TURKISH REPUBLIC OF NORTH CYPRUS
NEAR EAST UNIVERSITY
HEALTH SCIENCES INSTITUTE

INVESTIGATION OF ZIKA VIRUS PREVALENCE IN NORTHERN CYPRUS

FATHI SALEM ABUSHOUFA

MASTERS THESIS

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

MENTOR

ASSIST. PROF. DR. AYSE SARIOGLU

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The Directorate of Health Sciences Institute

The thesis committee has accepted this study for the degree of Master of Science in Medical Microbiology and Clinical Microbiology.

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Director of Institute of Health Sciences

DECLARATION

Hereby I declare that this thesis study is my own study, I had no unethical behavior in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behavior of breeching patent rights and copyright infringement during the study and writing of this thesis.

Fathi Saleem Aboushoufa

Signature

ACKNOWLEDGEMENT

Firstly, I would like to thank God almighty, for this blessed opportunity. I would like to express my best gratitude and appreciation to my family especially my parent and my wife who have been supported and encouraged me in all steps of life including my education journey.

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Last but not least, I would like to express many thanks to the Near East University for giving me the chance to do a master's thesis.

DEDICATION

To my parents…I dedicate this research for always trying to see the best come out from me, Also, this research dedicated to my wife for her understanding, caring and encouragement. And for all friends and everyone who supported and helped me in education journey.

ÖZET

Fathi Saleem Aboushoufa

Yrd. Doç. Dr. Ayşe Sarıoğlu

Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı

Coğrafi modifikasyonlar, özellikle iklim değişikliği ve nüfus artışı, sağlık açısından tüm dünya için potansiyel bir tehlike haline gelmektedir. Özellikle, Zika, Batı Nil, Dang, Chikungunya ve Sıtma gibi sivrisineklerin sebeb olduğu farklı enfeksiyonlardaki artış son dönemde önem kazanmaktadır. Vektör kaynaklı (çoğunlukla sivrisinekler ve keneler) viral hastalıklar, kıtadaki basit ve hızlı göçü nedeniyle büyük endişe kaynağıdır. Altmış yıl önce, insanlarda farklı öldürücü hastalık salgınları, Amerika Birleşik Devletleri'ndeki Hantavirüs ve Henipavirüsler gibi hayvanlardan kaynaklanan virüslerin genişlemesinden ve ortaya çıkmasından kaynaklanmaktaydı. Zika virüsü, serebral kalsifikasyonlar, ventrikülomegali, hidranensefali, mikrosefali, beyin yapılarında yokluk ve anormallikler ve göz kalsifikasyonları gibi fetal anormallikleri içeren birçok hastalıkla ilişkili olabilmektedir. Bu araştırmanın amacı, Kuzey Kıbrıs Türk Cumhuriyeti (K.K.T.C) vatandaşlarında Zika virus enfeksiyonunun varlığının araştırılmasıdır. Kuzey Kıbrıs Türk Cumhuriyeti vatandaşı olan veya K.K.T.C.'de 5 yıldan beridir ikamet etmiş toplam 91 hasta örneği bu çalışma dahl edilmişir.Örnekler Yakın Doğu Üniversitesi kan bankasından toplanmıştır. Serum örnekleri çalışılana kadar -80 ° C'de saklandı. Serum numunelerinde Zika virüs IgG antikorları ELISA (VERSAmax Ayarlanabilir Microplate Reader Operator.s Manual) tekniği ile araştırıldı. Test edilen 91 örnekten hiçbirinde Zika virüs Ig G pozitif olarak tespit edilmedi. Sonuç olarak, Kuzey Kıbrıs'taki Zika virüs enfeksiyonlarını gösterme özelliği taşıyan çalışmamızda, Zika virüsün olmadığı tespit edildi.

Anahtar kelimeler: Zika virüs, K.K.T.C., ELISA

SUMMARY

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Geographical modulations, especially in terms of climate change and population is now becoming a potential danger to the whole globe especially in the aspect of health. Specifically, due to increase in infection rates of different mosquitoes borne diseases like Zika, West Nile, Dengue, Chikungunya and Malaria. The vector borne (mostly mosquitoes and ticks) viral diseases are of great concern because of its simple and rapid migration throughout the continents. Sixty years ago, different killer diseases outbreaks in human aggravated from the wide spread and emergence of viruses that originated from animals such as Hantavirus in the United States of America and Henipaviruses. There is a strong relationship between Zika diseases and a rise in fetal abnormalities for example cerebral calcifications, ventriculomegaly, hydranencephaly, microcephaly, absence and anomalies in brain structures and calcifications of eye. The objectives of this research was to investigate the prevelance of Zika virus among citizens of Turkish Republic of Northern Cyprus (TRNC). A total of 91 patients's samples who are citizens of Northern Cyprus or resident in T.R.N.C. for at least 5 years were selected from the Near East University blood bank in this study. Serum samples was stored at -30°C until they use. Serum samples were tested by ELISA (VERSAmax Tunable Microplate Reader Operator.s Manual) for IgG antibodies against Zika virus. Out of 91 samples, we did not detect any Zika virus Ig G positivity. The result of the study shows that there is no risk for Zika virus infection in Northern Cyprus.

Key words: Zika virus, Northern Cyprus, Elisa

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Abbreviations

CHIK: Chikungunya

DENV: virus Dengue virus

EEA: European Economic Area

ELISA: Enzyme linked immunosorbent assay

EU: European Union

F: Female

FDA EU: Food and drug agency of European Union

GBS: Gullian-Barre Syndrom

JEV: Japanese Encephalite virus

JSHU: Joint Services Health Unit

M: Male

MERS: Moderate Eastern Respiratory Syndrome

NSB: Non-specific binding

OD: Optical density

PCR: Polymerase chain reaction

PRNT: Plaque-reduction neutralization test

RNA: Ribonuclecleic acid

SARS: Severe Acute Respıratory Syndrome

SPSS: Statistical Package for the Social Sciences

TBEV: Tick-borne encephalitis TBEV: virus

TRNC: Turkish Republic of North Cyprus

WHO: World Health Organization

WNV: West Nile

YFV: Yellow Fever virus

ZIKV:Zika virus

CHAPTER 1

1. INTRODUCTION

1.0 Zika Virus

Geographical modulations, especially in terms of climate change and population is now becoming a potential danger to the whole globe especially in the aspect of health. Specifically, due to increase in infection rates of different mosquitoes borne diseases like Zika, West Nile, Dengue, Chikungunya and Malaria (Singh et al., 2016). The vector borne (mostly mosquitoes and ticks) viral diseases are of great concern because of its simple and rapid migration throughout the continents. Sixty years ago, different killer diseases outbreaks in human aggravated from the wide spread and emergence of viruses that originated from animals such as Hantavirus in the United States of America and Henipaviruses (Cedar virus, Nipah, and Hendra) in Australia, different influenza subtypes such as swine flu, bird flu etc, Ebola virus from African countries, Severe Acute Respıratory Syndrome (SARS) and Moderate Eastern Respiratory Syndrome (MERS). Previously, Ebola was the killer disease that threatened the entire world due to its outrageous nature, but recently Zika virus (ZIKV) already sets the floor on fire because of its wider and rapid spread (Ioos et al., 2014). Its outbreaks were more challenging in the sense that they cannot be easily and quickly predicted as well due to their rapid, quick and wider dispersion among affected human beings. Furthermore, lack of proper prevention and less knowledge about the epidemiology of the disease resulted in making the situation worst (Hamel et al., 2016). Recently, there is a raising concern on Zika virus due to its world global wide spread not as in its initial incubation and emancipation, whereby its was limited and was only found in its dwelling regions, which are Asia and Africa (Tjaden, Caminade, Beierkuhnlein, & Thomas, 2018). People from North America were victimized due to the emergence of different arthropod borne diseases such as Chikungunya, Powassan, Jamestown Canyon and recently Zika (G. Calvet et al., 2016). Currently Zika virus is regarded as an infectious disease that has the ability of spreading over new areas in which *Aedes* mosquito vector can be found (D. Musso et al., 2014). This virus has a single stranded, unsegmented, positive sense RNA genome having an approximate 11kb with a single open reading frame (Atkinson et al., 2016).

There is a strong relationship between Zika diseases and a rise in fetal abnormalities for example cerebral calcifications, ventriculomegaly, hydranencephaly, microcephaly, absence and anomalies in brain structures and calcifications of eye etc. More than four thousand babies

(400) from Brazil since 2015 were born having anomalies having small and abnormal brain and head which is a condition known as microcephaly. The transmission of Zika virus generally occur via the perinatal transmission, *Aedes* mosquito, blood transfusion and sexual intercourse. Also, from affected mother, it could be transmitted through the transplacental way which can subsequently affects the undeveloped brain of the baby. Mostly, the methods used in diagnosing Zika virus comprises of polymerase chain reaction (PCR) and Ig M-Ig G based enzyme linked immunosorbent assay (ELISA)techniques. Though, there is no single effective and efficient treatment for such disease (Didier Musso et al., 2015). But vaccination can be employed as an effective alternative which can controls and reduce the negative effects of this disease. Therefore, control and prevention stand to be the only methods in our arsenal to fight against this disease. Other various techniques that can be used to control mosquito's population such as using chemicals, controlling mechanically and controlling genetically are equally available and effective for reducing the harm of this disease (Gourinat, O'Connor, Calvez, Goarant, & Dupont-Rouzeyrol, 2015).

Zika virus was first isolated in 1947 in Uganda. In addition, since the early Zika virus outbreak in Brazil in 2015, these viruses have been widely spread quickly across Mexico, Central and South America. These viruses are so challenging, as their outbreak was usually rapid and unpredictably spreading across different regions among affected and infected human beings. Regarding this situation, the World Health Organization (WHO) on first of February 2016 declared Zika virus as a 'Public Health Emergency of International Concern'.

Even though Zika virus is now regarded as a great as well a significant danger and threats to general public health around the world, there is no much research conducted on it in Africa especially in the North Africa and other parts of the world (Andrea, 2018.).

The aim of this research was to investigate the seroprevelance of the Zika virus infections among citizens of Turkish Republic of Northern Cyprus.

LITERATURE REVIEW

1.1 Zika virus

1.1.1 Zika lineages

Zoonotic Zika virus is a single stranded positive RNA virus which belongs to the genus flavivirus and a family of *Flaviviridae*.

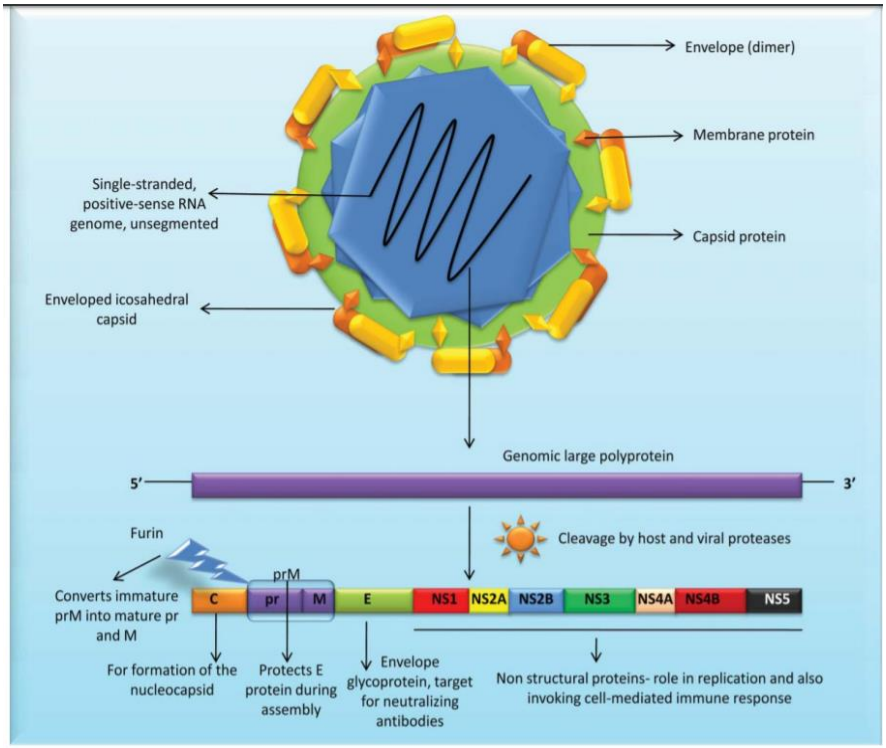

Fig. 1.1: Structure of Zika virus and its genome (Singh et al., 2016).

The medical significance of mosquitoes borne of the Flaviviruses are Zika virus, tick borne encephalitis, Yellow Fever virus (YFV), Japanese Encephalite virus (JEV), West Nile virus (WNV) and Dengue virus (DENV). Genomic and phylogenetic analysis have shown that Zika virus main lineages are African and Asian. And the research also showed that the African lineage is further sub-divided into two other sub divisions (Singh et al., 2016).

It has been reported by Faye et al. that the geographical distribution of Zika virus originated from Uganda strain which has been reported based on two independent Zika virus introductions from west and east Africa. Additionally, it is possible that a Malaysian strain has also originated from this Ugandan strain during the mid of the twentieth century, where by the Asian lineage subsequently originated from the initial Malaysian strain. Faye et al., added that whether the

Asian lineage has originated from the African strain due to the fact that there is a quick and rapid wide spread in the Asian region. Even though, other theories can be taken into consideration. The first one was Zika virus was first introduces to Asia during the mid of the 20th century. Secondly, both of the Zika virus lineages have parallel evolution prove resulting from an ancient and common ancestor. A detailed analysis based on the differences between the 2 lineages as a result of the possibility of the mutation that can help us in understanding the observable neuronal complications. Moreover, the yet un answered question still remains unresolved regarding the natural host of Zika virus. Though some studies showed some serological highlights that showed evidence in monkeys as well as other animals (Gong, Xu, & Han, 2017). Figure 1.2 below shows the phylogenetic analysis of the isolated ZIKV strains.

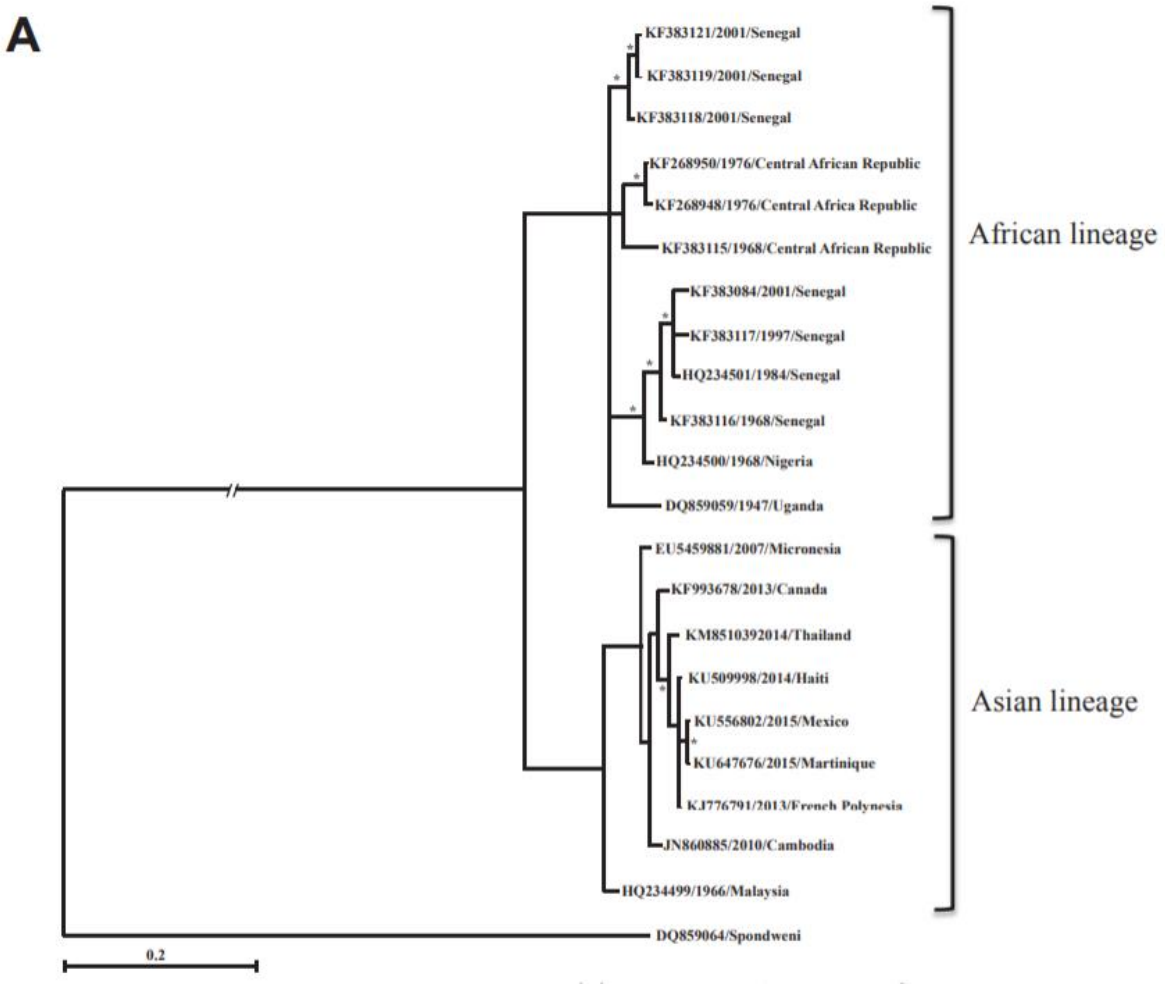

Fig. 1.2: Phylogenetic analysis of the isolated strains of ZIKV. Non-concatenated nucleic acid sequences of the NS5 (Gong, Xu, & Han, 2017)

1.2 History of Zika virus

This virus is a member of the genus Flavivirus and belongs to the family of *Flaviviridae*. which consists of other global significant human pathogens like tick-borne encephalitis (TBEV) virus, YFV, JEV, DENV and WNV. Zika virus was isolated from a febrile sentinel rhesus monkey in the year 1947 in Zika forest which is located in Entebbe city Uganda at the Experimental Center for East African Viruses' Research Institute. The virus was further isolated from *Aedes* africanus mosquitoes from the same country and in the same forest, and a lot of monkey species were found to be Zika virus positive (Lazear & Diamond, 2016). Lower animals in the Zika forest such as civets, giant pouched rats, tree rats and squirrels do not show any serological proof of Zika virus infection. Therefore, higher primates i.e monkeys and humans are the main vertebrate hosts for Zika virus (Failloux et al., 2017). Following the next decades after Zika discovery, it was equally isolated from human beings during an outbreak in Southeast Asia and Africa, though it remains obscure because of its fairly nature benign of the infection. Zika came into consideration globally in the year 2007, when it created a widely outbreak in Micronesia (Singh et al., 2016). It has been reported that approximately 75% of the population of the island of Yap became infected with this disease during a period of four months. In the beginning of 2015, it has been found in Brazil and then widely spread throughout Oceania. It was further dispersed and spread into Europe, United States and other places mostly via travelers from Caribbean and Latin America. The rate by which this virus was spread in Caribbean and Latin America since its emancipation can be comparably the same with the outbreak of Chikungunya virus through the western Hemisphere in 2013. (Failloux et al., 2017).

1.3 Geographic distribution of the emergence of Zika viruses in Africa

The African continents shows evidences of the emergence of Zika virus through serological studies in humans, sporadic cases and epidemics, as shown in figure 1.3.

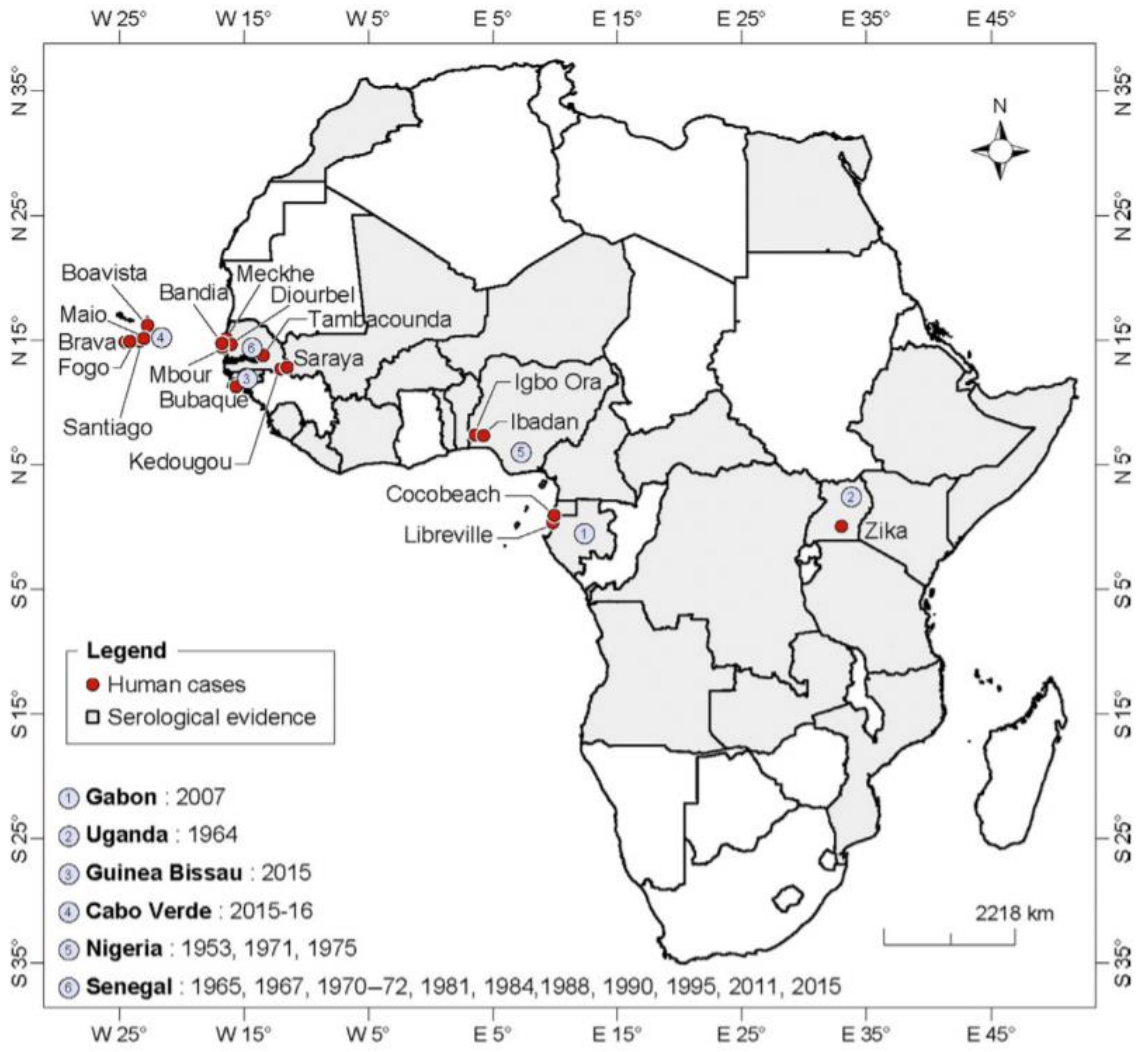

Fig. 1.3 Geographic distribution of ZIKV emergences in humans in Africa (Failloux et al., 2017)

1.3.1 West Africa

The early detection of Zika virus in Africa was in Senegal at Fleuve, Ferlo, Casamance and Dakar in 1962. Mostly, Zika virus was recorded more in the western part of this country than other parts especially on children below the age of ten. Serological studies have shown that the antibodies of children below the age of 4 was prone to be attacked by this virus in Bandia in the year 1967. A survey shows that approximately 59% of the populations of the children from Senegal oriental, Sine Saloum, Casamance and Diourbel are found to Zika disease positive. Recently, Senegal reported the first-time outbreak of Zika virus in 2016. The recent survey conducted at Ivory Coast in 1999 at Comoe National park location showed that about 49% of the population were Zika virus positive during the YF outbreak detection. In 1953, a young girl was isolated with Zika virus strain in Nigeria as the first reported case of Zika virus in the

country after conducting neutralizing antibodies experiments among children below the age of 16 from a city called Ilorin in 1951. Equally, it has been reported by Moore and his research group, who isolated this virus from 3 patients in 1975 in Ibadan state of Nigeria. In Sierra Leone, it has been reported that Zika virus was among the commonest killer disease in the region (Lanciotti, Lambert, Holodniy, Saavedra, & del Carmen Castillo Signor, 2016).

Lastly, in 2015, issues of prurit that is in association with fever, skin rash have been reported in women and adult in West Africa. Around May 2016 there was over 7000 suspected cases that were reported mainly in Fogo, Maio and Santiago Island. Whereby among the first 64 blood samples that were collected more than 15 patients have already been infected with Zika Virus disease (Grard et al., 2014).

1.3.2 Central Africa

In Gabon, human cases of Zika virus were first discovered in the year 2007. Similar to Chikungunya virus and dengue outbreak. Zika virus was found in four human blood samples which were collected from Coccobeach and Libreville. Prior to this outbreak, proof on human infection by Zika virus was limited (Grard et al., 2014).

A study was conducted on Zika virus infection to human in Cameroon in the year 2010 and the result showed that almost 12% of the tested subjects were infected with Zika virus disease. Recently, a study was conducted in the whole country and the result showed that about 18% of the subject to prevalence and prone to this disease (F.N. et al., 2012).

1.3.3 East Africa

As stated, Uganda is the first African country where Zika virus disease was firstly detected in 1947. During the studies it has been reported that 12.8% of the 261 patients which were from 4 different districts i.e Molambi-Gambo, Centre, Toro and Bwamba were found to be Zika virus positive.

It has also been reported in Kenya that a low seroprevalence for Zika virus was conducted in Kitui, Malindi and Nyanza which showed the prevalence of percentage of 1.3%, 52% and 3.3% respectively. Another research from Northern Kenya showed that about 13% of the population for the research ecology were infected with Zika virus (Report, 1987.).

1.3.4 Southern Africa

In 2013, a risk assessment test was conducted in Zambia, which showed that approximately 6% of the subjects were Zika virus positive which is based on IgG-IgM antibodies test performed in the country. In Angola a survey was conducted around 14 districts of the country in 1960 out of which 17% were found to be Zika virus positive (Jupp, 2005).

1.3.5 North Africa: Egypt and Morocco

In Egypt a neutralizing Zika virus antibodies have been found in every one out of two hundred tested subjects in 1950. Equally, it has also been reported to show lower percentage on subjects in which antibodies to Zika virus were conducted in Morocco in 1968 to 1969 (Diallo, Dia, Diagne, Gaye, & Diallo, 2018). Figure .1.4 shows the transmission cycle of zika virus among African regions.

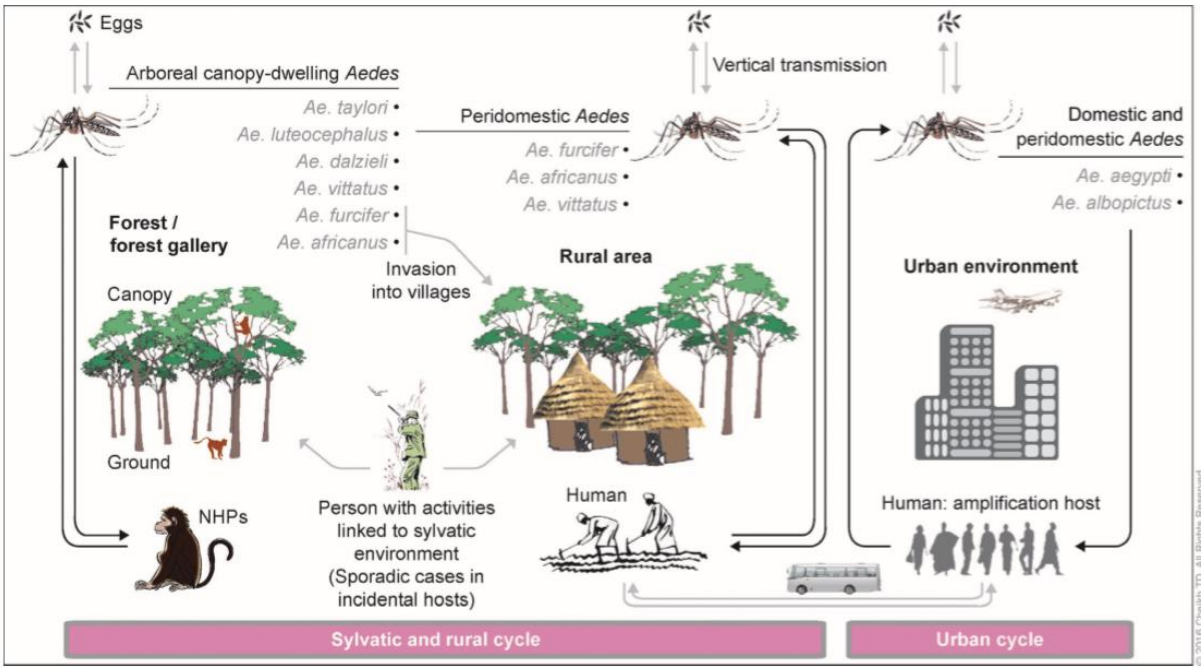

Fig. 1.4: Transmission cycles of Zika virus in Africa (Diallo, Dia, Diagne, Gaye, & Diallo, 2018)

1.4 Asia

As far as the relevance of climate change is concern for Zika virus and similar disease transmission. Climate is very vital as a determining factor for its transmission. Asian countries are expensive localities that accounts so much for the tropical and subtropical land mass. Even though, there is a large climatological variation that have effect on these area as far as Zika virus transmission is concern. The population of vectors capable of transmitting Zika virus are

widespread and persistent in even dense area in Asia than even the disease as currently or even historically (Plourde & Bloch, 2016).

These vulnerability leads from its inability in addressing shifting diseases patterns because of their changing climatic nature. This is more pronounce in areas having lower ability of absorbing shocks in the public and health care systems through severe and novel frequent outbreaks of Zika virus. Zika infection is known to be associated with various neurological orders such as microcephaly among others in this region. The burden of these diseases is already catastrophic due to the fact that the widespread of vaccine implementation has not come earlier and the vaccines and drugs are not approved quickly (Quam, n.d 1987).

Climate can be used in determining the baseline of conditions of an environmental conducive for disease transmission, which once its optimized and fulfilled, there will be an increase in the disease incidences. The figure 1.5 shows dependencies of temperature on other 5 parameters that can influence potentials of the endemic Zika transmission, which was done based on the models proposed by Liu-Helmersson and colleagues which was published inn 2014 (Plourde & Bloch, 2016).

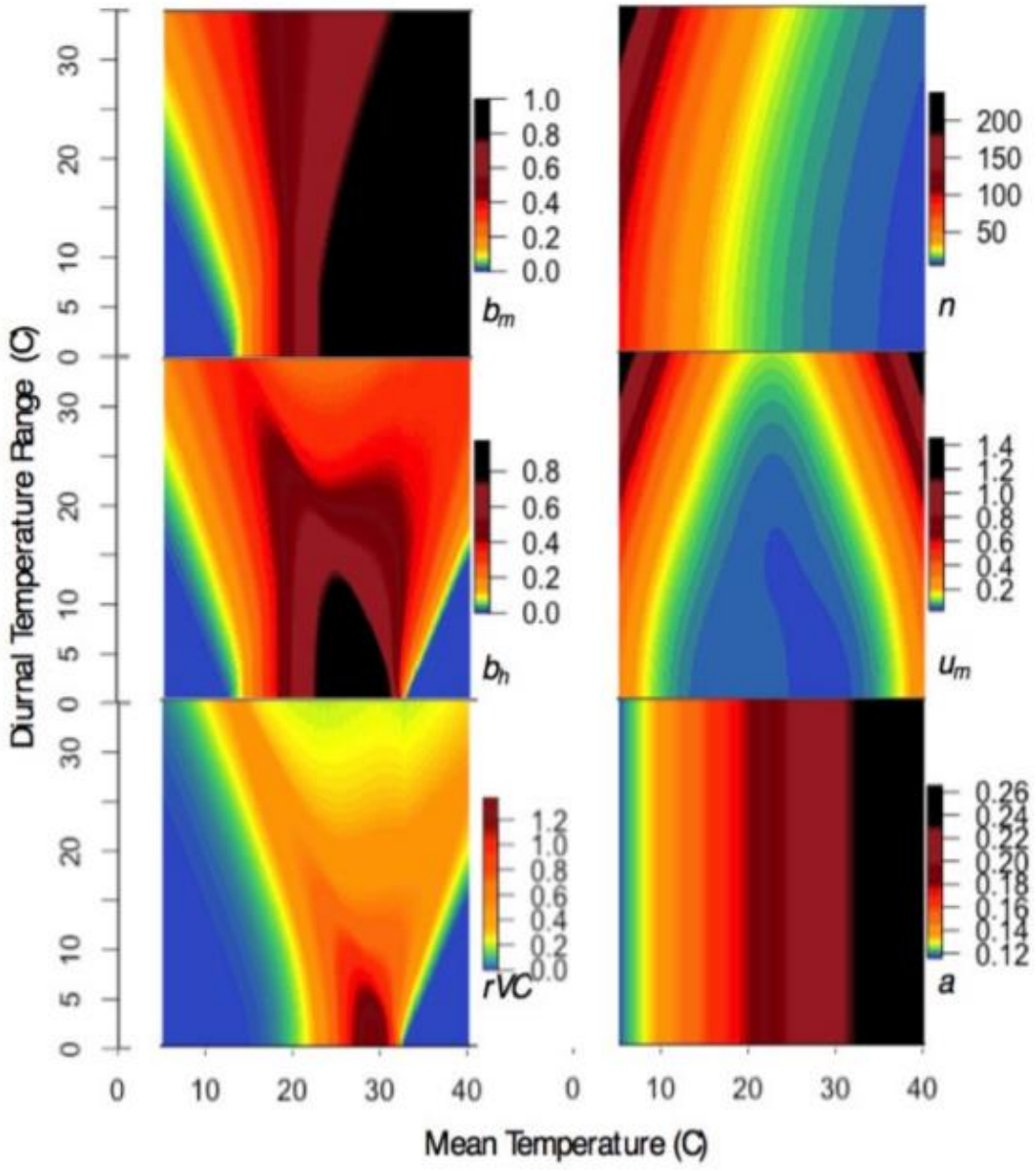

Fig. 1.5 Transmission of Zika virus infections and temperature dependency (Ritter, Martines, & Zaki, 2017)

1.5 Europe

The most important and relevant factor of changing climatic pattern for Zika solely depends on the extent by which climate can served as the determining factor in the ongoing transmission at a given region. This relevance is attributed to the extent that the public health and the health care systems can manage the diseases or can prevent the human population from receiving the negative menace or even being infected. However, because Europe is better equipped and

highly civilized in various aspects compared to other regions of the world like Asia, Africa and America. Therefore, local transmission of Zika occurred very rarely in Europe in the last and current century. The figure 1.6 shows the pattern of the expected changing suitability of the Zika virus epidemics in Europe for the last and current century (Zumla et al., 2016).

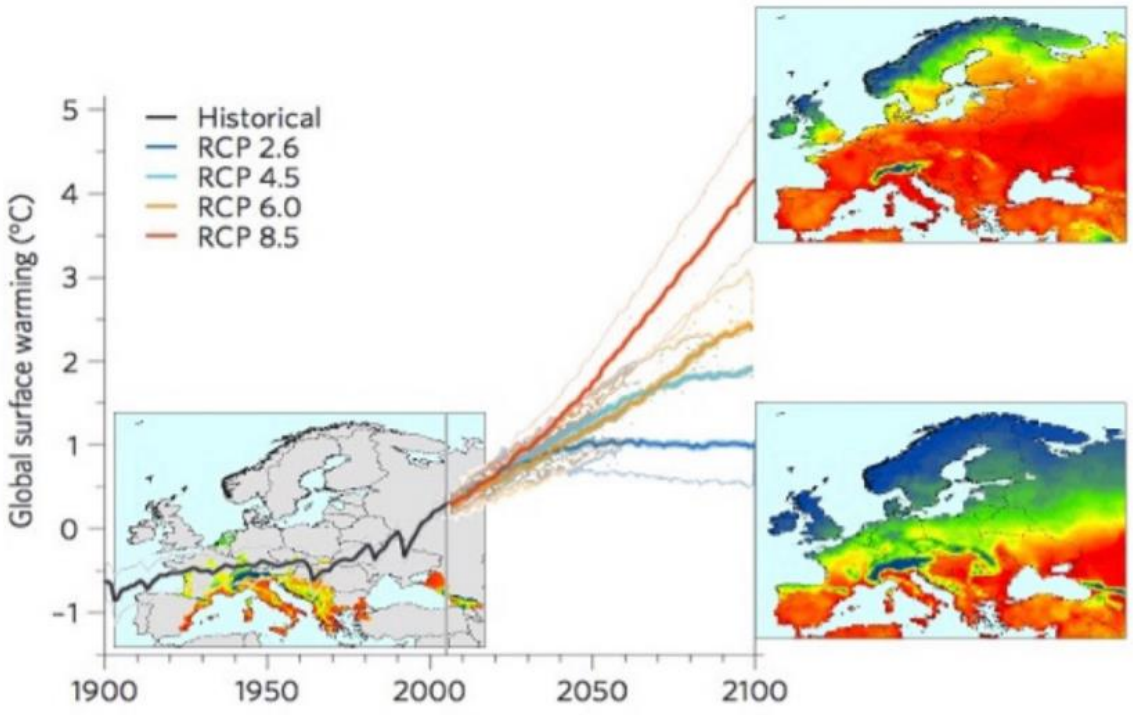

Fig. 1.6: Zika virus potential in Europe (Boeuf, Drummer, Richards, Scoullar, & Beeson, 2016)

Table 1.1 below shows the distribution of Zika virus disease cases that have been reported in various European countries ranging from 2015- 2017 by the European Union. In which Bulgaria, Cyprus, Iceland, Liechtenstein and Poland have not encountered the outbreak Zika virus disease in this years. These *Aedes* mosquito virus is generally the causative agent or the vector of this virus.

Table 1.1 Distribution of Zika virus disease cases by country, EU/EEA, 2015–2017 (Report, 2019)

Country	**2015**	**2016**	**2017**
Reported cases	**Reported cases**	**Reported cases**	Reported Cases
Austria	1	41	8

Belgium	1	120	42
Bulgaria	.	.	.
Croatia	.	.	0
Cyprus	.	.	.
Czech Republic	.	13	4
Denmark	.	8	6
Estonia	.	0	0
Finland	1	6	2
France	.	1 141	28
Germany	.	.	69
Greece	.	2	1
Hungary	.	2	0
Iceland	.	.	.
Ireland	1	15	4
Italy	.	101	25
Latvia	0	0	0
Liechtenstein	.	.	.
Lithuania	.	.	0
Luxembourg	.	2	0
Malta	.	2	0
Netherlands	11	98	6
Norway	.	8	4
Poland	.	.	.

Portugal	.	18	1
Romania	.	3	0
Slovakia	.	3	0
Slovenia	.	7	0
Spain	10	301	44
Sweden	1	34	16
United Kingdom	3	194	14
EU/EEA	29	119	27

1.6 Turkish Republic of North Cyprus (TRNC)

Generally, mosquitoes are the major vectors of parasitic viral pathogens, and helminths that causes diseases to humans. There is a growing interest for determining and examining the establishment as well as widespread of *Aedes* mosquitoes, which subsequently dispersed Zika virus. It has been reported that understanding native mosquitoes is very important due to concerns about white and black-striped mosquitoes that bites aggressively in the day. A survey was conducted at Southern part of the Cyprus at Limassol district. Cyprus is an island, which is divided into two different regions named as Northern and Southern Cyprus (Violaris et al., 2009). In that study, all adult mosquitoes were collected during June 2015 and these mosquitoes were identified as *Aede*s (Stegomyia) cretinus. At the same period (June 2015) the public health managements of the Cyprus Republic equally collected cretinus adult mosquitoes from local districts and villages. In conclusion, the study highlighted that there is a need to educate and raise awareness through seminar and workshop programmes concerning insects that have medical importance and the need for networking and collaboration between relevant authorities which involves the government, health care system authorities and the citizens (Martinou et al., 2016). Figure 1.7 below shows the location of the collection sites for *Aedes cretinus* in Cyprus.

Finally, to our best knowledge there has not been any published report in the literature related with Zika virus infections in Northern Cyprus.

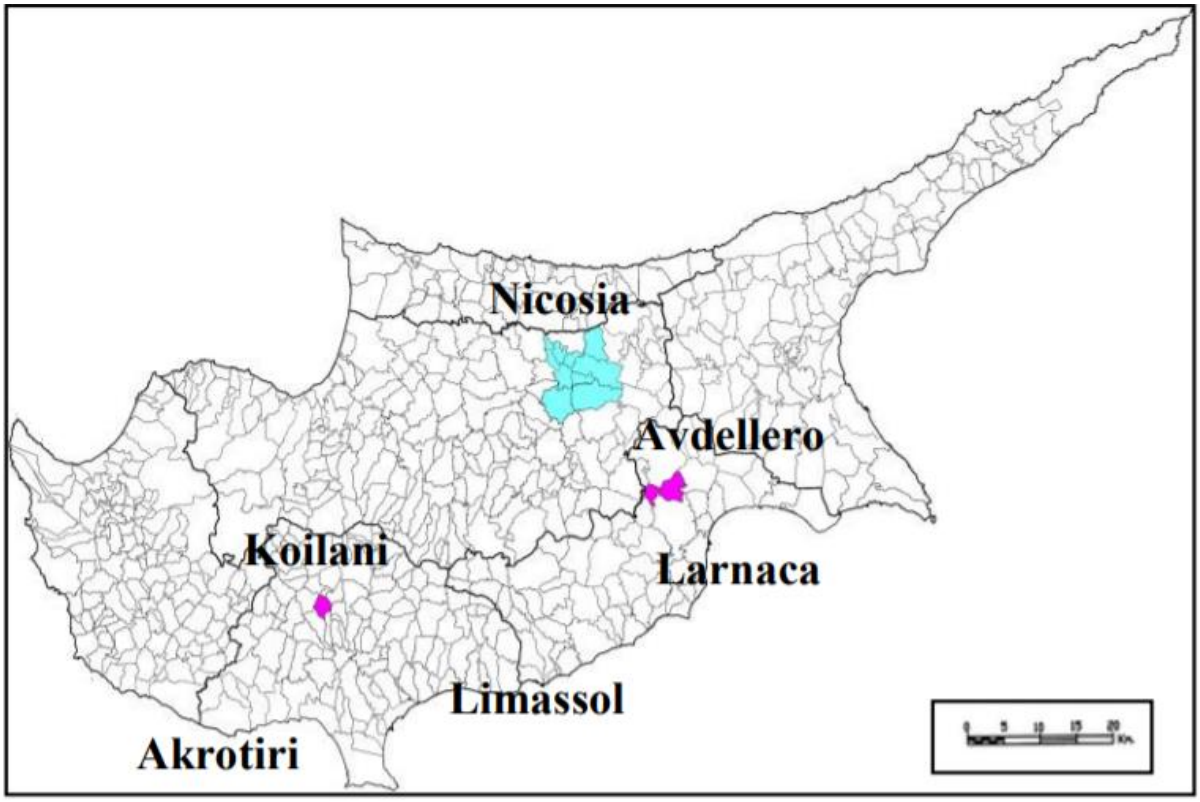

Fig. 1.7: Location of the collection sites for *Aedes cretinus* in Southern Cyprus. Blue: historical record (1949); Purple: contemporary records (2015). (Martinou et al., 2016).

1.7 Zika virus epidemiology

Zika virus is a flavivirus, which is significantly transmitted through daytime active *Aedes* mosquitoes. It has equally been reported to be transmitted sexually, having lower rates of transmission orally. Zika virus was first discovered in Zika forest in Uganda from a rhesus macaque in 1947. The early evidence that shows this virus can affect humans was from a serological survey, which was done in Uganda. Proof of sporadic infections on human was later shown in Africa and some parts Asia (Petersen, Jamieson, Powers, & Honein, 2016).

The first Zika outbreak was reported to have in in the federal republic of Micronesia on the island of Yap. During that epidemic, it has been reported that more than 74% of the total subjects' population have been infected with Zika virus (G. A. Calvet, Dos Santos, & Sequeira, 2016). Figure 1.8 below shows the Landmarks in Zika virus epidemiology.

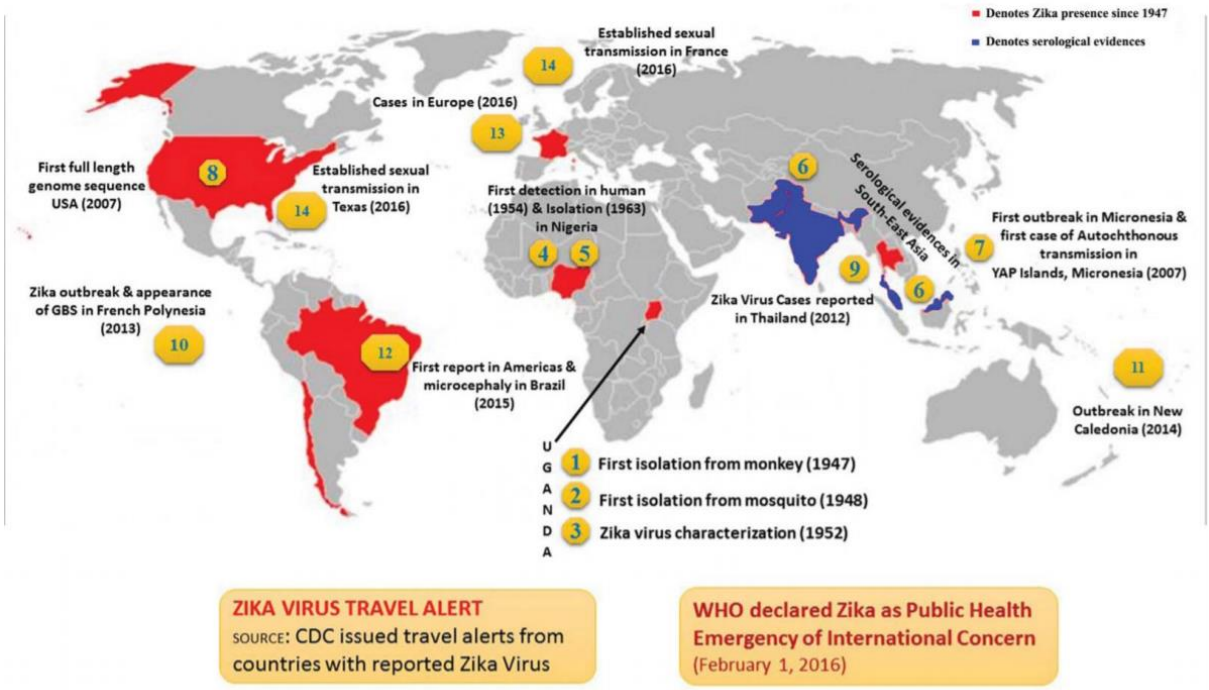

Fig. 1.8 Landmarks in Zika virus epidemiology. The red area depicts Zika virus presence and/or serological reports (Since 1947-2016) and in blue denoted serological evidences of Zika in Southeast Asia (G. A. Calvet, Dos Santos, & Sequeira, 2016).

Since then Zika virus continued with its eastward and wide spread migration, which was detected in French Polynesia around 2012 to 2014, then in 2014 to Chile and to Brazil around 2015. In the course of this time over 1.5 million Zika cases have been reported. Zika out break and transmission has been filed and documented in more than 66 territories and countries. Since its outbreak in 2015, over 49 countries whom were initially Zika virus negative have now experienced their first Zika virus outbreak (G. A. Calvet et al., 2016). Zika virus infection has rapidly emerged as a significant global threat which is demonstrated on figure1.9.

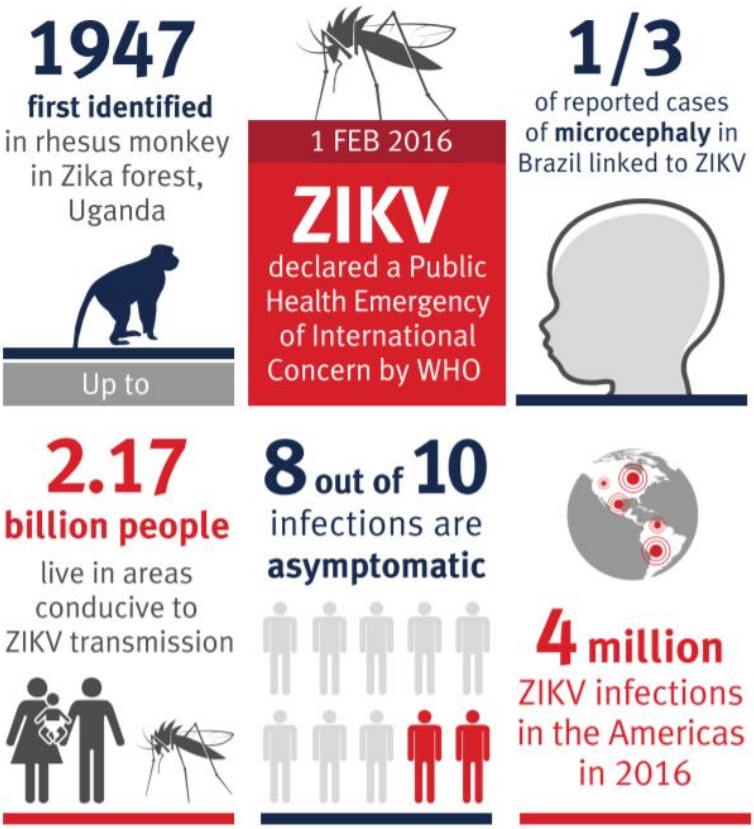

Fig. 1.9: Zika virus infection has rapidly emerged as a significant global threat (G. A. Calvet et al., 2016)

1.7.1 The Yap epidemic (Micronesia)

In Island of Yap which belongs to the federated states of Micronesia, situated along the Pacific Ocean. The population of this federation composed of more than twelve thousand inhabitants composing over seven thousand in island of Yap based on the population census of 2000. About 200 cases of Zika virus has been reported by some Health care organizations in the year 2007. Which consist of 108 real cases from Yap island. A study was designed and conducted around April 2007 which consist of active screening of different kind of cases which were done in hospitals and other healthcare centers, which involves the use of seroprevalence technique of the global population and done in a random sample of two hundred households (Boeuf, Drummer, Richards, Scoullar, & Beeson, 2016). More also, entomological survey was equally conducted in the same area at the same time. The result showed that out of the 185 cases of Zika virus infection, 26% (49) were confirmed cased and 32% (59) were under probability. In the 9 out of 10 communities of the Yap island in the end of the screening. It shows that the rate of the attack was found to be 14.6 out of the 1000 inhabitants. Whereby 61% of the cases were found to be female patients and the median age was found to be 36 years and the range was

between 1 to 76. Furthermore, symptoms of Zika virus infection for patients having biological confirmation (n= 31) which includes digestive disorders, edema, myalgia, retro orbital pain, conjunctivitis, rash, headaches, mild fever and arthralgia. There were no report of death and hospitalization (Ioos et al., 2014).

1.7.2. French Republic (FP) epidemic

This is an overseas country for the French republic, having 5 archipelagos and consists of almost 120 islands in which almost 75 of it are inhibited with people. The total population of FP was more than 270,000 inhabitants based on the 2012 census. French Republic has been reported to have been attacked to dengue epidemic because of their serotypes DEN1 as well as DEN3 for numerous weeks. On October 2013, the health care authorities together with the hospital managements reported Zika virus outbreak for the first time from the people of Tuamotu and Marquesas island which subsequently dispersed to all other islands of French Republic (Ioos et al., 2014). The evolution of the weekly number of suspected Zika cases in French Polynesia is shown in figure 1.10.

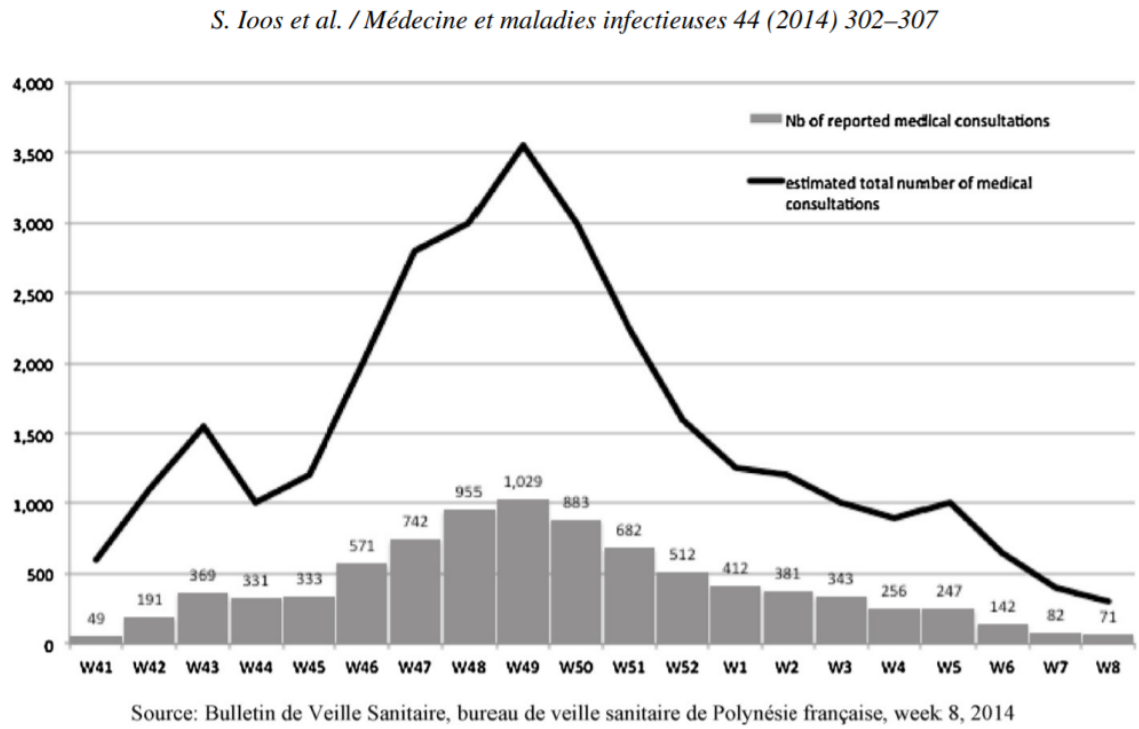

Fig. 1.10: Evolution of the weekly number of suspected Zika cases in French Polynesia, October 30, 2013 to February 14, 2014 (epidemic still ongoing) (Ioos et al., 2014).

Health workers and other health professionals were well informed and the community surveillance was equally reinforced through the network of sentinel doctors. 8510 suspected cases have been reported since the beginning of the outbreak up to February 2014, which leads to the estimation of about 10% 0f the population i.e 29,000 subjects (Ioos et al., 2014). Figure 1.11 below showed the number of cases with neurological complications by hospital admission day in French Polynesia.

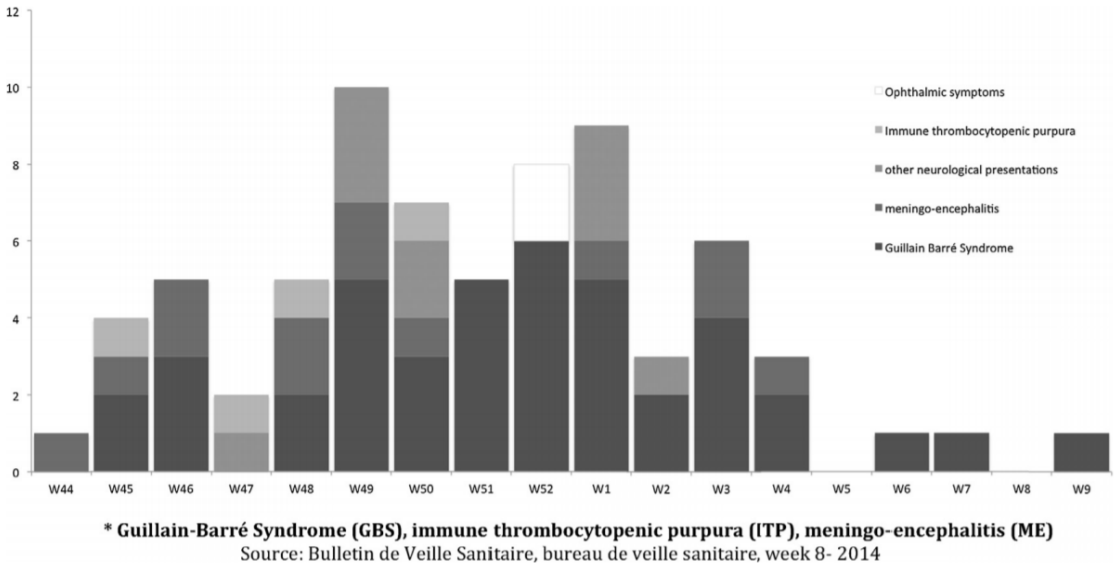

Fig. 1.11: Number of the cases with neurological complications by hospital admission day in French Polynesia, 2013–2014 (n = 73) (Ioos et al., 2014).

1.8 Vector of Zika virus

The first isolation of Zika virus was from after the first isolation of ZIKV from *Aedes* (Ae.) africanus in 1948. The virus was detected mainly from sylvatic *Aedes* genus mosquito, which includes apicoargenteus, vitattus, luteocephalus and *Aedes* furcifer. In many countries, Zika virus has been detected from antropophilic *Aedes* aegypti mosquitoes, which is the main vector of Chikungunya and Dengue viruses around the globe. It is also considered as the major vector of Zika virus in South East Asia. And in turns leads to the prevalence and outbreak of the disease in the region (Aid et al., 2017).

Moreover, during the major outbreaks that occurs in Pacific Islands, *Aedes* polynesiensis and *Aedes* hensilli are the major causative agents of Zika virus. Furthermore, the major Dengue

virus and chikungunya virus vector called *Aedes* albopictus has also been reported recently to be infected by Zika virus (Benelli & Romano, 2017).

Though the virus reservoir has not been identified clearly, some of the researchers suggested that Zika virus can be maintained naturally through sylvatic cycle, composing non-human primate and in wide range of *Aedes* mosquito specie (Ioos et al., 2014). In urban area usually the transmission could be ascertained through antropophilic mosquitoes Ae and Aegypti and Albopictus Ae. The *Aegyti* mosquito species is of significant worrisome due to its habitat and also its diurnal feeding to bite numerous hosts during its development cycles of its eggs that made it very efficient in the disease transmission. In contrast, serological reports have shown that the presence of certain antiviral antibodies in different animals such as elephants, felines and the rodents. And this clearly shows that some other reservoirs have the ability of playing a role in the virus transmission cycle (Wong, Li, Chong, Ng, & Tan, 2013).

Notably, the non- human primates cannot be found in the pacific islands in which the virus has been found as well the nature of the viral reservoir in this region happened to remain speculative.

More also, the need for identifying vectors and other potential vectors of Zika virus in vulnerable region for controlling the disease outbreaks is very significant and important.

1.9 Diseases of Zika virus

1.9.1. Clinical symptoms

Zika virus generally have an incubation period of about three to twelve days. Its clinical symptoms consist of pruritis, vertigo, diarrhea, vomiting, abdominal pain, edema of feets and hands, asthenia, myalgia, headache, arthralgia, non-purulent conjunctivitis, maculopapular skin rashes and acute fever. The symptoms are generally self-limiting which might last for 4 to 7 days. It has been reported that some cases have recorded haematospermia and haematuria. These symptoms are similar with that of Chikungunya and Dengue, though for Zika the signs are mild compared to others. Sudden appearance of rashes and onset of fever, seldom pruritic that disappear rapidly with time, are the characteristic features. Sometimes Zika may lead to nerve associated disorders which are called as Gullian-Barre Syndrom (GBS). It has been reported also that infected pregnant women that are travelling to a region where there is an outbreak of Zika virus recently, that there should be subjected to ultrasound routinely in order

to assess the development of the infant. And if the fetus is getting infection from the mother through uterus, this has been reported to be the evidence behind the infant's disorders such as eye abnormalities, hydraneancephaly, hydro fetalis, intracranial calcification and microcephaly (Baud et al., 2016).

A report shows that about twenty nine percent of the 42 pregnant women that were infected with Zika virus have fetal anomalies. Zika virus disease pathogenesis is observed in figure1.12.

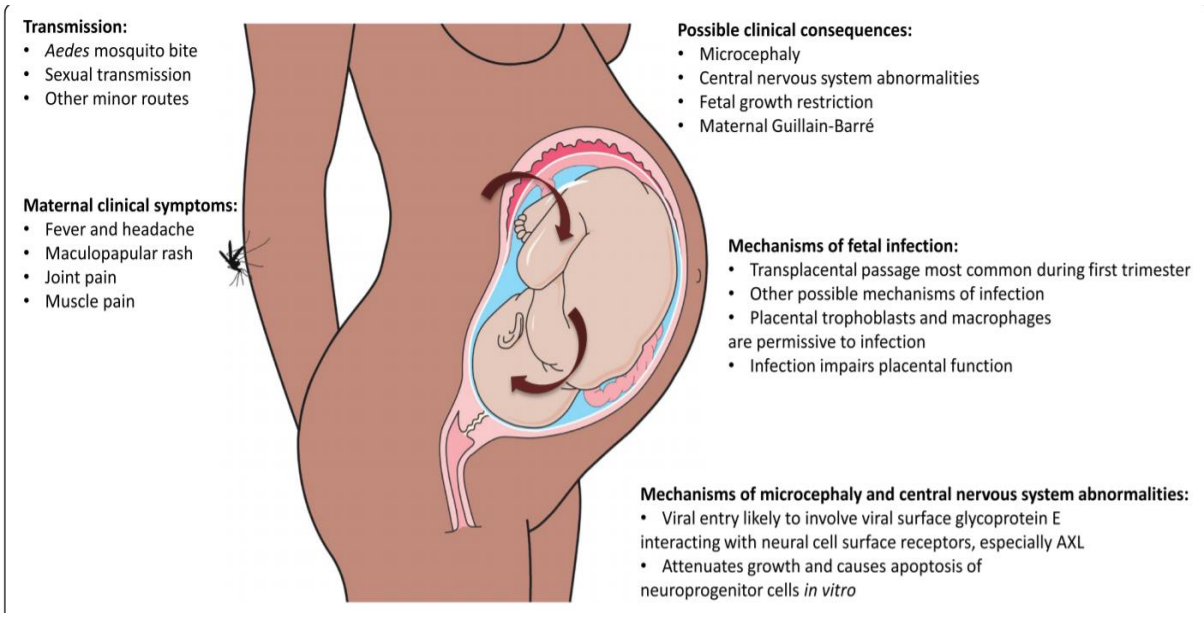

Fig. 1.12: Zika virus disease pathogenesis. The figure summarizes key points regarding ZIKV transmission to infants (Baud et al., 2016)

It has been reported by Ventura et al., that there was no any adverse effect on the retinal development in these fetuses. But in cases were no cranial abnormalities have been detected from the infected pregnant women, there is the need for checking periodically during the first year the cranial ultrasound, the hearing tests in order to diagnose the subclinical cases, frequent eye check-up. The case of Microcephaly has been detected in Latin America, though Asia and Africa are the hypothesis and endemic region that was suggested that pregnant women that are infected with Zika virus will have immunity against Zika, therefore can resist some infant disorders. In recent discoveries, it has been reported that hypertensive iridocyclitis by Zika infection is very possible. Ocular anomalies have also been reported, and it composed of gross pigment mottling, chorioretinal atrophy and hypoplasia of the nerves in the eye. The other two auxiliary Zika virus i.e. Dengue and Chikungunya virus has been seen in Columbia and been

reported. Figure 1.13 demonstrates a detailed overview of Zika virus clinical symptoms (Singh et al., 2016).

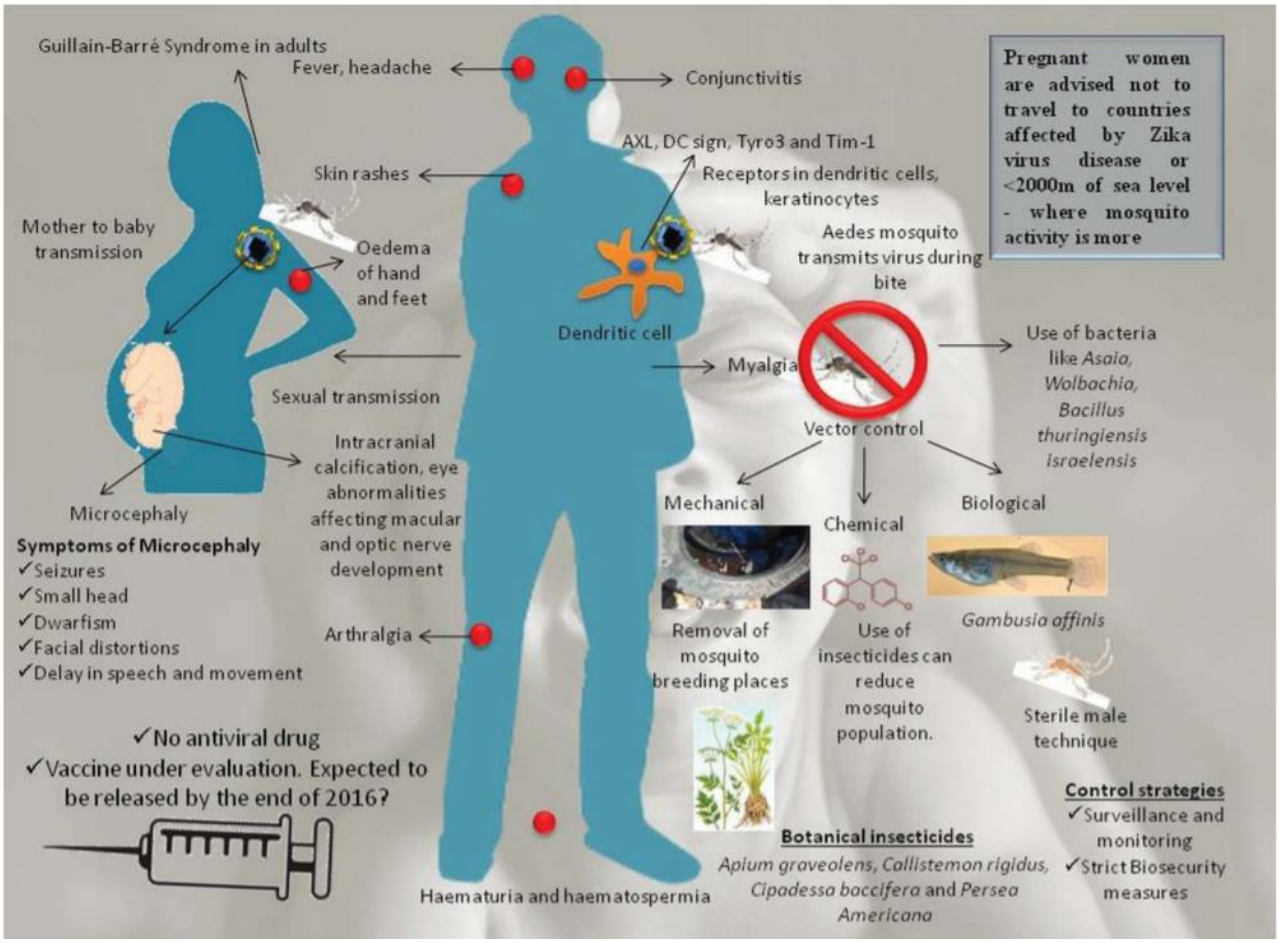

Fig. 1.13: Detailed overview of Zika virus clinical symptoms and some prevention tips (Singh et al., 2016).

1.9.2 Pathology and pathogenesis

The type of Zika virus which was isolated from French Polynesia shows its affinity to human immature dendritic cell, epidermal keratinocytes and dermal fibroblasts. These viruses can replicate in the human midgut when the *Aedes* mosquito suck the blood just like other groups of Flaviviruses and subsequently to the salivary glands. Moreover, the vertical transmission of the virus through transovarian route has also been reported. The *Aedes* mosquitoes pass the virus while sucking on human and the virus will enter human cell via receptors such as DC-SIGN, AXL, Tim-1 and tyro-3 which are found in the surface of both nerve and skin cells. In the cells, they applied the host machinery and subsequently causes autophagy and apoptosis of the cells which lead and resulted in entering into other cells.

The Zika virus stimulates the transcription of MDA5, TLR-3, RIG-I as well as interferon genes such as MX1, ISG15 and OAS2. The replication of the virus in the cells results in releasing of type I interferon. The replication of this virus is rise by inducing autoghaphy inside the host. Thereby, the agents inhibiting the autophagy will reduce the load of the virus in the cells. Subsequently, the virus is channeled to hearts, muscles and central nervous system.

Early research shows that Zika virus has shown affinity towards the cells in the human brain and it was demonstrated with the help of intraperitoneal injection of mice which shows that Zika virus has the ability of crossing the blood brain barrier (Lazear & Diamond, 2016).

1.10 Diagnosis of Zika virus

Recently, there is high rise in the number of cases regarding ZIKV, particularly in the developing countries has led to so many administrations and agencies for different unapproved diagnosis assays. it has been reported that the best method or assay to be used for asymptomatic pregnant woman that travelled to area with high risk of ZIKV is using polymerase chain reaction (PCR) during the first two weeks. These is in accordance with the prescription and authorization of FDA EUA. This technique can be conducted on different samples such as blood, serum, cerebrospinal fluid, amniotic fluid and urine. However, its major drawback is it has high chances of giving false negativities which subsequently leads to giving higher errors. It can also be detected using serological tests by IgM antibody capture enzyme-linked (MAC-ELISA) assay. Moreover, because of the molecular and epidemiological similarities of ZIKV and other forms of Flaviviruses. There it is recommended that IgM ELISA method should be performed to antibodies against DENV, CHIK and ZIKV. Generally, it is conducted by adding samples collected from patients using a well and pre-coated using antibodies in order to capture the human IgM. Then a specific virus antigen is added and then washed away, which then binds to the IgM of the patients. Typically, neutralizing antibodies to ZIKV develop in the human body within the first two week of symptoms and continue to remain at detectable levels for up to 12 weeks. However, the risk of false-positives is high for IgM and IgG assays. If ELISA testing is inconclusive or positive, plaque-reduction neutralization test (PRNT) should be performed to confirm the presence of ZIKV, then measures the ability of a patient's antibodies to neutralize a specific virus. Even though ELISA technique requires higher time, materials, labor and cost than PCR but it gives a promising result due to its sensitivity as well as its specificity than PCR (Nicolini, McCracken, & Yoon, 2017).

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Sample collection and storage

A total of 91 serum samples of the patients collected from blood bank of Near East University between 15 September 2019 and 10 December 2019 were involved in this study. All serum samples were randomly selected within Turkish Republic of Northern Cyprus citizens. The serum samples of the patients were collected in sterile tubes. About 1ml of serum sample was collected from each patient. Then, the serum samples were subsequently stored at -30°C until they use. For the detection of Zika virus, serum samples of the patients were tested by microplate reader (VERSAmax Tunable Microplate Reader Operator.s Manual) for Ig G antibodies against Zika virus.

Ethical approval of the study was taken from Health Sciences Institute Committee of Near East University (NEU/2019/73-914).

2.2 Sample and reagent preparation

All the samples and the reagents were stored at room temperature before use. According to the manufacturer instructions, the serum samples were required to be diluted before test. For this purpose, 100-fold dilution was achieved by adding 10 µL of unrepeated sample to 99 µL of sample dilution buffer. The sample preparation is shown on Figure.2.1.

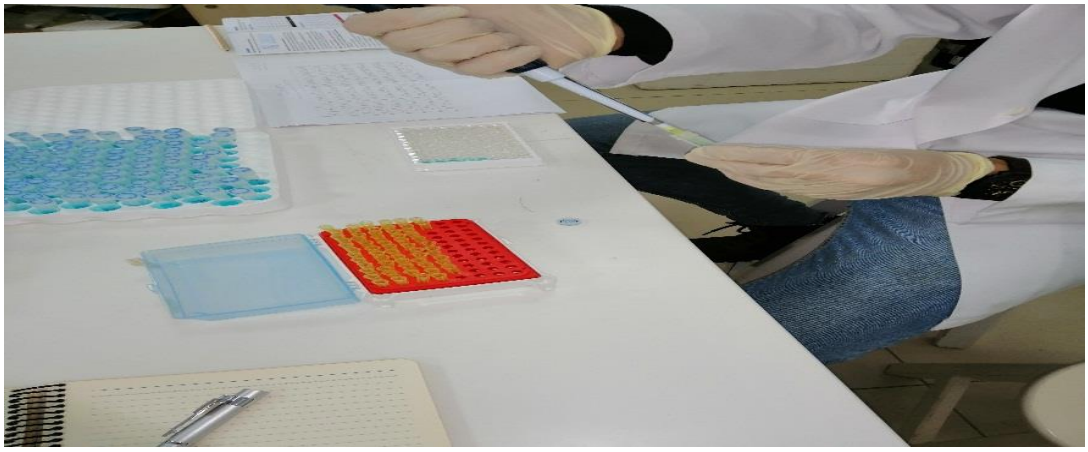

Fig. 2.1: Sample preparation

Furthermore, reagents of the human anti-Zika Ig G ELISA kit were prepared according to the manufacturer instructions. Treatment reagent, positive control, negative control, treatment control, wash buffer (1X) and the substrate solutions were prepared before Zika Ig G ELISA test. Firstly, treatment reagent was prepared. For this purpose, the treatment reagent was reconstituted with 14 mL of treatment diluent for at least 15 minutes prior to the assay. Then, the positive control and the negative control were reconstituted by adding 1.1 mL of sample dilution buffer to each tube respectively in to order to obtain positive and negative controls. For the preparation of the treatment control, 1.1 mL of sample dilution buffer was reconstituted with the treatment control. All reconstitutions were performed for a minimum of 15 minutes prior to assay.

The preparation of the 500 mL wash buffer (1X) was achieved by adding 20 mL of wash buffer concentrate to 480 mL of deionized or distilled water. In case of crystals formation in the concentrate, wash buffer was warmed at room temperature and mixed gently in order to dissolve all the crystals. Lastly, the substrate solution was prepared. Color reagents A and B are carefully mixed together in an equal proportion within 15 minutes of use and were further protected from light. Moreover, 100 µL of the resultant mixture was required per each well.

2.3 Assay procedure

Human anti-Zika virus Ig G ELISA (R&D Systems, USA) kit, which provides human Zika virus Ig G microplate, background plate and treatment microplate inside, is designed to measure Zika virus Ig G antibody in human serum. All microplates were immediately marked with the appropriate color marker after opening the plate bags to distinguish between the plates. The plates are not stacked; they were spread out as a single layer. This is very important for even temperature distribution. All the reagents were brought at room temperature before use.

2.4 Sample and control treatment (used for Treatment Plate)

The serum samples were diluted 100-fold in the sample dilution buffer (as discussed in the sample preparation section). Then, 125 µL reconstituted treatment reagent was added to the treatment plate to each well. After that, 125 µL of the diluted serum samples or the reconstituted controls was added to each well in duplicate well for each sample or control. Additionally, 125 µL of sample dilution buffer was added only per well in duplicate wells for non-specific

binding (NSB). The plates were covered with adhesive strip and incubated for one hour at room temperature.

2.5 Zika virus IgG detection (used for both Human Zika Virus IgG and Background plates)

From each well of the treatment plate, we transferred 100 µL to the human Zika virus IgG microplate and 100 µL to the background microplate according to the manufacturer instructions. Figure.2.2 shows transferring of samples from Zika virus treatment plate to Zika virus IgG microplateplate and background microplate.

Fig. 2.2: Transferring of the samples from Zika virus treatment microplate to Zika virus Ig G microplate and background microplate.

New tips were used for each transfer. The microplates were covered with adhesive strip and then incubated for 1 hour at room temperature. Each well was aspirated and washed three times using wash buffer (1X) (400µL/well) using (Multi Wash III) Tricontinent Model : 8441-11, . Excessive water was removed by using paper napkin. Subsequently we added 100 µL of Zika virus Ig G conjugate to each well and then covered by using adhesive strip. The microplates

were incubated for another one hour and then washed three times again. Furthermore, 100 µL of the substrate solution was added to each well and substrate solution was protected from light during experiment, and then the plates were incubated for 20 minutes at room temperature. Substrate solution changed from colorless to gradations of blue. Finally, 50 µL of stop solution was added to each well. The color of the wells was expected to change from blue to yellow upon addition of the stop solution. Wells that are green in color indicates that the stop solution has not mixed thoroughly with the substrate solution. If the green color or the color change does not appear uniform, it is recommended to gently tap the plates to ensure through mixing. The optical density (OD) of each well was determined by microplate reader (VERSA max microplate reader, USA) within 15 minutes at 450 nm with a correction wavelength at 540 nm for detecting human Zika virus Ig G antibody. The picture below shows the color change of the well after adding stop solution (Figure.2.3).

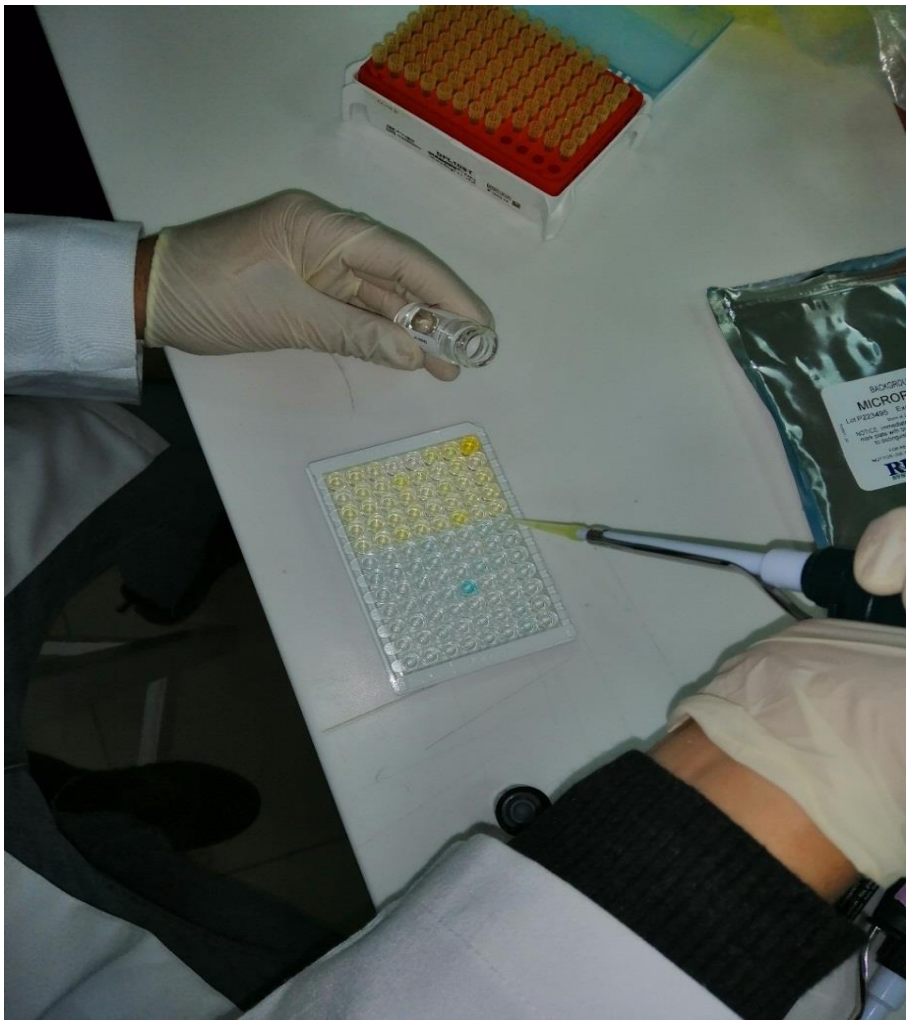

Fig. 2.3: The change of colour of the well from blue to yellow

2.6 Calculation of each sample and control net OD

According to the manufacturer instructions, the formulae below was used in order to calculate the control and each patient's ODs.

1.(Average OD from Zika virus IgG Plate – Average OD of NSB from Zika Virus IgG Plate) – (Average OD from Background plate – Average OD of NSB from Background Plate).

2. Non-Specific Binding for Background plate: Plus two values divided by 2.
3. Non Specfic Binding for Zika virus IgG plate: Plus two divided by 2.
4. Average OD from Background plate: Plus two divided by 2.
5. Average OD from Zika virus IgG plate: Plus two divided by 2.

OD values were calculated for steps 2, 3, 4, 5 for Background and Zika virus Ig G plates, after analyzing of the plates by using microplate reader.

2.7 Statistical analysis

Statistical analysis of the data was performed with SPSS Ver 13.0 (SPSS Inc., Chicago, IL, USA). Person Chi-Square and Fisher's Chi-Square tests were used to determine any statistical significance and the significance was evaluated at $p < 0.05$.

CHAPTER 3

3.0 RESULTS

3.1 Quality control

For a valid assay, NSB, negative control, treatment control and positive control should be within the range given below. Table 3.1 shows OD values of the control result for valid assay. In our study, the OD values of all the reagents were obtained within the given ranges which shows that the study was performed successfully.

Table 3.1: OD Values for quality control for valid assay

Calculated Net OD Values	**Control Result for Valid Assay**
<0.100	NSB, negative control, treatment control
>0.400	Positive control

3.2 Interpretation of the results

The interpretation of the net OD values obtained for NSB Zika virus microplate, NSB background microplate, positive control, negative control and the treatment control was calculated according to the human anti-Zika virus Ig G ELISA kit instructions. The calculated net OD values of these tests is given in the table 3.2. All OD values were within the range thus, the assay was accepted as valid.

Table 3.2: Calculated net OD values of the microplates and the reagents

Tests	**OD values**	**Range**	**Interpretation**
NSB for Zika virus microplate	0.054	<0.100	Valid
NSB for Backgrounds microplate	0.0525	<0.100	Valid
Positive control	0.826	≥ 0.400	Valid
Negative control	0.027	<0.100	Valid
Treatment control	0.0545	<0.100	Valid

The cut-offs were selected using values from a small set of field data and are only estimated. The recommended data interpretations are shown in the table 3.3 below.

Table 3.3: Interpretation of the results

Calculated O.D Values	**Result**	**Interpretation**
< 0.100	Negative	No detectable Zika virus Ig G antibody
0.100-0.200	Equivocal	The sample is suspected of Zika virus Ig G antibody. Therefore, repeat the test. If samples gives similar result, then Zika Virus Ig G antibody cannot be determined, and testing should be repeated by an alternative method or new sample should be collected
>0.200	Positive	Zika virus Ig G antibody detected

According to the OD values of the samples obtained from the study, 91 patients (100%) were obtained as negative for human anti-Zika virus Ig G ELISA test.

3.3 Sampling testing data

A total of 91 patients (n:60, 66% male and n:31, 34% female) were involved in this study. All serum samples of the patients were selected from citizens of T.R.N.C. The avarege age and the mean age value of the patients were calculated as of 42.96 ± 18.777 ($18 - 90$). The patient's net OD values were calculated according to the instructions. The characteristics information and the calculated net OD values of the patients are demonstrated in Table 3.4 and 3.6.

Table 3.4 Characteristics information of the patients and the calculated net OD values

Table 3.4: Sample data

	Age	Gender	Nationality	OD values	Range	Interpretation
1	18	M	TRNC	0.094	< 0.100	Negative
2	47	M	TRNC	0.018	< 0.100	Negative

3	37	M	TRNC	0.0305	< 0.100	Negative
4	40	M	TRNC	0,003	< 0.100	Negative
6	19	M	TRNC	0.02	< 0.100	Negative
7	82	M	TRNC	0.007	< 0.100	Negative
8	21	F	TRNC	0.007	< 0.100	Negative
9	90	M	TRNC	0.0005	< 0.100	Negative
10	51	M	TRNC	0.0445	< 0.100	Negative
11	63	M	TRNC	0.0095	< 0.100	Negative
12	34	M	TRNC	0.02	< 0.100	Negative
13	50	F	TRNC	0.0025	< 0.100	Negative
14	35	F	TRNC	0.0205	< 0.100	Negative
15	35	F	TRNC	0.0185	< 0.100	Negative
16	40	M	TRNC	0.0035	< 0.100	Negative
17	19	M	TRNC	0.034	< 0.100	Negative
18	54	M	TRNC	0.0015	< 0.100	Negative
19	48	M	TRNC	0.0175	< 0.100	Negative
20	41	M	TRNC	0.0005	< 0.100	Negative
21	40	M	TRNC	0.0055	< 0.100	Negative
22	37	M	TRNC	0.0095	< 0.100	Negative
23	40	M	TRNC	0.003	< 0.100	Negative
24	80	F	TRNC	0.021	< 0.100	Negative
25	45	F	TRNC	0.011	< 0.100	Negative
26	68	M	TRNC	0.0055	< 0.100	Negative
27	26	M	TRNC	0.004	< 0.100	Negative
28	48	M	TRNC	0.006	< 0.100	Negative
29	29	M	TRNC	0.001	< 0.100	Negative
30	23	F	TRNC	0.006	< 0.100	Negative
31	72	M	TRNC	0.021	< 0.100	Negative
32	26	M	TRNC	0.005	< 0.100	Negative
33	60	M	TRNC	0.012	< 0.100	Negative
34	43	F	TRNC	0.0105	< 0.100	Negative
35	57	M	TRNC	0.045	< 0.100	Negative

36	37	F	TRNC	0.006	< 0.100	Negative
37	37	M	TRNC	0.0135	< 0.100	Negative
38	31	F	TRNC	0.014	< 0.100	Negative
39	76	M	TRNC	0.011	< 0.100	Negative
40	32	M	TRNC	0.015	< 0.100	Negative
41	79	M	TRNC	0.0245	< 0.100	Negative
42	40	M	TRNC	0.0115	< 0.100	Negative
43	37	M	TRNC	0.0095	< 0.100	Negative
44	43	M	TRNC	0.014	< 0.100	Negative
45	47	F	TRNC	0.007	< 0.100	Negative
46	56	F	TRNC	0.0085	< 0.100	Negative
47	36	M	TRNC	0.001	< 0.100	Negative
48	29	M	TRNC	0.018	< 0.100	Negative
49	42	M	TRNC	0.012	< 0.100	Negative
50	76	M	TRNC	0.0165	< 0.100	Negative
51	45	M	TRNC	0.0135	< 0.100	Negative
52	77	M	TRNC	0.0195	< 0.100	Negative
53	33	M	TRNC	0.014	< 0.100	Negative
54	76	M	TRNC	0.009	< 0.100	Negative
55	22	F	TRNC	0.0075	< 0.100	Negative
56	73	F	TRNC	0.015	< 0.100	Negative
57	32	F	TRNC	0.0045	< 0.100	Negative
58	42	F	TRNC	0.0095	< 0.100	Negative
59	85	M	TRNC	0.0155	< 0.100	Negative
60	19	F	TRNC	0.005	< 0.100	Negative
61	21	F	TRNC	0	< 0.100	Negative
62	85	F	TRNC	0.012	< 0.100	Negative
63	35	M	TRNC	0.021	< 0.100	Negative
64	31	F	TRNC	0.008	< 0.100	Negative
65	37	F	TRNC	0.08255	< 0.100	Negative
66	33	M	TRNC	0.0115	< 0.100	Negative
67	58	M	TRNC	0.015	< 0.100	Negative

68	18	F	TRNC	0.007	< 0.100	Negative
69	21	F	TRNC	0.012	< 0.100	Negative
70	23	M	TRNC	0.0205	< 0.100	Negative
71	42	F	TRNC	0.017	< 0.100	Negative
72	51	M	TRNC	0.0125	< 0.100	Negative
73	51	M	TRNC	0.0045	< 0.100	Negative
74	80	M	TRNC	0.023	< 0.100	Negative
75	18	M	TRNC	0.008	< 0.100	Negative
76	27	F	TRNC	0.0115	< 0.100	Negative
77	31	F	TRNC	0.0075	< 0.100	Negative
78	31	F	TRNC	0.0085	< 0.100	Negative
79	30	F	TRNC	0.0105	< 0.100	Negative
80	50	M	TRNC	0.005	< 0.100	Negative
81	48	M	TRNC	0.0055	< 0.100	Negative
82	21	F	TRNC	0.023	< 0.100	Negative
83	38	M	TRNC	0.008	< 0.100	Negative
84	35	F	TRNC	0.009	< 0.100	Negative
85	45	M	TRNC	0.005	< 0.100	Negative
86	49	F	TRNC	0.007	< 0.100	Negative
87	41	M	TRNC	0.031	< 0.100	Negative
88	18	M	TRNC	0.012	< 0.100	Negative
89	27	M	TRNC	0.0105	< 0.100	Negative
90	21	M	TRNC	0.0085	< 0.100	Negative
91	52	M	TRNC	0.002	< 0.100	Negative

Abbreviations: M: male; F: female; TRNC: Turkish Republic of Northern Cyprus

Table 3.5: Various ages of the subjects involved in the study

	N	Minimum	Maximum	Mean	Std. Deviation
Age	91	18	90	42,96	18,777
Valid N (listwise)	91				

A total of 91 patients were involved in this study. The minimum and the maximum age of the patients were 18 and 90 respectively and the mean age of the patients involved in this study was calculated as 43. The standard deviation was calculated as 18,777.
All samples were found to be human anti-Zika Ig G ELISA negative and the frequency of the positivity and the negativity is shown in the Table 3.6.

Table 3.6: The analysis of the result

	Samples Tested	**Positive (n,%)**	**Negative (n,%)**	**Equivocal**
Anti-Zika virus Ig G ELISA	91	0 (0%)	91 (100%)	0

The serum samples of the patients were collected between 15 September 2019 and 10 December 2019 in Near East University, Turkish Republic of North Cyprus. About 1ml of serum was collected. The study shows that 100% of the patients were negative for Zika virus Ig G.

Out of 91 patients, 60 patients (66%) were male and 31 patients (34%) was female. The gender of the study group was demonstrated on Table 3.7.

Table 3.7: Gender of the study group

Gender	**N**	**%**
Male	60	66
Female	31	34
Total	91	100

Table 3.8: Calculated the minimum and the maximum OD values

ELISA results

	n	Minimum	Maximum
Result	91	,000	,094
Valid N (listwise)	1		

The Zika virus Ig G ELISA test results is shown in table 3.8. The calculated the minimum and the maximum Zika virus Ig G OD values were found as 0.00 and 0.09 respectively.

CHAPTER 4

4.0 DISCUSSION

To our knowledge, this study is the first to show the Zika virus infections in Northern Cyprus. From the result, it can be justified that there is no prevalence or outbreak of Zika virus in Northern Cyprus. The analysis involved in serum samples of 91 patients, who have various range of ages. The minimum age of the patients involved in this study was 18 and the maximum was 90, while the average age of the patients was found to be 43 among the 91 patients. As we had elderly people in our study group, this also confirms our findings that there has not been ZİKV infections for long years in Northern Cyprus. This research is a gender combine research having both male and female patients. The study composed of 60 male and 31 female patients having a percentage ratio of 66 and 34 respectively. The result was found to be 100% negative in all 91 patients involved in the study. The Zika Virus ELISA IgG results showed that the maximum Zika virus Ig G antibodies was 0.09 and the minimum was 0.00 for 91 patients in the study. For instance, Musso et al., reported in their studies conducted at French Polysenia, located at the Southern Pacific and their result showed that about 28,000 cases of ZIKV outbreak were reported during February 2014 which is almost 11% of the people population. In comparison to our result their result showed a significance outbreak and prevalence of Zika virus which will need urgent and immediate attention to reduce the epidemiological effect of the virus and to minimize the risk. Unlike in our study which showed 0% outbreak (D. Musso et al., 2014).

The authors' described in the discussion chapter that during this outbreak in some regions like Italy, Indian Ocean and Réunion Island, donation of blood was stopped while blood product was also imported from the blood bank elsewhere. But in areas like French Polynesia it is difficult to import the blood product from blood bank elsewhere due to its isolation geographically. They concluded their study by suggesting that Zika virus nucleic acid testing (ZIKV NAT) can be employed in order to minimize the blood-transmitted Zika virus disease based on the recommendation of the European center for the disease prevention and control (CDC). Blood safety management need to be cautious and should consider the fact that transfusion of blood from people that migrate from areas attacked with ZIKV infection outbreak (Musso et al., 2014).

It has also been reported by Calvet et al., that Zika virus can be detected from the pregnant women amniotic fluid. Their findings showed that the Zika virus can penetrate across the

placenta and affects the fetus. Their group was the first one to conduct research on the amniotic fluid and found the possible ways by which the mother can infect the unborn baby. Contrary to our studies in which we used serum as the sample (G. Calvet et al., 2016). In our study, we did not involve the pregnant women. However, investigation of pregnant women in Northern may also be important and can be performed as a further study.

More recently in the year 2017, it has been reported by Benelli et al., on the elucidation of Zika virus from different samples such as blood, saliva and amniotic fluid etc. Their research showed that their might be a tendency of finding this virus in one the sample type and then subsequently be absent in another sample types (Benelli & Romano, 2017).

It has been reported by Martinou *et al.*, regarding *Aedes* cretinus mosquitoes which was recorded first in the year 1949 in Nicosia, even though there were no sole and available records since then and also it has not been included in the list composed by Violaris et al. (2009) of mosquitoes found in Cyprus (Martinou et al., 2016).

An environmental scientist has also reported it in Republic of Cyprus during June 2015 and he stated that 'Various white and black mosquitoes that mimics tiger mosquiti specie bites people so much at the yards of the restaurants at St. Mavra". Further surveys were organized by the Joint Services Health Unit (JSHU) at the village of St. Mavra at Koilani (Limassol district) which was equally conducted in the same period i.e around June 2015. Moreover, they were also some complaints from some citizens at Aydellero village in Larnaca city who equally reported to the authorities of Public Health agencies of Republic of Cyprus that there is presence of some mosquitoes that resembles tiger mosquitoes (Violaris et al., 2009). Northern Cyprus is considered as a risk area for Zika virus disease because of the various people coming from many countries from all over the world, especially, from the regions which has previous medical evidences like Africa and Asia.

Our research showed that, all patients were negative for Zika virus Ig G however, it is very crucial to bear in mind that if any of the patient is positive for ZIKV, it should serve as a quick reminder especially on various consideration for blood donation safety measures. These measures will prevent the outbreak of these disease into other areas and regions. It has been suggested by Musso et al., that blood transmission should involve the use of ZIKAV NAT in order to prevent individuals from transmitting ZIKV. They further recommend that authorities need to be vigilant and should consider deferral of blood donors returning from areas with an outbreak of ZIKAV infection (Musso et al., 2014).

The reason for analyzing and testing different sample types is due to the significance of this disease. Zika virus analysis is very crucial considering its negative impacts on human health, which can lead to different health issues and subsequently can even leads to death. Therefore, it is very important to check and make analysis on countries and regions that have never encounter or even outbreak of this deadly disease. So as to prevents its outbreak. Because health care system believes that 'protection is better than cure'.

Therefore, it is crucial in order to test the accuracy of such studies to use different sample types. Though our study was the first one in Northern Cyprus and there is no history of Zika virus outbreak but we can increase our confidence limit by enhancing the number of the patients and using different sample types of various patients.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This research is the first one conducted in Northern Cyprus for our best knowledge, for the determination of Zika virus prevalence among local citizens of Northern Cyprus. The results clearly show that the Zika virus is not found in Northern Cyprus. In our study, we used the serum samples of the patients of different ages including elderly people. The purpose of this was to increase the chance of detecting the infection in Northern Cyprus. We did not detect any Zika Ig G positivity. Although, Northern Cyprus is considered as a risk area for vector borne disease because of various people coming from many countries from all over the world, there is no Zika virus infection up to now. Therefore, it is highly recommended to repeat the same study with different citizens by using higher number of samples.

As ZİKV is not found in Northern Cyprus, there is no need to test for blood donors for ZIKV.

5.2 RECOMMENDATION

Finally, it is recommended that this study may be carried out with a larger number of patients and that it is repeated with the participation of TRNC citizens and citizens of other countries. It will also be important to use different sample types such as saliva, urine, amniotic fluid (for pregnant women) and blood in order to enhance the accuracy and confidence limit of the result.

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