in Djibouti.

The choice of variables and statistical analysis were informed of by the study objectives. Two main groups of variables were obtained from the questionnaires used (see **Table 2**). First category constituted of socio-demographic parameters; namely, the subject profile (Age group, Gender, tribe, Occupation, education), the household profile (Region, Wealth index-SES, Household size, number of children), living environment (Living near river bank, Living near market, Living near dumpsite) and health situation (Sleeping out at night, Keeping animals, Khat, smoke, vaccination, Co-morbidity); and were used in risk assessment of infection status. Second group on general social behaviour; including individual access to media, risk communication message (and messenger), risk perception, health behaviour (vaccination, treatment, mask use, etc); and the subjective norms (attitudes and belief) towards H1N1p; and were used to analyse for predictor of health behaviour (see **appendix**).

2.7 Lessons Learnt in Field Operation in Djibouti

The objectives, design and implementation of Copanflu programs were stringent, based on the WHO-EHESP CoPanFlu International Consortium Core protocol. This was especially so for among nation with limited surveillance capacity and preventive medicine programs. Among the protocol provisions to be fulfilled, were the household inclusion criteria and detailed information collection. As mentioned above, the households were enrolled only after all members' consented participation (blood sampled and respond to questionnaire). With low literacy level among adults, the use of assisted completion of questionnaire for large family or majority of subjects with minimal skills of reading, writing and comprehension of English or French was quit involving. The translation of the script in local dialect was the only solution. The completion assisted enrolment, on average per subject took between 20-45minutes and per household up to 2-7 days.

The administrative and logistical preparation for supply of biologics was understandably time consuming due to procurement protocols and absence of local supplies for biologics and consumables. Rarely, local research activities take place with this magnitude. This study was the first of its kind to be implemented in the host country. The participation of local health care personnel's was also a challenge given they are

overly occupied and only a few volunteered time into our research team. The finalist medical students and community healthcare (social) workers were greater partners in meeting the human resource needs of the project.

In Djibouti, like many Arabian countries with hot ASAL eco-climate, work is half a day (08h00-11h30), before breaking for lunch and returning to work for 2hours (16h30-18h30), from Sunday to Thursday afternoon. Thursday afternoon to Saturday (included), are weekends according to their official Muslim calendar. Under this working schedule and with unreliable public transport, it meant that the actual field work per day only lasted less than 3hours a day and had to be in the afternoon. This translated to more than two days per households for each of the three research teams, including the need for the follow up of the incomplete households.

Cultural and lifestyle practices create some inhibition to enrolment pace. During enrolment, blood sampling could not be done after the sun downer, reason "*....the devil will drink their blood*". Similarly, in Ramadhan period, blood sampling was not allowed, because it was against the Islamic religious provision. In religious conservative households, only male were allowed to attend to male subjects and female to female subjects, if not so, the enrolment had to postponed till this provision was met.

As discussed earlier, Djibouti is mainly an ASAL eco-climate and temperature may soars to >40^{o}c and humidity to >80%. In traversing the harsh terrains in the afternoon, needed extreme caution, particularly in sample handling and preservation, to avoid haemolyses. The research team was also exposed to extreme harsh ambient environment and required frequent rehydration. Last but not least, In Djibouti, the majority of people are living in the informal settlement and this made it difficult to trace physical residence (compared to places with formal housing), therefore, a local, particularly, among the community health workers were our important point persons.

Against all the odds, this report presents exciting findings on infectious disease situation in Djibouti city, of the republic of Djibouti. As a project coordinator (Dr Andayi Fred), i appreciate the support granted by the host partner (MOH-DEIS) throughout the Phase I study period. Though the Phases II and Phase III of the study were planned but not realized due internal restructuring and re-organisation of the host institution. We belief future studies could learn more from our experience and improve in the approach.

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Figure 10: A walk through District 4, Balbala Quartier housing neighborhood in Djibouti City (courtesy of France Diplomatie 2009)

Figure 11: A walk through District 1, backstreet housing neighborhood in Djibouti City (courtesy of France Diplomatie 2009)

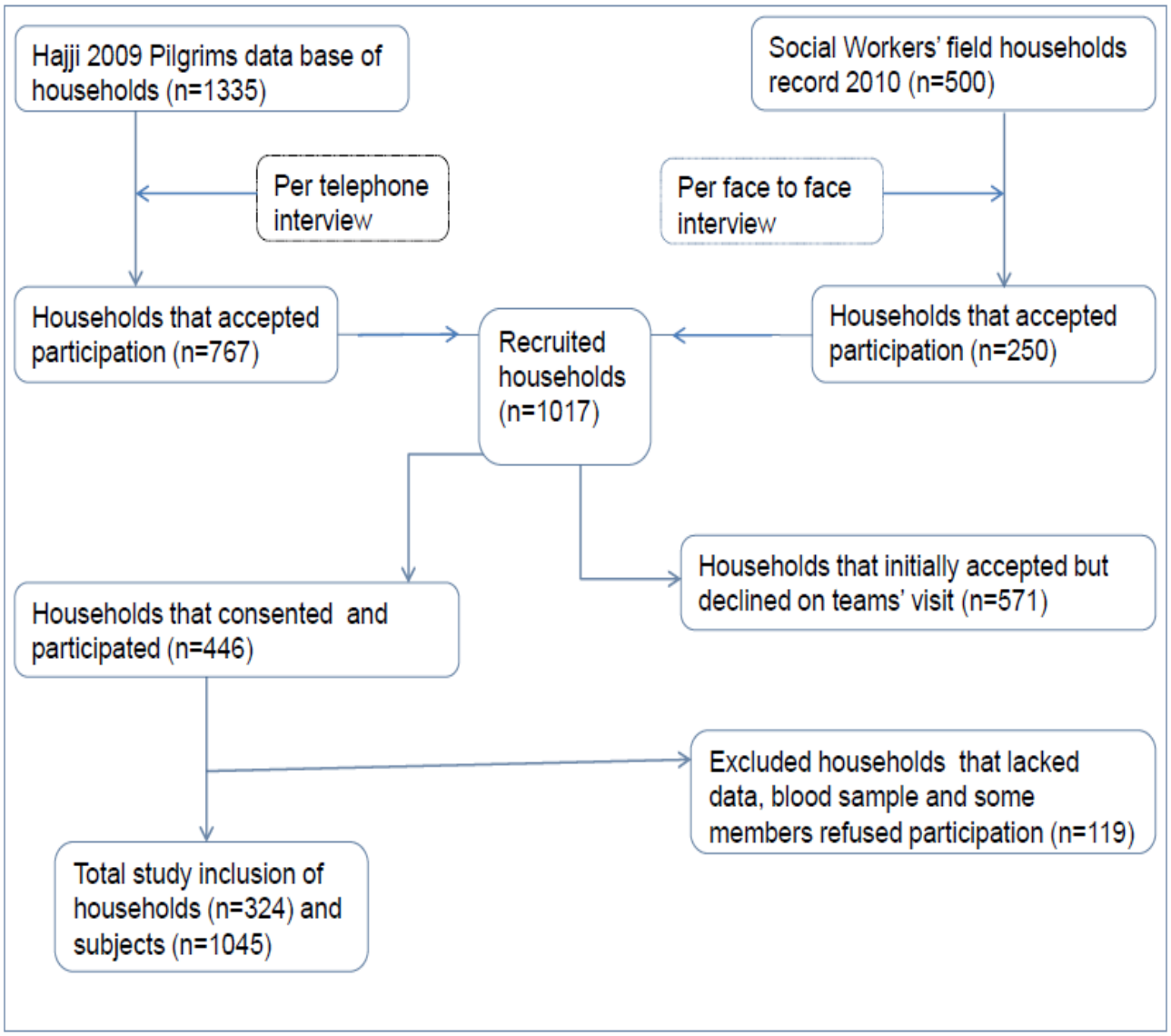

Figure 12: Flow chart of household enrollment in the Djibouti cohort

	Characteristics	Individuals in the cohort (n)	Proportion in the cohorte (%)
1	Résidence (District 1)	433	41.4
	District 2	340	32.5
	District 3	200	19.1
	District 4	72	6.9
2	Age group (0-14yrs)	332	32.1
	15-24yrs	166	16.0
	25-54yrs	472	45.6
	55-64yrs	40	3.9
	≥65yrs	25	2.4
	Missing values	10	
3	Gender (Male)	466	44.9
	Female	571	55.1
	Missing values	8	
4	Occupation (under 13yrs and not in school)	109	10.4
	Employed	162	15.5
	Student	301	28.8
	Jobless	473	45.3
5	Education (Under 5yrs)	88	13
	Primary	201	26.7
	Bac+	123	18.2
	Not schooled	265	39.1
6	Household size (1 or 2 persons)	330	31.6
	3-4 persons	152	14.6
	5≥ persons	563	53.8
7	Ethnicity (Afar)	101	10.2
	Arab	174	17.5
	Ethiopian	58	5.8
	Somali	645	65
	Immigrants	15	1.5
	Missing value	52	
8	Sleep out in open air at night	75	7.2
9	Living nearby market food	410	41.9
10	Living nearby the river bank	45	4.6
11	Living nearby open waste sewage	24	2.3
12	Living nearby dumpsite	140	14.5
13	Keep animal	149	15.2

Table 2: Demographic characteristics of the Djibouti study participants (n=1045)

CHAPTER 3

A PRELIMINARY STUDY

Influenza Surveillance System of Djibouti

3.1 Introduction

Syndromic surveillance is concerned with continuous monitoring of public health-related information sources and early detection of adverse disease events. It is touted as the greatest application and of relevancy to the low income developing countries. It uses the clinical symptoms (of cases yet to be confirmed in laboratory) as a proxy to evolution of certain disease syndromes in a population. A lot of focus is on the *Fever*, *Gastrointestinal*, *Hemorrhagic illness*, *Localized cutaneous lesion*, *Neurologic*, *Rash*, *Severe illness and death*, *Specific infection*, *Lymphadenitis*, *Botulism-like/botulism*, and *Respiratory* in the general population. Often, the surveyor utilizes the classical data from hospital clinic consultations and laboratory diagnosis reports, or from non-classical sources such as nurse calls, medication purchases, and school or work absenteeism.

According to CDC(Yan, Chen, & Zeng, 2008), the ideal system must be structured and synchronized to function optimally in hardware, software and human resource personnels to achieve the ultimate objective. Therefore due diligence at various phases in invaluable, start from the data sourcing, through various stages of data processing, to the data analysis and visualization, and in the final usage by the public health expert. In developed countries, these specialized systems are routinely in use for monitoring health event in the general public, military camps and in special mass gathering (hajji, Olympic, worlds summits). The gathered information is for advance detection and preparedness in prevention and control of infection diseases outbreak. Currently, there both commercial and opens source systems available for public use, which includes, the BIOSENSE, ESSENCE, ROD, BIOPORTAL SYSTEM, French Sentinel Network (FSN)system, LEADERS etc(Yan, Chen, & Zeng, 2008)In developing countries, particularly, South East Asia and Middle East similar system have been deployed, but with considerable success. Most of them have been installed in collaboration with US military, mainly to to help in monitoring emerging infectious disease in areas of their military operations.

Experts agree that these systems offer a promising opportunity for less costly infectious disease monitoring many low income countries of Asia and Africa, especially in the wake of pandemic influenza (**Chretien *et al* 2008**). Republic of Djibouti exhibited potential to benefit from this establishment of

syndromic surveillance system, given its limited use of laboratory diagnosis in clinical case management and constituent of a highly dynamic population, of which large proportion of international community (especially the military personnels). In retrospective, an evaluation of respiratory illness (ILI) in Djibouti was conducted to provide insight of the potential application of syndromic surveillance system, as well as, provide preliminary report of the first wave of pandemic influenza in the COPANFLU Study. This report lacks the algorithmic estimation of outbreak threshold for spatial, temporal or spatial-temporal ILI trends. It therefore cannot purport to accurately represent the ILI trend illness, but gives overview of what might have transpired prior to the second pandemic wave, based on descriptive statistics

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3.2 Research Article 1

Surveillance and monitoring of pandemic flu in a resource limited environment: a case of Djibouti and a WHO-Copanflu International Study preliminary Report

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Abstract

Background: A syndromic surveillance system using non-classical data sources for detection and monitoring evolution of influenza and influenza like illness (ILI) in Djibouti is reported here as part of the preliminary report of Djibouti WHO-Copanflu International Study (WCIS).

Methodology: Secondary ILI data of between September 2007 and September 2010 on *clinical reports, over-the-counter drug sales, laboratory diagnosis reports* and health promotion programs were obtained from *Department Epidemiology and Health Information* Djibouti, for an integrated statistical analysis.

Results: Results were indicative of a concomitant direct positive relationship between the first three variables and weather pattern; there was a progressive increase in cases towards winter months.

Conclusion: Our results showed that the current surveillance system was capable of detecting ILI trend, but more innovations in prompt reporting and instituting open source syndromic surveillance system software's, were needed to enhance its usefulness in monitoring pandemic evolution. Therefore, a successful implementation of WCIS in part, creates a platform, upon which some of the challenges could be addressed, so as to have a near-real time surveillance protocol.

Keywords: Djibouti, Copanflu, Multicentre study, Surveillance, Resource limited, Pandemic H1N1

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INTRODUCTION

The Spanish flu pandemic of 1918/19 infected and killed millions of people throughout the world and threatened to wipe humanity off the face of planet. It was the greatest pandemic recorded in human history. Based on known facts on pandemic genesis (antigenic drift), scientist had anticipated a high severity index pandemic around this period. However, the first wave of the 2009 pandemic influenza (H1N1p) fell short of this prediction. In spite of that, evident disease burden was putting pressure on health care facilities on most countries worldwide.

The WHO was therefore compelled to encourage continuous surveillance and monitoring of all influenza viruses in circulation. This was for better understanding of the pathogens' interaction characteristics and severity for mitigation preparedness [1]. In addition, it was to confirm earlier studies observations that showed H1N1p subtype had potential to evolve and cause unprecedented morbidities and mortalities. Another school of thought suggested that the observed moderate severity was but due to a quick respond and measures initiated by the WHO that have minimized the magnitude on global health and economy [1].

At that moment it was generally understood that, though H1N1p was marked as moderate, in areas with less developed preventive medicine (in universal health cover) and has less generous disaster emergency fund [2], the situation was likely to be different, and worse. To protect them, a priori surveillance and monitoring of influenza and influenza like illness (ILI) was important. The lack of adequate funding in these countries called for more innovative approach in establishing the surveillance system, based on the health information of the national primary health care facilities. Of interest being the records of pharmaco-vigilance, laboratory analysis, clinical cases and weather pattern.

Republic of Djibouti is a small nation located at the horn of Africa, bordering the Red Sea gulf of Eden, Somali, Ethiopia and Eritrea. It belongs to the low income countries of the world that are characterized by low human development index. In respond to the threat posed by the H1N1p to the local population, the WHO, *EHESP French School of Public Health Paris France*, *National Institute of Public Health of Ministry of Health Djibouti* launched a 350 household's longitudinal prospective cohort study in the four administrative districts of Djibouti city. This was part of the *WHO-EHESP Copanflu International Consortium Program* that was to investigate the pandemic influenza H1N1p events on themes of *viral molecular biology*, *epidemiology and sociology* risk factors; in six countries from five continents.

In Djibouti, there was no published report on ILI baseline statistics, this preliminary study was therefore set up, partly to provide this important information and secondly, to evaluate the potential of the current surveillance system in monitoring the pandemic H1N1p evolution. This study was based on the records from the local health information archives.

METHODOLOGY

Study Area and population

This study was conducted on data obtained from the health care system represented in the six administrative regions of the republic of Djibouti, namely; *Djibouti City*, *Ali Sabieh*, Dikhil, *Tadjourah*, *Obock* and *Arta*. In most facilities, the medical services charges are based on government subsidized out of pocket payment in public facilities or non subsidized fee payment in private health facilities. Of the estimated over 820,000 inhabitants, *Djibouti City* region at 475,322 has the highest, followed by *Ali Sabieh* 86,949; Dikhil 88,948; *Tadjourah* 86,704; *Obock* 37,856 and *Arta* 42,380, in that order. Majority them (70.6%), reside in urban environment, while the rest are either nomadic (19.7%) or enjoy the rural sedentary lifestyle (9.7%) [3].

Data Collection

The data used in this study were derived from Ministry of Health Djiboutis' *Annual Health Statistic Report*s of 2007, 2008, 2009 and 2010 (up to September). It constituted weekly or monthly record of Clinical case consultations, sales of pharmaceutical ILI drugs, laboratory analysis, vaccination activities and those of Health Promotion and Hygiene [MoH 2010]. The data had been obtained from 15 government facilities in Djibouti city (Balbala1, Balbala2, Hayableh, PK12, Doraleh, Einguella, Ambouli, Arhiba, Ibrahim Balala, Farahad, Khor Bourhan and General Peltier); six private hospitals (FNP, FAD and Gendarmerie); and 5 regional referral hospitals (Dikhil, Ali Sabieh, Arta, Obock and Tadjoura) [4].

Statistics analysis

Records from different sources were summarized in Excel 2007 spread sheet, stratified in three data categories of; (a) cases of ILI (b) Over counter ILI drug sales (c) laboratory diagnosis of ILI. Records that were either irrelevant to ILI or all together missing were excluded. The summarized information entailed the following variables; the dates, region, age, sex, vaccination, key health promotion message, nature of clinical syndrome and type of drug. A descriptive statistics was used to illustrate the trend, proportions, frequencies and distribution of data by variables mentioned above.

Copanflu Djibouti Program

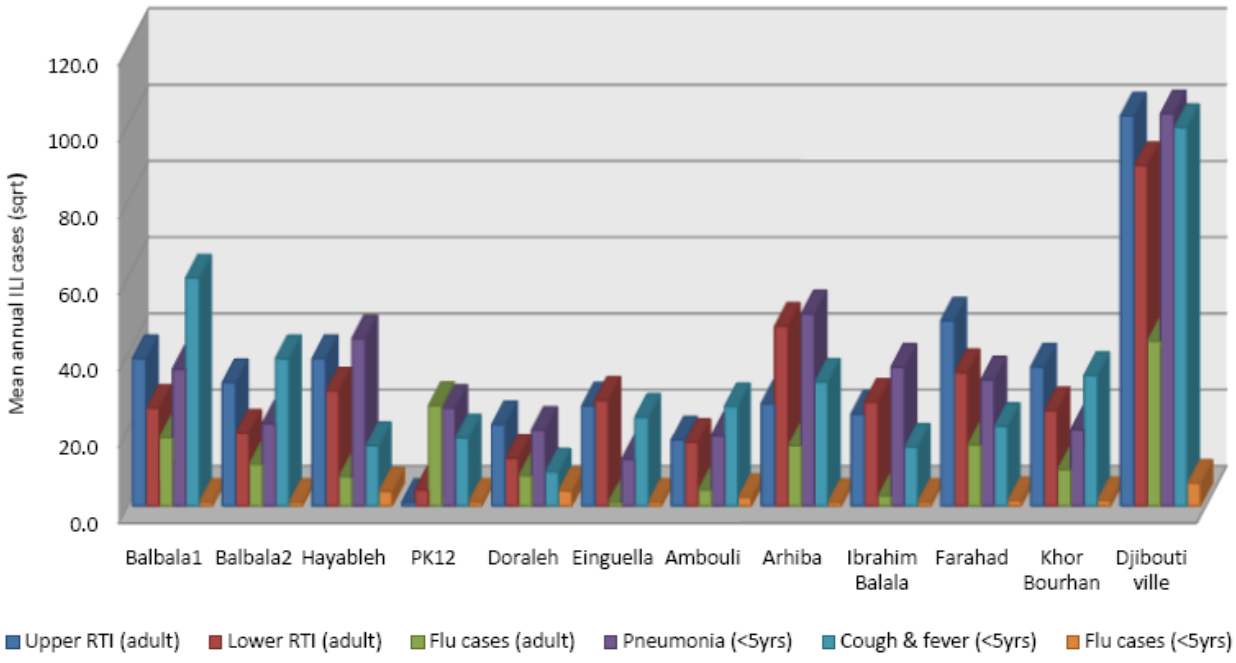
At the time of going to the press, the Copanflu Djibouti program was at constitution phase, which entailed the recruitment and enrollment of consenting participants from households meeting the entry criteria.

RESULTS

Data consideration

Only ILI relevant and complete records were included. It was observed that the data generated earlier than 2009 had a lot of incomplete records. For this reason, this study was therefore limited to the data obtained between January 2009 and June 2010.

Figure 1. Distribution of ILI cases in Djibouti City Health Centers

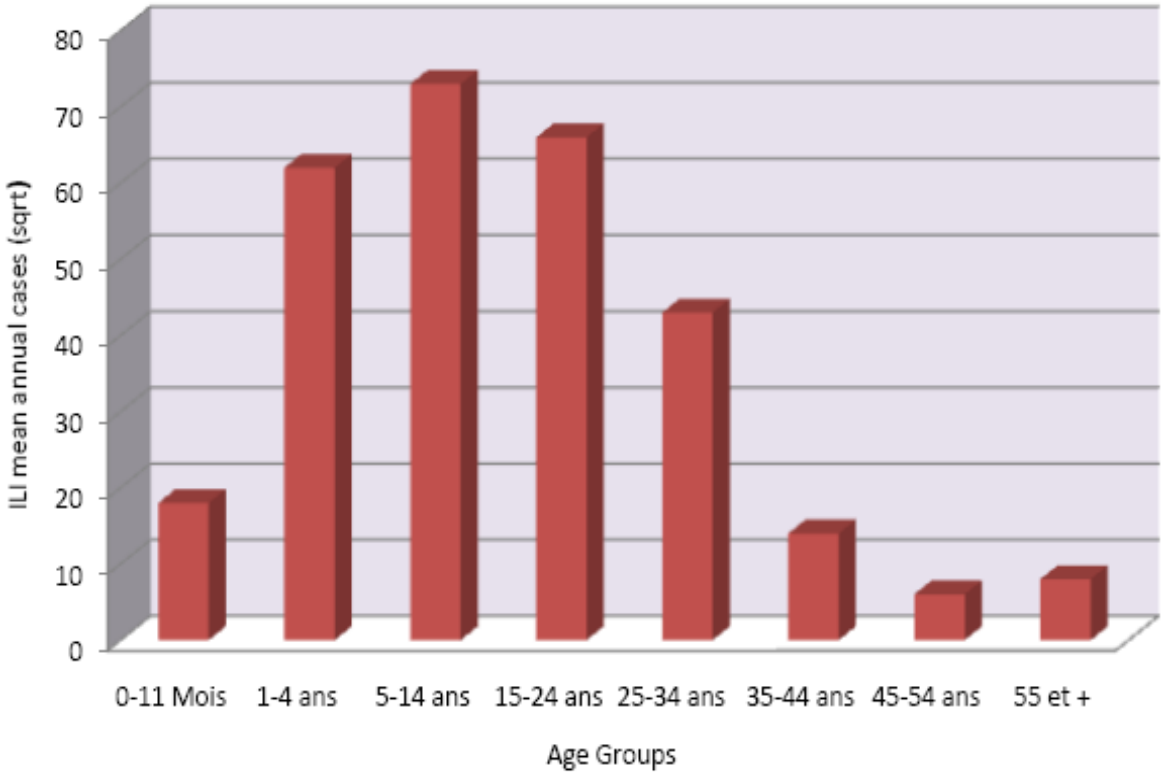


Findings on Clinical consultations

Up to 83% of the national health care system facilities were located in Djibouti city. From the consultation records, ILI accounted for up to 39.9% (326,445) of all the hospital visits (**Figure 1**). Of note was that, majority of the ILI cases, 58.2% (187,586) were from government facilities compared to the private clinics 11.9% (38,389). It was also observed, but not surprising that more than two thirds of these cases were from the Djibouti city region. By age group distribution, it showed that the young person's

below 25 years were disproportionately affected compared to other age categories (**Figure 2**). Interestingly, these infections were found to peak sharply toward colder months (peak of winter) season from October towards April(**Figure 3**).

Figure 2. Age group pattern of ILI cases in Djibouti



Findings on pharmaco-vigilance

Of all the over-counter drug sale report, 2% (99,350) of them were antipyretic and antiflu medication (such as painkillers, cough syrup, nasal drops) drugs. Surprisingly and of Interest was that 91.4% (90,765) of these flu medications were procured from the five peripheral administrative regions, with Djibouti city region accounting for less than 2% of the sales (**Figure 4**). In addition, the demand for these medications was found to increase toward the winter months of the year (result not shown), which corresponds to the findings in the ILI consultations. None of the surveyed pharmacies had record on sale of antivirals antiflu drugs (zanamivir or oseltamivir).

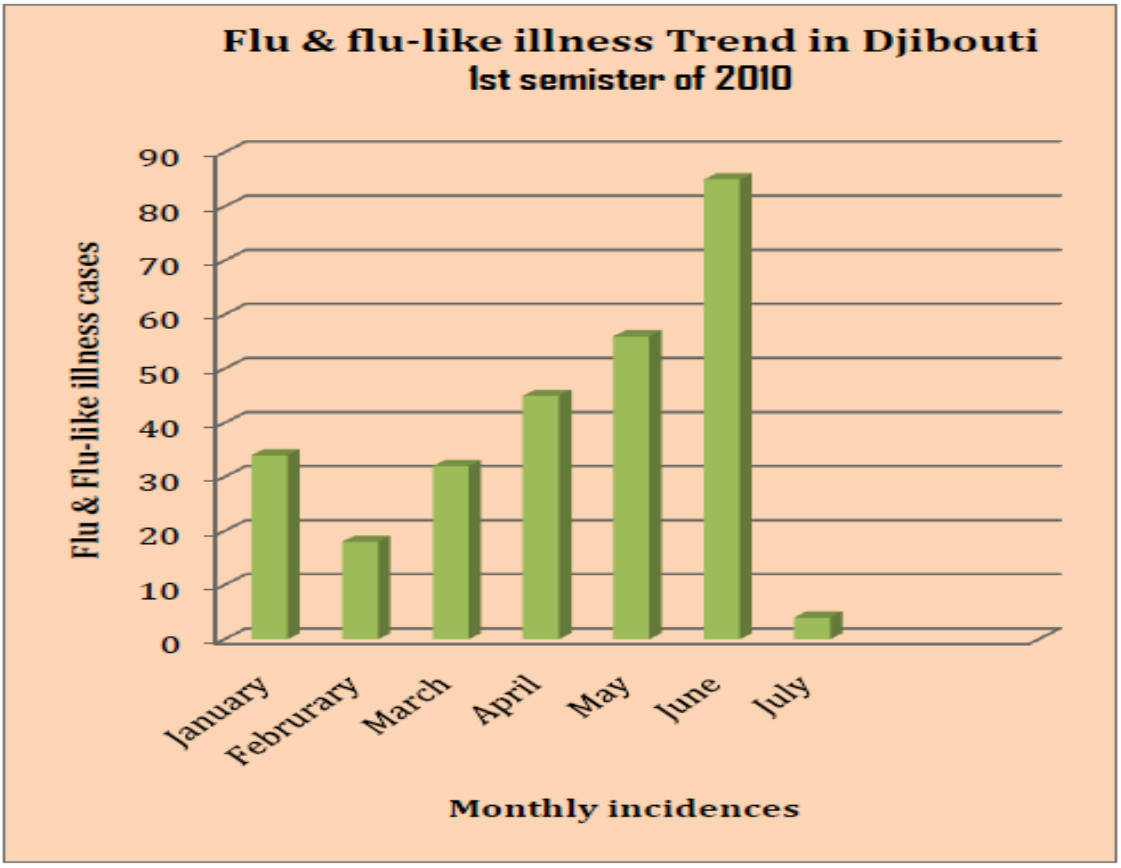


Figure 3: Monthly incidence cases of influenza and influenza like illness reported in the public health care facilities of the republic of Djibouti in 2010

Findings on laboratory analysis

Record on ILI laboratory analysis was very limited; most of it was missing or irrelevant. Of all the documented analysis, only 0.0072% (250) was complete and relevant to ILI. This was not unexpected, since diagnostic services in the health care system are not well funded, especially for viral analysis, which depends on external sponsorship. This technical cooperation was first explored following highly pathogenic avian influenza human incidence that was reported in 2006 [5]. The primary partners on this are the Egypt based USA Naval Army Medical Research Unit three (NAMRU 3) and the Kenyan Based KEMRI CDC.

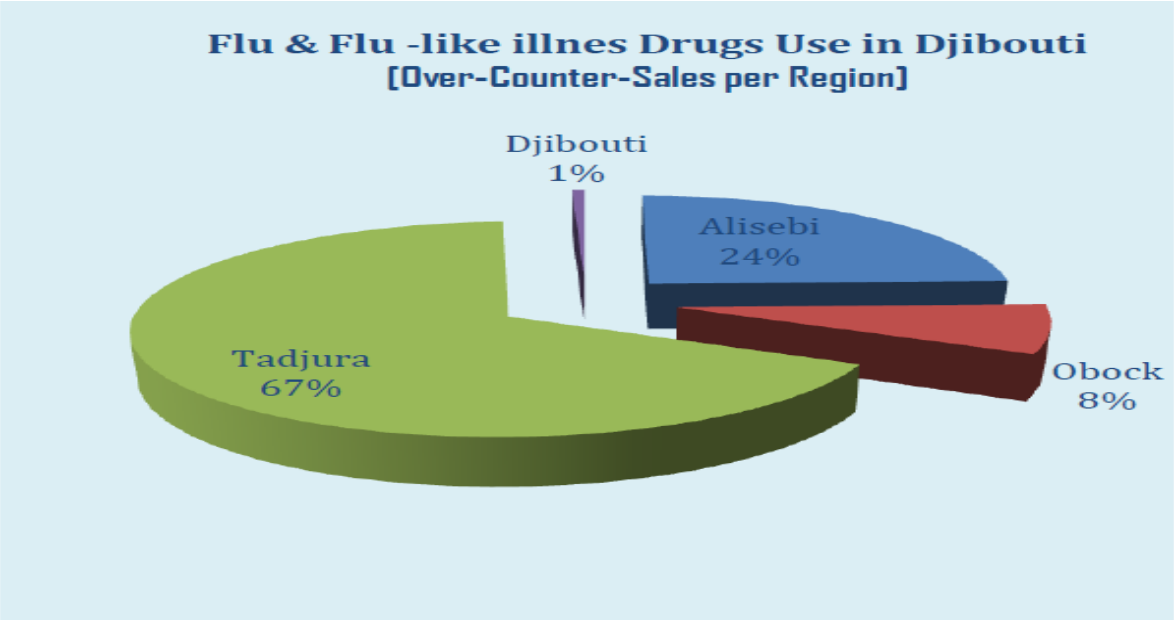


Figure 4: Distribution of over-counter consumption of the Influenza and Influenza like illness drugs in various regions of the Republic of Djibouti from January 2009 to September 2010

Findings on vaccination activities

No influenza vaccination record was documented for the period under review. However though not relevant to ILI, at least 0.4% (3,122) of the adult vaccinees were for yellow fever and meningitis. This was mainly due to the mandatory international travel requirement. Rest of vaccination recorded had been conducted on children under the UNICEF Expanded Program of immunization (EPI) program for childhood vaccine preventable infections. At the time of going to the press, the WHO had donated at least 80,000 vaccine doses of H1N1p virus that were yet to be administered, due to a logistic challenge (*Dr. Ammar Ahmed Abdo, Ministry of Health Djibouti, Personal communication, 2011*).

Findings on Health Promotion activities

Print and audiovisual media use in health promotion was common means of reaching out to urban masses. While for the rural and nomadic population, the face to face communication was preferred means. The health promotion activities were mostly reactory to an outbreak or encouraging EPI programs participation. Therefore no particular trend was observed (results not included). Activities targeting general population were limited to HIV/STD, Cholera and pandemic influenza, whose objectives were to increase public awareness, encourage case reporting and risk aversive health behavior. In the Djibouti Copanflu program, the study incorporated the basic training of participants on ILI infection and non-pharmaceutical intervention options.

DISCUSSION

Strength of the surveillance system

This study gives a basic description of the important features of respiratory illness surveillance and monitoring system of the republic of Djibouti. It has illustrated some of its strength, weakness and opportunities and threats that can be explored to consolidate its usefulness in decision making processes, with regard to ILI and the current pandemic H1N1p. This information was intended to lays fundamentals for consideration towards improving its sensitivity prescribed by the International Health Regulation [6].

Of note is the observed disproportionate ILI burden in young person's below 25 years compared to other age groups (Figure 2), which was consistent with the current pandemic H1N1p [9]. This essentially reflects a potential of the system to picking more than usual high number cases of ILI in the population. Efficiency of it could be enhanced by effective data collection and reducing the time between consultation and analyzed data usage. This findings are similar to the recent H1N1p studies elsewhere[1, 9], which also reported an increase of ILI cases that corresponded with the peaking of winter season, ie more and more cases occurring towards peak and decline gradually thereafter. Though not employed in this study, the use of modeling (ie time series) on quality data could improve output accuracy and allow for broader application.

From the pharmaco-vigilant data, the use of anti-flu drugs pattern was found to bear pattern similar to that of the evolution of the ILI consultation demands, which peaked towards the winter peak. Of interest was that there was a marked tendency of person staying in five rural regions to seek over counter medications compared to those in the capital city region. This suggested that existence of important inhibitory factors such as the distance from the health facility or the cost of accessing consultancy services. This observation is of great significance, particularly to policy makers, as it compares the disparity between the health care access of the capital city and the rest of the regions [7].

Weakness and opportunity of the surveillance system

There are several strengths in the current surveillance system, as mentioned above. However, some of the notable short comings associated included un-timeliness (takes long time, upto 6months to have summary statistics), incompleteness (vague codes for over-counter drug sales), entry errors (on incidence case reports) and missing records. Such challenges are common and tend to be improved on with time. Among the best ways overcome these inherent limitation is by innovativeness to improve promptness [7]. This may include the use of satellite phone handset for by regional health centers and mobile phone handset for city sentinel clinics reporting. In addition, the continuous evaluation and reframing of the

data entry process to capture the changing times and needs cannot be overemphasized [8]. A properly collected data is an invaluable asset, which besides alerting for impending outbreaks, can be a useful for predictive modeling of annual event of ILI diseases, including the H1N1p [9].

Contribution of the Copanflu Program

This project goal was to investigate the 2009 pandemic influenza H1N1p events in the republic of Djibouti. Additionally, the intended public health spin off benefit was to include creation of a demographic diseases sentinel surveillance (DSS) unit from the study cohort. DSS are sentinel herd used for future situational awareness on infectious diseases status of the general population. The important lessons to be learnt and findings observed to help formulate policy on ILI prevention and control, and form a platform for a refined syndromic surveillance protocol, similar to those used elsewhere in Asian and south American countries [7,10].

Application of open source surveillance system (softwares)

Although this study might have no direct association to softwares use, but it is in our view important to mention Syndromic surveillance software systems with algorithms for calculating basic statistics are becoming a standard practice in disease monitoring globally, because they make near-real time observation of syndromic cases evolution possible. As is the situation of current system, the lack of promptness and missing data, may benefit from their use. However, due to high commercial license fee required, such softwares remain far beyond the reach of many low income economies, like as Djibouti. An option of using a free open sourced program such as ESSENCE or RID etc with basic capabilities offers great opportunity [10]. Basic features of interest being the capacity to generate custom graphs, maps, plots, and temporal-spatial analysis output for specific syndromes. Such advancement should transform the current ILI surveillance system to a more prompt and accurate compared one compared to the current paper format or excel format in use [11,12].

CONCLUSION

In conclusion, the current surveillance system is capable of detecting ILI trend and pattern, but more innovations in prompt reporting and use of syndromic surveillance system (softwares), could enhance its usefulness for early detection and pandemic evolution monitoring. The ongoing WCIS project is hoped to create a platform upon which challenges faced by could be addressed and gainfully incorporated to achieve a near-real time detection of ILI.

ACKNOWLEDGEMENT

First author is grateful to Mr. Abdul Garad, head of Health information at the department of epidemiology and health information, ministry of health Djibouti, for availing this for the this study. He also thanks the EHESP Doctoral Network for the travel grant award to Hong Kong Conference on Influenza control option IV, in which this work was also presented. He is also grateful to the EHESP for award PhD scholarship. He wishes to acknowledge academic guidance from his doctoral advisors Prof Antoine Flahault (EHESP Dean), Prof Xavier De Lamballeria (U. of Aix Marseille II) and Dr Ammar Abdo. Thanks to the *France Institut de Microbiologie et Maladies infectieuses* (IMMI) for funding the WCIS project, and project administrators Imane El hadi, Ilana Levin and Regina Duroc for their support.

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CHAPTER 4

PANDEMIC INFLUENZA EPIDEMIOLOGY IN DJIBOUTI

4.1 Introduction

This article report the cohort of pandemic influenza (copanflu) Djibouti programs main findings, as initially envisaged in the WHO-EHESP COPANFLU International consortium Core protocol (Lapidus et al 2013). The main objective was to assess health and social impact of the pandemic and allow for an international comparison. The essence was to document a 2 years follow-up of individuals in a standardized way, from at least 1000 households in each country. It was expected to begin from July 2009 to until the end of 2010. In tropical countries, as noted by Viboud et al (2009), influenza seasonality is poorly defined, with cases occurring throughout the year (Radin et al 2013). Of concern in this project was to determine whether the pandemic was equally in circulation in the hot regions, like Djibouti, and if its profile differed significantly from the temperate ones.

To answer these questions and allow for international comparison, a standardized core protocol was used but adapted to local situation (Lapidus et al 2013), with a lot of adjustments made due to logistical challenges. Instead of three phases only first phase was accomplished, consequently, surrogate approach was adopted to realize the intended objectives. First, the use of single sera sampling, with highly specific screening cut point of HI titre ≥80 as surrogate for positive sero-conversion, a practice confirmed in our laboratory as an excellent estimator of infection status. Secondly, participants were opportunistically sampled from the Hajji 2009 pilgrims and community health service database of household earmarked for relief aid by the government. Under these situation, the study realized two general objective on sero-epidemiology (*reported here in chapter 3*) and socio-epidemiology (*reported in chapter 4*) of pandemic influenza in the republic of Djibouti.

Of interest in this report was that, the observed characteristics of the H1N1p in Djibouti were consistent with what was known then on the pathogen, which included the unique U-shaped curve of age group versus sero-prevalence, which indicated that the young and the elderly were the most affected (Viboud et al 2009). Despite the limited number of incident cases documented, the H1N1p virus was confirmed by this study to have circulated widely in the winter of 2010 and 2011, with about 30% attack rate. In lieu of the above, the future respiratory illness prevention and control should be tailed to specific vulnerable individuals such as student and those working in groups indoors. It was concluded that the lack of robust

data provided by surveillance systems in southern countries can be responsible for the underestimation of the epidemiological burden, although the main characteristics are essentially similar to what has been observed in developed countries. Although not all intended objectives were met, these study findings have significant contribution towards understanding H1N1p in tropics and this part of the world which remains under reported.

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Andayi et al. (2014) Determinants of Individuals' risks to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents - A CoPanFlu cross-sectional study. **Virology Journal 11:13**

VIROLOGY JOURNAL

RESEARCH Open Access

Determinants of individuals' risks to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents - A CoPanFlu cross-sectional study

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Abstract

Background: Following the 2009 swine flu pandemic, a *cohort for pandemic influenza* (CoPanFlu) study was established in Djibouti, the Horn of Africa, to investigate its case prevalence and risk predictors' at household level.

Methods: From the four city administrative districts, 1,045 subjects from 324 households were included during a face-to-face encounter between 11th November 2010 and 15th February 2011. Socio-demographic details were collected and blood samples were analysed in haemagglutination inhibition (HI) assays. Risk assessments were performed in a generalised estimating equation model.

Results: In this study, the indicator of positive infection status was set at an HI titre of ≥ 80, which was a relevant surrogate to the seroconversion criterion. All positive cases were considered to be either recent infections or past contact with an antigenically closely related virus in humans older than 65 years. An overall sero-prevalence of 29.1% and a geometrical mean titre (GMT) of 39.5% among the residents was observed. Youths, ≤ 25 years and the elderly, ≥65 years had the highest titres, with values of 35.9% and 29.5%, respectively. Significantly, risk was high amongst youths ≤ 25 years, (OR 1.5-2.2), residents of District 4(OR 2.9), students (OR 1.4) and individuals living near to river banks (OR 2.5). Belonging to a large household (OR 0.6), being employed (OR 0.5) and working in open space-outdoor (OR 0.4) were significantly protective. Only 1.4% of the cohort had vaccination against the pandemic virus and none were immunised against seasonal influenza.

Conclusion: Despite the limited number of incident cases detected by the surveillance system, A(H1N1)pdm09 virus circulated broadly in Djibouti in 2010 and 2011. Age-group distribution of cases was similar to what has been reported elsewhere, with youths at the greatest risk of infection. Future respiratory infection control should therefore be tailored to reach specific and vulnerable individuals such as students and those working in groups indoors. It is concluded that the lack of robust data provided by surveillance systems in southern countries could be responsible for the underestimation of the epidemiological burden, although the main characteristics are essentially similar to what has been observed in developed countries.

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Background

In April 2009, an acute febrile respiratory illness that spread rapidly across Mexico and the United States [1], was reported. This aetiological pathogenic virus was later identified as a new influenza A strain (referred to as A (*H1N1)pdm09 virus* in this article), a re-assorted variant of North American and Eurasian swine lineages which was immunologically distinct from the circulating seasonal influenza A strain H1N1s [2]. The geographic dispersion of this virus resulted in high numbers of new cases that overwhelmed laboratories and the clinical capacity of many nations, compelling the WHO to issue a pandemic alert on June, 11th 2009 [1]. A year later, more than one million cases and almost 20 thousands deaths had been reported from 214 countries [3]. These figures are likely to be an underestimate of the actual morbidity and mortality burden due to the *A(H1N1)pdm09 virus*, particularly amongst southern hemisphere nations [4]. The WHO further encouraged the scientific community to investigate the severity of this new pathogen and the associated risk factors. Amongst the notable observations were the high antibody titres mainly thought to be due to new infections amongst the young population (≤ 25 years) and previous contact with the antigenically related H1N1 strain amongst the elderly (above 65 years) [5,6]. More severe cases and fatalities were observed in young people, co-morbidity conditions [7], obese and pregnant women [6,7]. At that time, available data on Influenza burden estimation were mainly derived from North hemisphere countries, plus Australia and New Zealand but were severely lacking in many other southern countries. Disparities in the influenza funding programme, healthcare systems and research activities, were the other important significant contributory factors [8]. The southern group also contained a high prevalence of other infectious agents such as HIV, malaria, Tuberculosis, malnutrition and hygiene related gastroenteritis [9]. Under the WHO region classification, African and Eastern Mediterranean (WHO-EMRO) countries, have a high prevelance of these pathogens which has not been systematically documented. Djibouti, the country of interest in this study, is one of 22 member states belonging to the WHO-EMRO region. The other countries in the region include, *Afghanistan, Bahrain, Djibouti, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Qatar, Saudi Arabia, Somalia, Sudan, Syria, Tunisia, United Arab Emirates* and *Yemen.* In Djibouti, the ILI (influenza like illnesses) laboratory confirmed surveillance data are a work in progress and therefore syndromic reporting represents the backbone of disease monitoring. According to the recent annual health statistics report [10], influenza and ILI account for most of the consultations and incidence cases in health facilities, in particular accounting for 48% and 53% of all clinical consultations reported among adults and children. In the same category, 47% and 46% of all incidence cases reported, respectively [10]. These data therefore underscore the need for sound monitoring of ILI by identifying the aetiological agents and their associated risks, to allow early detection and advance preparedness against serious outbreaks such as the recent swine flu pandemic. Here, we have focussed on Djibouti, a subtropical country faced with the previously cited challenges. We report sero-prevalence data from a cohort of 1,045 subjects and uncover risk determinants of A(H1N1) pdm09 infection amongst Djibouti city residents during the A(H1N1)pdm09 pandemic.

Method

The Djibouti CoPanFlu *(Cohorts for Pandemic Influenza)* programme was part of an international project that performed sero-epidemiological investigations of influenza A(H1N1)pdm09 that was identified in six countries from five different continents (France [11,12], Laos [13], Djibouti [14], Mali [15], Bolivia (Delangue et al., manuscript in preparation), and the Indian Ocean (Reunion Island [16]) through longitudinal prospective household cohort studies. All centres used a standardised sampling and testing protocol, three phases and two years follow up, adapted to the local context of the host country [11]. Unlike other centres, Djibouti's study was limited to Phase one, and therefore is hereby reported as a cross-sectional study.

Study design and demographic characteristics

The study was conducted between 11th November 2010 to 15th February 2011 in four administrative districts (*arrondissements*) of Djibouti city, which is the largest urban agglomeration and capital city of the Republic of Djibouti, a country in the Horn of Africa. It covers about 23,200 km^2 and hosts 818,159 inhabitants, the majority of whom 58.1% (475,322) are inhabitants of Djibouti city [17]. It has two climatic seasons, the summer which lasts from May to September, and the winter from November to April [18]. After receiving authorisation from relevant government departments, 1,835 household heads were recruited from two sources: 1,335 were from the 2009 Hajj Pilgrim database and 500 from the community of health workers (CHW) cognisance list (Figure 1). The Hajj Database is an annual document constituted by the Djibouti Ministry of Religious Affairs and Immigration for participants to Muslim pilgrimage to Mecca, Saudi Arabia. The CHW database is a document constituted by the Djibouti Ministry of Health. It includes a list of vulnerable households earmarked for emergency government support in case of natural disaster or disease outbreaks. Information was given to all household members and enrolment was conducted when all members could be included. Participants or their legal representatives

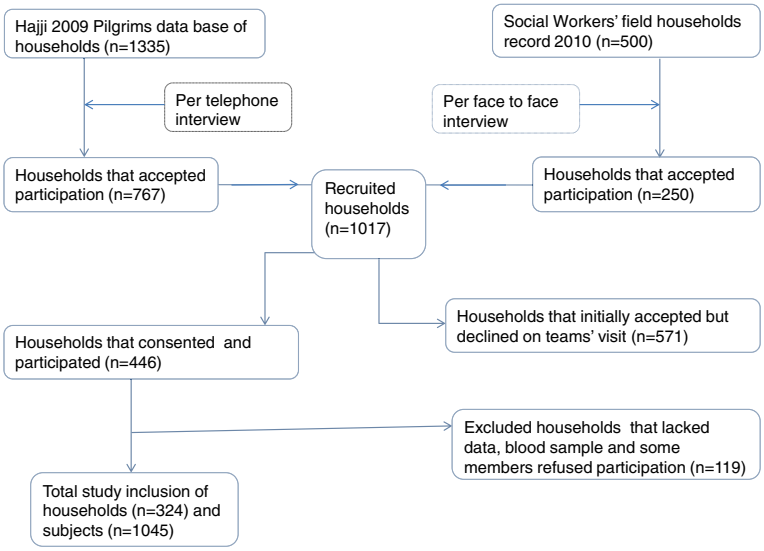

Figure 1 Inclusion chart showing the various stages included during the constitution of the 324 households and 1,045 subjects from amongst the residents of Djibouti city.

were *a priori* required to give informed consent. Only households meeting the following criteria were enrolled in our cohort; all members of the household shared one roof, they shared meals and living area, consented to participation (including blood sampling and responding to questionnaires), and were permanent residents of District 4. On an appointed date, the capillary blood samples (~100-500 μL) were collected and the assisted response to standardized French questionnaires was completed, using the local dialect to translate questionnaires whenever necessary. The fresh blood was allowed to clot at room temperature, before separating sera from clots by centrifugation. Separated sera were then stored at −20°C until the assay time. All assays were conducted in Biosafety level 3 laboratory environments at the EPV UMR_D 190 laboratory of the University of Aix Marseille 13005, France.

Laboratory analysis

Detection of antibodies to A(H1N1)pdm09 virus was performed according to CoPanFlu standardized HI protocols, as previously reported [13,14,19]. This entailed twofold automated dilution 10^{-1} to 10^{-7} of test samples and control (positive and negative) sera, performed in the presence of a serum non-specific agglutination inhibitor. A highly specific cut off of HI titre at ≥80 was used to identify positive samples.

For the detection of sero-neutralisation antibodies, we performed analysis on the HI positives (≥80) using a standard microneutralisation (VNT) assay protocol [12]. It entailed an automated twofold serial dilution 10^{-1} to $10^{-7}$of test samples and control sera in flat bottomed 96-well cell culture microplates (Nunc™). A 50 μL sample of titrated virus at 100$TCID_{50}$ was then added to an equal volume of serum and incubated at 37°C in a CO2 incubator for 60 minutes. Afterwards, a 50 μL aliquot of freshly prepared MDCK cell culture suspension at 2×10^5 cells/μl was added, and then incubated at 37°C in a CO2 incubator until the cytopathic effect (CPE) formed in the control, which was usually about 3-5 days. Absence of CPE was considered to reflect complete neutralisation (positive reaction). A serum with standard VNT titre at ≥10 was considered to be positive [20,21].

Data management and analysis

The wealth index (SES class) was determined on the basis of household ownership of nineteen different assets, by the *principal component analysis* as described by Vyas et al [22]. The GMT computation was conducted according to the SAS PROC LIFEREG, which is a survival analysis procedure in SAS 9.3 statistical software. The method avoids underestimation of the censored observations in the calculation of GMT as described by Nauta [23]. In brief, a titre of 5 was assigned to all HI tests resulting in negative observations, followed by a log transformation of log HI titre = log2 (HI titre/5), and estimation of the maximum likelihood of the GMT of truncated HI titres and their 95% confidence interval. To avoid potential bias, all the vaccinated subjects were excluded from all the prevalence and risk analyses. The risk analysis was performed in the generalised estimating equation model to determine the predictors of individuals' infection at household level. This model accounts for the existing correlation between subjects enrolled

from the same household. Infection status (HI titre ≥ 80) was the dependent variable that was evaluated against several independent variables from socio-demographic, housing environment and subject profile. Those variables found to have p-value <0.25 in bivariate analysis, were fitted in a multivariate model through a backward stepwise reduction process in accordance with the Bursac et al [24] method. In the final model, variables with p-value ≤ 0.05 were considered to be statistically significant.

Ethical statement

Ethical approval was received from the WHO-EHESP CoPanFlu International Consortium of the *French School of Public Health (EHESP)* in France [11] and the Ethical Review Committee of the Djiboutian Ministry of Health's *National Institute of Public Health (INSP)*. A written informed consent was obtained from each study participant, including the minors (<16 years) through their parents or guardians.

Results

Demographic characteristics

From the four administrative districts of the city, 1,045 subjects, belonging to 324 households, were included in the CoPanFlu cohort (Table 1 and Figure 1). The overall median age was 25.9 (range 0.3-100.9) years, and there were no significant differences in age distribution between sample and the national population census [17]. We had an average of 3 (range 1–10) members per household who were stratified into three groups: 563 subjects (53.9%) were living in *"large households"* (≥5 persons), 152 (14.6%) in *"medium households'* (3–4 persons) and 330 (31.6%) in *"small households"* (1–2 persons). Within these households, 72% (755) of the subjects belonged to a household with children. Subjects were distributed according to occupation, there were four different categories: (a) "jobless" (45.3% (473)) were defined as persons above 13 years, not in any gainful economic activities, including the retirees and those who were not attending school/university; (b) "students" (28.8% (301)) were persons attending school/university most of the day, no age limit; (c) the "employed" (15.5% (162)) were persons in gainful economic activities, public or private, as a means of livelihood; (d) "under 13 years and not student" (10.4% (109)) were persons below 13 years of age, who were neither employed nor schooling. Concerning literacy levels, 25.4% (265) had no formal training, 19.2% (201) had basic education, 11.7% (123) had high-school education; missing values: 43.7% (456). On wealth index (SES), three classes were computed, 27.3% (285) belonged to the lowest class, 12.2% (127) to the middle class and 37.5% (392) to the upper class; missing values: 23% (240). Of the constituted subject cohort, 1.4% (14) had been vaccinated against A(H1N1)pdm09 and none for seasonal influenza, 8.1% (85) had on-going chronic illness, 10.5% (110) had a recent respiratory infection (ILI) illness in the past three months from November 2010), 1.3% (14) were on an unspecified treatment and 2.3% (13) of the women were pregnant.

Influenza serological status in age groups

In this study, the infection status (seropositivity) was determined by our established HI assay protocol [13,16,19,25], in which, the HI titre at ≥ 80 is considered a cut off when the use of seroconversion criteria is impractical, since the two measures have been confirmed to approximate each other [13,16,19,25]. All positive cases were attributed to the recent infection or past contact with an antigenically closely related virus in those older than 65 years [5,6]. Of the 110 subjects with recent respiratory infections, 23 were seropositive for the 2009 pandemic influenza virus. Figure 2 shows the reverse cumulative distribution of HI titres for antibodies against A(H1N1)pdm09 by various age group categories. The relationship between age group and level of antibodies is further detailed in Figure 3, which reports (a) seroprevalence values in age classes according to ≥40 and ≥80 HI titre cut-off values, and HI GMT values and (b) distribution of neutralising antibodies seropositivity in subjects with HI titre ≥80 (according

Table 1 Demographic characteristics of the Djibouti CoPanFlu cohort

Demographic characteristics	*Number of individuals in the cohort*	*Proportion in the cohort*
Gender (female)	571	54.64%
Age		
0-14 year-old (yo)	332	31.77%
15-24 yo	166	15.89%
25-54 yo	472	45.17%
55-64 yo	40	3.83%
>65 yo	25	2.39%
Unknown	10	0.96%
Localisation		
District 1	433	41.44%
District 2	340	32.54%
District 3	200	19.14%
District 4	72	6.89%
Ethnic origin		
Afars	101	*14.64%*
Arabs	174	17.50%
Somalis	645	64.90%
Ethiopians	58	5.80%
Migrants	15	1.50%
Unknown	52	4.98%

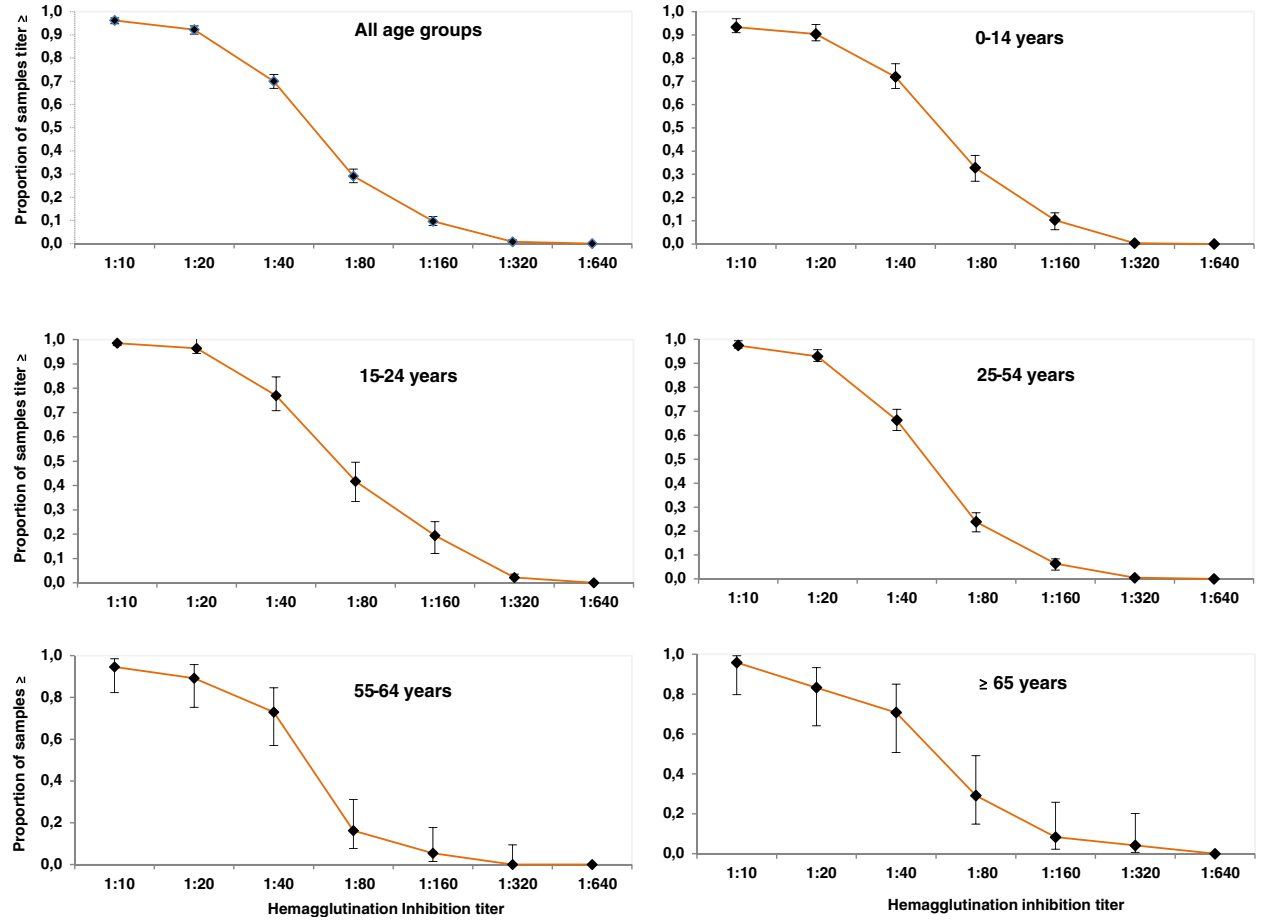

Figure 2 Reverse cumulative distribution curves of HI titres for antibodies and their 95% confidence intervals against H1N1pdm09 for different age groups amongst Djibouti city residents (Nov 2010-Feb 2011).

to ≥10 and ≥20 VNT titre cut-off values). The main observations are: **(i)** The poor discrimination in age groups when using an HI cut-off at 40, is in agreement with previous studies using the same experimental protocol [13]. HI results at titre 40 presumably aggregate the detection of low-titre specific antibodies and cross-reactive antibodies previously acquired following seasonal influenza infection [12]. The HI cut-off at ≥80 is more discriminative and provides distribution in age groups that faithfully follows that of neutralising antibodies (Figure 3). **(ii)** The global "U-shaped" distribution of antibodies in age groups, with a lower prevalence of antibodies in subjects belonging to the 25–60 age classes. Both the prevalence at HI ≥ 80 and the mean HI titre were significantly lower in this group, when compared to youths below 25years. As discussed elsewhere, this distribution may reflect, on the one hand the epidemiological exposure of the children and young adults to influenza infection, and on the other hand past exposure to H1N1 variants (that share more antigenic similarity with the novel pandemic variant than recent seasonal H1N1 strains) for patients over the age of 60 (*i.e.* 'pre-drift' Spanish flu-related H1N1 strains) [2,5].

Seroprevalence according to districts of Djibouti city

In a choroplethic map, seroprevalence of antibodies was highest in *District 4,* followed by *District 1, District 2* and *District 3,* in that order (Figure 4a). A refined analysis according to Locations "Quartiers" identified Quartiers 1, 6, Balbala and Damerjob as those with the highest observed seroprevalence values (≥40.7%) (Figure 4b).

Regression analyses

From the bivariate analysis, significant associations were confirmed between infection status and a number of risk factors, namely; *age group, place of residence, occupation, working environment, household size* and *living nearby river bank (*ie habitants living near River Balbala valley banks, drawn from *District 2, District 3* and *District 4.*); but not for others, such as, *recent respiratory illness, wealth index (SES), gender, having children in the*

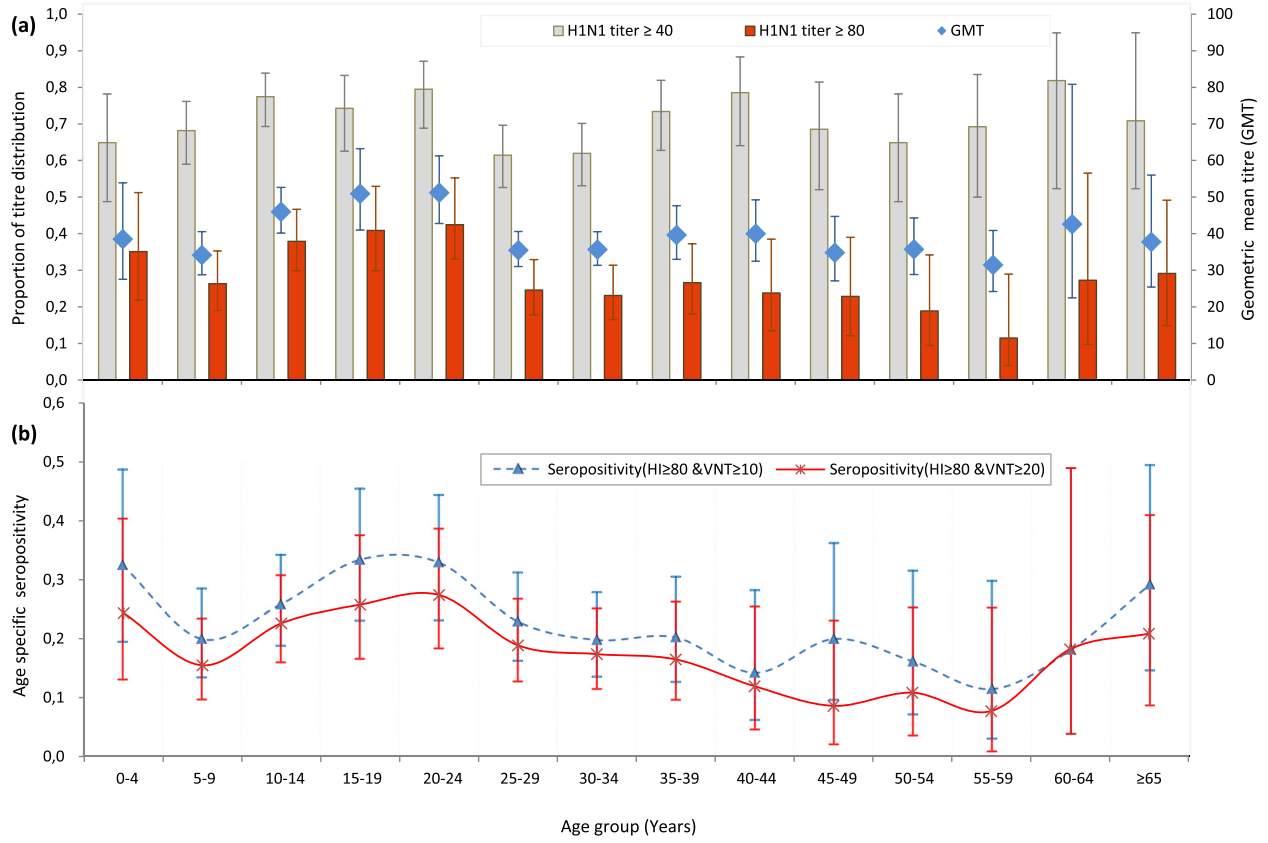

Figure 3 Relationship between Djiboutian age group and level of antibodies against H1N1pdm09 virus. The graphs illustrate the relationship between age groups to; **(a)** the specific antibodies (titres ≥40, ≥80 and GMT) and to that of **(b)** the neutralising antibodies (titres ≥10 and ≥20), with their 95% confidence interval.

household, literacy level, pregnancy in females, tribe, chronic illness and *other housing environment variables* (Table 2). In the final multivariate model, *working environment*, *occupation* and *wealth index (SES)* were significant risk predictors of A(H1N1)pdm09 infection. With the exception of *occupation-student* (aOR 2.2, p = 0.0075), the two others, working *in open air space* (aOR 0.4, p = 0.0253) and belonging to low SES class (aOR 0.4, p = 0.0348), were protective against the risk of infection (Table 2).

Discussion

Our results revealed that: the younger people were amongst the most affected; that certain regions of the city bore a disproportionately high risk for a pandemic burden compared with other regions and that the occupation and working environment of individuals were important A(H1N1)pdm09 infection risk predictors for Djibouti. We therefore report for the first time, the epidemiological characteristics of pandemic influenza A(H1N1)pdm09 in this nation, which is located in the WHO Eastern Mediterranean Region (EMR). EMR is comprised of 22 countries, including Djibouti (see introduction) and has little available epidemiological information for A(H1N1)pdm09, particularly relating to morbidity (incidence), mortality (fatalities) and sero-prevalence (sero-surveillance). The EMR is in the northern hemisphere. According to recent studies, the global pandemic occurred in two major waves in both the southern and northern hemispheres [4]. In many parts of the world, the first wave occurred between May and November 2009, followed by a gap and overlap by an ascending second wave that ran from October 2009 to February 2010 [4,26]. Once introduced, the A(H1N1) pdm09 virus spread rapidly across the country for 15 to 20 weeks, thereafter transmission was sustained at a much lower intensity [27]. This strain had the ability to predominate and replace other seasonal strains like the H3N2 and seasonal H1N1, with few exceptions.

As documented in temperate countries [27], in EMR the two waves seem to have followed and peaked in cold times during winter, but their peak magnitude varied between countries. The first pandemic wave is presumed to have begun with the reports of the first cases in the region, which were in Kuwait in May 2009 and in the United Arab Emirates (UAE) in July 2009 [28,29], before spreading to other member states. Once infection was

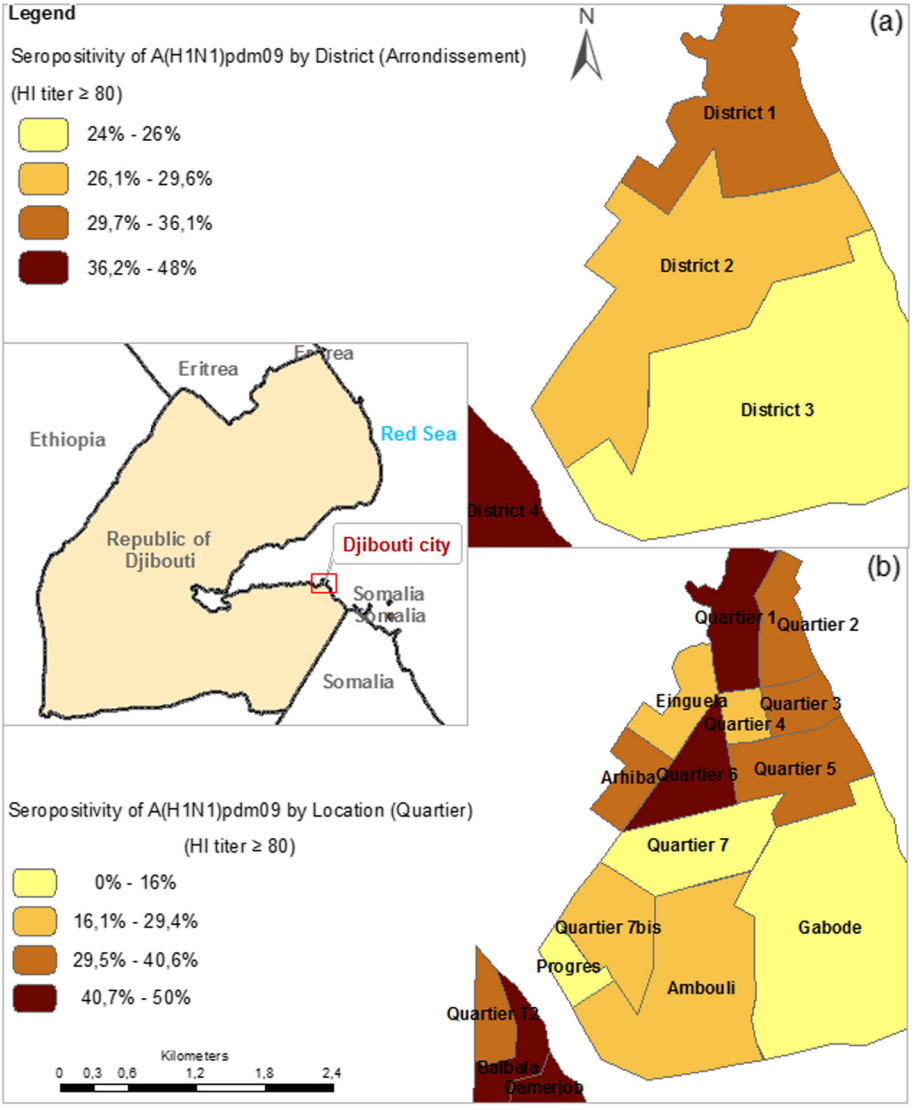

Figure 4 Map showing the geographical location of the study area, the Djibouti city within the republic of Djibouti and the prevalence of A(H1N1)pdm09 in administrative Districts (a) and locations (b) and as determined by a titre of ≥80.

introduced to these two nations it gradually progressed to allow community transmission, peaking in August 2009 for UAE, and in October 2009 for Kuwait [28,29]. The second wave was more pronounced and better documented than the first, probably due to more awareness of the infection. It begun in late October 2009, peaked in December 2009 and January 2010 and then declined from February to March 2010 [3], with a lag in Afghanistan, Iraq and Oman [3,26]. This corroborates the sentinel data in Egypt and Morocco [30], which confirmed that the peak of A(H1N1)pdm09 incidence occurred between November 2009 and January 2010. Similarly, to Djibouti's neighbour, Ethiopia, which reported its first two cases in June 2009 (first wave), followed by a lapse until early 2010 (second wave), when more cases were noticed [31]. Although this scenario was acceptable to the regional WHO EMR office, it should be considered carefully since countries with functional surveillance systems were likely to detect and report the pandemic circulation earlier than those without these surveillance systems.

The EMRs official statistics of 24th October 2009, indicated 17,150 incidences and 111 fatalities [32]; and later, as of 30th April 2010, reported 1,059 fatality cases [26]. Amongst the 1,059 fatalities reported, Egypt (277), Iran (147), Saudi Arabia (128), Syria (138) and Morocco (64) had the highest proportion in 20 of the 22 member states involved [26]. At the country level, limited Information was available from Djibouti, with only nine laboratory

Table 2 List of predictors of influenza H1N1pdm09 infection amongst Djibouti city residents

	Population characteristics	Univariate analysis (OR-odd ratio)			Multivariate analysis (adjusted aOR-odd ratio)		
		OR 95% CI	Std error	P-value	OR 95% CI	Std error	P-value
1	Age group (0-14 yrs)	1.5 (1.1-2.2)	1,18	**0.0105**	0.8 (0.1-6.1)	2,80	0,8413
	15-24 yrs	2.2 (1.5-3.3)	1,23	**<.0001**	1.2 (0.5-2.9)	1,55	0.6189
	25-54 yrs	Ref	Ref	Ref	Ref	Ref	Ref
	55-64 yrs	0.3 (0.3-1.5)	1,58	0.3002	0.4 (0.4-4.0)	3,26	0,4256
	>65 yrs	1.3 (0.5-3.3)	1,58	0.5339	-	-	-
2	District (1)	1.3 (0.8-2.0)	1,26	0.2500	2.0 (0.5-5.5)	1,67	0,1822
	2	1.2 (0.8-1.9)	1,26	0.3453	1.7 (0.7-4.0)	1,57	0,2668
	3	Ref	Ref	Ref	Ref	Ref	Ref
	4	**2.9 (1.4-5.8)**	1,42	**0.0027**	1.9 (0.7-5.5)	1,72	0.2358
3	SES class(Low)	0.6 (0.4-1.1)	1,32	0.1142	0.4 (0.2-0.9)	1,53	**0.0348**
	Upper	0.7 (0.4-1.2)	1,31	0.2347	-	-	-
	Middle	Ref	Ref	Ref	Ref	Ref	Ref
4	Household size: Large (≥5 persons)	**0.6 (0.4-0.9)**	1.20	**0.0105**	-	-	-
	Medium (3–4 persons)	0.8 (0.5-1.3)	1.30	0.3102	-	-	-
	Small (≤2 persons)	Ref	Ref	Ref	Ref	Ref	Ref
5	Occupation (<13 yrs and not student)	0.9 (0.5-1.5)	1,34	0.6157	-	-	-
	Employed	0.5 (0.3-0.8)	1,27	**0.0067**	-	-	-
	Students	**1.4 (1.0-2.0)**	1,18	**0.0313**	2.2 (1.2-3.9)	1,34	**0.0075**
	Jobless	Ref	Ref	Ref	Ref	Ref	Ref
6	Gender (Man)	0.9 (0.7-1.2)	1,16	0.5588	-	-	-
7	Have waste bin	0.7 (0.5-1.1)	1,23	0.1570	-	-	-
8	Live nearby large dumpsite	0.7 (0.4-1.1)	1,28	0.1264	-	-	-
9	Live nearby industry	4.8 (0.5-41.3)	2,99	0.1521	-	-	-
10	Live nearby river banks	**2.5 (1.2-5.1)**	1,44	**0.0119**	-	-	-
11	Live nearby small dumpsite	0.6 (0.4-1.1)	1,30	0.0883	-	-	-
12	Khat use	0.7 (0.5-1.1)	1,22	0.1381	-	-	-
13	Smoking	0.7 (0.5-1.0)	1,23	0.0826	-	-	-
14	Chronic illness	0.9 (0.5-1.6)	1,33	0.6938	-	-	-
15	Recent Respiratory illness	1.0 (0.6-1.7)	1,30	0.9554	-	-	-
16	Have children in household	1.2 (0.8-1.7)	1,20	0.3973	-	-	-
17	At night may sleep out, in the open	1.4 (0.8-2.6)	1,34	0.2154	-	-	-
18	Working in indoor space area	0.6 (0.3-1.1)	1,35	0.1132	-	-	-
19	Working in open space area	**0.4 (0.2-0.8)**	1,43	**0.0105**	0.4 (0.2-0.9)	1,44	**0.0253**

Estimation used generalised estimating equations. Predictors in univariate analysis with p-value ≤0.25 were included in the multivariate analysis. Seropositivity was based on an HI titre cut-off of ≥1:80. Predictors with p-value >0.05 were considered significant and are presented in bold.

confirmed cases and zero deaths cited in both reports. Unpublished influenza and influenza like illness data suggest that Djibouti city might have experienced a lagged second wave with a steady increase from January to June and a sharp decrease in July (Dr. Ammar Abdo Ahmed, personal communication). This is, however, based on a quite limited number of cases (n = 278). We therefore observed that it was difficult to propose an estimate of the actual epidemic impact, because the different patterns reported from neighbouring countries (*e.g.*, Ethiopia [31]) indicated that incident cases might have been underreported. We attempted to provide additional epidemiological information through this seroprevalence study of the Djiboutian population, which happened in the winter of 2010 (November 2010 to February 2011).

This study had several limitations. Firstly, late sampling did not enable distinction between individuals infected during the very first wave in summer 2009 and those infected during the second wave (autumn 2009 and during 2010). The general assumption was that the

infection occurred between the winter of 2009 (November 2009 to February 2010) and that of 2010 (November 2010 to February 2011), with the exception of subjects over 65 years old who could have been exposed to pandemic antigenically related strains before [5,6]. As a result, the study could only provide a rough cumulative estimate of the influenza A(H1N1)pdm09 burden during years 2009–2010. Secondly, our study potentially underestimated the prevalence of pandemic influenza since the cohort data collection ended one month before the end of the winter season. However this underestimation was unlikely to have any significant effect on epidemiological characteristics such as age, spatial distribution and risk factors. Thirdly, in the absence of pre-pandemic samples, sero-prevalence data were used as a surrogate for seronconversion data to estimate the number of infected cases. Of note is that this approach has been validated to be a robust estimation of seroconversion rate as is evident from previous studies [15,16]. Fourthly, the opportunistic study of individuals recruited from the 2009 Hajj Pilgrim and community health (social) workers databases cannot claim to provide a representative picture of the Djiboutian population. Hypothetically, we might have compromised the participation of the middle class households since the Hajj Pilgrimage to Mecca is costly and only those amongst the high-income bracket can afford to do it, and, conversely, it is the majority of low-income persons who depends on the community health (social) workers service. These two groups are therefore likely to have different exposures to infection; and international travel and participation in the Hajj could have elevated the risk to pandemic influenza to those who were involved [33]. However, with regard to influenza epidemiology, such a bias was expected to have a limited impact on the estimate of the global number of cases in the general population [34].

In our study, which happened soon after the second pandemic wave (November 2010- February 2011), we had a 29.1% overall seroprevalence and with most occurring amongst the young, 35.1% in 0–4 years old and 34.5% in 5–19 years old. These observations are consistent with the recent meta-analysis estimation of ten studies from nine countries (of Asia, Africa, Europe and North America) [4], which indicate that the overall prevalence, in the same period, was 32% (95% CI 26-39%) and more occurrences were in 5–19 year olds at 47% and in 0–4 year olds at 36%, as was the case of Djibouti [4]. We also compared the prevalence (34.4%) amongst young Djiboutian <20 years with two other countries, Laos [13] (20.8%) and Mali [15] (19.5%) that applied the study protocol. A brief overview of the EMRs status, based on the first 500 reported cases, indicates more young people (29 years) were disproportionately affected in the first wave and accounting for up to 59.6% of all incidences [26]. However, precise comparison with findings from other regions is hampered by the limited information reported [26], and variations in the occurrence of the first and second waves [35,36]. Our findings suggest that the country had a comparable higher disease burden than many of the other documented countries. This therefore usefully complements data from the Djibouti Ministry of health, the regional health agencies, the WHO and the CDC records.

In the risk assessment for A(H1N1)pdm09 infection, our results indicated that the seropositivity was significantly associated with individuals' age (<25 years), occupation (student), place of residence (District 4) and the living environment (living near to a riverbank). As mentioned earlier, people under 25 years old were immunologically naïve and vulnerable to infection with the new virus [5,6]. Amongst the elderly, cross-reactive antibodies against the 2009 pandemic influenza virus are likely to have originated from infections caused by the 1918 Spanish influenza virus and antigenically related descendants as suggested by haemagglutinin sequence comparison [6]. Similarly, students may be at specific risk of infection because of their age class and as a result of their close social interactions within the school environment that potentiates rapid spread of ILI [6]. By residential location, we observed that District 4 had the highest risk for infection compared to those residing elsewhere. This region is geographically separated from the three others, districts 1, 2 and 3, by a seasonal river. This population is largely made up of refugees or illegal migrants fleeing the civil unrest in neighbouring countries, living in close proximity and have high material deprivation. Such characteristics in part contributed to their underrepresentation (and that of the district) in our cohort, due to the very restrictive household enrollment protocol applied. In spite of this, the overall picture of seropositivity distribution by age group and risk factors was unlikely to be biased. Because, the age group proportions of the district was not significantly different from those of the three other districts. Whilst low wealth index alone was not associated with seropositivity (see below), it is possible that specific characteristics of this vulnerable population may represent an increased risk of infection [34]. Besides District 4, individuals living close to the River Balbala valley banks (see Figure 4, comprised of some residents from *District 2, District 3* and *District 4)*, had a three times greater risk of infection than those living further away. We have no specific explanation for this observation.

Of note is the observation of significant protection against the A(H1N1)pdm09 infection by those people having a low wealth index, large household size, being working class and working in open air spaces. A possible explanation is that the Djiboutian people belonging to a low wealth index class could have had limited exposure to the new pandemic strain, hence the lower incidence [34], a situation mostly associated with relative social isolation

compared to other groups such as the working class who would benefit from better living standards, access to medical treatment and information. Similarly, people working in areas which are well ventilated would have limited chance of sustained air contamination from infectious aerosols. According to Kieffer *et al.*, the lower risk amongst households with more members was attributed to the overcrowding, which results in reduced times that one member spends face-to-face with another, thereby decreasing exposure times between individuals, and thus limiting the infection risk [13].

Conclusion

Despite the limited number of incident cases detected by the surveillance system, A(H1N1)pdm09 virus circulated broadly in Djibouti in 2010 and 2011. Age-group distribution of cases was similar to what has been reported elsewhere, with youths at the greatest risk of infection. Future respiratory illness control should therefore be tailored to reach specific and vulnerable individuals such as students and those working in groups indoors. It is concluded that the lack of robust data provided by surveillance systems in southern countries could be responsible for the underestimation of the epidemiological burden, although the main characteristics are essentially similar to what has been observed in developed countries.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AF: field work, lab analysis, statistical analysis, and first draft manuscript writing; CP: statistical analysis, and manuscript writing; KA: field coordination, data management; SN: lab analysis; AAA: field coordination; FA: study design; and DLX: study design, and coordination. All authors read and approved the final draft.

Acknowledgements

The authors thank all three Djibouti CoPanFlu Program partners and personnel's. In particular, Regine Ducos, Maud Subtil, Sarah Kitar, Ilana Levin and Imane El Hamdi from EHESP Rennes. The authors also acknowledge contributions from Moulid Barkhat, Basra Ibrahim and the entire field research team from Djibouti Health Ministry and Institut National de Santé Publique (INSP), who facilitated the fieldwork operation and adaptation of the core protocol to the local situation. The authors are grateful to Nathalie Leblanc and Marie-Thérèse Aristodemo for their field support and assistance. AFN was financed by a doctoral fellowship awarded by EHESP. Finally, the authors are grateful to *Professor Ernest Gould* from the Centre for Ecology and Hydrology in Oxford, United Kingdom for the grammatical correction of the manuscript.

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Received: 21 October 2013 Accepted: 20 January 2014
Published: 27 January 2014

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Cite this article as: Andayi *et al.*: Determinants of individuals' risks to 2009 pandemic influenza virus infection at household level amongst Djibouti city residents - A CoPanFlu cross-sectional study. *Virology Journal* 2014 **11**:13.

CHAPTER 5

ARBOVIRUSES AND HEMORRHAGIC FEVERS

Arboviruses are RNA viruses transmitted by hematophagus arthropods such as mosquitoes, ticks, sandflies etc. Infection by these pathogens may result in severe human diseases of encephalitis and hemorrhagic fevers or mild form associated with transient fevers and poly-arthritis.

These viruses belongs to seven families, namely, the; (a) *Togaviridae family* - alphaviruses (Alphavirus, one of 2 genera); (b) *Flaviviridae family* (Flavivirus genus); (c) *Bunyaviridae family* (Bunyaviruses, nairoviruses and Phlebovirus genera); (d) Reoviridae family (Orbiviruses genus); (e) *Rhabdovirus family* (vesiculoviruses genus); and the (f) *Orthomyxoviridae family* (thogotoviruses genus)(ICTV, 2011).

Amongst them, the most important families for the region of Africa's and Eastern Mediterranean's regional, to which Djibouti belongs, are the *Togaviridae*, *Flaviviridae* and *Bunyaviridae* families. The Flaviviruses family was given more attention here, as most of its members belong to the emerging and re-emerging zoonosis.

5.1 Characteristics of Arboviruses

The Flaviviridae are a family of icosahedral enveloped RNA viruses that infect vertebrates and frequently cause serious, often lethal, infections in humans. In **Figure 1** below is a schematic representation of flavivirus particles (a), their genome organization (b) and the viral life cycle (c), (Heinsz and stiasny 2006). An Immature virion has two membrane-associated proteins; prM and E, which are in a compact heterodimeric complex. The prM protein is cleaved during maturation, resulting in the rearrangement of E into homodimers, as seen in the mature virions. M protien, a carboxy-terminal cleavage product of prM remains, proximal to the viral membrane. These surface proteins usually packed tightly around the virus particles, see **Figure 1a**. According to Stiasny and Heinz (2006), the flavivirus genome is a positive-stranded RNA of approximately 11000 nt and has a single, long ORF (open reading frame) that encodes the structural proteins C, prM/M and E and seven non-structural proteins. This ORF has on both ends non-coding regions (NCRs), see **Figure 1b**. A detailed genomic function and characterisation is provided for in **Figure 2** below.

The replication cycle of Flavivirus begin with virus entry, which is receptor-mediated endocytosis process, into endosomes. The acidic pH in the endosome cause structural alterations of E protein, leading to membrane fusion and the release of the nucleocapsid into the cytoplasm. After uncoating, the positive

stranded RNA genome is translated to initiate virus replication. The virus assembly occurs in the endoplasmic reticulum (ER) and produces immature virion particles (with prM), which are then transported through the exocytic pathway in the trans-Golgi network (TGN). The acidic pH in the TGN causes an irreversible conformational change in the prM–E complex that is required for the maturation cleavage by cellular furin or a related protease. Mature infectious virion particles are finally released by exocytosis, see **Figure 1c**. (Stiasny and Heinz 2006)

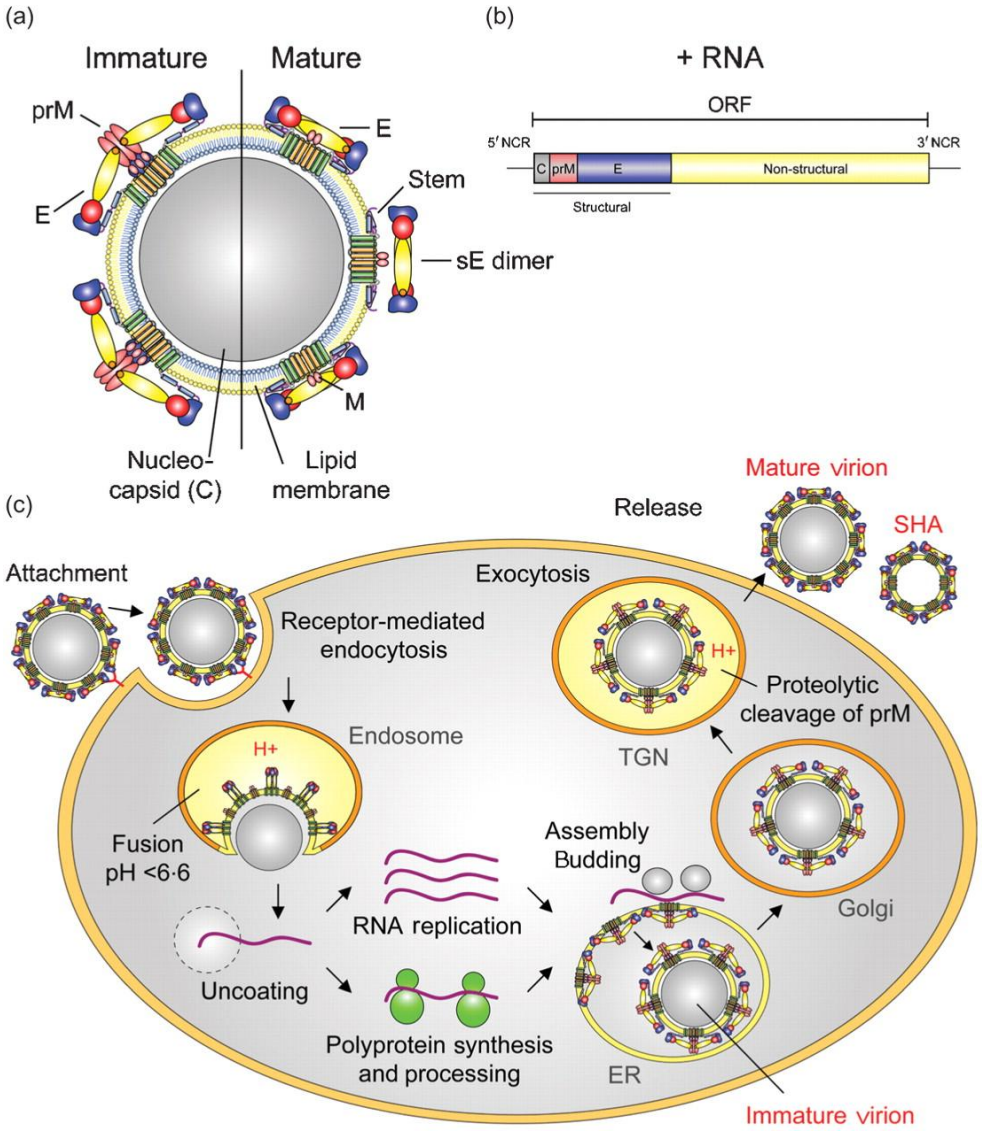

Figure 1 : Schematic representations of flavivirus particles (a), their genome organization (b) and the viral life cycle (c). (Heinsz and stiasny 2006)

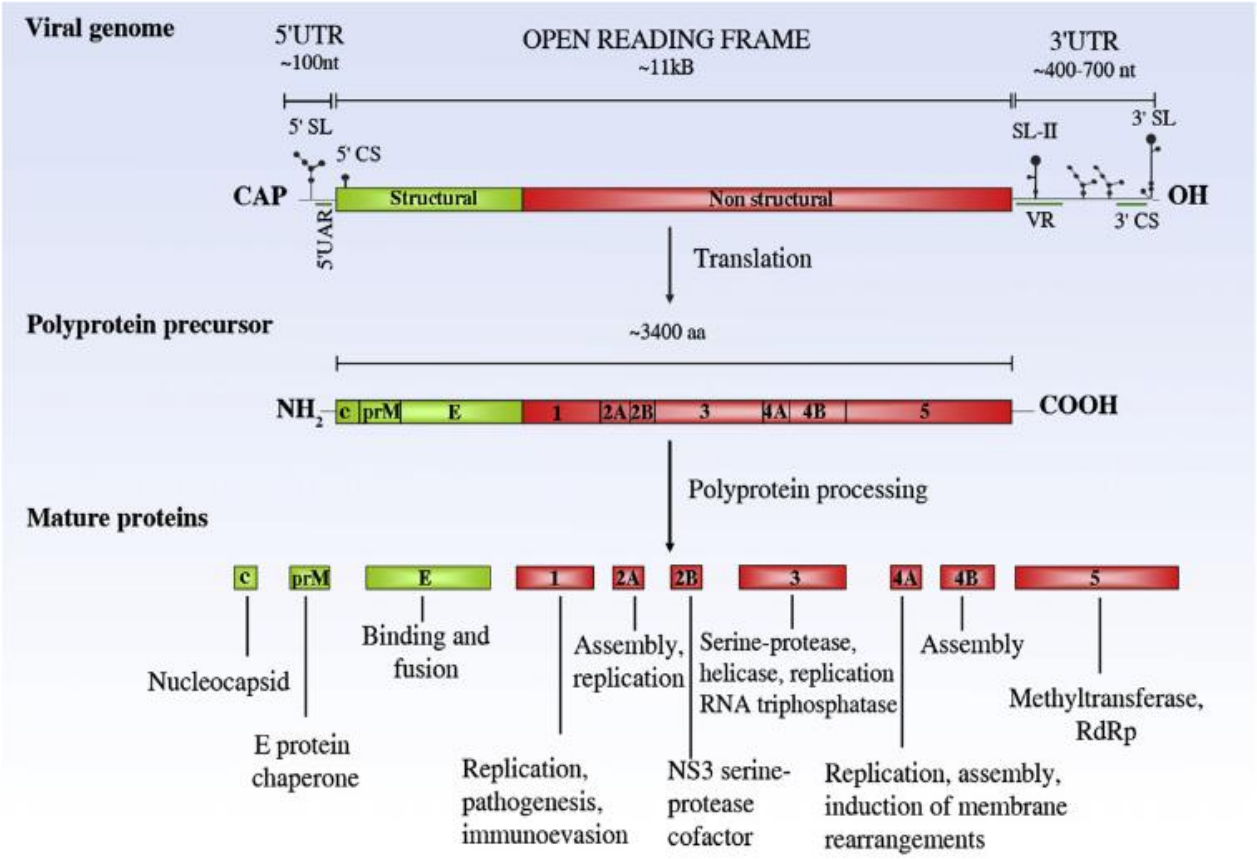

Figure 2: Flavivirus Genome Structure and functions (Fenardez-garcia et al 2009)

5.2 Transmission cycle

A lot of arboviruses are zoonotic and are maintained in an enzootic cycle involving birds, rodents or non-human primates as reservoir hosts. Human gets infected from a bite of infected Haematogus vectors such as mosquitoes. These insects acquire the virus by feeding on infected reservoirs, before transmitting to other primates (human or non-human). There are three transmission cycles known; the Enzootic (jungle or sylvatic), Rural epizootic cycle (intermediate or savannah) cycle and Urban epidemic cycle (**see Figure 3**). The enzootic (sylvatic) cycle entails the transmission of the virus between nonhuman primates (e.g. monkeys) and vector species found in the jungle. These viruses are also transmitted by vector from monkeys to humans when humans encroach into the jungle. In Africa, rural epizootic (savannah) cycle exists, which entails the transmission from vector to animals or humans living in rural, or working in jungle border areas. In this cycle, the virus can be transmitted from monkey to human or from human to human via vector. The urban epidemic cycle involves the transmission of the virus between humans and

urban vectors, primarily *Aedes aegypti*. The virus is usually brought to the urban setting by a viremic human who was infected in the jungle or savannah(Weaver & Barrett, 2004)

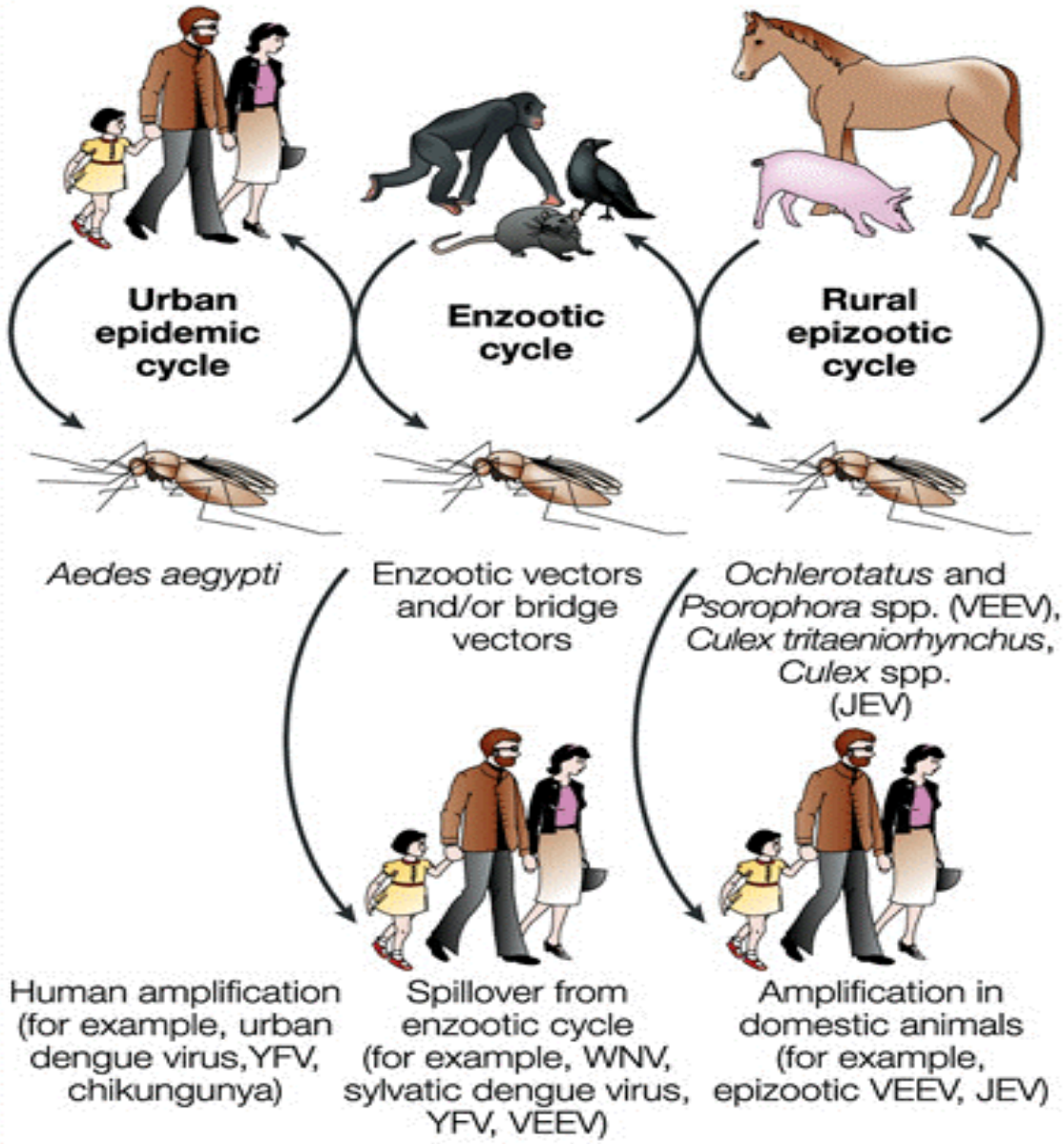

Figure 3: The three transmission cycle of arboviruses(Weaver & Barrett, 2004)

5.3 Host Range

(a) **Invertebrate Host**

Most arboviruses are maintained in nature and spread between susceptible vertebrate hosts by haematophagous arthropods; and between invertebrates' hosts by the trans-ovarian and possibly venereal means. This translocation is enabled by the virus ability to replicate efficiently in both the vertebrates resulting viremia, and in arthropods' tissues, that enable subsequent transmission (**Figure 4**).

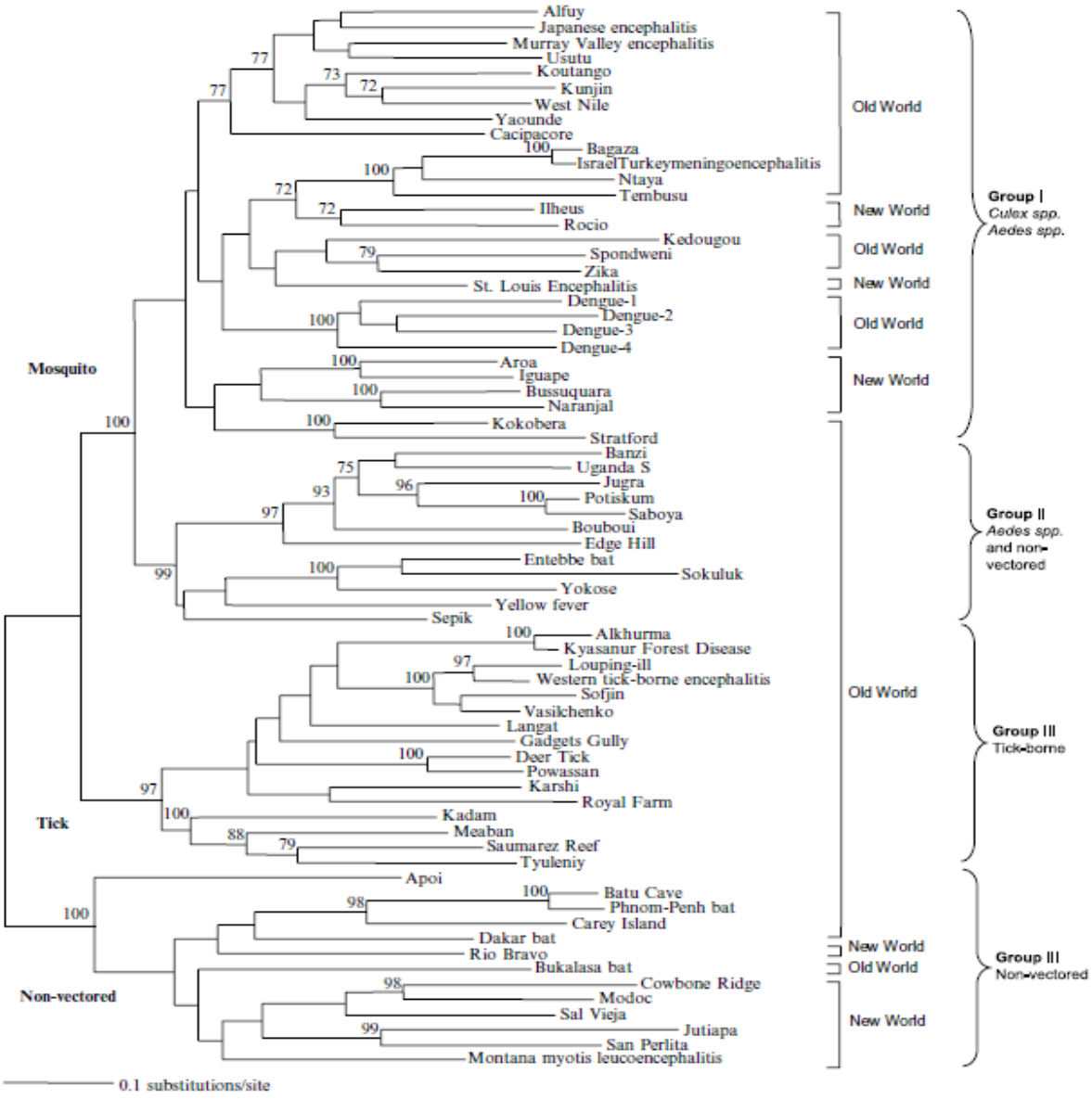

Figure 4: maximum likelihood phylogenetic tree of NS5 nucleotide sequences (990bp) from 70 flaviviruses, showing various categories by vectors of transmission (Gould et al 2003)

The vectors competence, population density, host preferences, infective biting rate and immunity are key determinants of their transmission potential (Fine 1981). Overall, the mosquitoes transmits over 50% of flaviviruses, followed by 28% ticks and the remaining 22% for no known arthropod vector (NKV) also thought to be transmitted by rodents or bats(G Kuno, Chang, Tsuchiya, Karabatsos, & Cropp, 1998).

(b) Vertebrate Host

Sustainable enzootic cycle in flaviviruses depends on vector biology, local ecology and nonhuman vertebrates host variables. Vertebrates contribution is somewhat limited to their; (a) abundance and dispersal, (b) seasonal breeding patterns, (c) attractiveness to mosquito vectors, (d) response to virus infection, (e) activity patterns, and (f) immune status(Ernest a Gould, de Lamballerie, Zanotto, & Holmes, 2003). The competency to support transmission is thought to be influenced by evolution and prevailing epidemiological factors(Gaunt et al., 2001).

(c) No Known vector Viruses (NKV)

The NKV viruses' emergence and dispersal follows the geographical distribution of bats and rodents from which they were isolated from, and this seem predictable from the phylogeny tree. The two species (bats and rodents) vertebrate are social beings; they stay in large groups and have a lot of physical contacts. This behavior promotes natural transmission and sustenance between hosts, and by extension, influences the geographical distribution of NKV(Ernest a Gould et al., 2003). In support of the above observations, many studies confirms that the rodent associated viruses (and one bat associated virus) are only found in the New World, and that the bat associated viruses are exclusively found in either the New or the Old World, but not in both.

5.4 Evolution

According to Kuno et al(G Kuno et al., 1998), the flaviviruses do exists in two major clades, the vector borne and the non known vector borne (NKV) clade). In the NKV clade there three clades, those associated to the (a) bats, (b) rodents; and (c) those linked to both the mosquitoes and bats. Of the two groups, the vector clade is the largest and has two sub-clades, the (a) mosquito and the (b) tick borne. Among the Tick borne group, the viruses are either associated to the (a) seabirds or (b) rodents (which also forms the tick borne encephalitis complex viruses). Members belonging to the vector clade viruses' shares a common evolutionary history. The phylogenic reports show that the tick borne clade were of mosquitoes borne derivative lineage which diverged later in evolution (Cook and Holmes 2006). Generally, all mosquito borne clade viruses can also be classified according to their clinical feature either

as a; (a) neurotrophic viruses which causes meningo-encephalitis in both human and livestock's, and are mainly transmitted by Culex species with birds as their reservoir. Or they may belong to (b) non-neurotrophic viruses which causes hemorrhagic fevers, transmitted by Aedes species and has the primates as their natural reservoir(Gaunt et al., 2001)

5.4 FLAVIVIRIDEA FAMILY

Of the 70 species of flavivirus identified, major outbreaks are caused by a few of the species which have been identified to be of great public health importance (see **Figure 5** below). These few viruses are part of the emerging viral zoonoses in both humans and veterinary medicine. In this review, the discussion will follow **Kunos et al** (G Kuno et al., 1998)classification that follows the vector and clinical syndrome of flavivirus infections, namely the (a) mosquitoes borne viruses causing Non-encephalitis, (b) mosquitoes borne viruses causing Encephalitis, (c) Tick borne viruses causing Encephalitis (d) Tick Borne causing Hemorrhagic fevers.

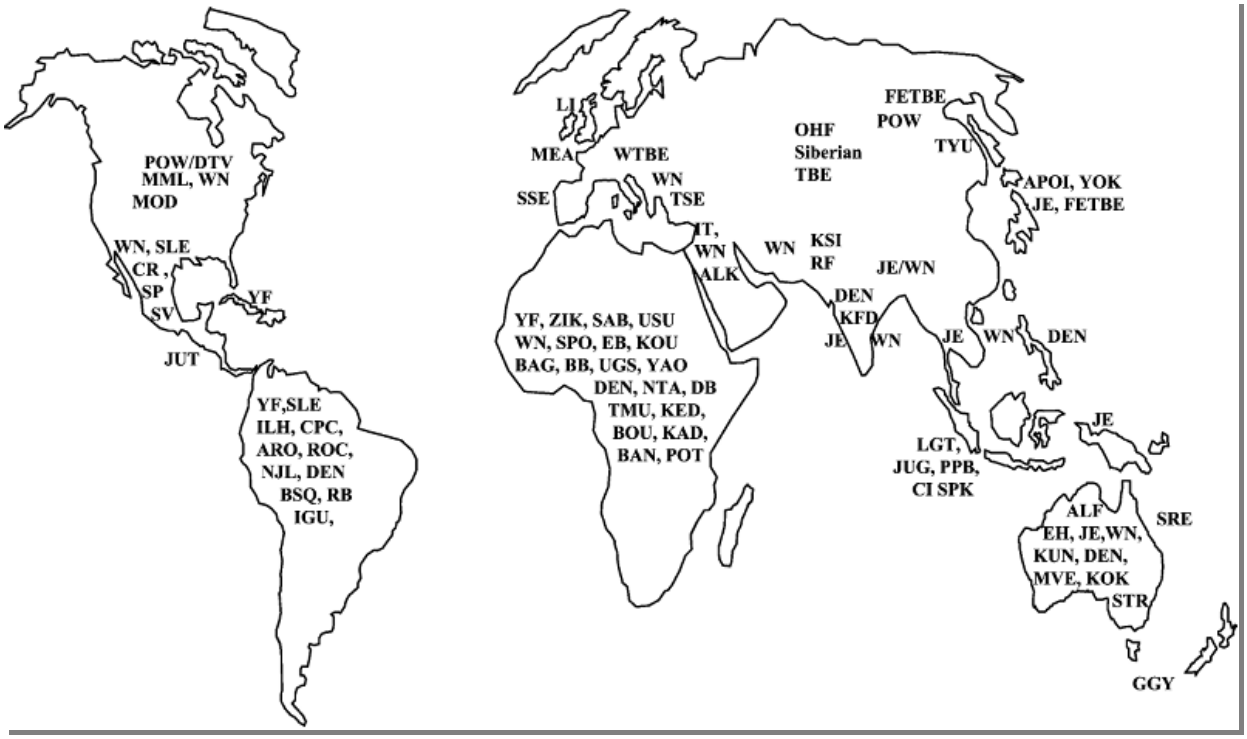

Figure 5: Approximated global location, by land mass, of recognized flaviviruses. Some viruses are shown more than once to emphasis their wide distribution (Gould et al 2003)

5.4.1 Mosquito Borne Virus Causing Non-Encephalitis

(a) Yellow fever virus (YFV)

YFV causes an acute severe hepatic syndrome characterised by jaundice in man. The virus is endemic in sub Saharan Africa, causing up to 200,000 cases and 30,000 deaths every year. Besides Africa, the South America, Central America, and the Caribbean are some of the most affected regions of the world. The pathway involves man, wild primates and *Aedes aegypti* mosquitoes in the tropical and subtropical region. This virus only escapes to infect human when they encroach the pristine forested areas in search of food, forage, expansion agricultural land and recreational activities. The infected humans, then introduce the pathogen to urban human settlement resulting in an outbreaks. In Europe and northern America the virus was probably introduced in ancient days during slave trade or in recent time through illegal wildlife trade and international transport(E. A. Gould & Solomon, 2008).

(b) **Dengue Virus**:

Dengue fever (DF) can be caused by any of the four serotypes, dengue 1-4. Infection to one serotype is not protective against the others, but instead increases the risk of severity, developing dengue hemorraghic fever (DHF) or dengue shock syndrome (DSS) if infected by another subtype. The enhanced risk is due to the Antibodies dependant enhancement (ADE) phenomenon(Rico-Hesse, 2003). Dengue is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes across the world tropics and subtropics, where it is endemic. In this region, yearly outbreaks occurs which coincide with high rainfall, high mosquito's population and large immune naive population. Dengue virus is thought to have originated in the past 100 and 800 years ago, with the four serotypes emerging from primates and spreading into humans in Africa or Southeast Asia independently. It then kept a low profile, covering a small geographical region, until 20th century when the World War II caused disruption and further spread of *Aedes spps* vector(E. A. Gould & Solomon, 2008). The Dengue severe form DHF remains rare compared to the mild form. Up to 40% (2.5billion) of the global population is at risk, including at least 100 countries from Asia, the Pacific, the Americas, Africa, and the Caribbean. Most DHF outbreaks are caused by dengue 2 serotype, whose first outbreak occurred in Philippines and Thailand in 1950, and in Caribbean and Latin America in 1980. According to the WHO, there are about 50-100 million dengue cases reported annually, among them is half million DHF cases and 22,000 deaths. Children are the most affected group(WHO, 2009).

5.4.2 Mosquito Borne Viruses Causing Encephalitis

(a) Japanese encephalitis virus (JEV)

Japanese encephalitis (JE) virus is the leading cause of vaccine-preventable encephalitis in Southeast Asia and Australasia in both humans and veterinary medicine. In humans alone up to 50,000 cases are reported annually with 25% case fatality(WHO, 2009)(E a Gould, Solomon, & Mackenzie, 2008). Its transmission cycle involves the *Culex species* mosquitoes and vertebrate hosts, mainly pigs and wading birds. Migratory birds, live animals trade and expansion of agricultural frontiers (for irrigation and rice farming) threaten to extent the geographical coverage of JEV. Human infection occurs following a viremic mosquito a bite causing an asymptomatic or result in only mild symptoms. However, a few of the cases progresses to brain encephalitis that is marked with sudden onset of headache, high fever, disorientation, coma, tremors and convulsions. One in every four encephalitis patients was likely to succumb to JEV, lack of specific treatment, other than palliative care is to blame. Vector control, vaccination and personal protection against mosquito bites are essential.

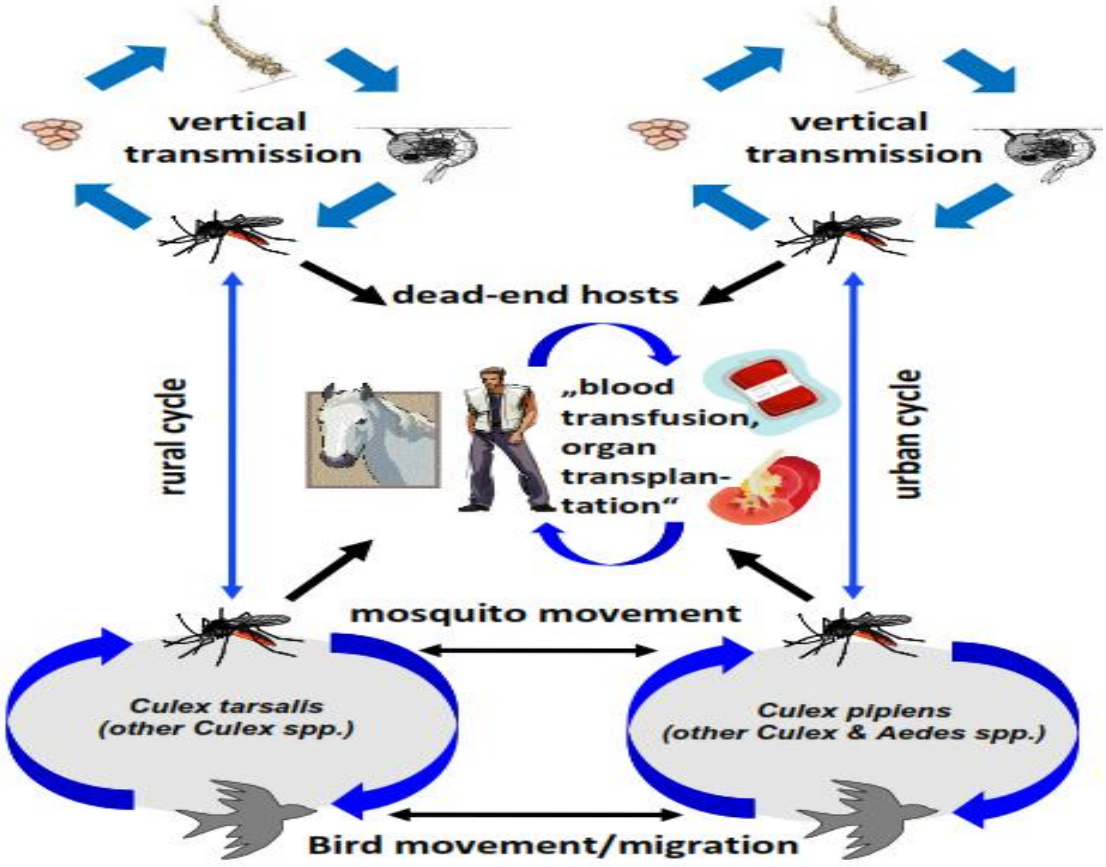

Figure 5: Schematic drawing of transmission cycle and potential dispersal modes of west nile virus (Pfieffer and Dobler 2010)

(b) West Nile virus (WNV):

WNV came from Africa and spread to the northern and eastwards by migratory birds. The diversity of WNV hosts vertebrates and vectors are the major contributor to its wider and ever increasing dispersion (see **Figure 5 and Figure 6** above). Major outbreaks in humans, birds and horses have been reported in Africa, Europe, Russia, India, USA and Australasia.

Outbreaks often occur in warm humid summers. In the 1999 there was a huge epidemic in the USA in both human and horses, 62 incidence cases and 7 deaths were confirmed, most severity occurring among the elderly. It's presumed the American WNV originated from the Middle East courtesy of migratory bird. On arrival, the vectors shifted feeding habit from birds to human after infection, this in part contributed to the human epidemic. Subsequent outbreaks in summers of 2004-2005 and 2011-2012 have also been reported.

Figure 6: Global distribution of west nile virus since 1960, those countries that have experienced outbreaks are shown in gray (Chevalier et al 2004)

5.4.3 Tick Borne viruses causing Encephalitis

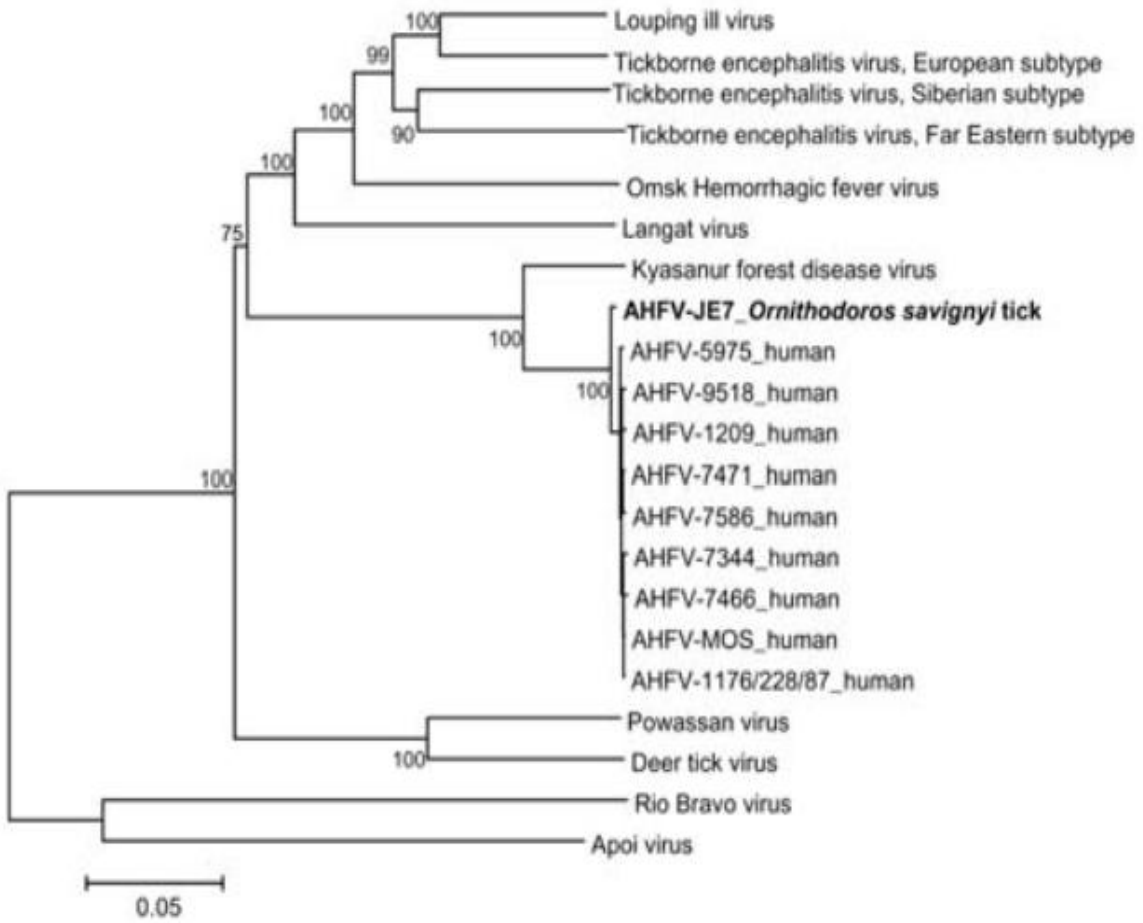

Figure 7: Phylogenetic analysis of AHFV-JE7 (shown in **boldface**)

AHFV-JE7 was detected in an Ornithodoros savignyi tick and homologous sequences of related mammalian tickborne flaviviruses based on colinearized nucleotide sequences (Charrel et al 2007)

(a) Tick born encephalitis virus (TBEV)

The TBEV outbreaks are serious public health problem in Europe and Russia, accounting for up to 3,000 and 10,000 cases annually, respectively. Three known subtypes of TBEV varies in locality, clinical presentation and virulence, the (a) European subtype, predominantly in Europe; the (b) Siberian subtype, found in Urals, Siberia and far-eastern Russia; and the (c) Far Eastern subtype, found in far-eastern Russia, China and Japan(Mansfield et al., 2009). Clinically, the Far Eastern subtype causes severe encephalitis (focal meningoencephalitis or polyencephalitis) that kills 5-35% of the subjects. The Siberian subtype is chronic, less severe with varying neuropsychiatric symptoms and with 1-3% case fatality rate. The last strain, the European subtype, causes acute biphasic disease that begins with

atypical influenza like illness and end with severe meningoencephalopathies (Mansfield et al., 2009). As mentioned above, TBEV is endemic in Europe, Russia and Asia. Forests, moorlands, or Steppe areas supports competent host tick vector survival, rodents and migratory birds that sustain transmission cycle(E. A. Gould & Solomon, 2008). Recent study indicates TBEV has been on the raise in Europe, from 1974 to 2003, over 400% cases increase was reported(Süss, 2008). Advances in diagnosis and surveillance in this region is possible a factor to explaining this surge. Other factors include the increase of colonisation by ticks into new territories as primed by the global warming and climate change; the socio-political changes; and deforestation for agriculture or habitation. Application of integrated vector controls measures, wide use of vaccine and active surveillance has resulted in slowing down the trend, much so in Europe but less in Russia and Czech Republic. Of interest too is the overall geographical pattern of virus virulence. TBEV Fatalities decreases westwards; from Japan and far-eastern Russia, at 40%, through central Europe to Western Europe, at <1% (Mansfield et al., 2009).

5.4.4 Tick Borne viruses causing hemorrhagic fever

(a) Omsk haemorrhagic fever (OHFV)

OHF is endemic in Russians' Western Siberia regions of Omsk, Novosibirsk, Kurgan and Tyumen, where it causes seasonal acute viral syndrome, characterized by flu-like illness to moderately severe hemorrhagic manifestations. Pneumonia, nephrosis, meningitis or both may also occur in 30% of patients(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007). There is no vaccine at the moment. However, palliative treatment of the sick, use of partial cross-protective TBEV vaccine in high risk and personal protection from biting insects in ticks' endemic regions are recommended. Large epidemics, localised outbreaks, and sporadic cases of OHF occurs, with 97% arising from northern forest-steppe regions. From 1946 to 2000, estimated 1344 cases were reported with 0.5% to 3% case fatalities (http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/omsk.htm. accessed on 10/10/2013). OHF has a wide host range that includes humans, ticks (*Dermacentor reticulatus*, *Dermacentor marginatus*, *Ixodes persulcatus*), mosquitoes (*Aedes flavenscens*, *Aedes subdiversus*) and rodents; muskrats (*Ondatra zibethicus*), water vole (*Arvicola terrestris*) and narrow-skulled voles (*Microtus gregalis*), that supports the enzootic cycle(Gritsun, Lashkevich, & Gould, 2003). Muskrats, was introduced from Canada in 1930s, and was of great significant to virus amplification in Siberia. Experimental and observational studies suggests that most human cases arise from infected tick bites, infected goat milk, virus contaminated water and or exposure to infected sick or dead rodents' to blood, feces, or urine (PHAC 2013 http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/omsk-eng.php . accessed on 10/10/2013)

(b) Kyansur Forest Diseases (KFDV)

Kyasanur forest disease (KFD) is a zoonotic febrile illness that was first identified in Shimoga, Karnataka state, southern India in 1957. Initially, it started as typhoid like illness outbreak around the forested Kyasanur areas that tested negative for widal. A follow up to outbreak investigation revealed that Humans and the two monkeys species of *Presbytis entellus* (Black faced langur) and *Macaca radiate* (Red faced bonnet monkey) to have been most affected. This also led to the first isolation of the virus from the monkeys' necropsy viscera and attaching *Haemaphysalis* spps(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007). There is new evidence indicating transmission seasonality which corresponds to active *Haemaphysalis spinigera* (forest tick) nymph activity in warm weather. While several rodents' species, such as porcupines, rats, squirrels, mice and shrews are thought to be natural reservoirs(Dobler, 2010). Monkeys and humans are most severely affected compared to most domestic and wild animals, which only experience mild subclinical manifestation. In human, clinical case of KDF is acute and biphasic syndrome lasting 1-2weeks. The Infection begins with chills, headache, fever, severe myalgia, cough, diarrhoea, vomiting and photophobia; and then may progresses to hemorrhagic fever and death or protracted recovery. Some of the patients may develop meningo-encephalitis then coma or bronchopneumonia prior to death. Case fatality rate is estimated at 2–10% and is estimated to affects 100-500 people annually(E. A. Gould & Solomon, 2008). No treatment exists, however, the use of inactivated KDF vaccine which has 70% protection and employing personal protective against biting insects. KFDV antibodies' cross reacts with members of Russian spring summer encephalitis (RSSE) virus group to which they belongs to the same serogroup. KFDV and Alkhurma (AHFV) share 89% sequence homology, suggesting common ancestral origin, and serological cross reaction suggests they are variants of the same pathogen(R N Charrel et al., 2001).

(c) Alkhurma virus (ALKV)

Alkhurma viral hemorrhagic fever virus (AHFV) is an endemic flavivirus in Saudi Arabia, causing febrile illness outbreaks associated with hemorrhagic and neurological manifestations. As mentioned above, AHFV is closely related to the Kyasanur Forest disease virus (KFDV), of which they shares up to 89% nucleotide sequence homology(R N Charrel et al., 2001). Based on phylogenic studies estimates, the two pathogens evolutionary diverged about 700years ago(R N Charrel, Zaki, Fagbo, & de Lamballerie, 2006). Since 1990 when first identified in Saudi Arabia, several outbreaks have been reports among animal industry workers and in hajji pilgrims. Overall case fatality rate has been estimated at 30% with male

youth of 24-39 years being the most affected. Upon exposure, human develops fever, headache, joint pain, muscle pain, vomiting and thrombocytopenia that progresses to hemorrhagic fever and encephalitis, and may be fatal(Madani & Tariq, 2005). AHFV mainly depends on camels and sheep as host reservoir to sustain their enzootic cycle. Bites from infected soft tick *Ornithodoros savignyi* and the hard tick *Hyalomma dromedarii*on allow the transmission to man. However, oral entry may occur through ingestion of un-pasteurized camel milk or mechanically entry via a skin wound contact(R N Charrel et al., 2006). There are a few cases reported outside of Saudi Arabia, such that of tourist returning from Egypt(Carletti, 2010). A wide geographical distribution of competent vectors in Persian Gulf region(R N Charrel et al., 2006), suggests a possible countrywide and region-wide AHFV distribution. This is of great public health concern. Other viruses genetically related to AHFV virus but with undefined public health implications are the Karshi virus (KSIV) and Royal Farm virus (RFV). These two,circulates in the Middle East (Uzbekistan and Afghanistan) and are thought to have hemorrhagic fever syndrome potential.

5.5 BUNYAVIRIDAE FAMILY

First isolated in Uganda during yellow fever study in 1943 as Bunyamwera, but later came to form Bunyaviridae family in 1975. This new family consists of four genera of arthropod transmitted animal-infecting viruses (Orthobunyavirus, Phlebovirus, Nairovirus, and Hantavirus genera) and one genus of plant-infecting viruses (Tospovirus). Hantaviruses was an exception, shown to be rodent-borne and aerosol transmitted vai rodent excreta.

***Orthobunyavirus Genus*:** Has over 150 viruses belonging to this genus, most of who are transmitted by mosquitoes (particularly, *Aedes species*) and amplified in a variety of vertebrate hosts. It is now confirmed that most of Orthobunyavirus genus members are endemic in sub-Saharan Africa, causing cases of febrile illness. There several serogroups in this genus known to exist, the *bunyamwera serogroups, Simbu serogroup viruses, group C, California serogroup* and *La Crosse virus serogroup*. Viruses in this genus infect both animals and humans, and are widely distributed across the globe.

Phlebovirus Genus: Phleboviruses are found in most parts of the world and are transmitted by diverse vectors, with Phlebotomine sandflies being the main one. Exception is for Rift Valley fever virus (RVFV), an important pathogen medically and agriculturally in Africa, and Uukuniemi virus (UUKV), which are transmitted by *Aedes species* mosquitoes and tick *Ixodes ricinus*, respectively. Of great medical importance

are the RVFV that cause large RVFV epizootics in various areas of sub-Saharan Africa; and phlebotomies fever viruses, Toscana Viruses (TOSV).

Nairovirus Genus: In this genus, the transmission is almost exclusively tick-borne, but a few isolations have been made from culicoides flies and mosquitoes. The most important pathogens are the Crimean-Congo hemorrhagic fever (CCHF), Hazara virus, the Nairobi sheep disease and Dugbe viruses.

Characteristics of Bunyaviruses

Members of Bunyaviridae are enveloped single stranded RNA viruses, with 3 segmented negative sense genome. It measures 90 to 100 nm in diameter and contains 4 structural proteins: 2 external glycoproteins (G1 and G2), nucleocapsid protein (N), and a large protein (L)-presumed to be a transcriptase(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007)

(a) Rift valley fever Virus

RVFV ecology and epidemiology is yet to be completely understood. The virus originated in Kenya and is now endemic in much of Africa and Arab peninsula (see **Figure 8** below). The periodic epizootics occur in southern and eastern Africa and follow the unusually high precipitation, which supports high *Aedes* mosquito population in flooded plains (dambos).

The virus is transmitted transovarially and is thought to stay inert in dry season in laid eggs prior to rainy season. The infected mosquitoes will then feed on nearby livestock to re-introduce the virus in circulation, resulting in an outbreak. The viremic animals, eventually amplifies the spread by introducing the virus to Aedes and other species of mosquitoes including *culicines* and *anophelines* in other flooded plains. The resultant impact is a widespread outbreak in livestock producing areas, marked with stormy abortion among the livestock,

and may result in hemorrhagic fevers incidence in humans. Less than 1% of human's infection results in severe syndrome. The risk of human infection is much more associated livestock occupational hazard, such as assisting birth or abortions of livestock, butchering animals, abattoir workers, than the mosquitoes bite(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007).

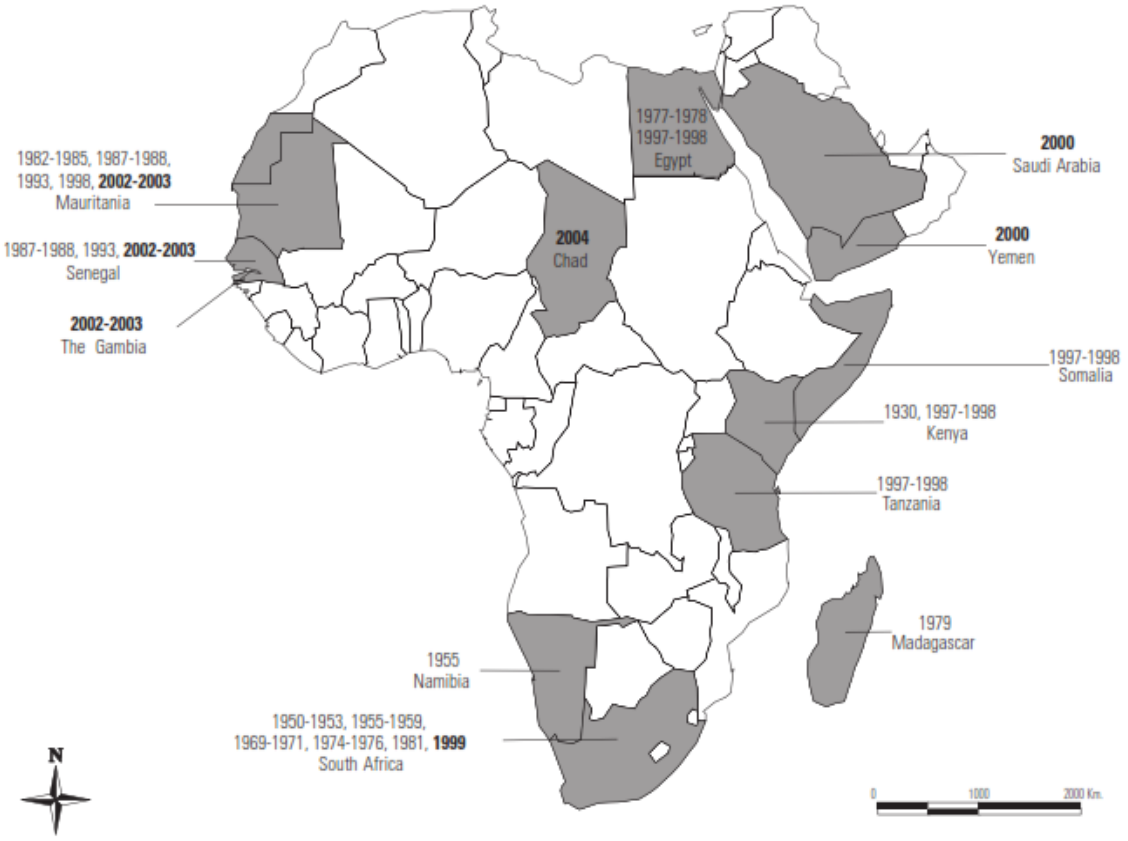

Figure 8: Rift valley fever outbreaks in Africa since 1930, recent episodes are in bold (Chevalier et al 2004)

(b) Toscana viruses

The sandflies vectors, particularly the *Phlebotomus papataci* geographical distribution directly determine the prevalence of Sicilian virus, sandfly fever Naples virus and Toscana virus. These viruses are endemic around the Mediterranean basin, Middle East, Arabian Peninsula, northwards Caucus Mountains, and toward Pakistan and India. The vector population increases in warm period (in temperate climate) and in winter period in ASALs areas. Sandflies are low flying, twilight feeders and their tininess enables them to pass through mosquito nets, making it difficult to contain them by convectional means. Outbreaks occur in summer, causing acute lymphocytic meningitis and meningoencephalitis syndrome especially among children. Many of the cases comes from central Italy, Cyprus, Portugal, and Spain, with the prevalence study showing that 20-25% of the population having antibodies against the virus. The TOSV virus is transmitted transstadial, transovarial or sexually among the sandflies. Vertebrates are amplifying hosts

that maintain the viruses in endemic areas, and the viremic mammals easily infect competent vectors. Attack rate in urban outbreaks has been reported to reach 75%(Alkan et al., 2013; Rémi N Charrel, Gallian, et al., 2005)

(c) Crimean-Congo hemorrhagic fever (CCHF)

CCHF is human tick borne arboviruses with the widest geographical distribution of all medically important arboviruses. Animals become infected by the bite of infected ticks and the virus remains in their bloodstream for about one week after infection, allowing the tick-animal-tick cycle to continue when another tick bites. Although a number of tick genera are capable of becoming infected with CCHF virus, ticks of the genus *Hyalomma species* are the principal vector. CCHF causes hemorrhagic fevers, with very high morbidity and mortality; the case fatality rate may exceed 80%.

Prior to 1970, most cases were limited in the former USSR, mainly in Crimea, Astrakhan, Rostov, Uzbekistan, Kazakhstan, Tajikistan and Bulgaria. Later, the virus was detected across the globe, to include Africa, Asia, Middle East and parts of Europe. After first outbreaks in Congo and Crimea, many more outbreaks were reported in china in 1965 with a case fatality rate of 80%, in Africa in 1960s in (South Africa, Congo, Mauritania, Burkina Faso, Tanzania and Senegal), in middle East in late 1900 (Iraq, United Arab Emirates, Saudi Arabia and Oman and Pakistan). Since 2000, more outbreaks have occurred in Pakistan, Iran, Senegal, Albania, Yugoslavia, Bulgaria, Turkey, Kenya and Mauritania(Ergönül, 2006).

5.6 TOGAVIRIDAE FAMILY

Togaviruses are spherical viruses with envelope and a genome with a positive sense single stranded RNA structure. The family has two distinct genera of *Alphaviruses* and the *Rubiviruses*. The genus of Alphavirus is the largest with over 40 species, including important emerging zoonosis like *Sindbis virus*, *Eastern equine encephalitis virus*, *Western equine encephalitis virus*, *Venezuelan equine encephalitis virus*, *Ross River virus*, *O'nyong'nyong virus*, *Chikungunya* and *Semliki Forest virus*. Alphaviruses causes variety of human and animal diseases, ranging from severe form- encephalitis, moderate form- arthritis and arthralgia, and to mild form- rash and fever. The Rubivirus genus has only one member, the rubella virus, which is a common childhood vaccine preventable diseases. In absence of vaccination, severe congenital defects occur in fetus born of pre-partum infected mothers(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007).

(a) Semliki Forest virus

Semliki Forest virus (SFV) is an alphavirus endemic in sub-Saharan Africa. It was first isolation from squashed 130 female *Aedes abnormalis* mosquitoes captured from Semliki Forest in Uganda in 1942. The virus is closely related to Chikungunya, Getah, and Mayaro viruses, and is principally transmitted by *Aedes africanus*, *Aedes aegypti* and *Aedes aegypti* mosquitoes. Incidence cases in horses, monkeys, and humans' has been reported, but the natural vertebrate reservoir is still unknown. Of note, is the epizootics of equine encephalitis that occurred in Senegal in 1974, and the laboratory accident that resulted in death of scientist in 1979. SFV clinical manifestation is characterized by fever, severe persistent headaches, myalgia, arthralgia and meningoencephalomyelitis(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007).

(b) Sindbis virus

Sindbis virus (SINV) was initially isolated in Egypt in 1952 from a pool of *Culex pipiens* and *Culex univittatus* mosquitoes. Its sero-prevalence was noted in wildlife's of Eurasia, Africa, and Oceania. Human cases are limit geographically to parts of Europe and Russia. Clinical cases of sandbis virus in Finland are referred to as *Pogosta disease*, in Sweden as *Ockelbo disease*, and in Russia as *Karelian fever*; where they manifest as acute fever, rash, arthritis, fatigue, and muscle pain, that may also evolve to chronic arthritis. In endemic area, the suspect SINV should be differentiate from other alphaviruses causing rash-arthritis such as chikungunya virus (CHIKV) and Ross River virus (RRV)(David M. Knipe, PhD; Peter M. Howley, MD; Diane E. Griffin, MD, PhD; Robert A. Lamb, PhD, ScD; Malcolm A. Martin, MD; Bernard Roizman, ScD; Stephen E. Straus, 2007).

(c) Chikungunya virus

CHIKV is a member of the SFV serocomplex that causes acute crippling arthritis. It was first discovered in an outbreak in Newala District of Tanzania in 1952 as a differential diagnosis for DENV fever. It was later isolated in serum and from *Aedes spp*. and *Culex spp* mosquitoes. Subsequent outbreaks followed across India, Southeast Asia and sub-Saharan Africa. A follow up studies on outbreak revealed the virus was maintained in similar transmission cycle as that of the yellow fever, in Africa. Its sylvatic cycle involved *Ae. africanus*, *Ae. Furcifer* and wild primates; while it's urban cycle that involved *Ae. aegypti* and humans. Sylvatic cycle occurred in rural areas and the virus was sustains in low circulation throughout the year. In urban areas, outbreaks occurred in rainy season, associated with increased *Ae. aegypti* population. They are of rare but very dramatic, infecting large proportion of susceptible population in a short period, and affecting all age groups. The prolonged inter-epidemic period may occur and is suspected to be a factor of

acquired immunity in the population. Interestingly, the lack of slyvatic cycle in Asia has seen the *Ae. Aegypti* maintain the urban cycle or depend on new introduction for outbreak to occur. Cases of iatrogenic infection in Laboratory have also been reported. Several phylogeographic studies concurs that CHIKV could in fact, have originated from Africa before finally spreading to the rest of the world. The three known clades of CHIKV lineages are defined by their initial geographic endemicity, namely the (a) West African, (b) Asian and (c) East-Central-South African ECSA(Powers, Brault, Tesh, & Weaver, 2000).

5.7 Laboratory Diagnosis

The laboratory diagnosis used in arboviruses detection are broadly classified into *(a) serogical techniques, (b) tissue culture viral isolation and (c) molecular techniques*.

(a) Serology Techniques

It detection of antibodies and other humeral factors of host immune system following viral infection forms the basis of most laboratory assay/diagnosis(Goro Kuno, 2003). This may focus on erythrocytes agglutination and or chromogenic enzyme labeled antibodies as markers for positive reaction, with up to six different assay protocols in use. First group of assay entails the use of blood and other humeral respond particles agglutination based tests. Example includes the *hemagglutination inhibition test, hemadsorption immunosorbent test, complement fixation test, immune adhesion hemagglutination test, reverse passive hemagglutination test, single radial hemolysis test and indirect hemagglutination test.* Second group is that of neutralization tests, designed to measure the presence of neutralizing antibodies against suspect virus. For example, the *plaque reduction neutralization test, Micro-neutralization assay, infectious titer reduction tests* and the *metabolic inhibition neutralization test.* Third group is of Enzyme Immunoassays. They are the commonest of all lab assays. The viral antigens are attached to a surface, upon which suspect serum (with specific antiviral antibody) is applied. It's followed by the addition of the enzyme linked anti-host antibody. In the final step, enzyme's substrate is added and reacts to produces a color as a detectable signal. All the ELISA steps are punctuated by buffer washings and incubation. In practices, the Elisa protocols varies a lot and may include the following; *Indirect ELISA; Sandwich ELISA; Competitive ELISA; Multiple and portable ELISA*. Forth group is of Western Immunoblot. These techniques are mainly used to detect specific proteins, including viral antigens in sample of tissue homogenate or extract. A gel electrophoresis is utilized to separate native proteins based on their structural properties. The resultant proteins will then

be transferred to a membrane (typically nitrocellulose or PVDF), for specific antibodies staining for target proteins. Fifth group is *that of* Immunofluorescent Antibody Test assay. It uses the specificity of antibodies to their antigen to direct the fluorescent dyes to specific biomolecule of interest within a cell. It therefore allows visualization of the distribution of the targeted biomolecule through the sample under the fluorescence microscope. Sixth and the last group entail the Recent Serological Methods. Most of these assays are in experimental stages and in use in research rather than diagnostics laboratories. They include the *high throughput rapid micro-neutralisation assays; lateral flow immunochromatographic assays; microsphere immunoassay; biosensors and micro-fluidic systems;* and *autologous red blood cell agglutination assay*(Goro Kuno, 2003)

(b) Tissue Culture Technique

This method allows for specific viral isolation using artificial cultivated either in mammalian cells, tissues or organs; or in arthropod vectors or cells; or in laboratory animals (such as neonate, embryonated eggs). Among the tissue cultures systems commonly used for isolation includes the: cells (vero, BHK 21, MDCK, C6/C36 etc), whole baby animals (rats, mice, guinea pig, hamster), embryonated eggs (chicken or goose) and arthropods (ticks, mosquitoes or their larvae). The implementation of tissue culture is very expensive and may require special biosafety facilities and highly specialized personnel.

(c) Molecular Techniques

This technique is the new gold standard for pathogen identification. The assay is built to characterize, isolate, and manipulate the molecular components (DNA and or RNA) of pathogens. Example of these assays includes the *DNA manipulation, sequencing, cloning, subcloning, library construction, screening, RNA isolation and characterization, analysis of expression, cDNA synthesis (RT-PCR) and analysis, microarrays and gene chips, and Real-Time-PCR.*

The commonest assay in this category is the polymerase chain reaction assay (PCR) and has three step processes. It begins with the RNA extraction (from viral culture, serum, CSF, tissues or insect pool), followed by the amplification and the final detection. In amplification process, which occurs in a thermocycler machine, RNA extracts, primers and probes are then used produce amplicons. The amplicons in quantitative assays, are detected using dye and displayed in a sigmoid graph eg *the Real Time 5' Exonuclease Fluorogenic Assays under TaqMan or SYBR green RT-PCR protocol*, while in a qualitative assay an agarose gel electrophoresis is used eg in the *standard RT-PCR Assays.* The amplicons can farther be treated in *Nucleic Acid Sequence–Based Amplification* assay to get more specific and precise traits of pathogen, such as mutation, lineage etc

5.8 Prevention and Control

(a) Vector Control

The world health organization (WHO) proposed the implementation of integrated vector management (IVM) as a means to overcome challenges experienced by the conventional single-intervention approaches to vector control. IVM is a multi-sectoral approach involving rational decision-making process for the optimal use of resources in vector control. It aims at improving the efficacy, cost-effectiveness, ecological soundness and sustainability of disease-vector control. This does not only ensure proper vector containment but also a generalized elimination of vector borne diseases such as *malaria, dengue, Japanese encephalitis, leishmaniasis, schistosomiasis and Chaggas disease.*

Emphasis of IVM is on established five key elements, namely the (a) Advocacy, social mobilization, regulatory control for public health and empowerment of communities; (b) Collaboration within the health sector and with other sectors through the optimal use of resources, planning, monitoring and decision-making; (c) Integration of non-chemical and chemical vector control methods, and integration with other disease control measures; (d) Evidence-based decision making guided by operational research and entomological and epidemiological surveillance and evaluation; (e) Development of adequate human resources, training and career structures at national and local level, hence reinforcing capacity building.

This approach is superior, comprehensive and incorporates the traditional control methods. Of note are of clearing or drainage of vector breeding areas, use of bed nets and screens treated with a pyrethroid insecticide against nocturnally active, anthropophilic arthropods and ectoparasites. Others approach includes the application of insecticide such as Etofenprox and deltamethrin on living area surface, use of insecticide-treated clothings, wearing of protective clothing, and application aerosol insecticide. The use of electric fans, mosquito coils/vaporising mats, and smoke, ar also common. Practical insect avoidance measures, based on an understanding of vector biology and the "Biological" vector control measures are useful but with limited success(WHO, 2004)

(b) Vaccination

Vaccination is the administration of purified antigenic material to stimulate an individual's immune system to develop adaptive immunity against a pathogen, ad prevent or ameliorate severity of an infection. Antigen used for vaccines development are either a full virion or a portion of it, if full, it could be a Live attenuated or dead (inactivated). The live vaccines are generated from serial passage attenuation or reverse genetics systems, as a chimera. For chimera vaccines, a stable attenuated virus forms a framework (backbone) upon which the targeted arbovirus subunits are mounted to be expressed and

generate immune-protection(Barrett, 2001; Koraka, Martina, & Osterhaus, 2010). Example includes the use of YF as a backbone for chimera vaccine of DENGUE, JEV, WNV and TBE, or the use of dengue as back bone for TBE and WNV (Barrett, 2001; Koraka et al., 2010). In subunit vaccines, parts of the whole virion, such as E protein, RNA and cDNA, are used as antigen source for immune activation. For Inactivated Vaccines, a whole virion is treated in radiation, heat or chemical to kill live viruses for vaccine, but the problem is it has very low immunogenicity. A lot of arboviral vaccines developments are in progress due to the continuous public health threat they pose. Many of the arbovirus candidate vaccines are in clinical trial phase, but only a few has been licensed or public use, including YFV, JEV, TBEV and WNV (for horse) (Chambers & Diamond, 2003; Leyssen, Charlier, Paeshuyse, De Clercq, & Neyts, 2003).

(c) Antivirals

At the moment there is no specific antiviral therapy available for the treatment of arboviruses infections. Most of them are in experimental phase. The use of recombinant Interferon and Ribavirin-Interferon Combination has been proposed, with varied outcomes. More prospective studies on antiviral agent are now focusing on creating inhibitors at various stages of viral cycle such as *viral entry inhibitors*, *replication inhibitors*, *nucleic acid based therapy*, *protease and helicases inhibitor*, *nucleoside analogs* and *viral assembly inhibitors.* However, more time will be needed before effective agents can be commercially available. Meanwhile, the palliative treatment and intensive medical care are advised to improve the patient clinical prognosis. The early detection of infection before overt clinical signs begins, and early start of symptomatic treatment, will give a good prognosis. At the moment, the World Health Organization (WHO) publishes regularly the technical guide for case management of various symptoms exhibited by patients, to help practicing clinicians(Leyssen et al., 2003)

5.9 Arboviral fevers epidemiology in the republic of Djibouti

Arboviral fevers are some of the least studied diseases in the republic of Djibouti. However, previous studies show that these pathogens are endemic in the neighbouring geographical region, that is the WHO-Eastern Mediterranean region (WHO-EMRO) and Africa. Some estimation shows that WHO-EMRO which hosts only 8% of the world's population, and contributes 11% global non malarial vector borne infectious diseases burden(WHO-EMRO, 2005a). Of great public health significant, are the *dengue fever*, *Rift Valley fever*, *Crimean-Congo haemorrhagic fever*, *yellow fever*, *West Nile fever and Japanese encephalitis*(WHO-EMRO, 2005a). These viruses' presence is reinforced by the availability of competent vectors, competent hosts and perennial flooding, which creates an enabling environment for amplification into outbreaks periodically (Weaver & Reisen, 2011). Several neighbouring countries to Djibouti have experienced

outbreaks before, including Sudan (Ebola, RVFV, YFV, CHIKV), Ethiopia(CHIKV), Somalia (RVF), Kenya (DENV, CHIKV, RVF), Uganda (CHIKV, Ebola, Marburg), Egypt (AHFV, WNV, RVF, CCHF)(Malik et al., 2013). Locally, Djibouti has, through few studies documented presence of TOSV, DENV-1, DENV-2, DENV-3, DENV-4, CHIKV, WNV, YFV, UGS, BAN, ZIK and HTNV(G. R. Rodier et al., 1996; G. R. Rodier, Parra, Kamil, Chakib, & Cope, 1995; G. Rodier, Solimani, Bouloumi, & Kremer, 1993; Salah et al., 1988; RB Tesh, Saidi, Gajdamovic, & Rodhain, 1976). It also reported a large outbreak of DENV-2 which occurred in 1991-1992(G. R. Rodier et al., 1996), that affected more than 12000 subjects; and a suspected CHIKV outbreak that occurred in 2011 (personal communication Dr Ammar Abdo Ministry of health Djibouti). The intermittent surveillance by the foreign military missions hosted in Djibouti in collaboration with ministry of health, confirms that the circulation of WNV and DENV viruses to be endemic(De Laval et al., 2012; Faulde & Ahmed, 2010). These studies have also demonstrated that through entomological studies(Faulde & Ahmed, 2010; Faulde, M Spiesberger, & Abbas, 2012; Fryauff et al., 1995), the endemic presence of competent vectors of arboviruses, including mosquitoes, ticks and sandflies. This overview shows the significance of inclusion of arboviruses investigation in this thesis. The study was aimed at investigating the epidemiology of major arboviral fevers of the republic of Djibouti at household level.

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CHAPTER 6

EPIDEMIOLOGY OF ARBOVIRAL FEVERS

6.1 Introduction

Infections by arboviruses are associated with syndromic encephalitis, hemorrhagic fevers and fevers of unknown origin. This situation is often compounded by lack of confirmatory laboratory diagnosis to inform case management choice of clinician, especially in low income countries.

In the situation in these environment, is marked by high prevalent of risk factors, including endemic competent vector control, lack of surveillance data and inadequate research activities, to address the recurrent challenges

For instance, the past research work in Djibouti, like many resource limited environment, did confirm endemicity of transmission vectors, and some of which were found to be infected by the dangerous pathogens. This translates into a great public health concern.

Such observation are not uncommon in urban centers in developing countries, these young and rapidly expanding cities suffers from infrastructural strains of unplanned urbanization, of which Djibouti city is no exception, indicated by burst open sewage, poor drainage, informal settlement and poor disposal of solid wastes. These settings are more attractive to most vectors, especially for mosquitoes'(*Aedes sp*, *Culex sp* and *Anopheles sp*) which accounts for more than 60% of all the arboviral transmission.

This study therefore was conceived to provides an overview of the prevalence and risk predictors of arboviruses infection in the city of Djibouti, with particular focus on the associated ecological actors influencing transmission vector distribution, such as socio-demography, housing environment and occupation of residents

This report forms a basis for policy formulation and research continuation that would inform future prevention and control measures.

Andayi et al. (2014) A sero-epidemiological study of arboviral fevers in Djibouti, horn of Africa. PLOS One Neglected Tropical Diseases (Submitted manuscript)

6.2 Research Article III

A sero-epidemiological study of arboviral fevers in Djibouti, horn of Africa

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KEYWORDS: arboviruses, vector-borne, epidemiology, Djibouti, horn of Africa

ABSTRACT

Arboviral infections have been repeatedly reported in the republic of Djibouti, consistent with the fact that essential vectors for arboviral diseases are endemic in the region. However, there is limited recent information regarding arbovirus circulation and the associated risk predictors to human exposure are mostly unknown. We performed, from November 2010 to February 2011 in the Djibouti city general population, a cross-sectional ELISA and sero-neutralisation-based sero-epidemiological analysis nested in a household cohort, which investigated the arboviral infection prevalence and risk factors, stratified by their vectors of transmission. Antibodies to dengue virus (21.8%) were the most frequent. Determinants of infection identified by multivariate analysis pointed to sociological and environmental exposure to the bite of *Aedes* mosquitoes. The population was broadly naïve against chikungunya (2.6%) with risk factors mostly shared with dengue. The detection of limited virus circulation was followed by a significant chikungunya outbreak a few months after our study. Antibodies to West Nile virus were infrequent (0.6%), but the distribution of cases faithfully followed previous mapping of infected *Culex* mosquitoes. The seroprevalence of Rift valley fever virus was 2.2% and non-arboviral transmission was suggested. Finally, the study indicated the circulation of Toscana-related viruses (3.7%) and a limited number of cases suggested infection by tick-borne encephalitis or Alkhumra related viruses, which deserve further investigations to identify the viruses and vectors implicated. Overall, most of arboviral cases predictors were statistically best described by individuals' housing space and neighbourhood environmental characteristics, which correlated with the ecological actors of their respective transmission vectors' survival in the local niche. This study has demonstrated autochthonous arboviral circulations in the republic of Djibouti and provides an epidemiological inventory, with useful findings for risk mapping and future prevention and control programs.

INTRODUCTION

Arboviral fevers are a threat to the global population and warrant a continuous surveillance and monitoring, especially in tropical and subtropical regions, where most of the low income countries are located.(Gould & Solomon, 2008) Viruses from families *Togaviridae* and *Bunyaviridae*, and from genus *Flavivirus* are responsible for the majority of human cases. The observed geographical dispersion of arboviral diseases is strongly correlated with ecological factors and human activities. [2] For example, dengue fever (DEN), Yellow fever (YF), and Chikungunya (CHIK) tend to spread to all regions where their *Aedes* transmission vectors are present (potentially affecting two thirds of the global human population [3]). The tick-borne encephalitis (TBEV) virus is endemic in Europe, Russia and Asia in forest, moorland and steppe ecosystems hosting abundant transmission rodent hosts and *Ixodid* vectors. The warm African eco-climates support abundant mammalian hosts, reservoir birds and vectors, which are favourable factors for arboviral transmission.(Gould & Solomon, 2008) To some extent, the same characteristics apply to the WHO Eastern Mediterranean region (WHO-EMR),(WHO-EMRO, 2005, 2007) to which our study area, Djibouti, belongs. A combination of limited surveillance capabilities for early detection and a lack of routine preventive medicine programs, in part explains why limited information regarding arboviral fevers is available in Djibouti. Nevertheless, the scientific literature provides evidence that essential vectors for arboviral diseases are endemic in the republic of Djibouti. These include some mosquito vectors (*e.g.*, *Aedes*, *Culex* and *Anopheles* species(G. R. Rodier et al., 1996; G. R. Rodier, Parra, Kamil, Chakib, & Cope, 1995; Salah et al., 1988)), ticks (*Ixodes*, *Rhipicephalus*, *Amblyomma*, *Hyalomma species*(Socolovschi et al., 2007)) and sandflies.(Faulde & Ahmed, 2010; Fryauff et al., 1995) In addition, potential animal reservoirs such as nomadic pastoralists' livestock,(FAO, 2005) migratory birds,(Magin, 2013) and rodents,(G. Rodier, Solimani, Bouloumi, & Kremer, 1993) are present. This evidence corroborates the existing risk of outbreaks, since a number of arboviral pathogens have been detected to be in local circulation.(De Laval, Plumet, Simon, Deparis, & Leparc-Goffart, 2012; G. R. Rodier et al., 1996, 1995; Salah et al., 1988; Tesh, Said, SJ, Rodhain F, & Vesenjak-Hirjan J, 1976) However, there is limited recent information and the associated risk predictors to human exposure are mostly unknown. This study therefore, is an attempt to bridge the existing knowledge gap, based on the Djibouti city general population. It is a cross-sectional analysis nested in a household cohort, which investigates the arboviral infection prevalence and risk factors, stratified by their vectors of transmission. Attention was given to *Culex*- (RVF and WNV), *Aedes*- (DENV, YFV and CHIKV), sandfly- (Toscana (TOSV) and related phleboviruses) and tick- (TBEV and related flaviviruses) borne viruses. The essential purpose was to provide an epidemiological inventory, with useful findings for risk mapping and future prevention and control programs.

METHODS

Study Area, Djibouti City

The study was conducted in four administrative districts of Djibouti city, Republic of Djibouti, which is one of the 22 member states of the WHO Eastern Mediterranean region.(WHO-EMRO, 2012) It is situated in the horn of Africa, at the Gulf of Eden of the Red Sea, bordering Somalia, Ethiopia, and Eritrea (Figure 1). It covers 23.200 km² with over 818,159 inhabitants, with majority of them, 70.6% (577 933) residing in urban areas.(DISED, 2012) Of those who live in urban, the largest proportion, 58.1% (475 322) are inhabitants of the capital, Djibouti city. Eco-geographically, the country is largely arid and semi arid, with perennial flooding during winter (November to April) and prolonged summers for the rest of the year.

Study Design (based on CoPanFlu Djibouti Program)

The protocol and samples used in the study were derived from the Djibouti Cohort of Pandemic Influenza (CoPanFlu) program, which previously had been part of investigation on the 2009 pandemic influenza (H1N1pdm09) sero-epidemiological,(Andayi, Crépey, et al., 2014) and vaccination intention(Andayi, Kieffer, et al., 2014) studies. It was an adaptation from the WHO-EHESP CoPanFlu International Consortium, whose protocol details are provided elsewhere.(Lapidus et al., 2012) Ethical approval was granted by both this Consortium, which was based at the EHESP French School of Public Health, Rennes France, and the Ethical Review Committee at the *National Institute of Public Health* (INSP) Ministry of Health, Republic of Djibouti. Households were enrolled into the study after the two institutional approvals. A household was defined as two or more persons staying in the same house, sharing meals and living room space, with or without familial relationship.(Kieffer et al., 2013) For a household to be enrolled, all subjects belonging to it were required to give a written consent before participation. Minors below 16 years were to give their consent through their parents or guardians. This consent also provided for specimen usage in other studies, apart from the COPANFLU programme. On enrolment day, all subjects participated in capillary blood sampling (100-300µL) and responded to French questionnaires which constituted of information on subjects' and households' profile, occupation and academic background, and residential environment characteristics (See Table 1 for details). A total of 324 households with 1,045 subjects were enrolled between 11th November 2010 and 15th February 2011, from the 2009 Hajj Pilgrim database and the community of health (social) workers (CHW) database.(Andayi, Crépey, et al., 2014)

Laboratory analyses

First intention detection of antibodies (IgG) against various pathogens was determined by two different enzyme-linked immunosorbent assay (ELISA) protocols. In the first protocol, in-house kits (in which antigen derived from whole-virion particles in non-inactivated cell culture supernatants) were used to test for YFV, TOSV, RVFV and CHIKV antibodies. In the second protocol, commercial kits were used for detection of DENV (PanBio®, Brisbane, Australia), WNV and TBEV (EuroImmun®, Lübeck, Germany) antibodies. Positive and negative control sera were provided by the *French National Reference Centre for Arboviruses* or by the kits' manufacturers. Additional sero-neutralisation experiments were conducted in which wild-type laboratory-adapted viral strains were used, with exception of the YFV, in which the D17 vaccine strain was used. Appropriate cell culture lines and reagents were used in accordance to the established Standard Operating procedures and Good Laboratory Practice protocols of the laboratory. All experiments were conducted in *Biosafety level 3* laboratory containment facilities, at the EPV UMR_D 190 research laboratory, or at the French National Reference Centre for Arboviruses, Marseille France.

ELISA protocol

In-house kits: Onto Maxisorp 96 well plates (Nunc™), a 100µL per well of 1:200 of virus supernatant at 10^5-10^7 pfu per ml, in PBS buffer, at pH 9.6, was added and incubated overnight at 4°C. The supernatant was discarded, and the plates blocked with 300µL of PBST-10% milk (containing 0.05% tween-20 (v/v), 10% non-fat dried milk (w/v) and PBS) and incubated at 4°C for 90-120 minutes. The plates were then washed thrice with phosphate-buffered saline (PBS; pH 7.4 and 0.05% tween-20 (v/v)). Afterward, a 1:200 of test sera in PBST-5% Milk was added in duplicate wells and incubated for 60 minutes at 37°C. The plates were washed as before, followed by the addition of 100µL of a 1:8000 dilution of goat F(ab')2 fragment anti-human IgG(H+L) peroxidase (Beckman Coulter™) in PBST-5% milk, and incubated for 90 minutes at 37°C. Plates were washed six times and a 100µL TMB substrate (SureBlue™) added to develop the reaction. This reaction was terminated by addition of 100µL Stop solution (1M Hydrochloric acid) after 30 minutes. The absorbance was read in a microplate ELISA reader (Bio-Rad Benchmark™) at 450 nm.

Commercial kits: ELISA was performed according to the manufacturer's instructions and the optical density (absorbance) was read in a microplate ELISA reader machine (Bio-Rad Benchmark™) at 450 nm.

ELISA screening cut point determination

For consistence, all samples were tested in duplicates using common sera controls (negative and positive) for all plates in specific pathogen assay. The values of all plates for a given test were subsequently normalized according to values of negative and positive controls. In addition, a panel of 176 true negative

samples was tested using the in-house and commercial kits protocols. This panel included sera from a previous study of French blood donors that tested negative for antibodies to all pathogens studied here using sero-neutralisation techniques. For both in-house and commercial assays, sera with normalised absorbance values above the cut off value (defined as [mean of normalised true negatives + two standard deviations]) were considered to be positive. The positivity ratio (normalized absorbance value of the sample / cut off) was used for ELISA interpretation with ratios ≤0.9 associated with negative results; ratios between 0.9 and 1.1 with equivocal results; and ratios ≥1.1 with positive results.

Micro-neutralization assay protocol

A virus neutralisation assay (VNT) was performed for all viruses but dengue to check the performance of the ELISA assays. In brief, 50µL of heat-inactivated (56°C, 30 minutes) serum dilution (10^{-1} to 10^{-7} in PBS) was added to 50 µL of viral suspension (representing 100 $TCID_{50}$) in flat bottomed 96-well cell culture microplates (Nunc™) followed by 100µL of Vero cells suspension (2 10^5 cells/ml) in MEM culture medium supplemented with 8% foetal bovine serum and antibiotics. The plates were then incubated at 37°C in CO_2 incubator and virus multiplication was measured after 3-5 days by observing a cytopathic effect (CPE) and by quantifying the amount of viral genome in the culture supernatant by using real-time RT-PCR techniques in the case of TBEV and Alkhumra virus (AHFV) (protocol available upon request to the corresponding author). Absence of CPE or real-time RT-PCR cycle threshold above 37 was considered a positive reaction. Sera with a sero-neutralising titre ≥10 were considered positive.

Statistical Analysis

Data entry and management was performed in the *FileMaker Pro Advanced 11*™ (FileMaker™) environment. From the 19 household ownership properties, the *principle component analysis* was used to create three socioeconomic level (Upper SES, Middle SES and Lower SES) levels.(Vyas & Kumaranayake, 2006) A descriptive analysis was performed on variables in preliminary evaluation. For public health importance, the infection status (seropositivity) was stratified for analyses as (a) *individual pathogens* or according to their (b) *transmission vectors*, namely: *Culex*-borne viruses (RVF and WNV), *Aedes*-borne viruses (YFV, DENV and CHIKV), sandfly-borne viruses (TOSV) and tick-borne viruses (TBEV and AHFV); or (c) *virus taxonomy*, namely: *Flaviviruses* (DENV, WNV, TBEV, AHFV, YFV), *Phleboviruses* (RVF and TOSV) and *Togaviruses* (CHIKV). Evaluation of heterogeneity of the seropositivity proportions in different independent variables such as districts, age groups, occupation and SES, was done by Chi square (χ^2) test or Fisher's exact test. Analysis of trend to establish potential systematic increase or decrease of infection status across the variable was also perfomed. The determination of socio-demographic and environmental

predictors to infection status were performed in the generalised estimating equation (GEE) models, which accounted for the household clustering effect among the enrolled subjects. Variables with p-value ≤0.25 in bivariate model were included in multivariate analysis in a backward stepwise reduction protocol, those with p-value ≤0.15 were retained in the final model and those with p-value ≤0.05 being considered statistical significance.(Bursac, Gauss, Williams, & Hosmer, 2008) Effect modification and interaction between variables on subjects' seropositivity were assessed. The use of GEE model in measurement of association, did not allow for the institution of post estimation validity evaluation.(Ballinger, 2004) All analyses were conducted in *Stata Statistical Software Release 13* (StataCorp College Station, TX: StataCorp LP).

RESULTS AND DISCUSSION

Demographic information for the 1,045 subjects belonging to 324 families involved in this study is shown in Table 1, a detailed profile has been provided elsewhere.(Andayi, Crépey, et al., 2014) Briefly, the participants were drawn from different age groups, gender, residential districts, ethnicity, occupation and socio-economic background in Djibouti city. Their diversity was manifested also in living conditions (housing space) and neighbourhood environ, which included: housing materials, domestication of animals, exposure to birds, sleeping habit (out in the open at night), and the proximity to the following: market, abattoir, open sewage, dumpsite and river bank, respectively.

The performance of the different ELISA tests was examined with reference to sero-neutralisation results for all viruses studied except dengue. For each test, a selection of ELISA negative samples and all available samples with a positive ELISA result were tested. However, due to capillary blood sampling, limited volumes of serum were available and some of the positives could not be further investigated.

For those viruses which had been previously identified in East Africa (YFV, WNV, CHIKV and RVFV) results are summarised hereafter and available in Table 2: *(i)* for YFV, 11 out of the 14 ELISA-positive samples were available for VNT. The Positive Predictive Value (PPV) of the ELISA test (ratio ≥1.1) was 0.64 and the Negative Predictive Value (NPV, calculating after gathering negative and equivocal results) was 1. We therefore tested other ratios for the definition of positives and identified an optimised ELISA ratio at 1.5, associated with a VPP at 0.91 and a VPN at 0.88. *(ii)* for WNV, 4 out of the 5 ELISA-positive samples were available for VNT. The ELISA PPV (ratio ≥1.1) was 0.56 and the NPV was 1; an optimised ELISA ratio at 1.3 was associated with a VPP at 0.75 and a VPN at 0.80; *(iii)* for CHIKV, 23 out of the 24 ELISA-positive samples were available for VNT. The ELISA PPV (ratio ≥1.1) was 1 and the NPV was 0.86;

(iv) for RVFV, 18 out of the 20 ELISA-positive samples were available for VNT. The ELISA PPV (ratio ≥1.1) was 0.83 and the NPV was 1.

For viruses never isolated in the region, results were as follows: *(i)* for TOSV, 33 out of the 34 ELISA-positive samples were available for VNT. The ELISA PPV and NPV (ratio ≥1.1) were 0.94 and 0.90, respectively, suggesting that some of the Djiboutian individuals tested had been in contact with genuine TOSV or a closely related phlebovirus (see below). *(ii)* for TBEV, all of the 5 ELISA-positive samples tested negative for other flaviviruses tested (DENV, YFV, WNV), which rules out the hypothesis of cross-reactivity with one of these pathogens. Since only two samples were available for additional seroneutralisation tests, reliable PPVs could not be calculated. The first sample was negative in seroneutralisation for TBEV and also for AHFV, a tick-borne flavivirus that has been previously shown to circulate in Saudi Arabia and in the south of Egypt.(Carletti, 2010; R N Charrel et al., 2001) The second sample was positive for TBEV (titre 20) and AHFV (titre 40), strongly suggesting contact with the latter virus. The sample was from a 13yo girl belonging to a family with a low socio-economic status, and living nearby an abattoir. His age and socio-economic status make unlikely a previous travel to Saudi Arabia (*e.g.* for the Hajj).

Using the aforementioned optimised ELISA positivity criteria, mosquito-borne virus infections were predominant, with 23.6% of the population testing positive for at least one *Aedes*-borne virus (DENV, YFV and/or CHIKV), and 2.6% testing positive for at least one *Culex*-borne virus (WNV and/or RVFV); in addition, 3.7% tested positive for sandfly-borne viruses (TOSV) and 0.6% for tick-borne viruses (TBEV). Detailed results are provided in Tables 2 and 3.

All subsequent statistical analyses were performed using ELISA results (with reference to optimised positivity ratios). First, we analysed double-positives (*i.e.*, samples with positive results for two different tests) and the issue of possible ELISA antigenic cross-reactivity between the different flaviviruses tested (DENV, YFV, WNV and TBEV) was addressed. No significant statistical association between serological results for flaviviral species was observed, confirming the good PPV of the ELISA tests for most of flaviviruses tested. The same analysis was performed for phleboviruses and a strong association was observed ($p<0.00001$) between TOSV and RVFV results. This is intriguing since TOSV and RVFV are antigenically very distant. A refined analysis of ELISA results for double-positives identified no relationship between the positivity ratios of TOSV and RVFV ELISA positives. Moreover, in double-positives, VNT geometric mean titres (GMTs) were high for both viruses (>20 for RVFV, >30 for TOSV).

Altogether, this suggests that an epidemiological relationship rather than an antigenic cross-reactivity should be invoked. Second, we examined possible associations that might be explained by exposure to a common vector. An obvious association ($p<0.00001$) was identified between DENV and CHIKV (70.8% of CHIKV positive samples are also DENV positive). This is evocative of a shared exposure to the bite of *Ae. aegypti*, which represents the vector of both CHIKV and DENV. The same link was not identified between YFV and CHIKV or DENV, despite their common vector. However, since YF is not endemic in Djibouti, the most probable explanation to YFV positive results is either immigration from an endemic country or vaccination (as recommended for Hajji Pilgrims and for travels in Ethiopia, see below). Third, a triangular significant association ($p<0.00001$) was observed between YFV, TOSV and RVFV. Since there is no antigenic relationship between YFV and phleboviruses, and different vectors transmit all three pathogens, the existence of a subpopulation gathering a variety of risk factors represents the most plausible explanation (see supplemental data in **Table S1**).

Univariate and –when authorised by numbers- multivariate analyses (UVA and MVA, respectively) were performed to assess relationship between serological results to subject and household profiles', occupation and academic background, and the residential environment characteristics (see **Table S2, Table S3 and Table 4**). Statistical analysis indicated that DENV and CHIKV positives share a number of risk factors: *(i)* living in District 1 (*i.e.*, city centre; MVA, $p<0.001$) and sleeping outside at night (MVA, $p<0.001$). Both are most probably linked with exposure to the common vector (*Ae. aegypti*), which is likely, in the warm and dry Djiboutian setting, to find a favourable environment in the urban areas of Djibouti, as previously reported in other locations(Sawabe et al., 2010) and can bite in the evening and the beginning of the night. *(ii)* living in large families (four or more persons; MVA, $p<0.001$). This may reflect favourable conditions for maintaining a population of *Aedes* mosquitoes in/around the household. In accordance with this observation, MVA indicates that keeping a domestic animal at home (which indeed may be part of the feeding resources available for female *Aedes* mosquitoes) is also associated with an increased risk for dengue (MVA, $p<0.001$). Amongst residential environment parameters, living nearby a river was associated with an increased risk for dengue (MVA, $p<0.001$). Running water is seasonal in Djibouti and, in the extreme context of the local climate, which is associated with limited vegetation, the presence of plant cover, puddles and water holes on the riverbanks is likely to offer an alternative to urban households as a source of larval sites for *Aedes* mosquitoes. Living nearby meat-markets (mostly located in peripheral poorly urbanised areas; MVA, $p<0.001$) and having a high socio-economic status (MVA, $p<0.001$) were found to be protective for DENV and CHIKV, respectively.

Finally, the distribution of sero-prevalence in age groups showed that the highest incidences were in young adults (20-39yo) for both DENV 96(22.2%) and CHIKV 13(3.0%), see Table 3. This indicates that dengue is not circulating in Djibouti under a regimen of hyper-endemicity, as observed in South-east Asia, but most probably under the form of episodic spillovers in a population that remains broadly naïve in all age groups. An important consequence is that dengue is not a specifically paediatric disease in Djibouti. Regarding CHIKV, these numbers indicate that, as this study was performed (November 2010 to February 2011), the Djiboutian population was massively naïve towards Chikungunya.

Regarding other viruses, the low sero-prevalence rates did not allow to identify major risk factors. *(i)* In the case of TBEV, UVA suggested migrants as a target population (p=0.01), which may reflect specific exposure to tick bites, presumably through long periods of time spent in a rural environment and/or contact with livestock. In the case of WNV, children under the age of 13, not sent to school nor employed appeared to be at risk (UVA, p=0.01), possibly reflecting low socio-economic status. *(ii)* In the case of YFV, no strong correlate was identified. *(iii)* In the case of TOSV, positives were more frequent in District 1 (MVA, p=0.004), and this may guide future investigations for identifying the vector and deciphering the transmission cycle in Djibouti. *(iv)* In the case of RVFV, MVA identified an elevated risk of infection amongst the young below 19yo (p=0.0150) and individuals of Arab descent (p=0.0160). Of note was that about a half (48.3%) of those in upper SES class were of Arabian ethnicity, and that the young Arabs were at least risk compared to their contemporaries (although not statistically significant). This may reflect a link with animal sacrifice related activities during Ramadan or travel in countries of high endemicity, since upper SES Arabian adult populations could afford such foreign trips, compared to majority of other tribes.(Davies, 2006) Regarding triple exposure to TOSV, RVFV and YFV, the best correlates identified by MVA were the age under 19yo (p=0.02) and the Afar ethnic origin (p=0.02), possibly reflecting specific risk factors such as transhuman pastoralism, contact with livestock (this age group is commonly in charge of the animals) and travels in Ethiopia (for which YF vaccination may be required) since the largest Afar populations reside in the Danakil Desert in Ethiopia.

CONCLUSION

This study reports arboviral sero-prevalence values and risk predictors in the winter of 2010, in Djibouti city. Of the total participants, over a quarter (27.4%) had evidence of infection to at least one of the eight arboviral infection. Studying simultaneously a variety of pathogens allowed us to weight serological cross-reactions. With reference to sero-neutralisation assays, it was minimal, reflecting our choice of

giving priority to the Positive Predictive Value of the tests used (with the possible consequence of slightly under-estimating actual prevalence rates).

Of interest was the conspicuously high burden of mosquito-borne viruses, especially, those transmitted by *Aedes* mosquitoes. DENV was found to have the largest and widest distribution across the different residential Districts of the city. It was first reported in the outbreak of 1991-1992,(G. R. Rodier et al., 1996) then remained steadily in circulation and was subsequently detected in survey studies.(De Laval et al., 2012) Our results suggest that dengue is present but still circulating at low levels compared with countries of high endemicity, resulting in limited immune protection of the population and infections distributed in age groups (*i.e.*, not predominantly impacting the paediatric population). Determinants of DENV infection identified by multivariate analysis point to sociological and environmental exposure to the bite of *Aedes* mosquitoes.

At the onset of this study, conducted in winter of 2010, contrary to DENV, CHIKV had never been reported in Djibouti. We report here a 2.6% sero-prevalence rate, with epidemiological determinants of infection very similar to those identified for dengue. It is worth noting that a CHIKV outbreak occurred in Djibouti during the year 2011 (personal communication Dr Ammar Ahmed Abdo, Ministry of Health Djibouti) and that, in our study, a majority of individuals with specific antibodies (>80%) were living in District 1. The most probable scenario is therefore that the virus has been circulating at low rate in 2010 in the city centre where exposure to *Aedes* bite appears to be the highest in Djibouti. The epidemic burst occurred in 2011 and this scenario is reminiscent of the Indian Ocean outbreak: CHIKV had been circulating at low level in 2005 in the naïve population of Reunion Island before an impressive burst in 2006. The predominance of DENV and CHIKV most probably reflects the fact that they are transmitted by the same peri-domestic vector, *Aedes aegypti*, which easily invades, spreads and colonises human habitation.(Sawabe et al., 2010) Regarding YFV (also transmitted by *Aedes aegypti*), the low prevalence numbers observed, the absence of epidemiological relationship with other *Aedes*-borne viruses and the lack of reported cases over the last decades in Djibouti suggest the identification of vaccinated individuals rather than the existence of local yellow fever foci.

Unlike *Aedes* borne viruses, viruses potentially transmitted by *Culex* mosquitoes (WNV and RVFV) were less represented in this study and no strong risk factors could be identified. The circulation of WNV in Djibouti has been previously documented *(i)* in horses, by the detection of specific antibodies (ELISA followed by PRNT or Western blot)(Cabre et al., 2006) and *(ii)* in mosquitoes, by the molecular

detection of WNV genotype 2 RNA in pools of *Culex pipiens* spp. *torridus* and *Culex quinquefasciatus*.(Faulde, M Spiesberger, & Abbas, 2012) Faulde and collaborators identified WNV RNA-positive mosquito pools in site ML4 (airport, positive pools were *Culex quinquefasciatus*) and ML5 (market place, positive pools were *Culex pipiens* spp. *torridus*). Remarkably, of the 5 individuals that tested positive for WNV antibody in the current study, one was living in the ML4 area and three in the ML5 area. Therefore, our results are in accordance with Faulde's findings, but they also confirm the classical discrepancy between the circulation of WNV in mosquitoes and birds and the number of cases of infection in dead-end hosts such as humans and horses (which in addition include a vast majority of asymptomatic or mild cases that do not draw medical attention). Regarding RVFV, its circulation in livestock has been repeatedly reported in the region.(Anyamba et al., 2009, 2010; Britch et al., 2013; CGRDS-Group, 2010) However it is noticeable, on the one hand, that Djibouti was not in previous studies recognised as a regional hot spot for transmission but a "potential epizootic area",(Anyamba et al., 2009, 2010) and on the other hand, that no human case has been reported in Djibouti. In our study, antibody to RVFV was not associated with any epidemiological or environmental parameter that would suggest the implication of *Culex* mosquitoes. This most probably reflects the predominance of non-arboviral transmission, due to contaminated aerosols (*e.g.*, from contact with livestock, in particular in case of miscarriage, manipulation of carcass, or ritual sacrifice).

Another important observation was the significant infection rate due to sandfly-borne viruses (3.7%). These infections accounted for the second most prevalent incidences, with a magnitude equal to that of CHIKV. This result, together with the high GMT titres observed using TOSV for neutralisation tests, is highly suggestive of the circulation in Djibouti of TOSV or a closely related virus. This is in agreement with the reference 1976 sero-survey(Tesh et al., 1976) by R.B. Tesh and collaborators which identified a 3.1% seroprevalence in Djibouti ("Territory of Afars and Issas") using a PRNT technique and the prototype Sandfly fever Naples virus strain. Juxtaposing 1976 and 2010 serological results indicates that viruses belonging to the Naples serocomplex have been circulating for decades in Djibouti and do not represent an emerging pathogen in the region. Further investigations may specify which specific virus(es) is (are) circulating, *i.e.* Sandfly fever Naples virus, TOSV or another variant possibly not yet identified.

In a 1995 article, D.J. Fryauff and collaborators proposed an inventory of sandflies in Djibouti.(Fryauff et al., 1995) In the coastal plain habitat zone (in which Djibouti city is located) the predominant phlebotomine flies were *Phlebotomus alexandri* and *Phlebotomus bergeroti*. *P. alexandri* belongs to subgenus *Paraphlebotomus* and is closely related to *P. sergenti*, which has been recently associated with TOSV in

Essaouira, Morocco;(Es-Sette N et al., 2014) it therefore represents a credible potential transmission vector for viruses of the Naples serogroup. In Fryauff and collaborators' study, it was found all year long, with a peak during the cool-wet season (Jan-Feb). *P. bergeroti* is related to *P. papatasi*, a vector of viruses belonging to the Naples and the Sicilian serogroup. It has never been associated with TOSV, but is the historical vector of Naples virus and Sicilian virus, that caused huge outbreak in military corps stationed in the Mediterranean, the North African and the Middle-East theatres of operations during World War II, and also proved to circulate in the 1970's in Sudan, Ethiopia and Somalia.(Alkan et al., 2013) Most individuals with specific antibodies to TOSV were living in District 1. Since sandflies occupy very focal habitats, ≤1 km from their breeding sites,(Moncaz, Faiman, Kirstein, & Warburg, 2012) this provides robust information for future investigations aiming at formally identifying virus(es) and vector(s) implicated.

Finally, the identification of one individual with high titre VNT antibody to the Alkhumra virus extends the potential distribution area of the virus. This probable constitutes the first identification of an autochthonous case in the horn of Africa. Contamination of humans may occur following the bite of ticks (*e.g.*, Ornithodoros savignyi,(Rémi N Charrel et al., 2007; Mahdi et al., 2011) Hyalomma dromedarii(Mahdi et al., 2011) but also as a consequence of non-arboviral transmission (*e.g.*, after manipulating carcass of infected animals or drinking contaminated raw milk.(Rémi N Charrel et al., 2005) A case-control study in Najran, Saudi Arabia, identified animal contact, neighbouring farms, and tick bites in the multivariate modelling whereas univariate analysis retrieved that contact with domestic animals, feeding and slaughtering animals, handling raw meat products, drinking unpasteurised milk, and being bitten by a tick were associated with Alkhumra virus infection.(Alzahrani et al., 2010) This deserves further investigations to clarify the epidemiological risk factors of infection in Djibouti.

In conclusion, a variety of arboviral pathogens is circulating in Djibouti. The impact of *Aedes*-borne viruses (DENV and CHIKV) is significant and justifies a reinforcement of vector control in urban areas. *Culex*-borne viruses (WNV and RVFV) circulate at low rate but deserve sustained surveillance because of their epidemic potential. Sandfly- and tick-borne viruses have never been isolated and described previously. This study provides evidence for their circulation in Djibouti and advocates for further investigations aiming at characterising them and deciphering their vectors and ecological cycles.

Competing Interests

None to be declared

Funding

Funding and support was obtained from: **(a).** WHO-EHESP Copanflu International Consortium of EHESP French School of Public Health, CS 74312 - 35043 Rennes, France; **(b)**. Aix Marseille University, IRD French Institute of Research for Development, EHESP French School of Public Health, UMR_D 190 "Emergence des Pathologies Virales", 13005, Marseille, France; **(c).** French Institute of Microbiology and Infectious diseases (IMMI) and **(d).** Ministère de la Santé, Institut National de Santé Publique, BP 1157, Djibouti, Republic of Djibouti. The work of RC was done under the frame of EurNegVec COST Action TD1303.

Acknowledgments

The authors thank Djibouti CoPanFlu Program partners. In particular, Regine Ducos, Sarah Kitar, and Imane El Hamdi from EHESP Rennes. They acknowledge contributions from Moulid Barkhat, Basra Ibrahim and the entire field team from Djibouti Health ministry and Institut National de Santé Publique, and are grateful to Nathalie Leblanc and Marie-Thérèse Aristodemo for their field support and assistance. FA was financed by a doctoral fellowship awarded by EHESP and Aix-Marseille University.

Authors' contribution

FA: field work, lab analysis, statistical analysis, and manuscript writing; **RC**: data analysis and manuscript writing; **AK**: field coordination and data management; **HR**: statistical analysis; **BP** and **ILG**: production and analysis of laboratory data; **AAA**: field coordination; **FC**: cohort coordination and data analysis; **AF:** study design and data analysis; **XdL**: study design and coordination, data analysis, and manuscript writing. All authors read and approved the manuscript.

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Figure 1: A map showing the geographical position of the study area, Djibouti city of the republic of Djibouti, in continental Africa.

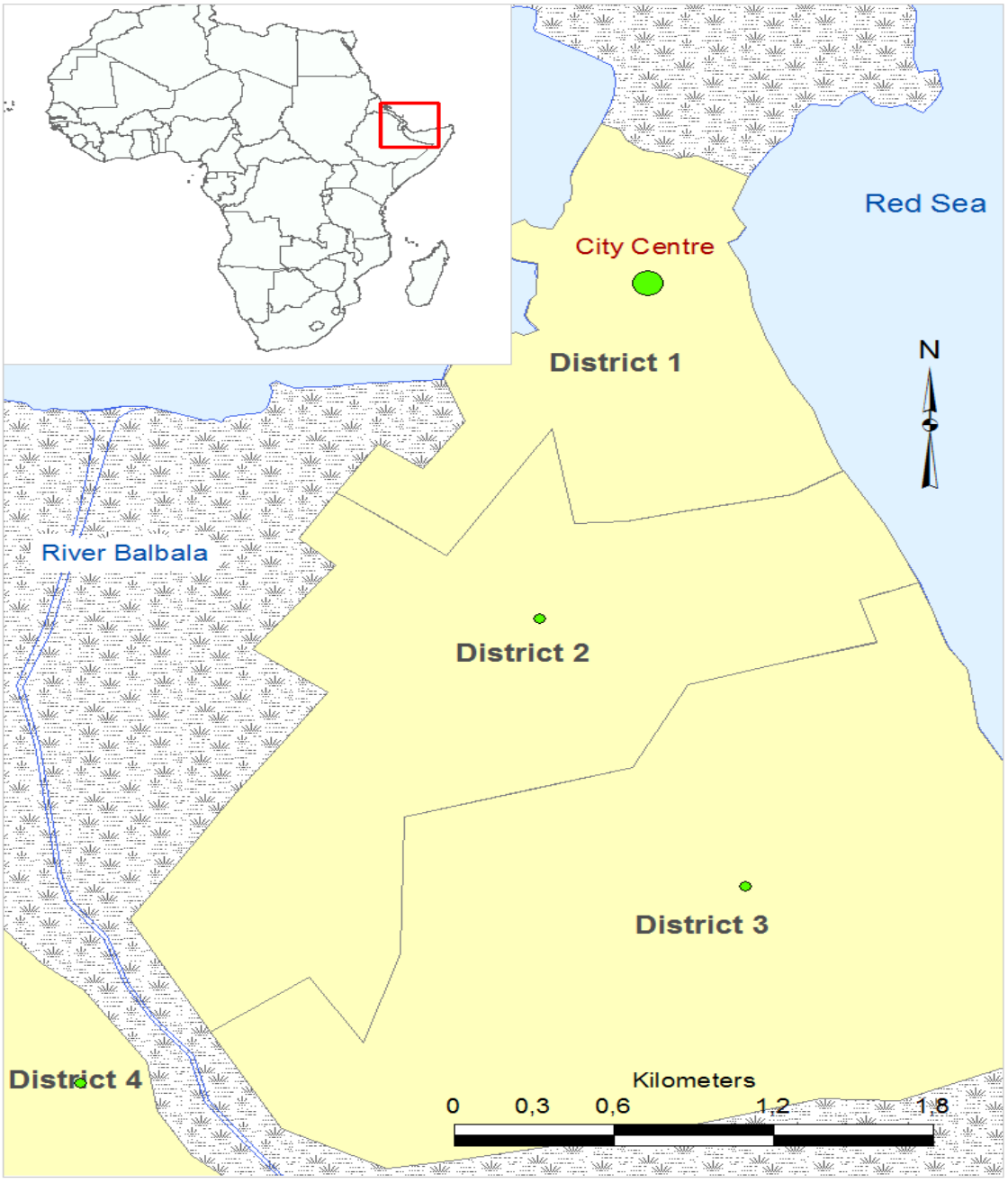

Table 1: The Djiboutian cohort demographic characteristics and study variables(N=1,045)

(a). Subject and household profile		n	%	(b). Residential environment		n	%	(c). Occupation and literacy		n	%
Age group:	≤19 years	409	39.1%	Residential District:	1	433	41.4%	Occupation:	≤13years	109	10.4%
	20-39years	435	41.6%		2	340	32.5%		Employed	162	15.5%
	40-59 years	155	14.8%		3	200	19.1%		Jobless	473	45.3%
	≥60 years	36	3.4%		4	72	6.9%		Student	301	28.8%
	Unknown	10	1.0%	Living nearby river bank:		45	4.3%	Education:	≤5years)	88	8.4%
Gender:	Women	571	54.6%	Living nearby dumpsite:		140	13.4%		Illiterate	265	25.4%
	Men	474	45.4%	Living nearby food market:		410	39.2%		Basic education	201	19.2%
Family size:	Small (≤3 persons)	563	53.9%	Living nearby vegetable market:		308	29.5%		Bac+	123	11.8%
	Medium (4 or 5 persons)	297	28.4%	Living nearby abattoir:		281	26.9%		Unknown	368	35.2%
	Large (≥6 persons)	185	17.7%	Living nearby open sewage:		24	2.3%	Working from outdoors:		77	7.4%
Children in household:	none	290	27.8%	Sleeping out in open at night:		75	7.2%	Working from indoors:		96	9.2%
	Few (≤3)	650	62.2%	Keeping animal(s):		149	14.3%				
	More (4 ≥)	105	10.0%	Exposure to birds:		11	1.1%				
Ethnicity:	Afar)	101	9.7%								
	Arab	174	16.7%								
	Ethiopia	58	5.6%								
	Migrants	15	1.4%								
	Somalis	645	61.7%								
	Unknown	52	5.0%								
SES Level:	Low	285	27.3%								
	Middle	127	12.2%								
	Upper	392	37.5%								
	Unknown	241	23.1%								

Table 2 : A matrix of various arboviruses sero-positivity profile for residents of Djibouti city in the winter of 2010

Arboviral pathogens (tested samples)	ELISA positive cut point	VNT Positive (titre ≥10)	ELISA seropositivity profile: number of ELISA assay(s) with positive result			Specific virus ELISA seropositivity profile						
			≥1	1	≥2	CHIKV	TOSV	RVF	YFV	WNV	TBE	DENV
CHIKV (914)	1.1	23	24	7	15	7	2	1	0	0	0	17
TOSV (915)	1.1	30	34	15	9	2	15	9	6	0	0	11
RVF (914)	1.1	15	20	8	5	1	9	8	7	1	0	3
YFV (903)	1.5	10	14	4	4	0	6	7	4	1	0	4
WNV (893)	1.3	3	5	2	3	0	0	1	1	2	0	1
TBE (893)	1.1	1	5	5	0	0	0	0	0	0	5	0
DENV (911)	1.1	n/a	199	160	23	17	11	3	4	1	0	160

Table 3: Prevalence of anti-arboviral IgG antibodies by administrative district of Djibouti city, age group and gender in the winter of 2010 (N=1,045)

Arboviral Pathogens	Sample tested (N=1045)	Positive cases (x=251)	Overall prevalence (%)	Gender		Age group (years)				Residential District			
				Male	Female	≤19	20-39	40-59	≥60	1	2	3	4
				n=466	n=571	n=409	n=435	n=155	n=36	n=433	n=340	n=200	n=72
				(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
DENV	911	199	21.8	20.0	18.4	15.9	22.1	17.4	30.6	25.9	13.8	13.5	18.1
WNV	893	5	0.6	0.4	0.5	0.5	0.5	0.6	0.0	0.7	0.0	0.5	1.4
TBE	893	5	0.6	0.4	0.5	0.7	0.0	1.3	0.0	0.2	0.9	0.5	0.0
YFV	903	14	1.5	1.5	1.2	2.2	0.9	0.6	0.0	1.4	1.8	0.0	2.8
CHIKV	914	24	2.6	2.6	2.1	2.0	3.0	0.6	5.6	4.6	0.6	0.5	1.4
RVF	914	20	2.2	2.1	1.8	3.4	0.7	1.9	0.0	2.1	2.1	1.5	1.4
TOSV	915	34	3.7	3.0	3.5	3.9	3.0	2.6	2.8	4.6	2.6	1.0	4.2
Any of the arboviruses	916	251	27.4	25.3	23.1	22.2	26.2	22.6	30.6	31.6	19.1	16.5	22.2
Flaviviruses	915	217	23.7	21.7	20.1	17.8	23.4	20.0	30.6	27.3	16.2	14.5	20.8
Phleboviruses	915	45	4.9	4.5	4.2	5.6	3.4	3.9	2.8	5.5	3.5	2.5	5.6
Alphaviruses	914	24	2.6	2.6	2.1	2.0	3.0	0.6	5.6	4.6	0.6	0.5	1.4
***Aedes*-borne**	915	216	23.6	21.7	19.8	17.8	23.7	18.1	30.6	27.7	15.6	13.5	20.8
***Culex*-borne**	914	24	2.6	2.1	2.3	3.7	1.1	1.9	0.0	2.5	2.1	1.5	2.8
Sandfly-borne	915	34	3.7	3.0	3.5	3.9	3.0	2.6	2.8	4.6	2.6	1.0	4.2

Table 4 : Determinants of arbovirus sero-positivity among Djibouti city residents in the winter of 2010

Risk factors for Dengue virus	aOR*	95% LCL	95% UCL	pvalue
(a) Subject and household profile				
Age group (≤19 yo)	0,7	0,5	1,0	**0,0290**
20-39yo	Ref	Ref	Ref	Ref
40-59 yo	0,7	0,5	1,1	0,1340
≥60 yo	1,2	0,7	2,0	0,4940
Sex (woman)	0,8	0,6	1,1	0,1230
Large family(6 or more persons)	1,7	1,3	2,2	**<0001**
(b) Occupation and literacy				
Employed	0,7	0,4	1,0	**0,0330**
Students	0,6	0,4	0,8	**0,0020**
Illiterate	0,7	0,5	0,9	**0,0170**
(c) Residential environment				
Residential District 1	1,7	1,3	2,2	**<0001**
Living nearby river bank	2,3	1,5	3,4	**<0001**
Living nearby dumpsite	0,5	0,3	0,8	**0,0100**
Living nearby abbattoir	0,4	0,3	0,6	**<0001**
Sleeping out in open at night	2,8	1,8	4,3	**<0001**
Keeping animal	1,9	1,4	2,5	**<0001**

Risk factors for Yellow fever virus	aOR	95% LCL	95% UCL	pvalue
(a) Subject and household profile				
Age group (≤19 yo)	2,3	0,7	7,3	0,1540
Sex (woman)	1,5	0,5	4,7	0,4800
Large family(6 or more persons)	2,5	0,8	8,0	0,1200
Upper SES status	10,1	1,3	80,6	0,0290
Middle SES status	9,5	0,9	104,4	0,0650
(b) Occupation and literacy				
Employed	1,3	0,1	20,5	0,8350
Working from indoors	5,3	0,7	43,3	0,1190
Student	2,7	0,2	29,4	0,4260
(c) Residential environment				
Living nearby food market	1,1	0,2	5,2	0,8720
Living nearby Vegetable market	0,2	0,0	2,0	0,1660

Risk factors for Chikungunya virus	aOR	95% LCL	95% UCL	pvalue
(a) Subject and household profile				
Sex (woman)	0,6	0,3	1,3	0,1920
Age group (20 to 39yo)	1,9	0,9	4,3	0,1020
Large family(6 or more persons)	5,7	2,6	12,5	**<0,0001**
Ethnic Afar	2,0	0,8	5,0	0,1180
Upper SES status	0,3	0,1	0,7	**0,0050**
Lower SES status	0,4	0,1	1,2	0,1100
(b) Occupation and literacy				
Employed	0,2	0,0	1,5	0,1220
Illiterate	0,4	0,1	1,3	0,1200
(c) Residential environment				
Residential District 1	8,2	2,8	23,9	**<0,0001**
Sleeping out in open at night	3,4	1,3	8,4	**0,0100**

Risk factors for Toscana virus	aOR	95% LCL	95% UCL	pvalue
(a) Subject and household profile				
Age group (≤19 yo)	1,3	0,6	2,8	0,4470
Sex (woman)	1,4	0,7	2,9	0,3050
Household with children	1,6	0,6	4,2	0,3200
(b) Occupation and literacy				
=None=				
(c) Residential environment				
Living nearby dumpsite	2,0	0,9	4,6	0,1000
Residential District 1	3,0	1,4	6,3	**0,0040**

Risk factors for Rift Valley Fever virus	aOR	95% LCL	95% UCL	pvalue
Gender (woman)	1,1	0,5	2,7	0,8040
Ethnic Arab	3,4	1,3	9,2	**0,0160**
Ethnic Arab & of Upper SES	0,2	0	1,5	0,1190
Age group (≤19 yo)	3,4	1,3	8,9	**0,0150**
Large family(6 or more persons)	2,1	0,8	5,5	0,1220

NOTE : *adjusted odd ratio from multivariate analyses | factors with statistically significant association at pvalue ≤ 0.05 are **Bolden**

Table S1: Predictors of multiple seropositivity to 3 arboviruses (YF, RVF and TOSV), among Djibouti city residents in winter of 2010.

Subject and houshold factors	Measure of association *aOR(95% CI)	pvalue
Seropositivity to YF, TOSV and RVF		
Age group: ≤19 yo	**11,9 (1,4 - 100,5)**	**0,0230**
Gender: women	6,1 (0,7 - 51,8)	0,0980
Ethnic: Afar	**6,5 (1,3 - 33,1)**	**0,0240**
Large family: 6 or more persons	5,3 (0,6 - 44,5)	0,1270
Seropositivity to TOSV and RVF		
Gender: women	3,2 (0,7 - 15,5)	0,1530
Age group: ≤19 yo	**7,7 (1,5 - 40,4)**	**0,0160**
Residential District: 2	**4,8 (1,0 - 21,7)**	**0,0430**
Large family: 6 or more persons	**6,9 (1,2 - 39,2)**	**0,0280**
Ethnic: Afar	4,7 (0,9 - 23,6)	0,0580
Seropositivity to TOSV and YF		
Gender: women	3,3 (0,7 - 16,1)	0,1470
Ethnic: Afar	4,0 (0,8 - 19,1)	0,0800
Large family: 6 or more persons	3,3 (0,7 - 16,3)	0,1410
Age group: ≤19 yo	**6,4 (1,3 - 31,9)**	**0,0240**
Seropositivity to RVF and YF		
Gender: women	2,7 (0,5 - 13,6)	0,2310
Age group: ≤19 yo	4,5 (0,9 - 23,2)	0,0710
SES level: Upper	3,5 (0,7 - 17,9)	0,1330
Large family: 6 or more persons	6,4 (0,8 - 52,7)	0,0840
Ethnic: Afar	3,9 (0,8 - 18,7)	0,0880

*adjusted odd ratio

NOTE: Factors with statistical significant association (at p<0.05) are bolden

Table S2: Univariate analyses of subject and household factors' as predictors of arboviruses sero-positivity among Djibouti city residents in winter of 2010

Subject and household factors	DENV OR 95% CI	TBE OR 95% CI	WNV OR 95% CI	YF OR 95% CI	CHIK OR 95% CI	TOSV OR 95% CI	RFV OR 95% CI
Age group: ≤19 years	0,8 (0,6 - 1,1)	1.7 (0.2 - 12.1)	0.6 (0.1 - 5.4)	2,3 (0,7 - 7,26)	0.9 (0.4 - 2.1)	1,5 (0,7 - 3,1)	**5,2 (1,5 - 18,0)***
20-39years	ref	n	1.2 (0.2 - 8.8)	0.6 (0.2 - 2.1)	1,94 (0,88 - 4,3)	ref	ref
40-59 years	0,8 (0,5 - 1,2)	5.6 (0.8 - 39.6)	1.9 (0.2 - 18.6)	0.5 (0.1 - 4.0)	n	1,0 (0,3 - 3,0)	3,0 (0,6 - 14,5)
≥60 years	1,3 (0,8 - 2,3)	n	n	n	2.3 (0.6 - 9.4)	0,9 (0,1 - 6,9)	n
Gender: women	0,8 (0,6 - 1,1)	0,8 (0,1 - 5,84)	2.4 (0.3 - 23.3)	1,5 (0,5 - 4,68)	0,6 (0,27 - 1,3)	1,4 (0,7 - 2,9)	1,0 (0,4 - 2,5)
Family: small ≤3 persons	ref	n	ref	ref	ref	ref	ref
medium (4 or 5 persons)	**1,8 (1,3 - 2,3)*** **	n	4,4 (0,4 - 47,9)	1,64 (0,4 - 7,27)	0,7 (0,2 - 2,7)	1,2 (0,5 - 2,5)	2,6 (0,9 - 7,5)
large (≥6 persons)	**1,4 (1,0 - 2,0)***	n	3,5 (0,2 - 56,2)	**4,3 (1,2 - 16,0)***	**4,2 (1,8 - 10,0 ****	1,2 (0,5 - 3,1)	**3,5 (1,1 - 10,6)***
Children in household: (0)	ref	n	n	ref	ref	ref	ref
few(≤3)	**0,7 (0,5 - 0,9)****	n	n	1,13 (0,3 - 4,33)	0,6 (0,2 - 1,4)	1,6 (0,6 - 4,2)	3,4 (0,8 - 14,8)
more(4 ≥)	1,0 (0,6 - 1,5)	n	n	2,14 (0,4 - 12,6)	1,1 (0,3 - 3,8)	2,1 (0,6 - 7,4)	4,8 (0,8 - 28,2)
Ethnicity: Afar	1,3 (0,9 - 1,8)	n	n	1.9 (0.4 - 8.5)	2,0 (0,83 - 5,0)	1.0 (0.3 - 3.1)	1.8 (0.5 - 5.9)
Arab	0,8 (0,6 - 1,2)	n	1.5 (0.17 - 15.1)	1.0 (0.2 - 4.3)	1,0 (0,3 - 3,4)	1.3 (0.6 - 3.0)	2.2 (0.9 - 5.7)
Ethiopia	0,7 (0,4 - 1,5)	n	n	n	2,2 (0,5 - 9,6)	1.2 (0.3 - 4.9)	1.0 (0.1 - 7.5)
Migrants	1,0 (0,3 - 2,6)	**19.6 (2.2 - 176.9)***	n	n	3,4 (0,5 - 24,8)	n	n
Somalis	ref	1.8 (0.19 - 17.6)	1.8 (0.2 - 17.9)	0.9 (0.3 - 2.7)	ref	0.9 (0.5 - 1.8)	0.5 (0.2 - 1.1)
SES Level: low	1,0 (0,6 - 1,6)	5.4 (0.6 - 52.2)	n	0,21 (0,0 - 2,3)	0,34 (0,11 - 1,2)	1,3 (0,3 - 4,5)	0,6 (0,2 - 2,2)
middle	ref	n	n	9,53 (0,9 - 104)	ref	ref	ref
upper	1,3 (0,8 - 2,2)	0.3 (0.0 - 3.2)	n	10,1 (1,3 - 80,6)	0,36 (0,1 - 1,2)	1,3 (0,4 - 4,5)	0,5 (0,2 - 1,8)

NOTE: Bolden crude odd ratio(OR) indicates a statistical significant association; with * for pvalue of 0.05 to ≤ 0.0051, ** for pvalue of 0.005 to ≤ 0.00011, and *** for ≤ 0.0001

A letter "n" represents an association that could not be estimated due to data limitation

Table S3: Univariate analyses of occupation and literacy, and the housing environmental factors as predictors of arboviruses sero-positivity among Djibouti city residents in winter of 2010

Occupation and literacy factors		DENV OR 95% CI	TBE OR 95% CI	WNV OR 95% CI	YF OR 95% CI	CHIK OR 95% CI	TOSV OR 95% CI	RFV OR 95% CI
Occupation: ≤13years		1,1 (0,7 - 1,7)	n	**13.6 (1.9 -94.5)***	3,4 (0,3 - 37,3)	1,0 (0,2 - 4,2)	0,9 (0,2 - 3,8)	n
Employed		0,7 (0,4 - 1,0)	1,4 (0,1 - 15,1)	1.6 (0.2 -15.6)	**5,5 (1,0 - 29,7)***	0,2 (0,0 - 1,5)	0,7 (0,2 - 2,2)	1.3 (0.4 - 3.9)
Jobless		ref	ref	0.4 (0,0 - 3.5)	ref	ref	ref	**0.2 (0.1 - 0.7)***
Student		**0,6 (0,4 - 0,8)***	0,8 (0,1 - 8,8)	n	3,9 (0,8 - 20,2)	0,7 (0,3 - 1,7)	1,2 (0,5 - 2,5)	**4,1 (1,6 - 10,2)****
Education: ≤5years		0,8 (0,4 - 1,4)	n	6.1 (0.6 -57.1)	2,0 (0,2 - 21,7)	n	0,7 (0,1 - 5,3)	**0,0 (0,0 - 0.0)*****
Illiterate		**0,7 (0,5 - 0,9)***	0,5 (0,0 - 7,4)	2.8 (0.4 -19.4)	0,4 (0,0 - 4,2)	0,4 (0,1 - 1,3)	0,8 (0,3 - 2,4)	0,4 (0,0 - 4,2)
Basic education		ref	n	1.2 (0.1 -12.2)	ref	1.7 (0.7 - 4.0)	ref	ref
Bac+		0,8 (0,5 - 1,2)	n	n	1,7 (0,2 - 11,7)	0.7 (0.2 - 2.8)	1,1 (0,3 - 3,9)	1,7 (0,2 - 11,7)
Working from outdoors:		0,7 (0,4 - 1,4)	n	n	n	1,8 (0,3 - 9,5)	1,0 (0,2 - 4,8)	1,2 (0,1 - 11,2)
Working from indoors:		0,7 (0,4 - 1,3)	1,6 (0,1 - 17,6)	3,4 (0 - 53)	5,0 (0,9 - 29,5)	0,8 (0,1 - 7,4)	0,8 (0,2 - 3,9)	**4,5 (1,0 - 19,5)***
Residential environ factors								
Residential District:	1	**2,2 (1,6 - 3,0)*****	n	5.3 (0.6 -51.0)	1.8 (0.6 - 5.4)	**9,6 (2,3 - 40,8)****	**2,1 (1,0 - 4,6)***	1,2 (0,4 - 3,1)
	2	ref	5.1 (0.5 - 48.6)	n	1.7 (0.6 - 5.3)	ref	ref	ref
	3	0,9 (0,6 - 1,5)	n	1.2 (0.1 -11.7)	n	0,9 (0,1 - 9,4)	0,4 (0,1 - 1,7)	0,7 (0,2 - 2,8)
	4	1,4 (0,7 - 2,5)	n	n	n	3,2 (0,3 - 34,6)	1,4 (0,3 - 6,3)	0,9 (0,1 - 7,3)
Living nearby river bank:		**1,7 (1,1 - 2,7)***	6,8 (0,7 - 64,2)	n	n	1,2 (0,2 - 9,0)	n	n
Living nearby dumpsite:		**0,5 (0,3 - 0,8)***	n	n	0,5 (0,1 - 3,7)	n	2,0 (0,9 - 4,6)	2,2 (0,8 - 6,2)
Living nearby food market:		**0,5 (0,3 - 0,6)*****	1,3 (0,2 - 9,42)	0,7 (0,1 - 7,4)	0,5 (0,1 - 1,7)	**0,1 (0,0 - 0,6)***	0,8 (0,4 - 1,6)	1,0 (0,4 - 2,5)
Living nearby Vegetable market:		**0,6 (0,4 - 0,8)****	2,4 (0,3 - 17,1)	0,8 (0,1 - 7,8)	0,2 (0,0 - 1,7)	n	0,8 (0,4 - 1,8)	0,9 (0,3 - 2,4)
Living nearby abbattoir:		**0,4 (0,3 - 0,6)*****	2,8 (0,4 - 19,7)	n	0,3 (0,0 - 2,0)	**0,1 (0,0 - 1,0)***	1,0 (0,4 - 2,1)	1,0 (0,4 - 2,8)
Living nearby open sewage:		0,2 (0,0 - 1,5)	n	n	n	n	1,3 (0,2 - 9,0)	**4,7 (1,2 - 18,9)***
Sleeping out in open at night:		**1,7 (1,2 - 2,4)****	n	4,2 (0,4 - 40)	n	**3,6 (1,4 - 9,2)***	0,9 (0,2 - 3,5)	1,5 (0,4 - 6,4)
Keeping animal(s):		**1,6 (1,2 - 2,2)****	1.7 (0.2 - 2.0)	2,6 (0,2 - 29)	n	0,3 (0,0 - 2,3)	0,6 (0,2 - 1,9)	0,7 (0,2 - 3,0)
Exposure to birds:		n	n	n	n	n	n	n

NOTE: Bolden crude odd ratio(OR) indicates a statistical significant association; with * for pvalue of 0.05 to ≤ 0.0051, ** for pvalue of 0.005 to ≤ 0.00011, and *** for ≤ 0.0001

A letter "n" represents an association that could not be estimated due to data limitation

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

A lot of interesting ideas of epidemiological and policy perspectives were generated from the works of this thesis, on republic of Djibouti. In this chapter, the significance of the main findings are presented, alongside the limitations, potential implications and proposed areas of future scientific work.

7.1 Discussion and Conclusion

Republic of Djibouti, like many developing countries, the data collection is relegated to a routine practice of profiling clinical record with no intention for practical use in immediate outbreak detection or future policy formulation. The first part of this work, has demonstrated that there are both enormous potential as well as limitations in the current influenza and influenza like illness survey system of this country. Of concern were the challenges in quality of data, timeliness, resources and expertise, which could be overcame by improvisation and minimum investment that would open a new avenue for the country's rapid detection of ILI outbreaks, and at a lesser cost(Vergu et al., 2006).

In spite of these, the local system captured profile of ILI trend that reflected that of the H1N1p(Itoh et al., 2009; Miller, Viboud, Simonsen, Olson, & Russell, 2009), and was highly suggestive of the first wave of pandemic influenza evolution in the country in the winter of 2009. Unfortunately, this insight was belatedly picked more than 12 months after, compared to situation of other refined syndromic surveillance systems integrated in health care systems in developed countries, such as the USA CDC s' Biosense system, France French Sentinel Network (FSN)system, and UKs' Real-time *Syndromic Surveillance* Team (ReSST) system(Ping Yan, Hsinchun Chen, 2008; Vergu et al., 2006), etc. These refined systems infact picked the aberration within days of events, enabled prompt public health sector respond, to the then, evolving pandemic influenza, for both first and second wave.

In Djibouti, the incomplete data from the system at the time of copanflu study completion could not allow for comparison or validation of the cohort finding during the second pandemic wave, which was unfotunate. However, the major findings of the cohort study were more robust, and clearly confirmed the prevalence and risk predictors of pandemic influenza which were consistent to those reported elsewhere in the world(Chowell et al., 2011; Miller et al., 2009; Cecile Viboud & Simonsen, 2012). These important observations were lacking in major outlets of influenza monitoring agencies, including the local health

department, CDC, WHO etc. Probably, it could be argued that, with improvement of surveillance system that incorporates algorithms for analysis and visual display at the department of epidemiology and health information would have primed stake holders on the onset of the first and second wave, and probably indicted whether the threat was significant or not.

At this time of reporting, though the actual significance of this work to the 2009 pandemic may be diminished, but this finding are invaluable for future use and adds to the ANISE (Africa National Influenza Surveillance and Epidemiology program) effort to generate information on influenza in the tropics(M. A. Katz et al., 2012). In the tropics, the past studies had suggested that the influenza A circulates throughout the year, but with less severity compared to the situation in the temperate region(Cécile Viboud, Alonso, & Simonsen, 2006). However, this notion is now being disputed as a biased observation, since most of the tropics constitute of low income nation that generate limited data for a credible comparison or analysis(Simonsen, 1999; Cécile Viboud et al., 2006).

Of interest to the local public health official was that the risk of infection was significantly associated with the populations' socio-economic situation. Those with high material deprivation (refugees, low SES level, illegal immigrants etc), were the most affected. The observed group vulnerability was not uncommon, but consistent with previous observation elsewhere(Tricco, Lillie, Soobiah, Perrier, & Straus, 2012). This finding in fact should inform international health relief agencies (UNHCR, WFP, GTZ, UNSCEF, CARE etc), working with these group locally, to consider the impact of influenza on respiratory illness (excluding TB) burden, in their service delivery.

Unlike the respiratory illness in this population, where the risk and prevalence that were influenced by mainly material deprivation associated factors; the arboviruses were influenced by the ecological actors supporting the transmission vectors survival. These actors existed either in natural or artificial settings that exists around human habitations. It should be realized that the harsh local climatic ambience(hot and dry) favors most vectors to live proximal to human habitation where sites with open wetness(water) or blood meal are ad lib (live animals, open sewage, near river banks or near dumpsite), and this pattern has been confirmed by the distribution of sero-positivity in the study area. Much of the cases were observed administrative district 1, which exhibits features of young and rapidly developing city, with enormous urbanization challenges. All these features are characteristics of challenges that encourages urban stable vector population, hence urban life cycle that entrenches endemicity of arboviruses(Sawabe et al., 2010; WHO-EMRO, 2005).

Not surprisingly, the mosquitoes and sandflies borne arboviruses were found pre-dominant, compared to ticks borne infection, which with caution could mean also the scale of their vectors distribution. Of note

was that Aedes borne viruses were notably the highest, marked by high prevalence of CHIK and DENV cases observed. This was consistent previous investigations elsewhere, which demonstrated that the invasion and colonization of poorly maintained urban centres by the Asian tiger mosquitoes (*Aedes aegyptea* and *Aedes alibopictus*), was to blame(de Lamballerie et al., 2008; Leroy et al., 2009). In fact these species has been documented to have instituted a shift from sylvatic to peridomestic habitation, with notable maintenance of stable circulation of DENV and CHIK in urban human population. .

The findings on Aedes borne viruses in this work indicates a possible stable circulation of the viruses locally, which is consistent with previous studies, in military personnel serosurvey(De Laval, Plumet, Simon, Deparis, & Leparc-Goffart, 2012), entomological survey(Faulde & Ahmed, 2010; Faulde, M Spiesberger, & Abbas, 2012) and general population(Rodier et al., 1996). of particular concern is that the DENV seropositivity distribution by age group in this study seems to suggests this pathogen is no endemic but likely to be sequestered in some parts of the city and occasionally spillover into largely naive population, and that circulation could be moving towards stability. Unlike DENV, the CHIK observation in this work is novel, and is highly suggestive a recent introduction and possibly an outbreak as indicated by Dr.Ammar Abdo Ahmed of INSP-Djibouti (personal communication).

Sandflie borne viruses (Toscana -TOSV and related group of viruses) as mentioned before was first documented in Djibouti in 1976(Tesh, Said, SJ, Rodhain F, & Vesenjak-Hirjan J, 1976), and seems to have remained in stable circulation ever since. This is consistent to the fact that sandfly insects inhabits less than 1km from the breeding point for their all of its lifetime(Moncaz, Faiman, Kirstein, & Warburg, 2012), it is therefore highly likely that these vector and so is the (TOSV and related) pathogens has remained endemic locally ever since. The continuous expansion of sandflies insects and sand flies fevers along the Mediterranean region and neighboring countries could also be suggestive of reintroduction from other regions(Alkan et al., 2013).

Observed sero-prevalence of rift valley fever -RVF, tick borne encephalitis-TBE and alkhurma hemorrhagic fevers-AHFV in the local population commonly involved in live stock economic activites, particularly the Afar who are the norrmadic pastoralists, the exposure is suggestive of strong association to individuals livestock industry occupational hazard. This includes the tick bites, exposure to infected blood aerosol or accidental contact with reminant arbotion materials. But because of their high dynamic lifestyle, it was impossible to rule out potential importation of cases. A further investigation is highly recommended.

This investigation has demonstrated autochthonous arboviral circulations in the republic of Djibouti and provides an epidemiological inventory, with useful findings for risk mapping and future prevention and control programs.

Overall the thesis work has confirmed that the respiratory illness and arboviral fevers are rampant in the republic of Djibouti, and that their risk factors are unique by subpopulation and geographical area. It is therefore concluded that formulating an effective intervention be based on local information, of which this thesis stands to contribute.

7.2 Limitations

The initial purpose of this work was to investigate the epidemiological, virological and sociological factors determining individual infection to pandemic influenza, at household level. However, the eventual adaptation of the Copanflu international protocol to the local situation altered several aspects, and partly contributed to some of the study limitations.

Firstly, on population sampling strategy, the opportunistic sampling of the cohort from Hajji Pilgrim data and community health workers database cannot claim to be a representative of Djibouti population. Because the Hajji Pilgrim is an expensive venture and there could only be afforded by the persons of high wealth index. While the community health workers list, is mostly constituted of person of low wealth index, who the government provides relief service. This implied that the mid wealth index group were likely to have been under represented, which could be a sources of biasness.

Secondly, by missing out on completion of Phase II and Phase III of the study was a big limitation. In Phase II the collection of viral specimen was to identify all the ILI etiological agent of co-circulating during the winter period, rather than only the pandemic strain which was capture in serological study. This was a big loss of important information. Given there was poor preventive medicine program in Djibouti and there could be other pathogens other than the pandemic H1N1 causing illness during that time that could have been reflected in the syndromic survey system as ILI. In addition, the exact transmission and evolution pattern of pandemic influenza at household level via social contacts network was not studied, which was part of phase ii, this could have elucidated some significant transmission pattern and enriched our findings. For Phase III, blood sampling would have aided in improving our classification of infection status, courtesy of sero-conversion estimation. Ideally, double sampling in at least two seasons was required, upon which fourfold sero-conversion would have been used as determinant of infection status. However, the use of stringent cut off criterion in both hemagglutination assay and microneutralization assay, were the best approach given the situation.

Thirdly, in the arbovirus study, the generalization of DENV virus was very limiting, in that the exact strain that was in circulation at that time could not be determined from the four known serotypes, if it was the DENV-2 that recently caused epidemic (1991-1992)(Rodier et al., 1996). Similarly, the Chikungunya and Onyonyong virus have very close antigenic properties(K, Besselaar, & Gibson, 1995; Powers, Brault, Tesh, & Weaver, 2000), by failing to test the CHIKV positive sera for ONNV virus cross reactivity, we missed an opportunity.

7.3 Perspective and Potential Implication

At national level, this study provides a unique pedestal to build on for betterment of infectious diseases surveillance and monitoring. As a preliminary work on this scope, it does indicate the potential areas to put emphasis on several specific areas. For examples the respiratory infection control, to focus more on District 4, and vector-borne disease control focus on District 1. Very narrow objectives can therefore be formulated to implement measures along other programs. For instance, to capitalize on Roll Back malaria to institute a integrated vector management program and entomological virus hunting.
The observed low prevalence of pathogens such as RVF, WNV, TBEV/AHFV suspect viruses, YFV and TOSV, should be a cause to worry, given no outbreak has ever been reported in Djibouti, yet the neighboring countries have been reported and in some they are endemic. Possibly, the introduction of screening of refugees and immigrants on arrival could help develop the transmission pattern. Since ILI cause about 50% of the medical consultation in both children and adults, understanding the exact distribution and their determinants as hinted in this thesis, will be important for prevention and control.

At regional level, this study gives credence to the risk and prevalence of important infectious disease of respiratory tract, arboviruses and hemorrhagic fevers. Through regional risk mapping, our study contributes vital information for application in international travel advisory and pre-deployment planning, given Djibouti host thousands of foreign military missions. In addition, this data should enable inter-national comparison of risk predictors and prevalence given the commonalities of eco-climate and socio-cultural behavior, particularly among the 22 members of the WHO Eastern Mediterranean region (WHO-EMRO, 2012).

For sustainability and organization of arboviruses surveillance; the regional agencies or cooperation's (such as WHO EMRO or CDC or French military, GTZ) could take advantage of the gains made by ANISE (Africa National Influenza Surveillance and Epidemiology program), which was instituted as pandemic influenza sentinel surveillance centers in 16 African states (Radin et al., 2012). The program

was instrumental in generating among the first general data in the region on pandemic influenza events(M. a Katz et al., 2012).

Lastly, this study offers unlimited opening to build on its findings and answers specific questions, of epidemiological and public health policy perspectives. For exemple:

- How much of the fevers of unknown origin can be attributed to arboviruses?
- What proportion of the circulating competent vectors are actually infected by arboviruses?
- How much of ILI classified as none-TB, actually are subclinical TB?
- Is it possible to institute functional effective diseases surveillance and monitoring unit amidst pressing demands resources in other areas, such as clinical service provision or perennial cholera outbreak?

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APPENDIX

APPENDIX I

This is an additional work that sought to supplement the investigation on the pandemic influenza social epidemiology. The contents of this section have only been mentioned in the main thesis text, the article below, is in the final preparation for submission for publication

RISK PERCEPTION AND HEALTH BEHAVIOR

Introduction

This article summarizes the socio-epidemiological module of the H1N1p of the Copanflu Djibouti study. In this module, the intent was to highlight risk perception and health behavior tendency, as a complementary investigation to sero-epidemiological study. Whereas in the first report (chapter 3), the main focus was on the prevalence and determinants of infection, in this report, the emphasis was on pragmatic anthropological approach to prevention and control of infection, using vaccination intention as a proxy.

A vaccination intention is used here as a measure of intended compliance to the proposed intervention strategy by lay people, and its future policy implication. To achieve this, the study applied psycho-cognitive theories in which health behavior can be predicted and explained. Of interest was the Protective motivation theory (PMT)(Rogers, 1975) . The PMT is a useful tool for health-related behaviors studies, in particularly for assessing the influencing factors and predictors of various behaviors. Exemplary application has been reported in studies of reducing alcohol use, enhancing healthy lifestyles, enhancing diagnostic health behaviors and preventing disease.

The core assumptions of PMT describe the adaptive and maladaptive coping with a health threat as a result of two appraisal processes (Leventhal (1970), in which the behavioral options to diminish the threat are evaluated. These two appraisal processes results in the intention to perform adaptive responses (protection motivation) or may lead to maladaptive responses. Maladaptive responses are those that place an individual at health risk with potential negative consequences, such as continue smoking, refuse cancer screening or refuse vaccination (Boer, Seydel, 1996). Overall, PMT is a result of threat appraisal and the coping appraisal. Threat appraisal is the assessment of the probability of vulnerability and severity, while coping appraisal is on response efficacy and self-efficacy. Response efficacy is the person's evaluation of the given recommendation to erase the threat. Self-efficacy is the personal confidence to successfully excute the recommendation. In brief, the protection motivation is a mediating variable whose function arouses, sustains and directs protective health behavior (Boer, Seydel, 1996).

In this study, population factors and modified illness perception questionnaire were used to investigate the vaccination intention in the second pandemic wave, up to 16th February 2011. A cross sectional survey on *cohort of pandemic influenza household (CoPanFlu)* was conducted on individuals aged above 15 years,

primarily by a face to face interview. Enrolled participant had previously been involved in a pandemic sero-epidemiological and whose finding are reported elsewhere, in chapter 4 (Andayi et al 2014).

A total of twelve psychosocial constructs were formulated for the PMT model, namely; *H1N1p Knowledge, Worry, Severity, Vulnerability, Self efficiency(control), Coherence (disease progression) and Prevalence, Response (vaccine) efficiency], Belief in health conspiracy, Belief in fatalism, and Belief in social support].* A mean score of psychosocial constructs of modified common sense model were calculated and analyzed for prediction of vaccination intention behavior.

Of the 703 individuals enrolled, 56.3% reported pandemic vaccination intention. Significantly, the perceived worry was the main predictor of high intention subjects; while those with subjective norm (belief in fatalism) and low socioeconomic status, were predictors for those with least interest. On social influence, clinicians were the greatest motivator for this intention, as opposed to politician, religious leaders or social workers.

This study gave insights into pandemic vaccination intention of Djiboutian, an example of a population from a low resource environment. It has also demonstrated that anxiety and socio-economic classes could influence future vaccination process. It was therefore concluded that massive public awareness should be prioritized to increase compliance, and the use of the less costly non-medical intervention should be proposed in future campaigns.

Andayi et al., (2014) Pandemic (H1N1) 2009 influenza vaccination intention; the determinants and implications on future vaccines uptake in Djibouti. *BMC Public Health* (submitted)

Research Article IV

Pandemic (H1N1) 2009 influenza vaccination intention; the determinants and implications on future vaccines uptake in Djibouti

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ABSTRACT

Background: The pandemic (H1N1) 2009 influenza caused huge socio-economic and health burden to the world population. At that time, vaccination was the preferred intervention; however, the general acceptability was unpredictable and a *priori* intention studies were desirable in forecasting coverage.

Objective: To assess the pandemic vaccination intention among Djiboutian during the second wave up to 16th February 2011.

Methods: A cross sectional survey based on the *cohort of pandemic influenza household (CoPanFlu)* program was conducted between 10th November 2010 and 16th February 2011. It targeted individuals of age above 15years for a face to face interview on validated questionnaires about risk perception and health behavior. A mean score of psychosocial constructs of protection motivation were calculated and subsequently applied in regression analysis to predict vaccination intention.

Results: Of the 703 individuals enrolled, 56.3% reported pandemic vaccination intention. Clinicians were the greatest motivator for this intention, as opposed to politician, religious leaders or social workers. Significantly, the perceived worry was the main predictor of individuals' declaring intention. While the subjective norm (belief in fatalism) and low socioeconomic status, were predictors for those with least interest.

Conclusion: This study provides insights of pandemic vaccination intention of a population from a low resource environment. It has demonstrated that anxiety and socio-economic classes could influence future vaccination process. It is therefore concluded that massive public awareness should be prioritized to increase compliance, and the use of the less costly non-medical intervention should be proposed in future campaigns.

Keywords: Pandemic *influenza*, *H1N1*, *risk perception*, *protection motivation theory*, *vaccination intention*, *Djibouti*, *Eastern Mediterranean*, *Africa*

BACKGROUND

In late April 2009 a new influenza subtype A(H1N1)pdm09 was reported in Mexico and USA that quickly spread to other parts of the world, causing the World Health Organization (WHO) to declare a pandemic alert on 11th June 2009. One year later, over 18,000 deaths and innumerable morbidities were reported in 214 countries(WHO, 2012a). In spite of the looses, the pandemic severity was rated moderate and equated to that of seasonal influenza(Dawood et al., 2012; Shrestha et al., 2011). For poor nations, this could have been an underestimation especially for those from Asia and Africa(Nair et al., 2011), even though many lacked active influenza surveillance(M. A. Katz, Schoub, Heraud, Breiman, Njenga, & Widdowson, 2012).

In Africa, among those affected, were from the Republic of Djibouti, whose first nine incidence cases were reported on 7th December 2009(WHO-EMRO, 2009). Presumably, the first pandemic wave coincided with the 2009 winter period (November 2009 to April 2010), and so was that of the second wave with the 2010 winter period (November 2010 to April 2011), as noted elsewhere(Miller et al., 2009). In these two waves many incidence and fatality cases occurred, but the exact numbers remain undocumented. Recent prevalence study that took place in late phase of second wave(F Andayi et al., 2014), suggests that more than 29.9% of the population could have been infected, with most incidents among the youth ≥ 25 years (35.9%) and the elderly ≥ 65 years (29.5%). In this environment, high malnutrition and infectious disease burden (TB, Malaria and HIV) in general population(WHO-EMRO, 2012), are significant risk for severity and susceptibility and this supports our assertion.

During the first wave, no vaccine was available for public use in many countries. At this time Djibouti public health sectors advocated for non-medical intervention in reducing the pace of transmission. This was by proposing the avoidance of public gathering, public transport and the practice of the respiratory and hands hygiene. Persons with clinical signs were advised to seek medical attention urgently. However, during the second wave, the distribution and use of pandemic vaccines had begun and many nations were obliging. The low resourced ones like Djibouti were also set to benefit from the WHO donations(Mihigo et al., 2012). At the time of the study, Djibouti's record on donated vaccines, supply or distribution to the public was unavailable(Mihigo et al., 2012; WHO-EMRO, 2012). For Djiboutian planning to go for Hajji Pilgrim to Mecca, Saudi Arabia in 2009 and 2010 sessions were among the few who made individual efforts for vaccination, as it was mandatory for participation.

The pandemic 2009 influenza vaccine coverage varied widely by region, country and subpopulation. High coverage was evident among European Union and North American nations than those from other parts of the world. At risk group, accounted for the most vaccinations led by the chronically ill, followed by the

pregnant women, healthcare workers, and with least covered was the general population. In several studies, male gender, young age, high literacy, being a doctor, high socio-economic status, previous seasonal influenza vaccination, trust in vaccine and use of official source of information; were significant predictors(Bish, Yardleyb, Nicollc, & Michie, 2011; Brien, Kwong, & Buckeridge, 2012). In some way, this is evident that not all who were offered vaccine accepted readily, and that there were many factors influencing uptake behaviour. Therefore identifying and understanding these determinants is important in making effective vaccination program strategies.

The use of the psycho-cognitive theories is one way through which these habits can be predicted and explained. Protective motivation theory is one such example(Rogers, 1975). It was a tool initially developed to explain the basis of fear appeals in health promotion. It was based on the observed human behavior of threat appraisals, which were found to determine their drive for self protection. This drive is influenced by judgment of illness severity and personal vulnerability. Upon weighing options, they develop a coping appraisal mechanism, which asseses the strength of various measures of avoiding danger (response efficacy) and their ability (self efficacy) to implement them. Individuals would then act on precaution (Adaptive behavior) if perceived level of fear is moderate, and will fail to act if it is extreme (mal-adaptation). Application of protection motivation theory in health intervention programs is not uncommon comply(Floyd, Pretince-Dunn, & Ronal, 2000). It has recently been used to assess the link between the psychological perception and socio-demography characteristics to pandemic vaccination intention and uptake(Bish et al., 2011; Brien et al., 2012)..

In Djibouti, there was an urgent need to administer the pandemic vaccine once the donation become available for the public. Since it was the first time such event was to being undertaken, many challenges were expected, including the fear of vaccine safety and efficacy. To develop a successful pandemic vaccination program with wide coverage was therefore important. The use of measurable psychological and demographic factors was needed to predict the pandemic precautionary behavior of vaccine use. To our knowledge no such work (vaccination intention study) had been conducted in the country that could have been used as reference. This was the first of its kind. A cross sectional study conducted on the *Cohort of Pandemic Influenza Household* (COPANFLU) Program participants', who previously had been involved in the pandemic sero-epidemiological study(F Andayi et al., 2014). The psychosocial theoretical constructs of protection motivation theory, socio-demographic factors and media access were used to investigate the general populations' attitude towards vaccination.

METHOD

Study area

Republic of Djibouti is a small subtropical country covering ≈23,200 km² and is located at the horn of Africa in the northern hemisphere. It has about 818,159 inhabitants, with the majority, 70.6% (577,933) of them residing in urban areas. Of those who live in urban, the largest proportion, 58.1% (475 322) are inhabitants of the capital, the Djibouti city(DISED, 2012).

Sampling and data collection

A cross sectional study investigating the determinants of vaccination intention towards pandemic influenza among Djibouti city residents was conducted in face to face encounter, towards the end of the second pandemic wave (10th November 2010 to 16th February 2011). Minimum sample size of 646 subjects was required for this study, assuming that 30% of them would declare vaccination intention, with projected accuracy of 0.05, design effect of 2, and that these intentions were normally distributed in the general population(Hosmer & Lemeshow, 2000).

Participants were recruited from the COPANFLU Djibouti's program that investigated the pandemic influenza sero-epidemiology(F Andayi et al., 2014). The initial cohort had a total of 1045 individuals from 324 families. The details on inclusion protocol and population characteristics are detailed elsewhere in earlier publication(F Andayi et al., 2014). In this survey, only subjects above 15 years of age, whose households met the following inclusion criteria were enrolled; i.e. all members stayed under one roof, shared meals and living area, consented and participated in blood sampling, responded to study questionnaires, and were permanent residents of Djibouti city.

The validated questionnaires used were in French language and were administered for ~10-30 minutes per family member on appointed date. The exercise was conducted by a team of at least 3 trained research interviewers (community health or social workers). Literate participants filled the questionnaires independently, while for those with limited reading and writing capabilities had an oral interview in a local dialect. Enrolled participants were neither enticed nor compensated for their involvement. However, they were explained to, that the study was on influenza and influenza like illness (ILI) and that they were required to give an informed legal consent for participation.

Measures

Questionnaires

The questionnaire used in this study had been standardized and validated previously, and is reported by Setbon and Raude(Setbon & Raude, 2010). Questionnaire design was principally built on the philosophy of protection motivation theory and illness perception(Moss-Morris et al., 2002; Rogers, 1975). Under this theory, the psychosocial theoretical constructs were used to determine health precautionary behavior against pandemic influenza in the general population. Of important to this study, were the twenty questions (20) used to measure the constructs, namely, for the threat appraisal (8), for coping appraisal (11) and for precautionary behavior (1). In *threat appraisal*, the influenza knowledge (3), perception of worry (2), perception of disease prevalence (1), perception of severity (1), and perception of vulnerability (1) were formulated. Whereas, for *coping appraisal*, the perception of self efficacy (perceived control) (1), perception of disease consequence (1), understanding of diseases progression (perceived coherence) (2), and perception of vaccine efficacy (1) were developed. Other *coping appraisal* generated, were those associated with subjective norms, which were basically the beliefs and attitudes that were not often scientifically true, such as belief in individual health situation (2), belief in fatalism (2) and the belief in social support (2). The precautionary behavior of interest to this study was the vaccination intention (1) against pandemic influenza. Of the twenty questions used, only vaccination intentions had a "YES" or "NO" respond, the rest were measured on a 5-point Likert scale (**Table 1**). On this scale, for example, the score of 1="completely disagree", 2="somewhat disagree", 3="neither agree nor disagree", 4= "somewhat Agree" and 5="Completely agree". All the nineteen items included, had an acceptable internal consistency of *Cronbach alpha* ≥ 0.65. The mean score of the items used to make a constructs were used as a measure of the construct(Setbon & Raude, 2010).

Table 1 Comparison of mean psychosocial perceptions towards the pandemic (H1N1) 2009 influenza by age and gender, among Djiboutian

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Threat Appraisal

The first measure of threat appraisal was on *influenza knowledge*. This was determined by the three questions administered. In which the subjects were asked if they knew the pandemic influenza, followed by, if they knew the difference between the seasonal and pandemic influenza, and lastly, if they knew

influenza illness was associate with fever and cough. The second measure was on the *perceived anxiety (worry)*. It was also evaluated by the two questions, one seeking to know if they were concerned getting sick from the pandemic, and another, if they were concerned about their family members getting sick of the same. Thirdly, the *perceived prevalence*, it was measured by a single question, which sought to determine how many cases, if reported to be of pandemic would motivate them into precautionary action. This ranged from 0-100; 101-1,000; 1,001-5,000; 5,001-10,000; and above 10,001, and with a score of 1, 2, 3, 4, and 5, respectively. Fourth measure was on *perceived severity*. It was based on a single question asking subjects if the pandemic exposure would results in a serious infection. Lastly, the *perception of vulnerability* was measured by one question that sought to know if they feared being infected by the pandemic.

Coping Appraisal

On coping appraisal, seven psycho-cognitive constructs were formulated. The *Perception of self efficacy* against pandemic was measured by a single question, which sought to determine if the subject had a protective measure around them in regard to the pandemic. The *perceived consequence* of pandemic infection was determined for by asking subjects how they have changed their lifestyle following the onset of pandemic influenza. To get idea on how the subjects comprehended pandemic diseases progression (*perceived coherence*), participants were asked two questions, if they believed that the diseases could reach their home town, and if by any chance, the disease was already in circulation in their town. Participants were also asked if they perceived *preventive measure (vaccine) was effictive* against pandemic infection. In addition, other coping appraisal associated with subjective norms, were determined. Among them was the *belief in individual health situation*. In this construct, two questions were asked to the subjects, if they can describe their health status as good, and if they are healthy because of doctors. *Belief in fatalism* was also evaluated, but through two queries that asked if sickness is a fate, and if healing was natural without medical intervention. Lastly, the participants were evaluated for their dependence on the *social support system*, by two questions, if their family took care of them last time they were sick, and if they family will do the same in future.

Precautionary Behavior Appraisal

The precautionary behavior of interested in this study was vaccination intention. Participants were asked if they intended to receive vaccine against pandemic influenza when available, and they were to give a "YES" or "NO" response. They were also asked of their past vaccination to seasonal and pandemic influenza.

Socio-demographic factors

Several socio-demographic characteristics were studied. Of note were the participants age groups, family size, wealth index (SES), gender, ethnicity, literacy level, occupation, district of residence and belonging to priority group of pandemic vaccination (i.e. pregnant women, chronic illness, recent ILI and households with children). Principle component analysis was used to determine the wealth index from 19 household ownership properties, as described by **Vyas et al**(Vyas & Kumaranayake, 2006). In addition, the information on pandemic risk communication and access to media were investigated

Statistical analysis

Data entry and management were conducted in the *FileMaker Pro Advanced version 11* software, and then transferred to the *STATA version 13* software for all the statistical analysis. In preliminary review, the descriptive statistics to visualize the frequency and distribution of various variables was done, followed by the calculation of the mean score for all the psychosocial constructs by age groups and gender, as described by Setbon and Raude(Setbon & Raude, 2010). Vaccination intention was the outcome of interest. To identify factors associated with it, both univariate and multivariate logistic regression analyses were performed. All the psychosocial constructs (of threat and coping appraisal) alongside the socio-demographic factors were used as independent variables. Chi square tests or Fischer exact tests for categorical variable and student t tests for continuous variable with normal distribution were performed. In a few occasion, the Mann-Whitney non-parametric test was applied. The variable entry criterion into multivariate analysis was set at Pvalue ≤ 0.25 and the variable retention criterion at Pvalue ≤ 0.1 so as to the minimize the discrepancies as a result of non-comparable parameters(Bursac, Gauss, Williams, & Hosmer, 2008). The optimal model was fitted by backward stepwise elimination. In the final model age and sex (potential confounder) were retained, and it was tested for the goodness of fit in Hosmer-Lemeshow and area under ROC curve methods. The observed associations were reported as odd ratio (OR) or adjusted odd ratio (aOR) with 95% confident interval, and those with pvalue ≤ 0.05 considered statistically significant.

Ethical consideration

This study received an Ethical approval from the WHO-EHESP CoPanFlu International Consortium of the *French School of Public Health (EHESP)* in France(Lapidus et al., 2012) and the Ethical Review Committee of the Djiboutian Ministry of Health's *National Institute of Public Health-INSP*(F Andayi et al., 2014; Lapidus et al., 2012). All participants gave informed written consent including the minors (<16 years) through their parents or guardians.

RESULTS

Participants

A total of 703 individuals from 324 households met the criteria and were included in this study, representing 67.3% participation. From the four administrative districts of Djibouti city, District 1 had the most, followed by District 2, District 3 and District 4, at 40.1%, 33.4%, 20.6% and 5.9%, respectively. Median age was 31.1 (range 15-100.9) years, with majority (79.4%) of them belonging to age group of 15 to 45 years old. Women were over-represented (60.9%) compared to men. Ethnic Somalis accounted for more than a half (63.3%) of the cohort, followed by the Arabs (17.5%) and the Afar (12.1%). Up to 20.5% of participants had Bac+, i.e. high school graduate qualification and above. When classified by occupation, the proportion of students was 14.5%, the employed 22.7% and the jobless 62.8%. Family sizes of participants varied with the number of subjects in each. Small families had ≤ 3 persons, medium had 4 persons and large had 5≥ persons, respectively. Most participants were from the small families (55.5%), followed by those from large (29.3%) and medium (15.2%). Three levels of socio-economic status (wealth index) were identified as the low (34.7%), middle (18.5%) and upper (46.8%). Lastly, the four at risk group to influenza infection were identified to be the pregnant women (3.1%), those with chronic illness (11.2%), those with recent respiratory illness (9.8%), and those with children in the household (59.3%).

Table 2 Socio-demographic factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

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Table 3 Media access and social motivation factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

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Socio-demographic characteristics

On vaccination history, 1.4% of participants reported to have had 2009 pandemic vaccination, but none had had a seasonal influenza vaccine. Of those enrolled, more than a half (56.3%) of them, reported vaccination intention against the 2009 pandemic influenza. This intention was highest (60-90%) in proportion among participants with Bac+ qualification, in employment, with chronic illness, residing in District 1 or District 4, of male gender, belonging to families of ≥ 4 persons, of Arab or Afar tribe, and of elderly age (≥60years).

In this study, the initial association between the socio-demographic factors and the vaccination intention was determined by the univariate analysis **(Table 2)**. This association was found to be influenced by participants' education level, socioeconomic status, occupation, ethnicity, household size and District of residence. But not, by their age group, gender and belonging to a household with children.

When asked on who would motivate them to pursue vaccination intention, most of them (70.4%) confessed that they looked upon clinicians, as opposed to the head of state, religious leader (sheikh) and social worker **(Table 3)**. This relationship with clinician was found to have significant (OR 2.95) association. On media access, majority of Djiboutian confessed their dependence on television and radio than newspaper and internet as source of information on pandemic influenza. Interesting, reliance on either audiovisual media or print was associated with less intention declaration.

Table 4 Psychosocial factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

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Psychosocial constructs

For each of the psychosocial construct, the overall cohort mean score and that of corresponding gender and age group categories found not to be statistically different from each other**(Table 1)**, except for a few. For example, a high mean score (≥3.5 on scale of 5) was reported for perceived worry, perceived severity, and subjective norms of belief in social support system and belief in individual health situation. The same applied for perceived vulnerability and perceived self efficiency, but only in two age groups at ≥45years old and 45-60years old, respectively.

Overall median score for most constructs were between 4 and 5, except for perceived influenza knowledge (1.0), perceived vulnerability (1.0), perceived consequences (2.0), perceived coherence (2.0) and the subjective norm of belief in fatalism (2.5) (**Table 4**). All the constructs had a significant association with the vaccination intention in bivariate analysis, but for one, the perceived coherence (**Table 4**).

Table 5 Predictors of pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

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Predictive model

The result of the final multivariate model is presented in **Table 5**, with prediction estimates of various covariates for pandemic vaccination intention. A Hosmer-Lemeshow goodness of fit test of pvalue of 0.9503 was obtained, suggesting an excellent predictive power. Further evaluation in the area under ROC curve, also confirmed a robust discriminatory power of 0.8615. This meant that the model could correctly discern up to 86% of participants who declared vaccination intention. Importantly, the perceived worry was found as the main predictor of vaccination intention declaration at aOR 3.22, while the low socio-economic status (aOR 0.17) or belief in fatalism (aOR 0.38) as important predictors for less intention. Other covariates in the model, but insignificant, were the perceived coherence, age group (15-30 years) and gender (women).

DISCUSSION

During the second pandemic wave, the use of WHO donated vaccines to protect the at risk population in poor nations was gaining momentum(Mihigo et al., 2012; WHO, 2012a). At that time, many of these countries were in preparation to receive and or rolling out the vaccination campaign. Understanding the general population orientation with regard to vaccination intention was necessary for wider coverage. This study was commissioned around this time and reflects the then mood of Djiboutian public towards pandemic influenza and vaccination intention. At least more than a half (56.3%) of them showed willingness to participate in future vaccination against the pandemic.

International comparison

These observations reported had a lot of similarities and differences to those conducted earlier and documented elsewhere in Asia, Australia, Europe and America(Bish et al., 2011; Brien et al., 2012). For example, in the recent vaccination intention studies, it indicates the proportion of 5-45% in Hong Kong(Lau et al., 2010), 11% in Turkey(Gaygısız, Gaygısız, Özkan, & Lajunen, 2010), 22-53% in Greece(Maltezou et al., 2010; Sypsa V, Livanios T, Psichogiou M, Malliori M, Tsiodras S, Nikolakopoulos I, 2009), 22% in Italy(Torre, Thiene, Cadeddu, Ricciardi, & Boccia, 2009), 44-67% in Australia(Eastwood, Durrheim, Jones, & Butler, 2010; Ferguson, Ferguson, Golledge, & McBride, 2010; Seale et al., 2011), 50-64% in USA(Horney, Moore, Davis, & MacDonald, 2010; Maurer, Harris, Parker, & Lurie, 2009; Tucker Edmonds, Coleman, Armstrong, & Shea, 2011), 56% UK(Rubin, Potts, & Michie, 2011), 61-62% in France(Schwarzinger, Flicoteaux, Cortarenoda, Obadia, & Moatti, 2010; Setbon & Raude, 2010), 68% in South Korea(Kwon, Cho, Lee, Bae, & Lee, 2011), 69% in Canada(Kaboli, Astrakianakis, Li, & Guzman, 2010), 70% in Malaysia(Wong & Sam, 2010) and 80% in Mexico(Esteves-Jaramillo et al., 2009).

However, this comparison should be with caution, since the timeline of these studies varied and opinions were likely influenced by other factors such as information and vaccine access. Of note is that, the intention was much higher at the beginning and declined towards the end of pandemic season, similar to the actual vaccination behavior(Bish et al., 2011; Brien et al., 2012). This trend has been explained to be a factor of progressive increase of knowledge that increase understanding of true risk, which then lowers the risk perception overtime(Bish et al., 2011). Though the intention studies, often do not translate to actual proportion of vaccination coverage, it allows a better insight on the potential inhibitors of vaccine uptake(Ibuka, Chapman, Meyers, Li, & Galvani, 2010).

In this cohort, at the time of the study, only 1.4% had had pandemic vaccination, which if with caution, is compared to those declaring intention at 56.3%, shows a big discrepancy retrospectively. However, this big difference between actual behavior and intention are not unique, but has also been reported elsewhere. For example, in recent studies reporting vaccine coverage versus intention declaration, indicates 5% versus 11% in Turkey(Gaygısız et al., 2010), 17% versus 53% in Greece(Rachiotis, Mouchtouri, Kremastinou, Gourgoulianis, & Hadjichristodoulou, 2010), 14% versus 22% in Italy(Torre et al., 2009), 14.5% versus 67% in Australia(Mak, Daly, Armstrong, & Effler, 2010), 20% versus 64% in USA(Maurer et al., 2009) and 11.1% versus 62% in France(Vaux et al., 2011).

Socio-demography and vaccination intention

For both vaccination intention and the actual behavior, as mentioned before are determined by both the individuals' psychosocial factors and socio-demographic dynamics. In this study, only 20.5% of the cohort had a Bac+ qualification, implying that nearly four fifth had some sort of reading and writing challenges. This collaborates other observations in this study that show lower pandemic influenza knowledge, compared to those reported in the USA, UK, Taiwan and morocco(Bish et al., 2011). In Djibouti, local media houses uses Somali and Arabic as the main language of communication, languages understood by more than 75% of the population. This, in a way, indicates if these channels were optimally used, the limitation of literacy would have been overcome. Lack of significant prediction of access to major mass media channels and declaration of intention, in part, adds credence to this argument.

In assessing social motivation to vaccination intention, we observed this could be also be another channel that could be exploited in future. This has been proven to work, particularly if persons in influential position demonstrate wiliness to participation or motivating participation, often results with high compliance(Fabry, Gagneur, & Pasquier, 2011; Tucker Edmonds et al., 2011). In this community, politician represented by the head of state (The President), religious leader (sheikh), and social workers; were found not to impress participation intention. However, clinicians were overwhelming quoted, and significantly found to motivate the vaccination interest. This is not uniquely of Djiboutian; but has been reported among the pregnant women of Quebec Canada and USA(Fabry et al., 2011; Tucker Edmonds et al., 2011).

Of note, was that the low socio-economic status participants were predictable as not willing to declare their intention. This is of obvious reason; the associated cost of getting vaccinated could inhibit participation of low income persons, for form majority of this population. Of interest, but not studies would have been to know whether proposing provision of free vaccines would have motivated participation or not. Future study on this aspect highly encouraged. However, this finding are contradictory to Taiwanese one, that observed high intention among persons of high SES status, citing engrave preventive medical practice attitude alongside access to disposable income, hence feeling "shameful" to confess(Huang, Miao, & Kuo, 2012).

Other parameters, such as age group and gender were found to be insignificant predictors of intention declaration. These two has in previous studies been found to have varied influence on intention declaration, with some studies showing strong association in elderly persons and others younger persons vice versa(Brien et al., 2012). But more elderly than young are reported to show high intention and has been linked to the routine seasonal influenza vaccination(Brien et al., 2012). The same case has been with gender, more women than men are reported to declare their intention of taking initiative, but it has been

linked to women worrying a lot more than men and by extension will readily seek precautionary behaviors than men(Sjoberg, 1998).

Psychosocial factors and vaccination intention

individual population perceptions of threat and coping appraisal are different so are their psychosocial factors determining their precautionary behavior(Rogers, 1975). Importantly is the level of knowledge, which remains central to other constructs. In this study, pandemic influenza knowledge varied from complete lack to somewhat lack of essential information (mean score of 1.5 on scale of 5). Observed low (‹3.5 mean score) perceived vulnerability, perceived consequence, perceived coherence and belief in fatalism; strongly correlated to depressed knowledge level. With caution though, least awareness might have been due to general low literacy and or ineffective pandemic awareness campaigns. Absentia of accurate information is often replaced by propaganda that exaggerates facts and skews the truth(Kata, 2010). This, in part, may explain the observed high perception on covariates measuring fear and anxiety among Djiboutian, such as perceived worry, perceived prevalence, and subjective norms (on health situation and social support system). If the drive of fear is enormous and no help is anticipated in worst case scenario, subjects give in to despair, ie they get maladapted, and have less desire to take precautionary behavior(Sjoberg, 1998). These observations are confirmed in the current study, in which the odds of declaring vaccination intentions were lesser among participants with belief in fatalism (extreme fear) (healing is natural and sickness is everyone's fate). But inverse to those with perceived worry (moderate fear) were willing to take precautionary behavior. The odds of declaring intention among this group, was about thrice more compared to the rest. This observation is consistent with French study that observed worry motivated pandemic vaccination intention(Setbon & Raude, 2010). However, more caution is warranted, given this cohort had oversampled women, who are known to worry more than men and hence likely to bias the outcome(Sjoberg, 1998). Lastly, unlike many studies before(Bish et al., 2011), in this study, the perceived severity, perceived coherence, perceived vulnerability, and subjective norm of self efficiency were not significantly associated to vaccination intention. This, as mentioned before, are attributable variance in population characteristics factors such as wealth index, media awareness campaign and literacy level.

Limitations of the study

This study has several limitations worth considering for fair interpretation. First, the study highlighted only the situational picture, as of, towards the end of the pandemic period, it excluded the important transitional phases of before and during, which in certain studies have shown shifts in perception of risk and illness representation(Ibuka et al., 2010). Secondly, the opportunistic study sampling of Hajji Pilgrim

data and Community health works (CHW) cannot claim to be representative of Djibouti population. There is a potential biasness in disproportionate sampling of persons from high wealth index (Hajji Pilgrims) and low wealth index (CHW database) with exclusion of the middle class. Thirdly, the study only focused on intention to vaccinate, and not the actual vaccination or vaccine accessibility; which are pertinent.

Implication of the study

The three significant parameters observed as predictors of pandemic vaccination intention, namely, perceived worry, subjective norm of belief in fatalism and low socioeconomic status, are strongly correlated and proper interpretations of this findings are essential for extrapolation in similar environment. Djiboutian like many cultures holds some beliefs(religious tenets, resilience to suffering, divine healing etc) which may not be true scientifically, and often interfere with obedience to recommended precaution health behavior(CDC, 2008). In this study, the measure of belief in fatalism is one such subjective norm. Here in entailing the perception of healing as a natural event and that sickness as everyone's fate. Such mind set happens in those who have resigned limited control of situation, and under such conditions they are bound to worry a lot about potential threat. The magnitude of this worry often depends on message communicated by the health department that is transmitted through media to the public. Lack of proper and accurate updates can lead to low information level and or misinformation that could probe excessive fear often known to result in mal-adaptation, refusal to comply(Floyd et al., 2000). Low income environment such as Djibouti, this is not uncommon. The authors are convinced that the this misfortune can be overcome by the use of audiovisual messages scripted in local dialect, which would positively increase disease awareness across SES and literacy levels of the population, with consequent increased participation intention. This study finding therefore provides insight for low income nations and encourages innovation on risk communication means as a way of increasing awareness and proxy to promoting compliance.

CONCLUSIONS

This study provides insights of pandemic vaccination intention of a population from a low resource environment. It has demonstrated that anxiety and socio-economic classes could influence future vaccination process. It is therefore concluded that massive public awareness should be prioritized to increase compliance, and the use of the less costly non-medical intervention should be proposed in future campaigns

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LIST OF TABLES AND FIGURES

Table 1 Comparison of mean psychosocial perceptions towards the pandemic (H1N1) 2009 influenza by age and gender, among Djiboutianr

Psychosocial covariates	Total	Male	Female	15-29yrs	30-44yrs	45-59yrs	≥60yrs
Sample size(N)	703	273	430	298	260	109	36
H1N1p Knowledge	1,50(±0,04)	1,46(±0,06)	1,53(±0,05)	1,45(±0,05)	1,45(±0,06)	1,68(±0,10)	1,71(±0,21)
Worry	3,95(±0,04)	3,96(±0,07)	3,95(±0,05)	3,84(±0,06)	3,99(±0,08)	4,11(±0,06)	3,9(±0,19)
Prevalence	4,28(±0,05)	4,31(±0,07)	4,26(±0,06)	4,29(±0,07)	4,29(±0,07)	4,28(±0,12)	4,21(±0,21)
Severity	3,06(±0,07)	3,17(±0,11)	2,98(±0,09)	3,17(±0,11)	2,87(±0,11)	3,27(±0,17)	3,03(±0,31)
Vulnerability	2,89(±0,08)	2,77(±0,13)	2,98(±0,10)	2,92(±0,13)	2,61(±0,13)	3,51(±0,19)	3,12(±0,35)
Self efficiency(control)	3,24(±0,79)	3,20(±0,13)	3,26(±0,10)	3,89(±0,12)	2,97(±0,13)	3,53(±0,19)	3,23(±0,34)
Consequences	2,21(±0,05)	2,17(±0,08)	2,24(±0,06)	2,24(±0,08)	2,10(±0,08)	2,36(±0,12)	2,39(±0,17)
Coherence (disease progression)	2,22(±0,04)	2,22(±0,07)	2,22(±0,05)	2,26(±0,07)	2,12(±0,07)	2,33(±0,10)	2,31(±0,18)
Response (vaccine) efficiency	3,07(±0,07)	3,13(±0,11)	3,02(±0,09)	3,13(±0,11)	2,94(±0,11)	3,32(±0,17)	2,88(±0,32)
Belief in health conspiracy	4,09(±0,03)	4,08(±0,05)	4,10(±0,04)	4,12(±0,05)	4,10(±0,05)	4,04(±0,07)	4,02(±0,11)
Belief in fatalism	2,53(±0,05)	2,45(±0,08)	2,58(±0,06)	2,47(±0,08)	2,44(±0,08)	2,81(±0,12)	2,72(±0,19)
Belief in social support	4,55(±0,03)	4,50(±0,05)	4,58(±0,04)	4,49(±0,05)	4,56(±0,05)	4,64(±0,07)	4,61(±0,13)

Table 2 Socio-demographic factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

Population characteristics		Sample	Vaccination intention		Odd ratio	p-value
		N	n	%	95% CI	
Overall		703	396	56,3%		
Age groups	15-29yrs	298	163	54,7%	1,37(0,97-1,96)	0,082
	30-44yrs	260	139	53,5%	Ref	Ref
	45-59yrs	109	70	64,2%	1,58(0,98-2,55)	0,059
	≥60yrs	36	23	63,9%	1,52(0,72-3,18)	0,271
Ethnic	Afar	85	52	61,2%	1,43(0,86-2,37)	0,166
	Arab	123	80	65,0%	1,86(1,18-2,93)	0,008
	Ethiopian	40	22	55,0%	1,17(0,58-2,35)	0,666
	Immigrants	10	4	40,0%	0,59(0,16-2,24)	0,442
	Somalis	445	229	51,5%	Ref	Ref
Persons in household	≤3	390	163	41,8%	0,20(0,13-0,30)	0,000
	4	107	80	74,8%	1,07(0,58-2,01)	0,821
	≥5	206	153	74,3%	Ref	Ref
Wealth Index	Low	244	81	33,2%	0,34(0,22-0,51)	0,000
	Middle	130	61	46,9%	0,84(0,50-1,43)	0,526
	Upper	329	167	50,8%	Ref	Ref
Gender	Female	429	151	35,2%	1,15(0,83-1,58)	0,406
	Male	274	244	89,1%	Ref	Ref
District	1	282	190	67,4%	2,84(1,94-4,16)	0,000
	2	235	107	45,5%	Ref	Ref
	3	145	66	45,5%	1,00(1,00-1,00)	0,678
	4	41	33	80,5%	3,84(1,75-8,40)	0,001
Pregnant women	1	13	7	53,8%	0,87(0,27-2,80)	0,817
Having children at home	1	417	234	56,1%	1,09(0,79-2,80)	0,613
Chronic illness	1	79	51	64,6%	1,89(1,08-3,32)	0,027
Recent ILI* illness	1	69	35	50,7%	1,05(0,60-1,85)	0,865
Education level	Bac+	144	103	71,5%	4,65(2,73-2,93)	0,025
	Below Bac+	559	257	46,0%	Ref	Ref
Occupation	student	102	49	48,0%	2,31(1,29-4,14)	0,005
	onjob	160	110	68,8%	1,88(1,27-2,77)	0,002
	Jobles	442	237	53,6%	Ref	Ref

*influenza and influenza like illness

Table 3 Media access and social motivation factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

(a) Who will motivate their intention to vaccination

Source of motivation	Sample	Vaccination intention		Odd ratio	p-value
	N	n	%	95% CI	
Clinicians	433	305	70,4%	2,95(2,10-4,15)	0,000
Head of state	65	26	40,0%	0,40(0,24-0,68)	0,001
Religious leader (sheikh)	180	77	42,8%	0,34(0,24-0,49)	0,000
Community social worker	32	9	28,1%	0,22(0,10-0,49)	0,000

(b) What was their main source of pandemic influenza information

Media channel	Sample	Vaccination intention		Odd ratio	p-value
	N	n	%	95% CI	
Newspaper	57	21	36,8%	0,33(0,19-0,58)	0,000
Radio	161	83	51,6%	0,57(0,40-0,81)	0,002
Internet	11	11	100,0%	-	-
Television	531	330	62,1%	1,08(0,71-1,63)	0,729

Table 4 Psychosocial factors associated with pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

Psychosocial covariates	Vaccination Intention	Mean Score	Std Err	Median	Odd Ratio 95% CI	p-value
(a) Threat Appraisal						
Influenza Knowledge	Overall (1 & 0)	1,5	0,04	1,00	2,36(1,83-3,03)	0,000
	Yes (1)	1,72	0,05			
	No (0)	1,15	0,04			
Worry	Overall (1 & 0)	3,95	0,04	4,00	2,77(1,48-5,19)	0,001
	Yes (1)	3,99	0,04			
	No (0)	3,5	0,18			
Prevalence	Overall (1 & 0)	4,28	0,47	5,00	0,93(0,81-1,07)	0,310
	Yes (1)	4,25	0,06			
	No (0)	4,34	0,07			
Severity	Overall (1 & 0)	3,06	0,07	4,00	1,90(1,71-2,11)	0,000
	Yes (1)	3,78	0,08			
	No (0)	1,95	0,10			
Vulnerability	Overall (1 & 0)	2,89	0,08	1,00	1,81(1,63-2,00)	0,000
	Yes (1)	3,68	0,10			
	No (0)	1,64	0,09			
(b) Coping Appraisal						
Self efficacy	Overall (1 & 0)	3,24	0,08	5,00	2,09(1,89-2,32)	0,000
	Yes (1)	4,19	0,08			
	No (0)	1,68	0,10			
Consequences	Overall (1 & 0)	2,21	0,05	2,00	2,16(1,81-2,57)	0,000
	Yes (1)	2,57	0,06			
	No (0)	1,64	0,06			
Coherence (Disease Progression)	Overall (1 & 0)	2,21	0,06	2,00	3,78(3,02-4,73)	0,000
	Yes (1)	2,66	0,05			
	No (0)	1,51	0,05			
Response (Vaccine) Efficacy	Overall (1 & 0)	3,07	0,07	4,00	1,89(1,70-2,11)	0,000
	Yes (1)	3,77	0,08			
	No (0)	1,96	0,1			
*Subjective Norms**						
Belief In Health situation	Overall (1 & 0)	4,09	0,03	4,50	2,43(1,95-3,03)	0,000
	Yes (1)	4,3	0,03			
	No (0)	3,77	0,06			
Belief In Fatalism	Overall (1 & 0)	2,53	0,04	2,50	1,45(1,26-1,67)	0,000
	Yes (1)	2,72	0,06			
	No (0)	2,2	0,08			
Belief In Social Support	Overall (1 & 0)	4,55	0,32	5,00	1,34(1,09-1,65)	0,005
	Yes (1)	4,61	0,04			
	No (0)	4,43	0,06			

*beliefs and attitudes, often not scientifically true

Table 5 Predictors of pandemic (H1N1) 2009 influenza vaccination intention among Djiboutian

Population characteristics	Odd ratio 95% CI	p-value
Age group (15-30yrs)	1,07(0,26-4,33)	0,929
Gender (Female)	1,46(0,33-6,36)	0,617
Low SES	0,17(0,04-0,75)	0,019
Perceived worry	3,22(1,40-7,40)	0,006
Perceived coherence	1,99(1,40-4,80)	0,125
Belief in fatalism	0,38(0,19-0,77)	0,007

APPENDIX II

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