SEROSURVEY OF WEST NILE VIRUS IN HOUSEHOLD–REARED PIGEONS IN BAUCHI METROPOLIS, NIGERIA

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DECEMBER, 2016

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P13MDCM8019

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PUBLIC HEALTH (MPH) FIELD EPIDEMIOLOGY DEGREE

DEPARTMENT OF COMMUNITY MEDICINE,

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DECEMBER, 2016

ATTESTATION

I hereby declare that the work in this dissertation "**SEROSURVEY OF WEST NILE VIRUS IN HOUSEHOLD-REARED PIGEONS IN BAUCHI METROPOLIS, NIGERIA**" was carried out by me under the supervision of Prof. Junaidu Kabir of the Department of Veterinary Public Health and Prevention Medicine, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma in any educational institution or university.

Istifanus James WAZIRI …… …….

Name of student Signature Date

CERTIFICATION

DEDICATION

To God the Father, the Son and the Holy Spirit. My Parents Mr. and Mrs. James A. Waziri, sisters Salamatu and Ruth.

ACKNOWLEDGEMENT

I would like to express my profound gratitude to the Nigeria Field Epidemiology and Laboratory Training Programme (NFELTP) for granting me the scholarship to enroll into this post graduate programme. Also my gratitude goes to my academic supervisors Prof Junaidu Kabir and Prof Jacob K.P. Kwaga for their assistance, advices, contributions and professional guidance to make this study a success, indeed it's an experience and an eye opener to be under your mentorships. Profs, i indeed appreciate.

My gratitude also to NFELTP Resident advisor Dr Patrick Nguku. Sir i thank you and appreciate your humility. My programme supervisors Drs Endie. N. Waziri, and Kabir Getso, despite your tight schedules you had time to look at my bound volume deliverables, Am indeed lucky being under your mentorships, I am saying thank you. To my field site supervisor Dr Philip Okewole ,sir i thank you for your fatherly advises, and the former Head of the Central Diagnosis laboratory Late Dr Peterside Kumbish, am indeed grateful ,though you left early, i indeed cherished my little time with you.

To the laboratory technicians of the Veterinary Public health Zoonoses Laboratory Mallam Yahuza Suleiman and Mallam Mahmood Abdulazeez, I am most grateful.

Also worthy of mentioning are my colleagues, Dr Nyampa David Barau, and his family, it's a privilege getting to know you, and you are indeed part of me now. Also to Mallam Isah Ali Hassan and Mrs. Wunmi Ajayi, I can't forget your kindness, God bless you all.

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LIST OF ACRONYMS

Acronyms…….Explanations

CDC…….....Centers for Disease Control and Prevention

CNS……..Central Nervous System

CSF……...…… …….Cerebrospinal fluid

CPE……..….Cytopathic effect

Ct…….……..……. Cycle threshold

dpi…….……. Day post-infection

DC-SIGN…….Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin

DENV…….Dengue virus

EEE ……Eastern equine encephalomyelitis

IF ……...Immunofluorescence

IFN……...….Interferon

JEV……...…….……Japanese encephalitis virus

NK……..…..Natural killer

NY99……..….New York 99

NS……Non-structural

PFU…….Plaque forming unit

Pr-E……..…Envelop protein

prM……..……Premembrane

RNA…….…...Ribonucleic Acid

SLEV…….. ……..….Saint Louis encephalitis virus

TBEV……..Tick-borne encephalomyelitis virus

WND…….....West Nile Disease

WEE…….……Western equine encephalomyelitis

WNE……..……West Nile Encephalitis

WNF……...…….West Nile Fever

WNV……..…….West Nile Virus

WWND……West Nile Virus Neuroinvasive Disease

YFV…….….. …Yellow fever virus.

SUMMARY

West Nile virus (WNV) is a mosquito-borne virus and belongs to the genus *Flavivirus* of the family *Flaviviridae*. It is an reemerging global pathogen, with transmission cycles involving a variety of birds and *Culex* species mosquitoes. Humans and most other non-avian species are incidental hosts. Not much is known about WNV and its potential zoonotic transmission from pigeons.

The study was a cross-sectional study designed to determine the occurrence of WNV antibodies in pigeons reared in households, the knowledge on mosquito-borne diseases, and associated risk factors related to WNV in households of Bauchi metropolis. The serological detection was done using competitive enzyme linked immunosorbent assay and a pretested interviewer administered questionnaire was administered to collect information regarding socio-demographics and history of pigeon keeping among owners, knowledge of mosquito-borne diseases, and risk factors related to WNV in household.

A total of 156 households were enumerated for the study and a total of 376 pigeon serum samples were collected and tested for WNV antibodies. Thirteen (3.5%) of the pigeon sera were positive for WNV antibodies. Highest seroprevalence of 14.3 %, 12.5 % and 11.8 % were found in Tura, Unguwan Jaja and Unguwan Ganjuwa areas of Bauchi metropolis respectively. Respondents of only 0.6% had knowledge on mosquito-borne diseases apart from malaria, 5.8 % knew that diseases could spread by mosquitoes to pigeons, 43.6% knew that diseases can cause high mortality in pigeons; while no respondent had knowledge on diseases of pigeons transmitted by mosquito. Risk factors of WNV in households indicated that 49.6% emptied all their containers around their houses that can hold water, 5.8 % had blocked or stagnant gutters that were not flowing, 73.7 % had mosquito nets at the windows and doors of their houses, and

13.5% of families regularly used mosquito repellent at night, and also while outdoor. Only 12.8 % respondents used any other mosquito control measures while outdoors. Discarded tires that may have accumulated on the property was admitted by 7.1 % whereas 60 % had drainage holes that were located on the outside of the house collecting enough water for mosquitoes to breed. Mosquitoes were seen in and around 17.3% and 16% of pigeon housing and drinkers respectively. Overall, respondents had low knowledge of mosquito-borne diseases. Not having blocked or stagnant gutter that is not flowing, and having mosquito nets at the windows and doors of respondents were found to be protective against WNV (OR=0.69 95% CI 0.21-2.29; OR= 0.46 95% CI 0.14-1.56). Having plastic containers, ceramic pots or similar water holding containers that have accumulated on properties, and having drainage holes located outside of the house collecting enough water for mosquitoes to breed were likely predisposing factors to WNV spread in the households surveyed, OR= 1.4 (95% CI 0.43-4.55); OR=2.14 (95% CI 0.56-8.25).

The study has established the presence of WNV antibodies in pigeons in Bauchi metropolis. There is need for further studies on WNV in other species of birds, and also to educate the populace on its zoonotic transmission.

Keywords: West Nile virus, Pigeons, Household-reared, Bauchi metropolis

CHAPTER ONE - INTRODUCTION

1.1 Background information

The name West Nile virus (WNV) derives from its initial isolation in the West Nile district of Uganda from the blood of a febrile patient in 1937. [(1)] West Nile virus is a member of the Japanese encephalitis antigenic complex within the family *Flaviviridae*, genus *Flavivirus.* [(2)] WNV is an arbovirus, which is an arthropod transmitted virus which is closely related to a group of viruses that cause disease around the globe such as dengue fever, yellow fever, Japanese encephalitis and tick-borne encephalitis. WNV shares a serocomplex with other mosquito-borne viruses that can cause encephalitis namely St. Louis, Murray Valley, the Kunjin subtype, and Japanese encephalitis viruses. [(3)] The first epidemics of West Nile fever were documented in Israel in the early 1950s.[(4)] The largest epidemic reported occurred in South Africa in 1974 after unusual heavy rainfall that resulted in thousands of hospital admissions.[(5)] Outbreaks of human West Nile virus infection have been reported throughout the Middle East, Sub-Saharan Africa, Europe, and Asia. [(5)] WNV is perhaps the widest distributed arbovirus globally, being now found on all the continents except Antarctica and from tropical to north temperate latitudes.[(6)] The epidemic in the USA was characterized by severe courses of disease in humans and horses and a high mortality in birds, especially in crows. Lethal outcomes of WNV infections have been observed in 326 bird species in the USA.[(7)] In the United States, a total of 48 states and the District of Columbia have reported West Nile virus infections in people, birds, or mosquitoes in 2015. Overall, 2,060 cases of West Nile virus disease in people have been reported to CDC. [(8)] Most of WNV infections in humans are asymptomatic, but approximately 20% of the infected people develop clinical symptoms, although severe neurological diseases are observed in less than 1% of them. West Nile Fever is characterized by several non-specific flu-like symptoms,

such as fatigue, fever, headache, muscle pain, weakness, rash, and neck-pain. Symptoms usually develop between 2 to 15 days after infection and last for 2 to 5 days.[9]

WNV cycles enzootically between mosquitoes and birds, however, it can infect and cause illness in a range of animal species including humans. Infection has been detected in many non-avian species including reptiles like the alligators, rodents (squirrels, chipmunks), ungulates (mountain sheep, goats, and cattle), equines (horses, mules, donkeys), skunks, rabbits, bears, dogs and cats.[10] Humans and to a greater degree, equines appear to be most severely affected by WNV, and may develop clinical disease. Infection with WNV has been reported to be responsible for human and equine morbidity and mortality.[11] Mosquitoes feeding on birds (Ornithophilic mosquitoes) are the principal vectors of WNV.[12] Among the many mosquito species found carrying the virus, *Culex* types are the most important in transmitting the disease to humans. Mosquitoes are the primary vector for transmission of WNV to humans. The *Culex* species of mosquito have their peak feeding during dusk and dawn, thus the risk for infection in humans is higher during these time periods.[13]

1.2 Problem Statement

WNV is geographically the most prevalent mosquito-transmitted virus, with a wider distribution throughout the tropical and temperate regions of the world.[7] However the distribution of this virus is poorly documented in Nigeria. Presently, It is endemic in Africa, the Americas, Asia, Australia, Eurasia, and the Middle East, where it infects birds, humans, horses and other mammals.[3] Infection with WNV has been responsible for human and equine morbidity and mortality [14] which has resulted in declines of bird populations. [15] Bird mortality from WNV infections is high in North America, with approximately 48,000 dead birds belonging to greater than 200 species reported as WNV positive. [16] Pigeons pose a considerable

health risk to the population, and are reservoirs of infectious diseases. [17] Therefore susceptibility of pigeons to WNV has contributed largely to the epidemiology of the disease. Domesticated pigeons infected with WNV were reported in the urban areas of South Korea were they were exposed to humans, which has raised concern on possible zoonotic transmission.[18]

As an reemerging global pathogen, WNV is shared between birds, and the mosquito vectors. The increase in population, coupled with the socioeconomic activities in Bauchi metropolis was reported to have implications for solid waste generation in open places closer to households,[19] thereby increasing the burden of mosquitoes and risk factors to WNV in the environment. With a growing population density in major cities in Nigeria including Bauchi metropolis, rearing of pigeons is seen as a hobby, and also a source of income to families, thereby increasing the exposure to diseases by pigeons within the households. WNV seroprevalence of 42.9% was reported in pigeons among other species of birds sampled from live bird markets (LBMs) in Kaduna metropolis, Nigeria.[20] In domesticated pigeons, the highest seroprevalence of 68% and 32 % was established during the surveillance after the 2010 and 2011 WNV epidemic season in Greece.[21] This has proven that there is high circulation of WNV in domesticated pigeons. Seroprevalence of WNV in domesticated pigeons within households in Nigeria is still unknown. Birds vary in their susceptibilities to WNV infection and clinical course of infection; they present with symptoms like neurologic disorders, anorexia and depression, rapid weight loss, and death.[22, 23] Humans present with neuroinvasive disease, meningitis which is characterized by fever and other signs of meningeal irritation, including headache, neck stiffness, and photophobia. [24] Weather conditions and climatic factors were found to be direct factors that influence vector population dynamics and competence enhancing their ability to acquire, maintain, and transmit WNV. The factors also create favorable ecological habitats for

vectors and host interaction, with permissive weather conditions and appropriate seasonal timing being crucial constituents for a successful transmission cycle. [25] WNV has been associated with sporadic disease in small numbers of other species, including squirrels, chipmunks, bats, dogs, cats, white-tailed deer, reindeer, sheep, alpacas, alligators and harbour seals during intense periods of local viral activity. [26] There were reported concerns that endangered species may be at risk of further population damage from a WNV outbreak. [27]

CDC guideline on surveillance reported that live-bird surveillance has been used traditionally both to detect and monitor arbovirus transmission. Two approaches documented were captive sentinel surveillance (typically using chickens, but other species have been used as well), and free-ranging bird surveillance.[13] Sentinel pigeon surveillance for West Nile virus was used by using lard-can traps at differing elevations and canopy cover classes in New York, which has indicated that seroconversion rate as significantly greater for pigeons in canopy-level traps. [28]

Veterinarians, veterinary associations /departments, and state agriculture departments are essential partners in any surveillance activities involving WNV disease. Medical and veterinary arbovirus disease surveillance system is not being conducted in Bauchi and Nigeria, which may be a dangerous sign of an impending WNV disease outbreak in future. WNV has been established in Nigeria in several animal species, birds and humans, [29-31] but none of the solutions suggested by several researches in Nigeria are seen to have been implemented despite the zoonotic implication of the disease. This is largely as result of paucity of knowledge regarding the disease and its risk factors. [32, 33]

1.3 Justification

WNV is reported as a non-specific infection that escapes medical attention in hospitals. [34] (Monath et al 2001). Different arboviruses are considered in the aetiology of acute febrile

illness in some parts of Africa except in Nigeria, [32](Baba et al.2006), and there are many illnesses in Africa that have signs of pyrexia that are said to be of unknown origin usually if malaria and typhoid fever are ruled out if they are non-responsive to antimicrobial drugs. [35](Olaleye.,1990). In 1990, studies done on WNV in Nigeria was reported among human and domestic animals sera collected from Ibadan and Maiduguri, this resulted in a higher prevalence in camels among other species.[35] High seroprevalence of 71% also found in horses in south western Nigeria. [31] Research into WNV and other emerging and reemerging diseases in avian species in Nigeria is scanty despite the outbreaks of avian influenza (H5N1) in different parts of Nigeria.

In Nigeria, a Previous serological survey of WNV in avian species focused on live bird markets in Kaduna metropolis, which showed that pigeons, local chickens, guinea fowls, layers and broilers were exposed to WNV infection. [20] Therefore it has become necessary to carry out survey on pigeons within households in the study area, to ascertain sources of infection as pigeons are of economic value to households rearing them. WNV is however considered a serious health problem due to the severity of symptoms and its mortality rate in outbreaks. Despite the high mortality rate in birds and the public health importance of the infection, information on the persistence of antibodies to WNV in avian species is currently limited.[36] From the study area, seasonal mortality of pigeons due to unconfirmed causative agents in frequently reported to the veterinary clinics, this informed the choice of Bauchi metropolis for the study. Surveillance of dead birds for WNV has proven useful for the early detection of WNV in the United States. It has also proven useful for the early detection of highly pathogenic H5N1.[36] From the outcome of this study, using pigeons in sentinel surveillance for early detection of WNV could be suggested for the study area and Nigeria. The highest WNV seroprevalence rate of 42.9% documented in pigeon at LBMs in

Kaduna metropolis .[20] spur the need for further research within households. Potential risk factors to household members and pigeons by mosquitoes will also be assessed. The result from this study will provide a base-line data for further epidemiologic study, and for the improved prevention and control of pigeons and other birds infected with WNV. Also, the study will increase awareness of the disease among pigeon keepers.

Therefore, the purpose of this study was to determine the seroprevalence, and the risk factors associated with WNV in households that were keeping pigeons. Given that there were no data on WNV circulation in household-reared pigeons prior to this study, the methodology used for this study can be adapted to research on other diseases of pigeon that are of veterinary and public health importance. Information regarding knowledge of the disease in domesticated pigeons is not readily available and it's not yet known what role pigeons play in the epidemiology of WNV in Nigeria.

The information from this study will also be used to enlighten pigeon keepers in the households on the necessary preventive measures to adopt in other to reduce the risk of exposure and possible transmission in Bauchi metropolis. This will help pigeon keepers to be informed on the mode of transmission and the necessary action to take when infection is detected.

1.4 Research Questions

I. Is there of WNV antibodies among pigeons reared in household in Bauchi Metropolis?

II. What are the risk factors associated with WNV in pigeons reared within households?

III. How knowledgeable are pigeon keepers on mosquito-borne diseases?

1.5 General and Specific objectives

1.5.1 General Objective

To determine the seroprevalence and risk factors associated with occurrence of West Nile Virus anti-pr-E antibodies in pigeons reared in households in Bauchi metropolis.

1.5.2 Specific Objectives:

I. Determine the seroprevalence of WNV anti-pr-E antibodies in households–reared pigeons.

II. To determine the risk factors associated with WNV infection in pigeons among owners of household-reared pigeons.

III. To assess the level of knowledge on mosquito-borne diseases among pigeon owners in their households.

CHAPTER TWO - LITERATURE REVIEW

2.1 History

West Nile Virus was first isolated in 1937 from the blood of a febrile patient in the West Nile district of Uganda[37] during routine surveillance for yellow fever virus (YFV).[1] West Nile Virus (WNV; *Flaviviridae: Flavivirus*) is an "Old World" virus which re-emerged in the mid-1990s to international concern as a major public health threat. However, infection was not linked to overt clinical disease until the 1950s when epidemics in Israel and Egypt stimulated detailed studies on the virus and serological distribution of WNV-specific antibodies in northern and Sub-Saharan Africa. [5, 38] The first epidemic neuroinvasive disease caused by of WNV was documented among the elderly in Israel in 1957. [39, 40] During the 1950s, an estimated 40% of the human population in Egypt's Nile Delta was reported to be seropositive for the virus. [12] The largest human epidemic occurred in Cape Province, South Africa, in 1974, when approximately 3,000 clinical cases of the virus were recorded. [12] Incidence of severe WNV-related disease in both humans and equines gained momentum in the mid-1990s with reported epidemics in northern Africa, the Middle East, and central Europe: Algeria (1994 and 1997), Morocco (1996), Romania (1996), Tunisia (1997), Russia (1999), Israel (1998–2000) and France (2000). [41, 42] The 1996 Romanian epidemic was reported as the first human epidemic associated with significant incidence of encephalitic disease. [43] Emergence of WNV in the Western hemisphere in 1999 resulted in its rapid expansion in naïve enzootic transmission cycles throughout North America and into Central America with occasional evidence of viral isolates, viral RNA detection, and/or seroconversions in some areas of South America.[43] Cumulative efforts since 1999 estimate over 3 million human WNV infections were reported in the US with >780,000 WN fever cases, 16,000 neurologic disease (WNND) cases, and over 1,500 associated fatalities. [44] Similar to the situation in the US is the WNV epidemics in Europe which have undergone a paradigm shift

with unprecedented levels of mortality and human neurologic disease during outbreaks. The first case of West Nile fever was reported on 23 June 2015 at Sofia in Bulgaria. Since the beginning of the 2015 transmission season, 108 cases of West Nile fever in humans have been reported in the EU, 60 in Italy, 18 in Hungary, 19 in Romania, seven in Austria, two in Bulgaria, one in France and one in Portugal. [45] Possible explanations for this shift in global WNV disease are multi-factorial and are dependent on both host and environmental stimuli. [43]

In 2000, the epizootic extended to 12 states of USA and the District of Columbia, [46] and WNV can now be found in many avian and mosquito species throughout North America.[47, 48] From 1999 to 2010, more than 2.5 million people were documented to be infected, with over 12,000 reported cases of encephalitis or meningitis and over 1,300 deaths.[49] In Canada WNV was first detected in August 2001. During that year, 128 dead birds and 11 mosquito pools tested positive in Ontario. As a response to the emergence of WNV in the US, surveillance for the virus was first introduced in Canada during 2000.

2.2 Virology

2.2.1 Flaviviridae

The family *Flaviviridae* contains 3 genera: the flaviviruses, which include WNV, dengue virus (DENV), and yellow fever virus (YFV); the hepaciviruses, which include hepatitis B and C viruses; and the pestiviruses, which affect hoofed mammals. Within the *Flavivirus* genus, which contains more than 70 viruses, viruses can be further classified into tick-borne and mosquito-borne virus groups. The mosquito-borne viruses may be roughly sorted into the encephalitic clade, or the Japanese encephalitis serocomplex, which includes WNV and Japanese encephalitis virus (JEV), and the non-encephalitic or hemorrhagic fever clade, which includes DENV and

YFV and there are 10 serologic/genetic complexes. [50-52] The geographic distribution of the mosquito-borne flaviviruses largely depends on the habitat of the preferred mosquito vector, with *Culex* mosquitoes transmitting encephalitic flaviviruses mainly in the Northern Hemisphere. [53] WNV is a single-stranded, positive-sense RNA virus that is surrounded by a capsid. The capsid is enclosed by a host derived envelope that has been modified by two membrane glycoproteins, envelop (E) and premembrane (prM). Glycoprotein E is the most immunologically important structural protein. Glycoprotein E mediates virus-host cell binding, elicits most of the virus neutralizing antibodies, and acts as the virus hemagglutinin. WNV can be divided genetically into two lineages. Lineage I strains have been documented isolated from Africa, India, Europe, Asia, and North America. [11] These WNV strains have been associated with avian and human morbidity and mortality. Lineage II strains have only been isolated from Africa and have not been associated with human encephalitis cases.[11]

2.2.2 Characteristics of West Nile Virus

WNV is an arbovirus in the family *Flaviridae*. Its spherical, enveloped capsid has a diameter of approximately 50 nm and contains single-stranded RNA that encodes the capsid (C), envelope (E), and premembrane (prM) proteins, as well as 7 nonstructural proteins that likely contribute to viral replication. The virus has 2 genetic lineages: Lineage 1 strains have been further divided into 4 clades: Kunjin, Indian, A, and B (which includes an Indian isolate). [54] The isolates in clade B, which includes strains from the United States, are all virulent in mice; lineage 2 and other clades in lineage 1comprise both virulent and attenuated strains.[54] Differences in pathogenicity may be related to nucleotides that code for specific regions in the prM, E, or nonstructural proteins of the virus. [54, 55] WNV strains from the United States are reported to be closely related to strains from Israel, with 99.7% homology in nucleotide sequences, indicating

that the strains in the United States almost certainly originated from the Middle East. [56] The strain isolated in New York in 1999 are said to be more virulent in American crows (*Corvus brachyrynchos*) than strains from Kenya and Australia (Kunjin virus, a subtype of WNV), and both the New York strain and the Kenyan strain experimentally killed house sparrows whereas the Australian strain did not. [57] Two genetic variants of the North American WNV strain were isolated in Texas in 2002; the major variant differed from the New York 1999 isolate by 0.18% of nucleotides, and the minor variant by 0.35%. [54] The 2 variants differed from each other by 0.5% of nucleotides, and their neuroinvasiveness in mice was similar to that of the New York 1999 isolate. In 2003, attenuated WNV strains were found in birds in Texas and Mexico, providing the first evidence of phenotypic variation of WNV strains in the Western Hemisphere.[55, 58] The reduced neuroinvasiveness and smaller plaque size of the Texas strains may be due to mutations in nonstructural proteins that result in lower levels of viremia; the attenuated strain from Mexico had a mutation in the gene encoding the E protein. [55, 58]

2.2.3 Structure of WNV and Proteins

WNV is an enveloped virion containing a single-stranded, positive-sense RNA genome. The genome consists of a single open reading frame of approximately 11 kb with no polyadenylation tail at the 3′ end. Both the 5′ and 3′ noncoding regions of the genome form stem-loop structures that aid in replication, transcription, translation, and packaging. [59-61] The viral RNA is translated as a single polyprotein that is post- and cotranslationally cleaved by both host and viral proteases, resulting in three structural (capsid, envelope, and premembrane) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins.[62] In 2001,it was reported that the 5′ end of the genome encodes the structural proteins, which are necessary for virus entry and fusion as well as encapsidation of the viral genome during assembly.[52] The

nonstructural proteins have many diverse functions, which is understandable as the virus has a very limited number of proteins and they must each serve multiple purposes during infection. NS1 has both a "cellular" form and a secreted form and is highly immunogenic. The structural proteins include a capsid protein (C) that binds viral RNA, a premembrane (prM) protein that blocks premature viral fusion and may chaperone envelope (E)-protein folding, and an E protein that mediates viral attachment, membrane fusion, and viral assembly. [63] The viral nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A NS4B, and NS5) regulate viral transcription and replication and attenuate host antiviral responses. [64-66] NS1 has cofactor activity for the viral replicase and is secreted from infected cells, NS2A inhibits interferon (IFN) responses and may participate in virus assembly and NS3 has protease, NT Pase, and helicase activities. [67, 68] NS2B is a cofactor required for NS3 proteolytic activity, NS4A and NS4B modulate IFN signaling, and NS5 encodes the RNA-dependent RNA polymerase and a methyltransferase. [69, 70] Several molecules have been implicated in the attachment of WNV to cells in vitro, including DC-SIGN, DC-SIGN-R, and the integrin _v_3. [71, 72] However, attachment receptors in vivo for physiologically important cell types, such as neurons, remain uncharacterized. After binding, WNV enters cells via receptor-mediated endocytosis that may involve clathrin-coated pits.[73] Following a pH-dependent conformational change in the E protein, the viral and endosomal membranes fuse, releasing the viral nucleocapsid into the cytoplasm. [3] WNV is believed to replicate on endoplasmic reticulum- associated membranes to generate a negative-strand RNA intermediate that serves as a template for nascent positive- strand RNA synthesis. [74] Positive-strand RNA is either packaged within progeny virions or used to translate additional viral proteins. WNV assembles and buds into the endoplasmic reticulum to form immature particles that contain the prM protein. Following transport through the trans-Golgi network, furin-

mediated cleavage of prM to M generates mature, infectious virions that are released by exocytosis. [75, 76]

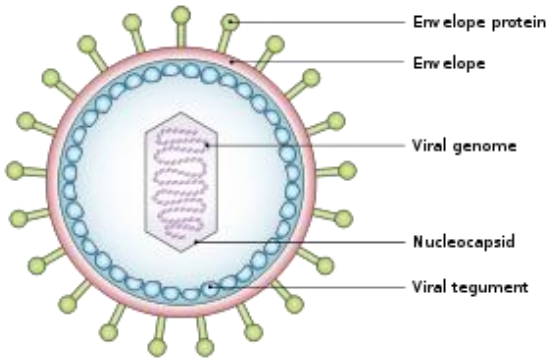


Figure 2.1: General Structure of West Nile Virus

(Source: *study.com/academy/lesson/west-nile-virus-structure-and-function.html)*

2.3 Pathogenesis

Most of the information on the pathogenesis of WNV infection is derived from experimental studies in mammals, mainly rodents. In these hosts, after mosquito blood-feeding, WNV replicates in the skin and is transported by Langerhans dendritic cells to draining lymph nodes. There the virus replicates and primary viremia and peripheral organ dissemination take place.[77] On the contrary, the exact mechanism and sites of WNV replication in avian hosts are still not well understood. It was later documented that in mammals, the virus replicates locally at the inoculation site and is rapidly distributed to all organ systems. [78] However, it has been demonstrated that the virus can be detected in the blood as early as 30–45 min after the mosquito feeding period, suggesting that in birds local replication is not necessary for the primary viremia. [79] In general, WNV can be isolated from the blood of infected birds at one day post-infection (dpi) (one day later in less susceptible species such as chicken or turkeys or in the case of oral infection) [80-82] Viremia can peak as early as 2–3 dpi in geese and some Passeriformes such as

crows and jays, or 4–6 dpi in raptors, owls and chicken. [83] Higher mean peak viremia has been reported in those birds that finally succumb to the disease. [57] WNV can be detected in the blood until day 6–7 pi in geese, passerines and owls and up to 10 dpi in raptors and turkeys. [84, 85] Little is known about innate avian host defenses against WNV infection, but in mammals, this includes inferon (IFN) production, complement activation, phagocytosis and cytotoxicity, with the participation of macrophages, neutrophils, natural killer (NK) cells and γδ T cells. [77, 86] WNV is thought to replicate at the site of inoculation and then spread to lymph nodes and the bloodstream.[87] Viral penetration of the central nervous system appears to follow stimulation of toll-like receptors and increased levels of tumor necrosis factor-α, which increases permeability of the blood-brain barrier. [88] WNV directly infects neurons, particularly in deep nuclei and gray matter of the brain, brainstem, and spinal cord. [89-91] Collateral destruction of bystander nerve cells may contribute to paralysis. [92] It was suggested that immune-mediated tissue damage may also contribute to pathologic changes in some cases.[93] Genetic susceptibility for severe disease in mice has been postulated to involve a deficiency in production of 2′–5′- oligoadenylate synthetase, but this genetic susceptibility has not been elucidated in humans. [91] Although most nonfatal WNV infections appear to be cleared by the host immune response, the virus is reported to persist in some vertebrate hosts. [91, 94]

Mosquito salivary components introduced at the site of infection in vertebrates modulate initial infection of target cells such as keratinocytes,[95] and skin-resident dendritic cells through several mechanisms including focalized suppression of immune effector cell trafficking to the site of inoculation.[96] Infected dendritic cells or keratinocytes migrate to draining lymph nodes from which a serum viremia is generated that then relays infection to visceral organs and potentially to the central nervous system .West Nile virus is capable of replicating and eliciting pathology in the brain, that is neurovirulence; however, a critical prerequisite to generating

neuroinvasive disease in humans is the virus' capacity to gain access to the central nervous system, which is the neuroinvasiveness.[97] Regardless of how the virus enters the central nervous system, murine models of infection have shown persistent viral replication in various tissues, including the central nervous system, suggesting a potential etiology for long-term neurological sequelae observed in patients with neuroinvasive disease. [97]

2.4 Epidemiology

2.4.1 Transmission

WNV transmission is maintained through a cycle between bird reservoirs and mosquito vectors, primarily of the genus *Culex*, with humans, horses, and other mammals incidentally infected. [98] Infection with WNV has been responsible for human and equine morbidity and mortality.[11, 14] Indeed, prevalence of WNV infection in mosquito and bird populations may be modulated by heterogeneity in vector or reservoir competence and contact rates between birds and mosquitoes [98-100]

West Nile virus can also be transmitted via transfused platelets, red blood cells, and fresh frozen plasma [101] as well as through heart, liver, lung, and kidney transplants.[102] Transmission via organ transplant has occurred from donors without detectable viremia, suggesting viral sequestration in organs shortly after viremia has cleared. One possible transplacental transmission following a second trimester infection was reported. It resulted in an infant with chorioretinitis, lissencephaly, and cerebral white matter loss.[103] Other rare or suspected modes of transmission include breast milk transmission, percutaneous or conjunctival exposure to laboratory workers, and by unknown means in patients undergoing dialysis and workers at a turkey breeder farm.[104] Wild birds were central to the transmission cycle of WNV because they

serve as amplifying hosts for the virus in nature. In the U.S., over 110 avian species, mostly songbirds, have been found to be susceptible to infection. Members of the family *Corvidae* (crows, jays and magpies) were especially important because they developed severe illness and a high mortality rate. This makes them useful as sentinels for the presence of virus in new endemic areas. Fortunately most other bird species show few symptoms of infection, but are viremic for several days after exposure and then develop lifelong immunity.[(11)] The virus was passed from one bird to another by the bite of ornithophilic mosquitoes, generally *Culex* spp. Numerous other mosquito species have been documented to be competent vectors of infection.[(105)] The mechanism for introduction of WNV to the New World is not known with certainty. One plausible scenario suggested is that infected mosquitoes arrived in the U.S on commercial aircraft or ships. This was however thought to be unlikely since the percentage of WNV-positive mosquitoes is extraordinarily low, even in areas of active transmission.[(106)] It is more likely that the initial outbreak was associated with the arrival of infected birds in the U.S, which could have occurred by normal migration, since several gull and duck species have transatlantic migration routes.[(107)] Other possibilities suggested include displacement of birds from their normal range by tropical storms or from legal or illegal importation.[(12)] It seems less likely that an infected human or domestic animal was the source of introduction. Mammals infected with WNV generally develop low levels of viremia and are incapable of sustaining a transmission cycle in nature.[(108)] After a mosquito feeds on an infected competent host, the arbovirus replicates within the mosquito and can then be transmitted to a susceptible host by injecting it's infected saliva .WNV can be transmitted by a variety of mosquitoes with different host-feeding preferences with up to 45 species and 8 genera reported to be positive in the U.S between 2004 and 2008. [(109)] WNV is however maintained in nature by transmission among ornithophagic *Culex* mosquitoes and a wide-variety of birds, especially those in the order

Passeriformes. Several *Culex* species have been implicated as vectors based mostly on laboratory vector competence studies, including *Culex univittatus, Culex neavei* and the *Culex pipiens* complex in Africa,[110, 111] *Culex pipiens* complex and perhaps *Culex modestus* and *Culex perexiguus* in Europe ,[112] *Culex annulirostris* and the *Culex pipiens* complex in Australia, and *Culex bitaeniorhynchus, Culex vishnui, Culex pseudovishnui, Culex tritaeniorhynchus* and *Culex quinquefasciatus* in India and Pakistan. [113, 114] However, it was suggested that the exact role of these species in virus epidemiology has been confusing due to their frequent blood feeding on large mammals and the limited numbers of isolations made during outbreaks and ecological investigations. [115] A large variety of migratory and resident bird species have been found naturally infected [116], but few host competence studies have been conducted to ascertain their importance in transmission.[6]

A study done in Italy reported a significant number of virological positive birds found in 2008 among magpies (Pica pica), carrion crows (Corvus corone) and rock pigeons (*Columba livia*) [117] which suggests a possible role of these resident species in the maintenance of local virus transmission. Mosquito bites account for nearly all human infections.

In 2002, intrauterine WNV transmission was documented for the first time in a 20-year-old woman who had onset of WNV disease in week 27 of gestation.[118] Probable WNV transmission through breast milk was also reported in 2002 [118] in a 40-year-old woman who acquired WNV infection from blood transfused shortly after she delivered a healthy infant. WNV nucleic acid was detected in her breast milk, and immunoglobulin (Ig) M antibody was found in her infant, who remained healthy. Transmission of WNV through blood transfusion was first documented during the 2002 WNV epidemic in North America [118]. In June 2003, blood collection agencies in the United States and Canada enhanced donor deferral and began

screening blood donations with experimental nucleic acid amplification tests. During 2003 and 2004, >1,000 potentially WNV-viremic blood donations were identified, and the corresponding blood components were sequestered.[119] Nevertheless, 6 WNV cases due to transfusion were documented in 2003, and at least 1 was documented in 2004, indicating that infectious blood components with low concentrations of WNV may escape current screening tests. [119] One instance of possible WNV transmission through dialysis has been reported.[120] WNV transmission through organ transplantation was also first described during the 2002 epidemic [118] It was also reported that chronically immunosuppressed organ transplant patients appear to have an increased risk for severe WNV disease, even after mosquito-acquired infection. [121]

During 2002, the estimated risk of neuroinvasive WNV disease in solid organ transplant patients in Toronto, Canada, was approximately 40 times greater than in the general population. [121]. WNV infection has been occupationally acquired by laboratory workers through percutaneous inoculation and possibly through aerosol exposure [122]. An outbreak of WNV disease among turkey handlers at a turkey farm have been reported, which raised the possibility of aerosol exposure. [123]

Birds are presumed to be the most important amplifying hosts of WNV. In laboratory studies, species in the orders *Passeriformes* (song birds), *Charadriiformes* (shorebirds), *Strigiformes* (owls), and *Falconiformes* (hawks) developed viremia levels sufficient to infect most feeding mosquitoes, whereas species of *Columbiformes* (pigeons), *Piciformes* (woodpeckers), and *Anseriformes* (ducks) did not. [98, 124] Certain passerines, including common grackles (*Quiscalus quiscula*), various corvids (crows, jays, magpies), house finches (*Carpodacus mexicanus*), and house sparrows (*Passer domesticus*) were documented to be highly infectious to mosquitoes and had mortality rates >40%. [124] Reported field studies during and after WNV outbreaks in several

areas of the United States have confirmed that house sparrows were abundant and frequently infected with WNV, this however is a characteristic that would allow them to serve as important amplifying hosts. [124-126] Local movements of resident, non-migratory birds and long range travel of migratory birds are thought to contribute to the spread of WNV. [81, 127] Although WNV was isolated from rodents in Nigeria and a bat in India, most mammals do not appear to generate viremia levels of sufficient titer to contribute to transmission. [26, 128, 129] Viremia levels of sufficient titer to infect mosquitoes were found after experimental infection of young alligators (*Alligator mississippiensis*).[130] Non mosquito borne WNV transmission has been strongly suspected among farmed alligators**,** domestic turkeys in Wisconsin, and domestic geese in Canada. [123, 131, 132] Transmission through close contact has been confirmed in both birds and alligators in laboratory conditions but this are yet to be documented in wild vertebrate populations.[98, 124, 130] Vertical transmission, overwintering, and survival of adult mosquitoes with some low activity during winter time have been reported in temperate regions. [133, 134]

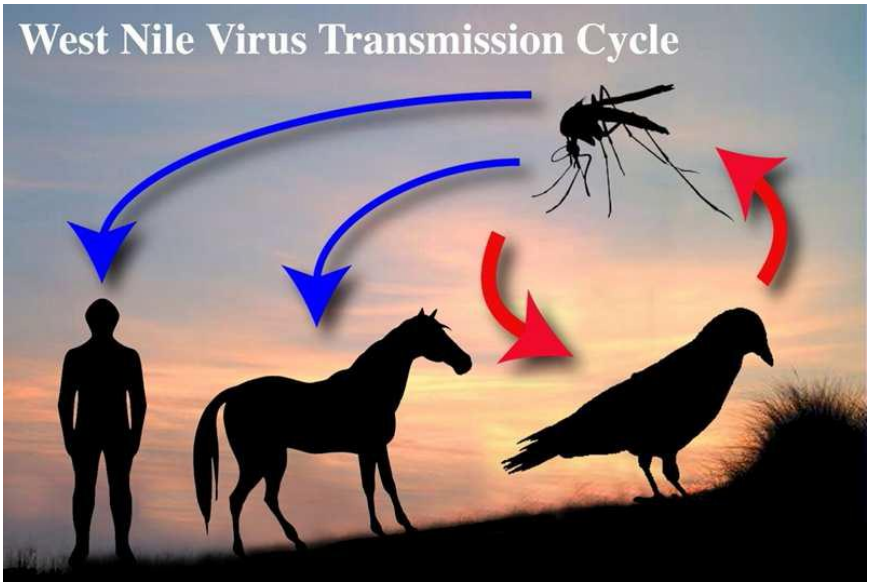


Figure 2.2: West Nile Virus Transmission Cyle

(Source: Tamas Bakonyi and Norbert Nowotny: West Nile epidemiology: global situation and recent outbreaks in Europe .Workshop on West Nile virus Berlin, 13th -17th of February, 2012)

WNV is transmitted primarily by the bite of infected mosquitoes that acquire the virus by feeding on infected birds. The intensity of transmission to humans is dependent on abundance and feeding patterns of infected mosquitoes and on local ecology and behavior that influence human exposure to mosquitoes. Although up to 55% of affected populations became infected during epidemics in Africa, reported outbreaks in Europe and North America have yielded much lower attack rates.[135] In the area of most intense WNV transmission in Queens, New York, in 1999, approximately 2.6% of residents were reported to be infected; that is most of these were asymptomatic infections, and similarly low prevalence of infection has been seen in other areas of the United States. In human, WNV disease in the Eastern Hemisphere has been reported mostly from areas in the Mediterranean Basin: in Algeria in 1994, Morocco in 1996, Tunisia in

1997 and 2003, Romania in 1996 through 2000, the Czech. Republic in 1997, Israel in 1999 and 2000, Russia in 1999 through 2001, and France in 2003.[136, 137] The incidence of WNV disease is seasonal in the temperate zones of North America, transmission of WNV in southern Africa and of Kunjin virus in Australia increases in the early months of the year after heavy spring and summer rainfall. [138].

West Nile virus is spreading in Europe at an increasing rate, with new lineages and variants emerging into new territories.[139] Several factors contribute to the current epidemiological picture, amongst which, urbanisation, variation in land use and climate are considered the most important.[140, 141] Perhaps because of the far longer history of WNV circulation and the recognition of at least five co-circulating lineages with variable pathogenicity. It is well established that climate affects many components of the WNV biological cycle [49, 142], and that the carrying capacity for both vector and host populations differs with land use.[143] Climatic anomalies are considered among the most important drivers of WNV emergence, and ambient temperature can be a determinant of outbreaks. [25] Permissive meteorological conditions are necessary for the persistence of active transmission,[140] and empirical studies suggest that temperature is a key factor influencing WNV evolution and dissemination. [144] Indeed, the relationship between temperature and increased WNV incidence in humans has repeatedly been confirmed both at national and multinational scales.[145, 146] The effect of temperature is likely to be mediated through its impact on the distribution, behaviour and survival of the mosquito vector, via direct impacts on the virus and on it's extrinsic incubation period in the competent vectors, [144] and via impacts on host distribution and behaviour. The nature of the association with temperature is therefore complex, for example, although warmer temperatures may have a negative effect on mosquito survival, they increase mosquito biting and development rates, and pathogen replication, [144] and can lead to increased human exposure risk through altered human

activity patterns.[6] Standing water is a prerequisite for larval development of the mosquito vector, without which they cannot complete their biological cycle.[147] WNV transmission to humans is inefficient and infrequent,[6] and cases of WNF occurs more often when mosquito population density rises. [148] Researches suggest that drought may lead to subsequent localised increases in mosquito numbers and disease outbreaks.[149] Drought conditions may encourage the aggregation of both hosts (birds) and vectors (mosquitoes) at remaining water bodies, increasing rates of transmission, [150] while potentially also influencing vector competence [151]. *Culex pipiens* thrives during drought conditions by exploiting larval habitats with high organic matter and artificial containers not reliant on precipitation.[152] Furthermore, drought conditions may force amplifier bird species into urban and suburban areas where water is more freely available, thereby bringing competent hosts into contact with competent urban vectors. [6] Associations between dry summers and increased WNV outbreaks have been shown in a number of US studies.[153] Wetlands, [154] agricultural area, [155] and urban infrastructure [156] have previously been reported to influence vector populations or WNV transmission.[157] In a study done in the US, the detailed resolution of land use classes allows a distinction to be made between irrigated, and rain-fed crop lands. Irrigated croplands were positively associated with WNF, where an increased human incidence of WNF was found to be associated with crop land cover and water catchment depressions in the otherwise semi-arid environment of Texas.[158] Indeed, US suburban areas are often characterized by assemblages of houses surrounded by vegetation (gardens, public parks, trees) which provide the optimal habitat for interaction of synantropic birds, mosquitoes and humans.[139] The occurrence of natural and protected areas was not significantly associated with WNF, but it is likely that increased sprawl of urban settlements causes fragmentation of previously pristine forest systems, increasing contact rates between

vectors, competent reservoir hosts and dead-end hosts such as humans, [157] therefore enhancing transmission from the sylvatic cycle. [100]

The introduction of WNV to Europe is hypothesized to be mediated by migratory birds, which may be infected in Africa, and then carry the virus northward during spring migrations to European locations.[12, 159] Migrating birds were reported to have played a key role in the geographic spread of WNV in the United States.[160] They are thought to be primarily responsible for virus dispersal, including reintroduction of WNV from endemic areas into regions that experience sporadic outbreaks. Each autumn an estimated 5 billion birds, representing over 300 species, migrate from North America to Central and South America, and similar numbers travel from Eastern Europe to Africa.[99] Long-distance migratory birds were not the only way to explain the spread of WNV over large distances; the virus could have been moved shorter distances sequentially by multiple individuals of short and long-distance migratory species [12] Wind patterns may also contribute to virus spread by wind-blown mosquitoes. [41]

2.4.2 Risk factors for infection with WNV in Humans and Animals

There are numerous risk factors, both known and unknown, that contribute to viral amplification in the environment and subsequent epizootic WNV activity [25]. Climate conditions, particularly ambient temperature and rainfall, are critical drivers of mosquito abundance and amplification of WNV[161, 162]. The most important risk factor for acquisition of WNV infection is exposure to infected mosquitoes.[163] *Culex pipiens* have been found to be direct correlation between increases in ambient temperature and increases in vector populations.[164] Interestingly, elevated temperatures also affect the rate of virus replication, leading to more rapid infectivity of the mosquito.[144] Precipitation is another key component to promoting vector abundance by providing ample breeding environments for mosquitoes,[165] although heavy rains and flood

waters have also been documented to reduce mosquito breeding grounds by flushing out their preferred stagnant water sources. [166] In contrast, drought conditions can promote WNV transmission by forcing birds and mosquitoes to share the same habitat through competition for scarce water sources; it was believed that the severe drought in Texas in 2011–2012 was the driver for the 2012 outbreak that resulted in 1868 human cases.[167, 168]

People living in close proximity to stagnant water sources where mosquitoes breed have been found to be at higher risk for infection. In Houston, Texas, where *Culex quinquefasciatus* mosquitoes serve as the primary vector, it was found that WNV case patients were significantly more likely to reside near slow-moving/stagnant water sources with heavier vegetation [169]. Similarly, in El Paso, Texas, where *Culex tarsalis* is the primary vector, WNV case-patients were close to yards that were flooded regularly by irrigation canals. [170] In northern Colorado, where *Cx. tarsalis* is also primary, irrigation and manual control of water on the landscape was also found to promote mosquito abundance.[171] In addition to being in areas with high vector abundance, risk for becoming infected can be influenced by time spent outdoors and decisions on whether to adopt personal precautions against mosquito bites. In 2003, a study done in Wyoming showed that those spending > or =3 hours outside per day were more likely to be seropositive than those spending less time outside per day. [172] In Houston, a serosurvey of homeless individuals found that time spent outdoors greatly influenced infectivity, with 12.5% of those who reported spending >12 h outdoors being positive for WNV, compared with only 2% of those who reported spending <6 h outdoors. [173] Increased time outdoors was associated with infection, [172, 174] in addition to inconsistent mosquito repellant use [172] and younger age, with children being five-times more likely to be infected with WNV when compared with adults. [175]In Romania, the risk for WNV infection was documented to be higher among persons with mosquitoes in their homes and with flooded basements.[176] Locations of WNV disease cases

during the 1999 outbreak in New York was analysed, however it was found that cases were clustered in an area with higher vegetation cover, indicating favorable mosquito habitat.[177] A study of the outbreak in Chicago in 2002 indicated that human disease cases tended to occur in areas with more vegetation, older housing, lower population density, predominance of older Caucasian residents, and proximity to dead birds.[178] Genetic predisposition for severe disease has been described in mice but has not yet been replicated in humans. [91] Dead-crow density (number of dead crows/square mile) was reported to be highly associated with the number of human cases by county in New York State and other areas.[179] Persons living in towns near areas of current or recent dead-crow clusters were suggested to be at increased risk of infection.[180, 181] The most important risk factor for acquisition of WNV infection was exposure to infected mosquitoes.[163] To identify risk factors for complications of WNV disease in humans, those with West Nile Encephalitis (WNE) were more likely to have a history of advanced age, alcohol abuse, and diabetes mellitus, compared to patients with West Nile meningitis or non-neuroinvasive WNF [182] Advanced age, immunosuppression, requirement of mechanical ventilation, and history of stroke were all associated with death in patients with WNE.[182] Advanced age has also been suggested to be a predictor of poor prognosis among humans with non-neuroinvasive WNF.[183] However, all ages are at risk of WNV infection; ages of reported cases ranged from 2 to 96 years.[184] In 2002, 105 cases of WNND (which includes WNE and meningitis) and 45 cases of WNF among children (< 19 years old) in the United States were reported. [118] Host immunity also appears to play a role in risk of developing WNV infection.[185] Low-lying places with poor drainage, urban catch basins, roadside ditches, sewage treatment lagoons, and man-made containers around houses provide good larval development sites for *Culex* spp. mosquitoes to deposit their eggs .[186]

As seen with the rapid expansion of WNV across North America, birds spread the virus into new geographic areas [12] and *Culex pipiens* species mosquitoes are predominately ornithophilic, in that they prefer feeding on avian hosts, particularly *Passeriform*es. [187] Passerine birds, including blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), house finch (*Carpodacus mexicanus*), American crow (*Corvus brachyrhynchos*), house sparrow (*Passer domesticus*), cardinal (*Cardinalis cardinalis*), and American Robin (*Turdus migratorius*) have been found to contribute most to viral amplification and transmission of WNV to mosquitoes. [188] In a study on spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex in Illinois found that 80% of blood meals from field collected, *Culex pipiens* mosquitoes were from avian sources, with 25 different avian species identified; American robin (*Turdus migratorius*) was the most over represented with 48% of avian blood meal sources. [189] In California, *Cx. tarsalis* species mosquitoes were found to have a much higher diversity in its host-seeking behaviors when compared with *Cx. Pipiens* complex mosquitoes, [190] though both species of mosquitoes were found again to be predominately ornithophilic. In contrast to the study in Illinois, the California mosquitoes rarely fed on humans, with human blood meal sources only found in 0.4% of *Culex pipiens* and 0.2% of *Culex tarsalis* mosquitoes, suggesting that human cases only occur when there is substantial epizootic activity.[191] In central and eastern Texas, the most common vector for WNV is *Culex quinquefasiatus*, which has a remarkably different host seeking pattern. Blood meal analyses found that only 39% of blood meals came from an avian source [192]. Canines were the most common blood meal identified (41%), whereas humans were the least common (0.4%).[192] WNV reservoir hosts are predominately ornithophilic, in that they prefer feeding on avian hosts, particularly *Passeriformes*.[187, 189] Passerine birds, including blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), house finch (*Carpodacus mexicanus*), American crow (*Corvus*

brachyrhynchos), house sparrow (*Passer domesticus*), cardinal (*Cardinalis cardinalis*), and American Robin (*Turdus migratorius*) have been found to contribute most to viral amplification and transmission of WNV to mosquitoes.[98, 188] In California, *Culex tarsalis* species mosquitoes were found to have a much higher diversity in its host-seeking behaviors when compared with *Cx. Pipiens* complex mosquitoes, [190] though both species of mosquitoes were found again to be predominately ornithophilic.

In a serologic study of dogs and cats during an outbreak of WNV infection among humans in Louisiana, factors such as residing exclusively outdoors and not receiving heartworm medication were associated with seropositivity in pet dogs. [193] In addition, stray dogs were more likely to be seropositive, compared with owned dogs, and dogs were more likely to be seropositive for WNV than cats; no risk factors for seropositivity in cats were identified.[193]

2.5 Seroprevalence

Field surveys were conducted in Far East Russia in 2005 and 2006 to know the Seroprevalence of WN virus in wild birds using micro-focus reduction neutralization test (FRNT). Neutralizing antibody to WN virus was identified in 21 serum samples taken from 145 wild birds (14.5%) [194]. Birds that were positive for antibodies to WN virus were in the families *Anseriformes*, *Charadriiformes*, *Columbiformes*, and *Pelecaniformes*. Birds in these orders have been reported to support WN virus propagation with high levels of viremia, and to serve as efficient amplifying hosts for the transmission of WN virus to mosquitoes. [98] The other WN virus-positive birds identified in the study were spotbills (*Anas poecilorhyncha*), harlequin ducks (*Histrionicus histrionicus*), red-breasted mergansers (*Mergus serrator*), black-headed gulls (*Larus ridibundus*), and common terns (*Sterna hirundo*), which are all migratory birds. Therefore it is possible that these birds were infected with the WN virus in Far Eastern Russia and carried the virus into other

regions of East Asia. In the Asia-Pacific region, migratory water birds typically display north-south flying patterns.[195] Studies carried out in Illinois on prevalence of West Nile Virus antibody in birds, reported *Columbiformes* (Mourning doves and Rock doves) to be positive for WNV; Mourning doves having a prevalence of 9.1% (1/11) and Rock doves 55% (11/20).[196] American Crows were reported to be rarely seropositive, despite the collection of crows exhibiting WNV symptoms. Several of these crows were subsequently found to be WNV positive on necropsy, supporting the findings that American Crows and Blue Jays frequently die 4–6 days post infection, which is before antibodies are detectable in some specimens [98] A reported finding on WNV in New York during an outbreak and resulted in13.3% positive for WNV antibodies in 120 Rock Doves (*Columba livia)* tested, and 66.7 % in 3 Mourning Doves (*Zenaida macroura)* tested.[197] Other families tested in the outbreak were *Anseriformes, Galliformes, and Passeriformes*. In an outbreak of WNV in a New York City among captive, wildlife population, serum or plasma samples taken from 485 individual animals representing 124 species, 50 families, and 24 orders were tested for the presence of WNV-neutralizing antibodies. Among the birds, samples from 89 species representing 32 families and 17 orders were collected and tested. Seroprevalence among all avian species tested was 34% (125 of 368) and seropositive birds were identified from 42 of 89 species tested. Only one (3%) of 32 birds housed in indoor enclosures was found to be seropositive, while 37% (124 of 336) of the outdoor birds tested were seropositive. [198] Seroprevalence among the different orders represented in the sampled population ranged from 0% to 100%.[198] However, among orders from which five or more birds were sampled, seroprevalence ranged from 20% to 83%. The owls had the highest seroprevalence (83%) and the pigeons the lowest (20%).[198] Seroconversion detection has been used for monitoring the spread of WNV as well as other arboviruses such as Eastern equine encephalomyelitis (EEE) and western equine encephalomyelitis (WEE).[199] WNV

seroprevalence, the number of individuals in a population with WNV antibodies, could also be used as a measure of host exposure and survival. It was reported that bird species with lower WNV-associated mortality rates would have higher antibody prevalence such as the higher seroprevalence they found in fish crows (*Corvus ossifragus*) compared to American crows.[200] Northern cardinals and gray catbirds were reported to be among the highest seroprevalent species in Georgia.[201] Cardinals were reported also with the highest seroprevalent species in a Florida survey along with house sparrows.[125] Prevalence of 1.5% West Nile virus–neutralizing antibodies was found in *Columbidae* sampled in Chaco, Córdoba, and Tucumán Provinces, Argentina, 2004–2006 (4/270) [202]

Across Illinois, over the course of a study from 2001 through 2004 sampled 5,236 wild, free-ranging birds belonging to 11 orders, 36 families, and 145 species. Positive wild birds represented 6 orders, 19 families, and 38 species. Despite the diversity of species exposed to WNV, the top five species (of those with more than 15 individuals sampled) throughout the state, in terms of the percentage that were seropositive, were Wild Turkey (40.5%, n = 37), Mourning Dove (38.1%, n = 97), Northern Cardinal (24.7%, n = 389), American Robin (15.2%, n = 341), and House Sparrow (9.6%, n = 1,207).[203] In that study, several species never tested positive for antibodies to WNV, including, Downy Woodpecker (n = 39), Tufted Titmouse (n = 49), and Black Capped or Carolina Chickadee (n = 76).[203] It was further suggested that the importance of these species in WNV transmission may be offset by its abundance.[203] However, in 2006 it was found that while host selection may be influenced by abundance, certain species of birds appear to be overused, such as the American robin or underused like the house sparrow relative to their abundance, suggesting that other factors may be involved in host selection. [100, 189] In the 1950s in the Egyptian Nile Delta, it was found that of 420 domestic and wild birds sampled, the hooded crow (*Corvus cornix*) and house sparrow had the highest WNV antibody seroprevalence

at 65% and 42%, respectively. [204] At the end of the 1974, WNV epidemic in South Africa, a high rate of seroprevalence was again found in the house sparrow (50%), a species with extremely large roosts observed at the time. [205] The most abundant seropositive hatch-year birds in the Chicago study site in 2005 were house sparrows, northern cardinals, American robins, and gray catbirds. [206] Furthermore, it was found that house sparrows appeared to be underused in relation to their high abundance in urban settings.[189] Vector host were later examined, and the rate at which susceptible hosts acquire infection or seroconvert at a fine spatial scale in the Chicago area, and found that American robins and house sparrows produced 95.8% of the infectious *Culex pipiens* mosquitoes. [207] In a serologic study on WNV viremia in wild rock pigeons from 2002–2003, on the WNV exposure rates of rock pigeons from a single locale adjacent to metropolitan Atlanta, WNV-specific antibodies were detected in 128 (25.7%) of 499 rock pigeons tested. Overall seroprevalence rates per collection for 2002 were 16%–45% and 11%–50% in 2003.[208] Using indirect ELISA to test anti-WNV antibodies in 12 species of bird, a seroprevalence of 2.9%, 2.5%, and 7.6% respectively were reported in Rock doves (*Columba livia*). [209] With PRNT, a seroprevalence of 10% (7/70) in *Columbiformes* out of over one thousand birds sampled in Central Iowa, USA was reported [210]

2.6 Clinical presentations

2.6.1 Birds

Typical clinical signs in birds are neurological, such as ataxia and paralysis, as well as non-neurological, such as depression, lethargy, ruffled feathers, weight loss and myocarditis [98]. Other signs of WNV-associated neurologic disease can be clinically inapparent or fatal, with considerable variability among species. These include sudden onset of recumbency, mild ataxia, abnormal head posture, circling, swimming in circles, tetraparesis, tremors, nystagmus,

seizures, and disorientation; additionally, signs of depression, mental dullness, anorexia, rapid weight loss, impaired vision, and sudden death may be evident.[23, 211, 212] The case fatality rate in American crows appears to be close to 100%.[98] When birds die from WND, it is within the first 24 h after the onset of clinical signs. WNV infection also causes damage in multiple bird organs, as brain, kidney, heart, lungs, liver, gonads, spleen, intestines, esophagus, and skin. As mentioned above, crows and blue jays are highly susceptible to WNV infection [98] and, thus, monitoring of crow mortality has been successfully adopted as an epidemiological indicator for tracking WNV activity in the USA, predicting an increase of the risk for human infections. [213, 214]

In dead birds, affected organs reported include the spleen and liver (evidence of enlargement, necrosis, and hemorrhage), heart (evidence of myocardial degeneration, inflammation, and pericardial lesions), pancreas (pancreatitis), and adrenal gland (chronic inflammation).[23, 215] Less frequent pathologic signs are pulmonary and intestinal hemorrhage, lymphoplasmacytic enteritis, renal congestion and nephritic foci, ovarian necrosis, and disseminated intravascular coagulation.[23, 211]

2.6.2 Humans

Most WNV infections in humans are asymptomatic, but approximately 20% of these infected develop clinical symptoms, although severe neurological diseases are observed in less than 1% of those infected. WNF is characterized by several non-specific flu-like symptoms, such as fatigue, fever, headache, muscle pain, weakness, rash, and neck-pain. Symptoms usually develop between 2 to 15 days after infection and last for 2 to 5 days;[9] however, in some instances, they can result in hospitalization and many patients with WNV encephalitis have movement disorders, including severe tremors and parkinsonism [216, 217]. Older age has been

associated with poor prognosis, but neuroinvasive diseases have also been reported in young people and children.[13, 218] Hypertension and diabetes have been suggested to be a potential risk factor for severe WND, but the most important factor, beside old age, seem to be immunosuppression, such as transplant patients or those who are human immunodeficiency virus infected.[13] In fact, a fully functional immune response has been shown to be essential to fight WNV infection in animal models.[87]

2.6.3 Horses

As in humans, the majority of WNV infections in horses are usually not accompanied by presentation of clinical signs, which are only observed in about 10% of the animals[26] and are characterized by fever and, sometimes, by ataxia and muscular weakness; however, some other signs like dysmetria, somnolence, or hyper excitation have also been observed.[219] Illness usually lasts for 3 weeks.[220] Even though outbreaks have resulted in 25% to 45% of mortality rates among affected horses,[42, 220] implementation of vaccination campaigns has strongly reduced these mortality rates, [221] and treatment guidelines for horses with WND have been published. Lesions associated with WNV infection in horses are mainly limited to the CNS and, in the most severe cases, neuronal degeneration. [219] Contrary to other *Flavivirus* infections, clinical cases of WND in horses do not precede that in humans, discarding their use as sentinels.[222]

2.6.4 Other animals

Beside birds, horses and humans, WND is unusual in other vertebrates, but neurological manifestations have been reported in squirrels, [223] dogs and cats, [128] and alligators.[130] West Nile virus infection does not appear to cause extensive illness in dogs or cats,[224] Hamsters [225]

and mice [226] but are frequently used as experimental models, as they present some disease signs parallel those exhibited by humans with severe neuroinvasive disease, such as confusion, tremor of extremities and paralysis.

2.7 Diagnosis

2.7.1 Virus isolation

WNV is not readily isolatable from tissues, plasma, serum and CSF samples in cell culture using either mammalian or mosquito derived cell lines, such as Vero E6, RK-13, AP61 or C6/36 [227, 228]. Moreover, these procedures must be performed under biosafety level 3 conditions [229] and it generally takes up to 7 to days before the appearance of CPE. Virus isolation is most readily accomplished if the following procedures for collecting the samples are adhered to; whenever possible, the samples should be collected under sterile conditions, in collecting media containing antibiotics and immediately refrigerated at 4 °C. Under optimal conditions the samples should be inoculated onto susceptible cells in culture within minutes of collection but when this is not possible, the time interval should be kept to a minimum and always less than 24 hours. In general these conditions can be met in reference laboratories and dedicated research facilities.[230] The isolation of WNV strains provides the added value of allowing further studies and research on pathogenesis, genetic variation, evolution and epidemiology.

Specimens for virus isolation include brain (particularly hindbrain and medulla) and spinal cord from deceased encephalitic horses; [231] a variety of bird tissues including brain, heart or liver may be used with success. [212] WNV can also be isolated from mosquitoes. Virus isolates are reported to be obtained more easily from avian specimens and to a lesser extent from mosquitoes and horses. Virus may be propagated in susceptible cell cultures, such as rabbit kidney (RK-13),

African green monkey kidney (Vero), or pig kidney cells. Primary isolation in embryonated chicken eggs or *Aedes albopictus* (C6/36) cell lines followed by passages in mammalian cells can also be used. More than one cell culture passage may be required to observe cytopathic effect (CPE). Confirmation of WNV isolates is achieved by indirect fluorescent antibody staining of infected cultures or nucleic acid detection methods.

2.7.2 Molecular methods

WNV genomes in peripheral blood are usually detectable from 2–3 days to 14–18 days post-infection.[232] It is usually possible to identify the viral RNA in serum and/or plasma samples. In addition the detection of virus using molecular methods has been demonstrated to be a useful tool that enables the detection of WNV genomes, even after prolonged times post-infection.[233, 234] For the routine detection of WNV RNA using molecular techniques there are two distinct diagnostic settings: the first involves blood and organ donation screening from subjects living in an area where WNV circulation is known to be occurring, and the second involves the identification of viral genomes in serum, plasma and CSF samples from patients presenting with a clinical picture typical of WNV infection. [235]

2.7.2.1 Reverse Transcription PCR (RT-PCR)

The sensitivity of conventional RT-PCR for WNV detection depends mainly on the target sequence. A test based on primers that target the C gene (3') and the prM gene (5') detected viral RNA equivalent to approximately 0.1 plaque forming units (PFU) derived from a cell grown NY99 isolate. [236, 237] Nested RT-PCR techniques can increase by up to 10 fold the RNA detection threshold but these methods are more prone to contamination, require a longer time to perform and increased involvement of laboratory personnel.[237] The use of pan-specific RT-PCR

techniques, capable of identifying most of the flaviviruses and mainly based on nested assays,[238] can potentially differentiate WNV related disease from other infections caused by flaviviruses. The amplicons obtained using these labour intensive methods need to be sequenced and the obtained electropherograms analysed in order to identify the infecting virus.[237]

2.7.2.2 Real-time reverse transcription PCR

Real-time reverse transcription PCR was developed for concurrent identification of lineage 1 and lineage 2 WNV. [239] Strain identification may be achieved by sequencing of the resultant amplicon and alignment with WNV reference strains. The procedure has been slightly modified and included here are the primers and probe directed to the NS2A region of the WNV genome. The assay may be performed with a commercial kit of choice that provides the expected amplification of the controls.

Real-Time RT PCR are generally rapid and reliable and can be used for detection of WNV on a large variety of samples, including non-human specimens, such as animal tissues and mosquitoes.[240] In general, the TaqMan probe-based assays are quite sensitive but sometimes incapable of detecting emerging genomic variants of the virus.[241] The SYBR Green based tests are as sensitive as the TaqMan tests, but less specific.[242] The decreased specificity could be both a disadvantage, since false positive results can be generated by diverse viruses, and an advantage, given the broader spectrum of WNV variants that can be identified.[242] Another potential disadvantage of real-time RT-PCR is represented by the generally short amplicons obtained, that are not suitable for sequencing and molecular characterization. Given the strain variation of WNV from season to season and in different geographical locations, new diagnostic molecular approaches have been proposed.[237] A multiplex PCR-ligase detection reaction assay (RT-PCR/LDR) based on the contemporary amplification of three different genomic regions

(one in the coding sequence of NS2 and two in the nonstructural gene NS5) is reported to minimized the risk of false negative results due to WNV genetic variants.[243] Another method, based on genome-wide RT-PCR overcame the undetectability of WNV isolates with genomic differences.[242] WNV isolates have been phylogenetically grouped into 7 different lineages and lineages 1 and 2 are documented to overlap in some areas of Europe. [244, 245] In order to distinguish between lineage 1 and 2, a novel real-time quantitative RT-PCR test has been developed. This test showed an extremely high differentiation capacity on different cell derived WNV isolates at the level of 2–4 genome copies. [239] WNV overlaps geographically with several other arboviruses including flaviviruses, alphaviruses and some bunya viruses and as the number of recognised emerging viruses increases in both tropical and sub-tropical regions, [246, 247] new multiplex RT-PCR assays have been developed to facilitate simultaneous identification. As these techniques improve, the early detection of multiple arboviruses is becoming a practical reality with the potential for rapid and cost-effective differential diagnosis and epidemiological surveillance.

2.7.2.3 Conventional reverse transcription PCR

Conventional reverse transcription PCR was developed for detection of lineage 1 WNV.[248] The sensitivity of conventional RT-PCR for WNV detection depends mainly on the target sequence. A test based on primers that target the C gene (3') and the prM gene (5') detected viral RNA equivalent to approximately 0.1 plaque forming units (PFU) derived from a cell grown NY99 isolate [236]. Nested RT-PCR techniques can increase by up to 10 fold the RNA detection threshold but these methods are more prone to contamination, require a longer time to perform and increased involvement of laboratory personnel.[236] The use of pan-specific RT-PCR techniques, capable of identifying most of the flaviviruses and mainly based on nested

assays,[238] can potentially differentiate WNV related disease from other infections caused by flaviviruses. The amplicons obtained using these labour intensive methods need to be sequenced for viral identification

2.7.3 Immunohistochemistry

The detection of WNV antigen using histochemical protocols in tissues obtained from fatal encephalitis cases has been available for diagnostic purposes for many years. In today's laboratories this procedure is performed rarely and usually in order to improve the certainty of a clinical diagnosis in cases where laboratory data are minimal.[249] Data on the diagnostic performance of this assay have been mainly obtained in animals and the specificity of this method depends largely on the specificity and quality of the antiserum used. The sensitivity in many cases can be low and is often dependent on the amount of WNV present in the tissue and the quality of the tissue under investigation.[250]

2.7.4 Serological test

Specific antibody detection still remains the most widely used approach for the diagnosis of WNV infection in humans. In general, the procedures for animal serology also follow those used for humans. [251]In order to understand the application of serology for WNV diagnosis, it is useful to remember that the mean times from the detection of viral RNA to IgM and IgG seroconversion are approximately 4 and 8 days respectively, as it was determined in a study performed amongst more than 200 WNV viraemic blood donors in the USA [232] Principal serological methods can be subdivided into two main groups: the first includes the enzyme-linked immunosorbent assays (ELISAs) and immunofluorescence (IF) based tests; the second includes the Plaque Reduction Neutralization Test which can be carried out using a highly

sensitive 50% or less-sensitive 90% endpoint (PRNT50, and PRNT90 respectively), both of which require the constant availability of standardised-validated infectious viruses and appropriate cell cultures. [237]

2.7.4.1 Plague Reduction Neutralisation Test (PRNT)

The PRN test is performed in Vero cell cultures in either 25 cm2 flasks or 6-well plates. The sera can be screened at a 1/10 and 1/100 final dilution or may be titrated to establish an endpoint. This test however is generally performed following standard protocols.[252] The method defined for Dengue by the WHO could be adapted for WNV, [253] which describes a standardised protocol for determining PRNT90 titres of sera from patients. The same protocol could be used also to determine a PRNT50 titre in sera obtained from sub-clinical infections. While being a valuable tool in the diagnosis of WNV infection and being the most specific of any of the *Flavivirus* serological assays, PRNT exhibits a number of limitations, which restrict the test's capacity to be exploited for high-volume measurement of antibody against WNV.[254] First, PRNT does not differentiate between IgM and IgG; thus, without prior measurement of both IgM and IgG by another method, it is difficult to determine if the assay is detecting current or past infection. PRNT also requires cell culture, viable (infectious) virus, and careful titration of the test virus.[254]

2.7.4.2 Hemagglutination Inhibition Tests (HITs) and Complement Fixation Tests (CFTs)

HITs are still used in some laboratories, but CFTs are hardly ever performed because it is difficult to maintain the satisfactory quality controls they require.[255] HITs exploit the ability of the E protein to aggregate avian erythrocytes in the absence of anti-E neutralizing antibodies. Test results are affected by the presence of non-specific inhibitors.[256] HITs was said to be less

sensitive than ELISAs and present the same drawbacks, a high level of antibody cross-reactivity. (199, 257)

2.7.4.3 Enzyme Immunoassays (EIAs)

These methods can be subdivided into three main categories: (1) the IgM antibody-capture EIA (MAC-EIA), (2) the indirect IgG EIA and (3) the epitope blocking EIA. All of these methods have the advantage of rapidity and reproducibility when compared with those detecting NAbs and with HIA and CFT. (237)

2.7.4.4 IgM antibody-capture enzyme immunoassay (MAC-EIA)

This test is suitable for routine detection of immune responses resulting from an acute viral infection. The IgM response is usually detectable within the first week of WNV infection, sometimes during the pre-symptomatic stage, in plasma, serum or cerebrospinal fluid (CSF) and generally persists for up to 47 days in plasma/serum with prolonged persistence in the CSF up to 100–199 days post infection and up to 1 year in plasma/serum of viraemic blood donors.(258) The standard commercially available MAC-EIAs use plates coated with antibodies, anti-human IgM and WNV antigens obtained from the brain of experimentally infected mice.(237) Although in general, results of MAC-EIAs correlate well with the PRNT90 and the cross-reactivity with IgM Abs resulting from infection by JEV serocomplex viruses is generally lower than for that of IgG antibody responses, (259) false positive results have been reported with the routine use of MAC-EIA.(260)

2.7.4.5 Indirect IgG EIA

The detection of WNV IgG by EIAs is now standardized and these methods are widely used for the determination of immune status to WNV either in suspected patients or in healthy asymptomatic populations.[261] A major limitation of these tests is, firstly, their restricted clinical specificity due to extensive cross-reactions with flaviviruses.[237] However, it is recommended that the detection of IgG should be performed in combination with the MAC-EIA in all cases of suspected infection, since persistence of IgM may last for up to 6 weeks after disease onset.[258]

2.7.4.6 Epitope blocking EIA

A technical variant of the EIAs for the identification of IgG is the so called "Epitope-Blocking EIA". This method is based on competition between the tested sera and WNV specific MAbs for binding to WNV antigens and was originally developed for diagnosis and prevalence studies in animals since it is species-independent.[262] This technique has also recently been evaluated for the diagnosis of human infection. The results obtained showed that this method is only applicable in populations not previously exposed to flaviviruses other than WNV [263] which results in a lack of correlation with the PRNT90 findings.

2.7.4.7 Immunofluorescent assay (IF)

The use of the IF assay in routine diagnosis of WNV infection is mainly dependent on the number of samples that need to be evaluated and the quality of training of laboratory personnel in interpreting the test results. Most of the tests used are developed "in-house" by individual laboratories using WNV infected cell cultures. Consequently this technique suffers from a lack of standardization when results are compared between laboratories and would greatly benefit from an External Quality Assurance (EQA) evaluation as shown later.[237] A commercially

available IF test (Euroimmun, Germany) was shown to have about 94% specificity when compared with the PRNT90 test, when it was performed on 200 sera collected during a human WNV outbreak in South Africa.[264]. In addition, new protocols for the IF testing have been developed for the detection of IgM and IgG antibodies. These methods have been reported to exhibit higher specificity than MAC EIA.[265] To reduce the non-specific reactivity (against related vector transmitted viruses or against cellular antigens) defined quantities of uninfected cells should be mixed with WNV-infected cells: the uninfected cells are obviously expected to remain non-reactive with tested sera.

2.7.4.8 Indirect and competitive ELISAs

Numerous indirect and competitive commercial and in-house ELISAs have been developed and are used to detect WNV antibodies. While competitive assays are applicable for sera of all species, indirect assays require enzyme-labelled species-specific antibodies. Both ELISA techniques lack specificity as they cross react with antibodies directed to other flaviviruses especially those of the Japanese encephalitis serogroup. They are very useful for epidemiological and surveillance purposes as well as a screening method. A positive ELISA result however should be confirmed by a more specific test such as VN or PRNT. Most indirect or competitive ELISAs detect antibodies of any immunoglobulin class (IgM, IgG). Vaccinated horses often test positive in the indirect or competitive ELISAs

2.8 Surveillance

To reduce the risks of WNV, adequate surveillance programmes must be put in place. Whether it is mosquito control activities or the promotion of personal protective measures, operational and communication elements of all approaches must be informed by appropriate surveillance of the

vectors, virus, and animal hosts, including humans. [24] Surveillance of vector populations can be done in a number of ways but should be focused on the collection of primarily *Culex* spp. mosquitoes. A combination of adult host-seeking and oviposition traps are likely to collect sufficient data to assist local management of WNV risk. [266] The first evidence of WNV activity may be identified through serosurveillance of sentinel animals, as part of arboviral surveillance programs, by the death of wild bird populations or by identification of symptomatic disease in other mammalian species, particularly horses.[267] Screening of donated blood products in endemic areas has also emerged as an important surveillance tool for human WNV activity.[268] Surveillance of dead birds for WNV has proven useful for the early detection of WNV in the United States. It has also proven useful for the early detection of highly pathogenic H5N1 avian influenza A (HPAI H5N1) in Europe.[269] In North America, during the initial bird surveillance in 1999, 17,339 dead birds were reported of which 5,697 (33%) were crows; 295 of 671 (44%) dead birds tested were laboratory confirmed WNV positive, and of these 269 (89%) were crows. [213] A multi-state surveillance network was established to track the movement of the virus. [270] This surveillance programme consisted of enhanced passive reporting of human clinical cases, enhanced passive reporting of equine clinical cases, mosquito collection and testing, regular antibody testing of captive sentinel birds, and dead bird reporting and dead bird collection and testing.

Birds are reported to be reservoir hosts for a number of arboviruses and are therefore useful to environmental surveillance efforts. But for arboviruses like WNV that cause higher mortality in some bird species, dead bird surveillance can be a useful risk indicator.[271]

2.9 Prevention and Control

With regard to WNV, an important focus of primary prevention is environmental control of mosquito vectors. These measures include removal of any potential mosquito breeding sites, such as containers or old tires that collect standing water. Depending on regulations, ponds or water tanks can be stocked with mosquito fish (*Gambusia affinis*) that feed on mosquito larvae.[272] Local mosquito and vector-control agencies in the United states often provide the public with mosquito fish free of charge as well as consultation on mosquito-control issues.[272] These agencies are tasked with monitoring mosquito populations at the community level and with the control of larval and adult mosquitoes through the use of larvicides and pesticides, respectively. Mosquito avoidance and repellents are another line of defense against infection. By remaining indoors during dawn and dusk, humans and other animals will avoid exposure to *Culex* mosquitoes during their peak feeding periods. The use of fans and mosquito traps can also reduce mosquito exposure. Topical applications of insect repellents containing permethrin, pyrethrins, or butoxypolypropylene glycol are documented to be effective for repelling mosquitoes when used on dogs and horses. [273, 274] It is important to select a product that has been approved for use in the intended species. Products approved for topical use in humans, such as N, N-diethyl-meta-toluamide (DEET) or oil of lemon eucalyptus (p-menthane- 3,8 diol) were reported not be used on other animals because of the potential for toxic effects. [274]

At the primary level of prevention, a range of personal protective measures are available, including the use of bed nets, insect repellents, as well as mosquito traps and other devices. The effectiveness of personal protective measures to prevent mosquito bites has been reviewed. [275, 276] Insect repellents containing N, N-diethyl-3-methylbenzamide(DEET),-(2-hydroxyethyl)-1-piperidinecarboxylicacid1 methylpropyl ester (icaridin), and p-methane 3, 8-diol (PMD) have all been demonstrated, in laboratory and field testing, to prevent bites from a range of mosquito

species. These products have been shown to have minimal adverse health effects on humans.[277] Even though these products can prevent mosquito bites, a recent study from North America indicated that repellent use was not a significant factor influencing WNV risk. [278] This result contrasts with earlier studies that indicated a positive relationship between insect repellent use and lower WNV seroprevalence.[279] However there is strong evidence that repellents do prevent mosquito bites provides sufficient support that personal protective measures should be encouraged, to assist in reducing the risks of WNV [24] West Nile virus prevention relies in part on methods to reduce the numbers of West Nile virus–infected mosquitoes. Community-based mosquito control programs using integrated pest management principles were reported to proactively identify the sources of vector mosquitoes and use several methods such as elimination of breeding sites, larviciding, and targeted adult mosquito control to prevent adult mosquito populations from achieving levels that increase human infection risk. [280] When increasing human case incidence or surveillance of vector, mosquito populations indicate an impending human epidemic, the immediate goal is to reduce rapidly the number of infected adult mosquitoes by widespread ultra-low volume application of organophosphate or synthetic pyrethroid insecticides. [280] Although the efficacy of these control measures is difficult to measure, strategically timed early-season control of adult mosquitoes in the Coachella Valley of California using ultra-low volume insecticide applications was documented to decrease subsequent West Nile virus transmission.[281] In addition, aerial ultra-low volume insecticide application decreased infected mosquito abundance and reduced human-case incidence during a West Nile virus outbreak in the Sacramento area.[282] Human health risks associated with ultra-low volume organophosphate or synthetic pyrethroid use appear negligible, largely because the timing of application and low volume of pesticide used result in minimal human exposure.[283, 284]

2.9.1 Treatment

Treatment of West Nile virus infection remains supportive. It is a level of secondary prevention to reduce the impact of WNV infected persons. Several investigated therapeutic approaches include immune γ-globulin, West Nile virus–specific neutralizing monoclonal antibodies, corticosteroids, ribavirin, interferon α-2b, and antisense oligomers.[285, 286] In India, clinically overt cases, specific methods are not available for the treatment of WNV infection. However, as a tertiary level of prevention in encephalitis cases, supportive therapy was recommended.[108] In India, since no studies have been carried out for the control of WNV, the strategies recommended for control of *Culex* mosquitoes. The integrated vector control strategies include the use of personal protection measures like protective clothing, bed nets, both chemical and neem based repellants, insecticides, insecticide impregnated curtains, and biological control methods by larvivourus fish, introducing natural parasites and predators and bacterial agents.[287]

2.9.2 Vaccination

In Europe, a few human vaccines against *flaviviruses* are available, specifically those against Tick-borne encephalitis Virus (TBEV), Japanese Encephalitis Virus (JEV), and Yellow Fever Virus (YFV). [288] it is noteworthy that no human vaccine against WNV has yet been developed. [289] WNV can induce severe neurological symptoms in older or immunosuppressed patients, which renders vaccine development more challenging since the safe and efficient introduction of vaccinal antigens into weakened immune systems requires innovative strategies. [290] In the case of WNV, only veterinary vaccines are currently used in Europe and have proven to be very effective in protecting horses from meningoencephalitis. Outside of Europe, WNV vaccination programs have been implemented to protect highly susceptible domestic bird species, e.g., geese [291] and endangered bird species [292] or inactivated and canarypox recombinant vaccines

commercialized for horses used in scrub-jays (*Aphelocoma insularis*).[293] DNA vaccines have been also used to experimentally reduce WNV viraemia in bird reservoir hosts, such as crows (*Corvus brachyrhynchos and ossifragus*) [294] and American robins (*Turdus migratorius*) [295], nonetheless, birds have never been vaccinated against WNV, nor against other flaviviruses, in Europe.

Two vaccines are available for vaccinating equines: an inactivated WNV vaccine and a recombinant vaccine that uses canary pox virus to express WNV antigens. [296, 297] An inactivated vaccine is also being studied for use in humans. [298] A chimeric live virus vaccine incorporating the genetic sequences for E and prM antigens into a 17-D yellow fever virus backbone has been shown to be efficacious in hamsters and is undergoing initial clinical trials in humans. [298]Another chimeric vaccine incorporating WNV genetic sequences into a backbone of attenuated serotype-4 dengue virus, induced protective immunity in monkeys.[296] A DNA vaccine that elicits expression of WNV E and prM antigens has been tested in mice, horses, and birds. [296] Vaccination of crows with Kunjin virus, a subtype of WNV, protected against WNV, and a DNA vector, which elicited expression of attenuated Kunjin virus, provided protective immunity against WNV in mice. [298, 299] Preliminary analysis was suggested that universal West Nile virus vaccine coverage would not be cost-effective; [300] however, the cost-effectiveness of vaccination of specific target groups such as elderly individuals has not yet been established. Because humans are dead-end hosts, a human vaccination program is thought not influence viral amplification in nature.[280]

2.10 Knowledge on Mosquito-borne Diseases

Mosquito-borne diseases are having high capacity to transmit pathogens to humans and constitute an important cause of morbidity and mortality. Mosquitoes are not only a nuisance, but are also responsible for the spread of a wide range of diseases including Malaria, Yellow fever, Dengue, Chikungunya, West Nile virus, Japanese encephalitis, Lymphatic filariasis and Rift Valley fever. The mosquito borne diseases of public health importance are complex and their occurrence depends on the interaction of various biological, ecological, social and economic factors. Mosquito-borne diseases had always and also will remain as an important public health problem. Each year, more than one billion people are infected and more than one million die from a mosquito-borne disease.[301]

Knowledge of mosquito borne diseases therefore differ across countries and continents. Study carried out in south western Virginia found the general knowledge at a low level of awareness on mosquito-borne diseases, but found that knowledge of WNV was highest than Malaria and other mosquito-borne disease.[302] Study on the Predictors of knowledge of selected mosquito-borne diseases among adults of selected peri-urban areas of Puducherry reported 57.4% level of knowledge on malaria, Dengue fever (19%), Chikungunya (53%),filarial (33%) among other diseases.[303] Malaria also was reported with the highest knowledge in Karnataka State, India and in Tanzania.[304, 305] Malaria causes the most number of deaths among the vector-borne diseases. WHO estimated that there were 627,000 deaths and 207 million cases in 2012, mainly in Sub-Saharan Africa followed by South East Asia. .[306] Findings from a study showed that majority 171(69.23%) knew that malaria is transmitted by mosquito followed by 68 (27.53%) Filarial and 08(3.24%) did not knew any diseases that are transmitted by mosquito.[307] A household survey

in Yemen reported good knowledge of Dengue, the could be because of the increasing incidence of dengue outbreaks in Yemen.[308] Malaria remains one of the major causes of morbidity and mortality in Sub-Saharan Africa because the environment favours the multiplication and sustenance of the parasite causing the disease. Malaria, a vector borne disease, transmitted by anopheles mosquito is an endemic disease in Nigeria.[309, 310] where 80-90% have the knowledge of the cause of malaria.

In Nigeria where malaria is highly endemic; most cases of febrile illnesses are likely to be treated as presumptive malaria. [311] Arboviruses are widespread in Nigeria considering that the mosquito vectors responsible for the transmission of dengue, yellow fever, WNV, chikungunya (*Aedes spp*) and those responsible for malaria (*Plasmodium spp*) are well established in this country. In a household survey in Ottawa, 77% are aware of WNV, while 41.7% feel its not an important health issue, [312] this illustrate the need to educate the general public about WNV. Similarly, in a household survey in New York, all the respondents have heard of WNV and most knew that people (92%) or animals (91%) could be infected with the virus, with birds as the most cited animal (61%).[313] In Nigeria, knowledge. and experience of general practitioners, pediatricians and other specialists in regarding the nature and management of arbovirus diseases seem inadequate.[32]

2.11 West Nile Virus in Nigeria

A survey for West Nile Virus (WNV) Hemagglutination-inhibition (HI) antibody was carried out in humans and domestic animals. Human sera were collected from Ibadan, while the animal sera were collected from both Ibadan and Maiduguri. Out of 304 human sera tested, 123 were positive (40%).[35] There was a higher prevalence of HI antibody in adults than children. Sex distribution of positive sera showed that 37% of males and 43% of females had WNV HI

antibody.[35] A study was designed to determine the peak season for WNV activity in a semi-arid zone of Nigeria. A total of 973 sera from patients with febrile illness suspected of malaria/typhoid fever were used for the study. The sera were assessed for IgM and IgG antibodies to West Nile Virus (WNV) and Yellow Fever Virus (YFV) by IgM capture ELISA (MAC-ELISA).[32] The IgM positive sera for WNV were also tested by Plaque Reduction Neutralization Test (PRNT) and RT-PCR. Twelve (1.2%) of the 973 sera tested had WNV IgM antibodies.[32] The prevalence of these antibodies during the cold harmattan period (IgM-2.9%) was reported to be significantly different from the dry (IgM-0.0%) and rainy season (0.1%). Overall, PRNT and ELISA results were in concordance. The peak of WNV activity in the semi-arid zone in Nigeria appeared to be November. A significant number of the patients tested (80.2%) had WNV IgG and the prevalence of WNV IgG was significantly different according to seasons.[32] In another report of WNV infection among humans in semiarid northeastern Nigeria, blood samples were tested from humans in Maiduguri, Borno State, during the rainy (July to September) and dry harmattan (October to March) seasons and obtained a WNV seroprevalence level of 25%.[33] A survey was carried out for West Nile Complement Fixing (CF) antibody in humans and domestic animals in Nigeria. Human sera were obtained from two communities namely Ibadan and Ogbomosho, but animal sera were collected from Ibadan and Maiduguri.[314] The overall CF antibody to West Nile virus in the two localities surveyed was 65%. Of 170 persons tested, 53% and 75% were positive in Ibadan and Ogbomosho respectively.[314] Antibody prevalence increased with age in both communities. Tests for antibody against other flaviviruses revealed that monotypic complement fixation reactions were found frequently in young people, but broadly reacting sera were common among the older age groups. Sex distribution of West Nile CF antibody showed that 49/82 (60%) of females and 62/88 (75%) of males had West Nile CF antibody.[314] Tests on animal sera showed that 33% contained CF antibody to West Nile

virus. Prevalence of CF antibody in different animal species was 62% in camels, 4% in cattle and 0% in goats.[314] Furthermore, total of 200 animal sera were examined, 50 camels, 50 goats, 49 cattle and 51 sheep. The highest prevalence of HI antibody was found in camels (26%), followed by sheep (20%).[35] Percentage of positive sera in other species were: goat (18%) and cattle (6%).[35] High seroprevalence of WNV antibodies of 90.8% was reported in horses in south western Nigeria.[30] An analysis of *Culex* and *Aedes* mosquitoes in southwestern Nigeria revealed no West Nile virus activity. The surveillance study was conducted to investigate WNV infection in mosquitoes in the rainforest ecological zone of south western Nigeria. It was noticeable that greater proportion of *Culex* mosquitoes which is the maintenance/bridge vector in enzootic or epidemic cycle of WNV than *Aedes* were collected.[315] Greater proportion of *Culex* mosquitoes were collected in Sudan/Sahel savannah of Maiduguri and rain forest of Ibadan.[32] Sequence analysis of mosquitoes 28S rRNA showed the consensus sequence as belonging to *Culex Quinquefasciatus.* [315] In a recent study in Kaduna metropolis, out of the 420 avian sera tested, a seroprevalence of 42.9%, 10.6%, 5.3%, 4.7% and 0.7% were detected in pigeons, local chickens, guinea fowl, layers and broilers respectively. In the same study , cockerel, turkey, and duck had no detectable WNV antibodies, and all the mosquitoes tested were all negative for WNV.[20]

CHAPTER THREE- METHODOLOGY

3.1 Study Area

The study was carried out in Bauchi metropolis which is the capital and the largest city in Bauchi state, North-east Nigeria. Bauchi State lies between latitude 09^{o} 52^{I} and 09^{o} 86^{I} North of the Equator and longitude 10^{o} 45^{I} and 10^{o} 45^{I} East of the Prime Meridian Bauchi city was formerly called Yakoba, and is located on the northeastern edge of the Jos plateau. The town covers a total land area of 3,687 square kilometers, and a population of 493,810, according to the 2006 National population census (NPC,2006). It is the most populous part of the state, and it serves as the seat of the state government. Housing nature in Bauchi metropolis are usually extended family compounds, with government estates and lowcost housing situated outside the center of the metropolis. The Sudan savannah type of vegetation covers the southern part of state were Bauchi metropolis is located. Here, the vegetation gets richer towards the south, but generally the vegetation is less uniform and grasses are shorter than what grows even further south, that is, in the forest zone of the middle belt. The vegetation type as described above is conditioned by the climatic factors, which in turn determine the amount of rainfall received in the area. The increase in population, coupled with the socioeconomic activities in the metropolis results in keeping large number of pigeons in households either for economic purposes or as a hobby. Like in other parts of Nigeria, domesticated pigeons are reared together with other species of birds in household. The Veterinary services in the metropolis and beyond is provided by the Area Veterinary Clinic at Kofar Ran in Bauchi metropolis which is the major state owned Clinic, with National Veterinary Research institute (NVRI) Diagnostic laboratory and extension services, and the College of Agriculture Veterinary Clinic.

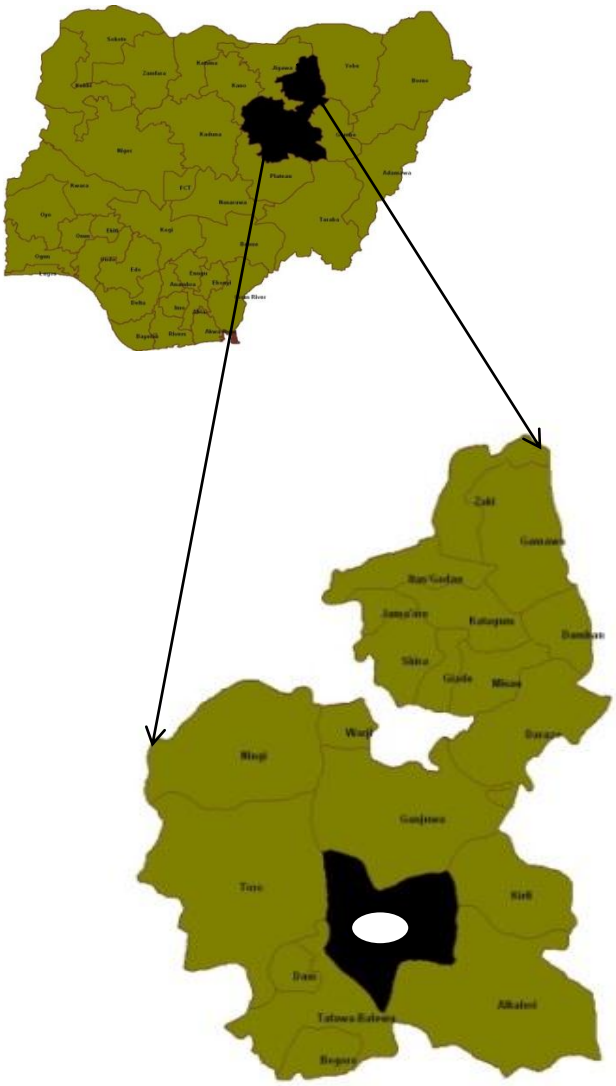


Figure 3.1: Map of Nigeria highlighting Bauchi State/Map of Bauchi State highlighting Bauchi Metropolis.

(Source: Designed using Health Mapper software)

3.2 Study Design

A cross-sectional study design was employed

3.3 Duration of the Study

The study period covered twenty months from May 2015 to December 2016. (Appendix 5)

3.4 Study Population

List of households rearing pigeons within Bauchi metropolis was drawn from the Area Veterinary clinic register, where records of households presenting pigeons to the clinic were documented. Live bird markets (LBMs) were visited to list out all the sources of pigeons in the market. Enumeration of households rearing pigeons within Bauchi metropolis was also done. This was done so as to have a sample frame to be used for the study. A total of 156 households were however enumerated and sampled.

3.4.1 Inclusion criteria

- All households within the sample frame rearing pigeons.
- Pigeons kept over the night in their housing prior to selection and sampling.

3.5 Sample size determination

Sample size was calculated from the formula for cross sectional survey.[316]

$$n=\frac{Z^2 1\text{-}\alpha/2 pq}{d^2}$$

Where;

N= Sample size,

Z= Standard deviation at 95% confidence interval that is, 1.96,

p = Prevalence of WNV infection in pigeons from LBM 42.9% [20]

q = 1-p (Complementary probability)

d = 5% desired level of precision at 95% confidence interval.

Therefore, $n = \frac{1.96^2 \times 0.429 \times 0.571}{0.05 \times 0.05} = 376$

3.6 Sampling Technique

Households used for the study were selected using snow-ball sampling technique for the study. The total number of pigeons in each household was determined prior to sampling. The number of pigeons sampled per household was based on the probability proportional to size sampling. Selection of pigeons was done using systematic random sampling for blood sample collection in each household. Prior to sampling all the birds were placed in a cage, and were retrieved one at a time. One bird is skipped each time thereby collecting blood samples from alternate birds each bird sample and the subsequently skipped birds were released and not returned into the cage.

3.7 Study Instruments

1. Needles and syringes, sample bottles (Cryovials), hand gloves, cotton wool, marker, masking tape, face masks and cooler for storing samples.
2. A pretested structured interviewer-administered questionnaires for households rearing pigeons.

3.8 Data Collection Methods

Verbal informed consent from pigeon owners was requested prior to the start of the study. Sample collection was done at the early hours of the day for easy asses to the pigeons, as pigeon housings were usually opened during the day time. Blood samples were collected from randomly selected pigeons. 1.5 to 2 milliliters (ml) of blood was collected by qualified veterinary personnel from the wing (brachial) vein of the pigeons under very strict hygienic conditions using sterile syringes and needles into a five milliliter (ml) plain Cryovials, and was kept in slanted position on ice after labelling, and within a few hours transported to the laboratory. In the laboratory, blood samples after coagulation were centrifuged and sera were kept frozen at -20°C until use. Pigeon sera (376) were collected and taken to the Bacterial Zoonoses Laboratory, Faculty of Veterinary medicine, ABU Zaria for analysis

To collect information regarding the study, a questionnaire was developed by referring to relevant literature. Prior to the study, fifteen questionnaires were pre-tested with a small scale pilot study outside the sample frame in Bauchi metropolis. The questionnaire was revised for assessing content validity, appropriateness and question comprehensibility. A face-to-face interview was adopted. Four research assistants were recruited from the Area Veterinary Clinic, Bauchi. They were trained Veterinary technicians and assistants who take blood samples in diverse species of animals as part of their routine duty in the Veterinary clinic. Prior to the start of the study, a one-day training was delivered to the research assistants in the local language of the respondents (Hausa) using a translated template so as to avoid giving different interpretations to the questions.

The socio-demographic information collected in the questionnaire included: age, gender, marital status, and educational attainment, occupation of the respondents, number of people in the

household and number of rooms in the house. In addition to the socio-demographic data, the questionnaire included: ten questions that explored history related to keeping of pigeons (Ownership of pigeons, reasons for keeping pigeons, number of years keeping pigeons in the house, current number of pigeons, family member assisting in rearing the pigeons, other species of birds kept in the household, source of pigeons and the type of housing pigeons are kept). Eight questions focused on knowledge related to mosquito-borne disease within the households (Knowledge of the diseases spread by mosquito apart from malaria, diseases transmitted to pigeons by mosquitoes, diseases of pigeons transmitted to man by mosquitoes, disease spread by mosquito to pigeons, diseases that can spread to man from pigeons, diseases that can cause high mortality in pigeons, duration of mortality in pigeons and month of the year with noticed high mortality). Fourteen questions focused risk factors related to WNV within the households (Empty all containers around the household that can hold water, how often are the containers emptied, empty containers around the neighbourhood, how often are they emptied around the neighbourhood, blocked stagnant water that is not flowing, duration of change of pigeons drinking water, how dead pigeons are disposed, how dead pigeons are handled, number of hours spent outdoor, or in the house premises at night, having mosquito nets in doors and windows, family members wearing shoes, socks, trousers, long sleeve shirts whenever outdoor or within house premises at night, family members regularly using mosquito repellents at night when outdoor or house premises, The last section of the questionnaire was a checklist observing some of the risk factors (Seen plastic containers ceramic pots, or similar water holding containers that have accumulated on properties, mosquitoes seen around the containers, discarded tires seen that have accumulated water on the properties, seen drainage holds that are located outside of the house collecting enough water for mosquitoes to breed, seen mosquitoes in the pigeon housing, and drinkers).(Appendix 1)

3.9 Data Management

3.9.1 Measurement of Variables

All results were presented in tables for the: Sociodemographic characteristics of pigeons owners in households in Bauchi metropolis, practices of pigeons reared in households of Bauchi metropolis, seroprevalence of WNV antibodies in pigeons reared in households of Bauchi metropolis, responses to risk factors related to WNV within households of Bauchi metropolis, bivariate analysis of the risk factors associated with WNV antibodies in households of Bauchi metropolis and knowledge related to mosquito-Borne diseases within households in Bauchi metropolis.

3.9.2 Statistical Analysis

ID Screen® West Nile Competition Multi-species kit for the detection of West Nile Virus anti-pr-E antibodies in horses and an avian serum by competitive ELISA was used.

3.9.2.1 Testing Procedure for Competitive ELISA (c-ELISA)

The test was carried out using 96 well microtiter plate c-ELISA in accordance to the manufacturer's instruction. All the reagents were allowed to come to room temperature (21^{o}C ± 5^{o}C) before use. All reagents were harmonized by inversion or vortex. The following steps were used:[317]

1. 50 µl of Dilution Buffer 2 (A complex protein solution confidential solution) was added to each micro well
2. 50 µl of the Positive control (dilution of a positive horse serum in a stabilizing buffer) was added to wells A1 and B1,which contain a purified WNV anti-pr-E antibody (Standard)

3. 50 µl of the Negative control (dilution of a negative horse serum in a stabilizing buffer) was added to well C1 and D1
4. 50 µl of each sample to be tested (Pigeon serum samples collected from households) was added in the remaining wells
5. Micro titer plates were incubated for 90 minutes ± 6 minutes at 21^{o}C (± 5^{o}C)
6. The wells were emptied and each well washed 3 times with approximately 300 µl of the wash solution (0.3M, NaCl, 0.05M KCl, 0. 13M,Na_2 $HPO_{4,}$0.03M $NaH_2PO_{4,}$ Tween 20 0.005%, pH 7.2). Drying of wells was avoided between washings.
7. Conjugate 1X (anti-pr-antibody peroxidase, horse radish peroxidase in PBS with 10% fetal bovine serum) was prepared by diluting the concentrated conjugates 10X to 1/10 in dilution buffer 2.
8. 100 µl of the conjugate 1X was added to each micro well
9. Microtiter plate were incubated for 30 minutes ± 3minutes at 21^{o}C (± 5^{o}C)
10. The micro wells were emptied and each well washed 3 times with approximately 300 µl of the wash solution. Drying of the micro wells between washings was avoided
11. 100 µl of substrate solution (Tetramethylbenzidine (TMB)) was added to each micro well.
12. Micro titer plates were again incubated for another 15 minutes ± 2 minutes at 21^{o}C (± 5^{o}C) in the dark
13. 100 µl of the stop solution (Sulphuric acid solution 0.5M) was added to each well in order to stop the reaction
14. The results were read and recorded using the ELISA reader at an O.D (optimal density) of 450 nm.

3.9.2.2 Validation

The test was validated when:

- The mean value of the Negative control O.D (OD_{NC}) is greater than 0.700

  $OD_{NC} > 0.700$

- The mean value of the Positive control (OD_{PC}) is less than 30% of the OD_{NC}

  $OD_{FC}/OD_{NC} < 0.3$

3.9.2.3 Interpretation

For each sample, the S/N percentage (S/N%) was calculated:

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

Sample presenting a S/N%:

- Less than or equal to 40% were considered positive
- Less than or equal to 50% and greater than 40% were considered doubtful
- Greater than 50% were considered negative

3.9.3 Questionnaire Analysis

Prevalence of WNV antibodies were calculated using the formular outlined by below. [318]

$$\frac{\text{Number of positive antibodies}}{\text{Total number of serum samples examined}} \times 100\ \%$$

Questionnaires were entered into Epi info 7.1.3.10 and analysed. Frequencies, proportions, and bivariate analysis were done to determine association risk factors. Knowledge response was scored as ‘yes’ or ‘no’. The scoring range of the questionnaire was 1 (maximum) to 0 (minimum). Each Knowledge question was assessed by giving 1 point to a correct or positive

answer and 0 points to incorrect or negative answers. Each question had only one correct or positive answer. Knowledge scores for individuals were calculated and summed up to give the total knowledge score. The knowledge scores of each respondent were classified into poor, fair, and good. Four correctly answered questions were scored as good knowledge, three correctly answered questions were scored as fair knowledge, while two or less correctly answered questions were scored as poor.

Descriptive analysis was carried out to illustrate socio-demographic characteristic of respondents. Categorical variables were measured as percentages and continuous variables were expressed as Mean ± Standard Deviation (SD).Univariate analysis and bivariate analysis was carried out to determine the risk factors. Risk factors were interpreted using odd ratios (OR) and 95% confidence interval (95% CI)

3.10 Ethical Considerations

Ethical clearance was approved by the Veterinary Department, Ministry of Animal Resources and Nomadic Resettlement, Bauchi state. Verbal informed consent from each respondent was requested before the start of the study. Respondents were free to decline to participate. Respondents were also assured of the non-harmfulness of the study on them or their pigeons. The safety of the respondents and the research assistants was of priority, and where this could not be guaranteed, the interview did not take place No names were requested on the questionnaire as the questionnaire numbers were used as identity. All questionnaires were kept under protective custody. The computer used for data entry was password-protected. Confidentiality was maintained throughout and after the study.

3.11 Limitations

The study may not be a true representation of the WNV in the study area, due to the short duration of the study, the study covered only one season, and the use of non-probability sampling to select households. The ELISA test could not establish whether the infection is recent or due to previous exposure of the pigeons to the virus. The ELISA kit might have detected cross reacting antibodies possibly due to Japanese Encephalitis and other tick-borne encephalitis viruses, that have not been previously studied in the study area. More sensitive and specific tests like Plague Reduction Neutralisation Test (PRNT) could have been able to detect more positive pigeons than the ELISA used in this study.

CHAPTER FOUR- RESULTS

A total of 156 households were enumerated for this survey. Total sera collected from the household-reared pigeons were 374 and analysed. Pre- tested questionnaires administered and analysed was 156 that is one questionnaire per household. Using competitive ELISA kit 13 of the 376 sera was positive for WNV antibodies. A seroprevalence of 3.5% was found from the study. From the questionnaire analysis, 96.2% of the respondents were male, and the age range of respondents was between 13 to 54 years. Respondents have practical experience keeping pigeons for an average of 1 to 5 years. Having blocked or stagnant gutter that is not flowing was reported in 5.8% of respondents, and discarded tires that have accumulated water on their properties was reported among 7% respondents. Knowledge of respondents on mosquito borne diseases was only 0.6%.

Table 4.1: Socio-Demographic characteristics of the pigeon owners in households of Bauchi metropolis (N=156)

Variables	Category	Number of Respondents n=156	Proportion within Each Category (%)
Age groups	≤14	07	4.5
	15-24	74	47.4
	25-34	48	30.8
	35-44	21	13.5
	≥45	06	3.9
Gender	Male	150	96.2
	Female	06	3.9
Educational level	Secondary	97	62.2
	Tertiary	46	29.5
	Koranic	07	4.5
	Primary	06	3.8
	No formal education	0	0
Occupation	Artisan	79	50.6
	Others (Student, Retirees)	30	19.2
	Trader	24	15.4
	Civil servant	22	14.1
	Farmer	1	0.6

The socio-demographic characteristics of the 156 participants interviewed in Bauchi metropolis are shown in the table above. Most of the respondents were males (96.2%) and with most of them (62.2%) having a secondary education and are mostly artisans (mechanics, welders, carpenters, tailors) (50.6%). The mean age of the respondents was 25.1 (SD ± 9.2) years (range 13 to 54 years) with the great majority of participants (47%) being between 14 and 24 years old.

Table 4.2: Practice of pigeon keeping in households of Bauchi metropolis (N=156)

Variable	Category	Proportion in each Category (%)
Owner of pigeons in the house	Self	124 (79)
	The entire family	21 (13.5)
	A relation	07 (4.5)
	Others	04 (2.6)
Reason for rearing pigeons in the house	Hobby	96 (34.3)
	Food	93 (33.2)
	Income	91 (32.5)
Duration (in years) of keeping/rearing pigeons in the household	1 -5	72 (46.2)
	6-10	27 (17.3)
	11-15	27 (17.3)
	16-20	17 (11)
	21-25	02 (1.3)
	≥26	11 (7)
Source of the pigeons	Neighbours	79 (37.7)
	Friends/acquaintances	70 (33.5)
	Live bird markets	50 (23.9)
	Others	10 (4.8)
Housing system for the pigeons	Cages	100 (55)
	Cartons/wooden nests fixed on house walls	39 (22)
	Pigeon lofts	38 (21)
	Large scale confined/ Indoor husbandry	03 (1.7)
	House ceiling	01(0.6)
	Scavenging /free range	0 (0)

Seventy two respondents had experience of keeping pigeons in their households for an average of 1 to 5 years. Beside pigeons, 67.9% kept mostly chickens among other species of birds (ducks, guinea fowls, turkeys, geese, parrots, canary). Majority of respondents sourced their pigeons from neighbours (37.7%) and house them in cages (55%).(Table 4.2)

Table 4.3: Seroprevalence of West Nile Virus antibodies in pigeons within household locations in Bauchi metropolis (N=156)

Location(Areas)	Number of Serum samples tested	No of positive samples	Percentage (%)
Gwabba	32	0	0
Illelah	6	0	0
Gwallaga	67	3	4.5
Tura	7	1	14.3
Nasarawa	17	0	0
Emir's palace	19	0	0
Gwan Gwan Gwan	15	0	0
Karofi	11	0	0
Bakin kura	14	1	0.07
Kobi	19	0	0
Nufawa	19	0	0
Bayan Bata	16	0	0
Unguwan Ganjuwa	34	4	11.8
Jahun	20	0	0
Unguwan Bauchi	15	0	0
Unguwan Borno	23	1	4.3
Unguwan Jaki	8	0	0
Unguwan Gado	10	1	10
Kofar Dumi	8	0	0
Unguwan Jaja	16	2	12.5
	376	**13**	**3.5**

Total households enumerated for the study were 156 and a total of 376 pigeon serum samples were collected and tested for WNV antibodies. Thirteen of the pigeon sera were positive for WNV antibodies (3.5%), seropositivity in the different household locations were 4.5% (3/67) in Unguwan Gwallaga, 11.8% (4/34) at Unguwan Ganjuwa, 12.5% (2/16) at Jaja. Tura had 14.3% (1/7) positive sera, 10% (1/10) at Unguwan gado, 0.07% (1/14) at Bakin kura and 4.3% (1/23) at Unguwan Borno areas. (Table 4.3)

Table 4.4: Risk factors related to West Nile Virus within the households of Bauchi metropolis (N=156)

Variables	Frequency (%)	
	Yes	No
Empty all containers around the house that can hold water	77 (49.6)	79 (50.4)
Having blocked or stagnant gutters that is not flowing	09 (5.8)	147 (94.2)
Having mosquito nets at the windows and doors of the house	115 (73.7)	41 (26.3)
Family regularly use mosquito repellent at night, when it is necessary to be outdoors within house premises	21 (13.5)	135 (86.5)
Having any mosquito control measure outdoor in the house premises	20 (12.8)	136 (87.2)
Plastic containers, ceramic pots or similar water holding containers that have accumulated on properties	66 (42.3)	90 (57.7)
Presence of discarded tires that may have accumulated on the property	11 (7.1)	145(92.9)
Presence of drainage holes that are located on the outside of the house collecting enough water for mosquitoes to breed	93 (60)	63 (40)
Noticed mosquitoes in the pigeon housing (Cages, Carton, lofts)	27 (17.3)	129 (82.7)
Noticed mosquitoes at the pigeon drinkers	25 (16)	131 (84)
Family members wear shoes socks trousers and long sleeves when out door at night	07 (4.5)	149 (95.5)

As to the risk factors related to WNV in households, 49.4% of respondents empty all containers around their houses that can hold water, fortunately only 5.8% had blocked or stagnant gutters that is not flowing . Respondents with mosquito nets on the windows and doors of their houses were 73.7%, 12% responded have undertaken mosquito control measures outdoor in the house premises, and only 7% had discarded tires that accumulated on their property and majority had drainage holes that were located on the outside of the house collecting enough water for mosquitoes to breed.(Table 4.4)

Table 4. 5: Bivariate analysis on the risk factors associated with West Nile Virus antibodies in households in Bauchi metropolis

Variables	OR	(95% CI)
Not having blocked or stagnant gutter that is not flowing	0.69	(0.21-2.29)
Having plastic containers, ceramic pots or similar water holding containers that have accumulated on properties	1.40	(0.43-4.55)
Having mosquito nets at the windows and doors of the house	0.46	(0.14-1.56)
Less mosquitoes in the pigeon housing	0.95	(0.20-4.61)
Presence drainage holes that are located on the outside of the house collecting enough water for mosquitoes to breed	2.14	(0.56-8.25)
Sourcing pigeons from LBM	2.3	0.69-7.44

From the bivariate analysis of the risk factors shown in the above table, having blocked or stagnant gutter that was not flowing in the household had an OR= 0.69; 95% CI (0.21-2.29); having mosquito nets at the windows and doors of the houses (OR=0.46; 95% CI (0.14-1.56) and having drainage holes located on the outside of the house collecting enough water for mosquitoes to breed (OR=2.14; CI ((0.56-8.25). Sourcing pigeons from the LBMs had an OR=2.3; 95% CI (0.69-7.44).(Table 4.5)

Table 4.6: Knowledge of respondents related to Mosquito-Borne diseases within the household in Bauchi metropolis (N=156)

Variable	Frequency (%)	
	Yes	No
Diseases spread by mosquitoes apart from malaria	1 (0.6)	155 (99.4)
Diseases of pigeons transmitted by mosquito	0 (0	156 (100)
Diseases of pigeons transmitted by mosquitoes to man	0 (0)	156 (100)
Diseases that can spread to you from birds like pigeons	0 (0)	156 (100)
Disease that can cause high mortality in pigeons	68 (43.6)	88 (56.4)

The positive rate of responses to knowledge questions concerning mosquito-borne diseases in households is shown in the table above. Knowledge response was scored as 'yes' or 'no'. The scoring range of the questionnaire was 1 (maximum) to 0 (minimum). Knowledge scores for individuals were calculated and summed up to give the total knowledge score. Among the 156 respondents, only 0.6% had knowledge of mosquito-borne diseases apart from malaria, and 43.6% of respondents were aware of diseases that can cause high mortality in pigeons.

None of the respondents have heard of diseases of pigeons transmitted by mosquitoes, diseases of pigeons transmitted by mosquitoes to man, diseases spread by mosquitoes to pigeons, and diseases that can spread to man from birds like pigeons.(Table 4.6)

CHAPTER FIVE-DISCUSSION

This serologic study was conducted in pigeons in Bauchi metropolis. Overall seroprevalence of WNV in the pigeons tested was 3.5%, that is only 13 of the 376 sera tested positive by c-ELISA for WNV envelop protein (E). This is consistent to what was reported among *Columbiformes* sampled in free ranging sites of Chaco, Córdoba, and Tucumán Provinces, Argentina, 2004–2006,[202] and in an outbreak of WNV in a new York city captive, wildlife population.[198] Using indirect ELISA, a lower seroprevalence of 2.5% was repoted in Rock doves.[209] However, this study is not in agreement with studies documenting high prevalence of WNV antibodies in *Colimbiformes*.[197, 198, 203, 208] In a study on serologic evidence for West Nile Virus infection in birds during an Outbreak in 1999 reported a high prevalence was documented in Rock doves *(Columbiformes)*.[319] The differences between these rates is likely because of sampling locations as the pigeons in this study were domesticated in the households, also that some of the studies were conducted during outbreaks. There were seven locations within the metropolis (Gwallaga, Tura, Bakin kura, Unguwan Ganjuwa, Unguwan Borno, Unguwan gado and Unguwan Jaja) out of the twenty that were positive for WNV antibodies.Though there is a close proximity between Unguwan ganjuwa and Jaja areas, the other locations were farther from each other. The reason why other pigeons tested negative in some sampled location is not clear, as practice of keeping pigeons in all household locatons appeared to be largely similar. It is suggested that age difference of the sampled pigeons, introducing new pigeons to the old flock, and the length of stay of the newly acquired pigeons could be factors for the seropositivity. Type of pigeon rearing could also be a factor, as some pigeons fly further away from the households during the day, and return late in the evening back in the cages, cartons or lofts. This might also contribute to the seropositivity in the different locations at the period of the study. The low seroprevalence could likely be as a result

of fewer mosquito bites on the pigeons that might cause high viraemia for transmission. Pigeons and possibly other species of birds that are domesticated are likely carriers of low level WNV antibodies. Though no outbreak of WNV has been reported both in human, horses and other species of birds in Bauchi metropolis and Nigeria; it is a pointer to a future emerging epidemic if preventive and control measures are not put in place. Most households keeping pigeons in Bauchi metropolis reported keeping other species of birds.

In this study, pigeons were sampled from households in the months of November 2015 to January 2016. Low mosquitoes population density was reported in the months of November and December in Calabar, due to the dry climatic condition and season of the year.[320] A study also found lower WNV seroprevalence in birds sampled in the cooler mountainous regions of Georgia versus nearby warmer, low-elevation sites. [201] The percent seropositivity in this study is lower than the seroprevalence of 49.2% reported in pigeons at LBMs in Kaduna metropolis,Nigeria [20] Findings from this study also showed that pigeons sourced from the live bird markets by household owners were more likely to have WNV antibodies than those from other sources (OR=2.3; 95% CL (0.69-7.44). Conditions of LBMs in Nigeria is reported to provide a favourable environment for the spread of diseases,because of the diverse domesticated and wild species of birds of different ages kept together over a long period of time.[20]

Though majority of the respondents had practiced the rearing of pigeons for a at least 1 to 5 years, others have reared them for more than two decades,which is mostly seen as a hobby, a source of food and for income generation. This is similar to findings in Bangladesh were it was documented to be a hobby, and source of alleviating poverty.[321] Besides pigeons, respondents admitted rearing other species of birds, in which family members assist in rearing. Pigeons can be said to be second most commonly reared poultry in terms of protein production for the

families.[321] Pigeon lofts, cages, and cartons were reeported to be common form of housing systems in Bauchi metropolis, even though a repondents admitted housing pigeons in the house ceiling. Allowing pigeons to be housed in ceiling could serve as a health risk to households. Pigeon feathers and droppings may cause allergic respiratory diseases to humans. Allergic alveolitis is reported in 1-10% of pigeon breeders. [322]

Based on questionnaires analysed, the number of respondents that admitted to have emptied containers and those that have not emptied all containers around their households are almost equal, whereas most of the containers within the neighbourhoods were not emptied. Majority of respondents did not have specific times when the containers were emptied. This creates a potential breeding site for the mosquitoes as resident-based management of water filled containers has been an effective and affordable means of decreasing numbers of biting adult mosquitoes,[323] and it is recommended by the World Health Organization for control of urban vector species worldwide. [324]

Questions regarding blocked or stagnant gutters that were not flowing showed that most respondents (94.2%) in Bauchi metropolis reported not having blocked or stagnant gutters in their compounds at the time of the study. The bivariate analysis indicated that they were less likely to have WNV infection in those households (OR=0.6; 95% CI (0.21-2.29)). Notably, through observation, it was found that the number of discarded tires that may have accumulated on the property were lower. But drainage holes that were located outside of the house collecting enough water for mosquitoes to breed were higher in proportion as observed in many of the studied households. There was an increased likelihood (OR=2.14; 95% CI (0.56-8.25) of WNV transmission, as people living in close proximity to stagnant water sources where mosquitoes breed. Residing close to areas that are flooded regularly by irrigation canals was found to be a

risk factor. [169-171] Based on observations of the households from this study, plastic containers, ceramic pots or similar water holding containers that have accumulated on properties, and mosquitoes around the containers were seen to be low. Community-based mosquito control programs using integrated pest management principles were reported to proactively identify the sources of vector mosquitoes and used several methods such as elimination of breeding sites, larviciding, and targeted adult mosquito control to prevent adult mosquito populations from achieving levels that increase human infection risk. [280]

Interestingly in this study, a high rate of use of mosquito nets at the windows and doors was reported by the households. This agrees with the finding in the bivariate analysis that showed the use of nets at doors and windows against mosquito as protective against mosquito-borne diseases (OR=0.46; 95% CI (0.14-1.56)). Mosquito netting was one of the frequently used practice in regards to mosquito avoidance reported in north Western Australia.[325] From this study, very few respondents were found to use mosquito repellent at night, or when necessary to stay outdoors or within house premises. Family members wearing shoes, socks, trousers and long sleeve shirts when outdoor at night were reported to be very low. This is similar to what was reported in Kansas in 2003. [326] Those that used outdoor mosquito control measures reported using only mosquito coils, despite the climate differences with Bauchi and Nigeria, it is consistent with what was reported in north Western Australia, including the Kimberley and Pilbara regions. [325] These findings may be due to low knowledge of WNV and its preventive and control measures in Bauchi metropolis, and Nigeria as a whole. It has been reported that WNV infection is nonspecific and that it often escapes medical attention. [327] Knowledge and awareness are insufficient to give much impact as risk perceptions may also be a moderating factor.[326]

The present study revealed that majority of respondents stayed longer outdoor at night (≥3 hours). The risk for becoming infected can be influenced by time spent outdoors. This agrees with the findings among health-fair participants, in Wyoming.[172] It has been found that increased time spent outdoors was associated with WNV infection. [174] Age and gender of respondents could be a factor for staying longer outdoors at night, as most of the respondents who stayed longer outdoors were young (15 to 24) and are mostly male. This pattern had been reported in Western Australia.[325]

Low burden of mosquitoes within and around the pigeon housing (cages, cartons, pigeon lofts) as well as the pigeon drinkers was observed in the period of study (November 2015 to January 2016) . This may likely be due to the frequency at which pigeons' drinking water was changed (at least once a week) and also the climatic condition. Questionnaire analysis also revealed majority of respondents (98.7%) handled dead pigeons using bare hands. Although WNV is not transmitted by handling infected birds, it is always best to take this precaution when handling any dead animal.[328] The practice of disposing dead pigeons directly into the trash was found to be common. This could be a result of the poor knowledge of zoonotic infections carried by the dead birds. Dead birds are usually used as indicators during WNV sentinel surveillance. [251]

This study brought out certain important perceptions of the knowledge of the population on mosquito-borne diseases with emphasis on pigeons, the understanding of which is deemed vital. The study showed that majority of the people interviewed had poor knowledge of any disease caused by mosquitoes apart from malaria. Only one respondent mentioned fowl pox. It is known that mosquitoes are carriers of the Pox virus, leading to chicken or fowl pox disease in poultry. [329] Although majority of the respondents have reared pigeons for an average of 1 to 5 years, none of them was aware of any disease transmitted to pigeons by mosquitoes. Knowledge of any

disease transmitted from pigeon to man was however poor. None of the respondents knew that pigeons can spread any disease to humans. Majority of the respondents admitted knowing Newcastle disease and very few knew bird flu (Highly pathogenic avian influenza (HPAI)) as major causes of mortality in pigeons. This however is in agreement with literature reporting that mortality due to HPAI can be as high as 50 to 100%,[330] and 90% to 100% in Newcastle disease.[331] Most of the respondents reported mortality that lasted less than one week. Case fatality rate due to WNV in birds can be up to 70% .[198]

CHAPTER SIX- CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study, the seroprevalence of 3.5% was found for WNV anti-pr-E antibodies among pigeons kept within households of Bauchi metropolis. Metropolitan locations with positive samples were Unguwan Ganjuwa, Gwallaga, Tura, Unguwan gado, Jaja, Bakin Kura and Unguwan Borno.

Overall knowledge related to mosquito-borne diseases other than malaria in households the study area was poor.

From the study, associated risk factors to WNV in the households were having plastic containers, ceramic pots or similar water holding containers, presence of drainage holes that are located on the outside of the house collecting enough water for mosquito to breed and sourcing pigeons from LBM.

6.2 Recommendations

As an outcome of the present study, it is recommended that.

1. Bauchi State department of epidemiology and the veterinary epidemiology unit should improve surveillance by including WNV routine screening in humans, mammals and other avian species.
2. Veterinary personnel at the live bird markets in Bauchi metropolis should be involved in active surveillance on a routine bases as a way of checking sources and history of birds brought into the markets.

3. The Veterinary public health unit of the ministry of Agriculture Bauchi should promote health sensitization campaigns using jingles and fliers so as to improve knowledge of emerging and re-emerging mosquito-borne diseases among poultry farmers
4. Bauchi state veterinary service directorate should organize regular meetings with the pigeon sellers association to inform them on possible preventive measures to adopt so as to minimize transmission of zoonotic infections
5. Bauchi state veterinary directorate through the Area Veterinary offices in Bauchi should train the pigeon owners on the best housing methods for rearing pigeons far away from the households.
6. Veterinary services in Bauchi metropolis should be targeted to pigeon owners so as to enlighten them on possible mosquito-borne diseases transmitted by pigeons and other species of birds
7. Post graduate students working on WNV should use other serologic, molecular and virus isolation methods to test for WNV in pigeons and other avian species so as to learn more on the epidemiology of West Nile Virus infection in Nigeria

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APPENDICES

APPENDIX 1

Questionnaire on Knowledge and Risk Factors Associated with West Nile Virus Among Pigeon Keepers in Households at Bauchi Metropolis, Nigeria, 2016

Date: ..... Location……

Good day. I am carrying out a Study on Pigeons in Households to identify factors associated with West Nile virus in Pigeons within Bauchi Metropolis. Also I will be assessing risk factors related to West Nile virus among pigeon keepers within Bauchi Metropolis. The aim of the study is to determine the prevalence of the disease and risk factors that can lead to zoonotic infections within households as well as the general public. You are not compelled to participate, however we would be grateful for your participation. The interview will last about 15 minutes. All information supplied will be treated strictly as confidential.

SECTION A: SOCIO-DEMOGRAPHIC DATA

1. Age in years as at last birthday: _____
2. Sex (***do not ask, tick as appropriate***): ☐ Male ☐ Female
3. Marital status (***Tick whichever applies***)

   a) Married ☐

   b) Single (never married) ☐

   c) Divorced ☐

   d) Separated ☐

4. What is your highest educational attainment?

   a) No formal education ☐

   b) Primary ☐

   c) Secondary ☐

   d) Tertiary ☐

   e) Qur'anic ☐

5. What kind of work do you do?

   a) Trader

   b) Civil servant

   c) Artisan

   d) Farmer

   e) Others (specify)…….

6. How many people live in your household? ≤5 ☐6-10 ☐11-15 ☐≥16 ☐

7. How many rooms are in your house? ≤5 ☐-10 ☐11-15 ☐≥16 ☐

SECTION B. PRACTICE OF PIGEON KEEPING

1. Who owns the Pigeons you rear in the house?

   a) My self ☐

   b) My relation ☐

   c) For the entire family ☐

   d).Others (Specify)……..

2. Why do you rear pigeons in the house?

   a. For income ☐

   b. kept as a hobby ☐

   c. For Food ☐

   d. Others (specify)……

3. How long (in years) have you been keeping/rearing pigeons in the house?

   .....

4. How many pigeons do you currently have? .....

5. Do your family members assist you with the rearing of the pigeons? Yes ☐ No ☐

6. In addition to pigeons, what other species of birds do you raise in your House? (***Tick all appropriate species and their number***)

Bird species	Total number
a) Chicken	……
b) Ducks	……
c) Guinea fowl	……
d) Turkeys	…….
e) Geese	…….

f) Wild birds …….

g) Parrots ……

h) Canary ……..

i) Others (specify)…….

7. What is the source of your pigeons?

a. Live bird market

b. Neighbours

c .Friends/Acquaintance

d. Others (specify)……

8. What Housing system do you use for your pigeons?

a) Scavenging/free range production ☐

b) Carton fixed on House walls ☐

c) Large scale confined/indoor husbandry ☐

d) Cages ☐

e) Others (specify)……

SECTION C: <u>RISK FACTORS RELATED TO WEST NILE VIRUS WITHIN THE HOUSEHOLD</u>

1. Do you empty all containers around your house that can hold water?

Yes ☐ No ☐ ***(if No skip question 1i)***

1i.How often do you empty all containers around your house that can hold water?

a) Daily b)Twice weekly c) At least once a week d)No specific time

2. Do you empty all containers around your neighborhood that can hold water?

   Yes ☐ No ☐ ***(if No skip question 2i)***

   2i. How often do you empty all containers around your house that can hold water?

   a) Daily b) Twice weekly c) At least once a week d) No specific time

3. Do you have blocked or stagnant gutters that is not flowing? Yes ☐ No ☐
4. How long does it take you to change your pigeon's drinking water?

   a) Daily b) At least once a week c) Monthly d) No specific time

5. How do you dispose your dead pigeons?

   Throw it direct on the trash ☐ Take it to the veterinarian for test ☐ Bury it ☐

   Double bagging and placing it in the trash ☐

6. How do you handle dead pigeons?

   Using bare hands ☐ Using hand gloves ☐ Put hands in a polythene bag ☐

7. How many hours do you spend outdoor or in the house premises at night?

   ≤ 3hours ☐ ≥3hours ☐ ≥ 4 hours ☐ ≥5hours ☐

8. Do you have mosquito nets at the windows and doors of your house? Yes ☐ ☐
9. Do all your family members wear shoes, socks, trousers and a long-sleeved shirt when outdoors or within the house premises for long periods of time, especially at night?

   Yes ☐ No ☐

10. Does your family regularly use mosquito repellent at night, when it is necessary to be outdoors or within house premises? Yes ☐ No ☐

11. Do you have any mosquito control measure outdoor in the house premises?

Yes ☐ No ☐

If Yes,what are the control measures?.....

SECTION D: KNOWLEDGE RELATED TO MOSQUITO-BORNE DISEASES WITHIN THE HOUSEHOLD

Do you know diseases spread by mosquitoes apart from malaria?

Yes ☐ No ☐

If yes to question *1* list them:

……
……
……

Do you know diseases of pigeons transmitted by mosquito? Yes ☐ ☐

If yes to *question 2* list them

……

……

Do you know diseases of pigeons transmitted by mosquitoes to man? Yes ☐ ☐

If yes to *question 3* list them

……..

……..

Do you know diseases that can spread to you from birds like pigeons?

Yes ☐ No ☐

If yes to question 5 list them:

……..

……..

Do you know disease that can cause high mortality in pigeons?

Yes ☐ No ☐

If yes to *questions 6* list them

……

……

How long did the mortality in the pigeons take?

≤1 week ☐ 2 weeks ☐ 3 weeks ☐ ≥ 4 weeks ☐

At which month (s) of the year do you usually notice high mortality of your pigeons? .....

(*Question 9-15 should be observed by the interviewer and ticked*)

Are there plastic containers, ceramic pots or similar water holding containers that have accumulated on properties ?Yes ☐ No ☐

Are there mosquitoes around the containers? Yes ☐ ☐

Are there discarded tires that may have accumulated on your property

Yes ☐ No ☐

Are there drainage holes that are located on the outside of the house collecting enough water for mosquitoes to breed? Yes ☐ No ☐

Are there mosquitoes in the pigeon housing (Cages, Carton, lofts)

Yes ☐ No ☐

Are there mosquitoes at the pigeon drinkers?

Yes ☐ No ☐

Thank You

APPENDIX 2

Practice of pigeon keeping in households of Bauchi metropolis

Variable	Category	Proportion in each Category (%)
Number of pigeons currently present in the house	≤10	50 (32.1)
	11-30	67(43)
	31-60	28 (18)
	61-90	07 (4.5)
	≥91	04 (2.5)
Family members assist with rearing of the pigeons	Yes	127 (81.4)
	No	29 (18.6)
Other species of bird in the house	Chicken (local chicken, broiler, layers)	100 (64.2)
	Ducks	23 (14.7)
	Guinea fowl	03 (1.9)
	Turkey	24 (15.4)
	Geese	02 (1.3)
	Wild birds	0 (0)
	Parrots	02 (1.3)
	Canary	6 (3.8)

APPENDIX 3

Risk factors related to West Nile Virus within the Households of Bauchi metropolis

Variables	Category	Frequency (%)
Empty all containers around the house that can hold water	Daily	09 (14)
	Twice weekly	13 (20)
	Once a week	10 (15)
	No specific time	34 (51)
Empty all containers around the neighbourhoods that can hold water	Daily	02 (4)
	Twice weekly	16 (33)
	Once a week	14 (28)
	No specific time	17 (35)
How long it takes to change the pigeon's drinking water	Daily	29 (19)
	At least once a week	36 (23)
	Monthly	26 (16)
	No specific time	65 (42)
Number of hours spent outdoor or in the house premises at night	< 3hours	26(17)
	≥3hour	130(83)
	≥ 4 hours	0(0)
	≥5hours	0(0)
How dead pigeons are disposed	Throw it direct on the trash	148 (95)
	Take it to the veterinarian for test	03 (2)
	Bury it	0 (0)
	Double bagging and placing it in the trash	5 (3)
Mosquito control measures	Use of mosquito coils	17 (11)
	None	139 (89)
Handling dead pigeons	Using bare hands	147 (94)
	Using hand gloves	04 (3)
	Put hands in a polythene bag	05 (3)
Empty all containers around the neighborhood that can hold water	Yes	13 (8.4)
	No	143 (91.6)
Are there mosquitoes around the containers?	Yes	13 (8.3)
	No	143 (91.7)

APPENDIX 4

Knowledge of respondents related to Mosquito-Borne diseases within the household in Bauchi metropolis (Sub-questions that were answered 'Yes' from the main question)

Variables	Category	Frequency (%)
How long mortality in the pigeons last	≤1 week	38 (55)
	2 weeks	26 (38)
	3 weeks	03 (4)
	≥ 4 weeks	02 (3)
List of diseases caused by mosquitoes apart from malaria to man	Fowl pox	1(0.6)
Diseases that can cause high mortality in birds	Newcastle disease	63 (94)
	Bird flu	04 (6)
List of diseases caused by mosquitoes apart from malaria to man	Fowl pox	1(0.6)
Diseases that can cause high mortality in birds	Newcastle disease	63 (94)
	Bird flu	04 (6)

APPENDIX 5



A: Pigeon lofts at a household in Gwallaga, Bauchi metropolis



B: Pigeon cages and cartons fixed to the house walls at Kobi area of Bauchi metropolis

APPENDIX 6

Gantts charts 2015/2016

Activity	May 15	Ju 15	Jy 15	Ag 15	Sep 15	Oct 15	Nov 15	Dec 15	Jan 16	Feb 16	Mar 16	Ap 16	May 16	Ju 16	Jy 16	Ag 16	Sep 16	Oct 16	Nov 16	Dec 16
Proposal defense/ Literature Review	→	→	→	→	→	→														
Sampling							→	→	→											
Laboratory/ Questionnaire Analysis										→	→	→	→	→						
Dissertation Writing															→	→	→			
Internal defense																		→	→	
External defense																				→

PLATES

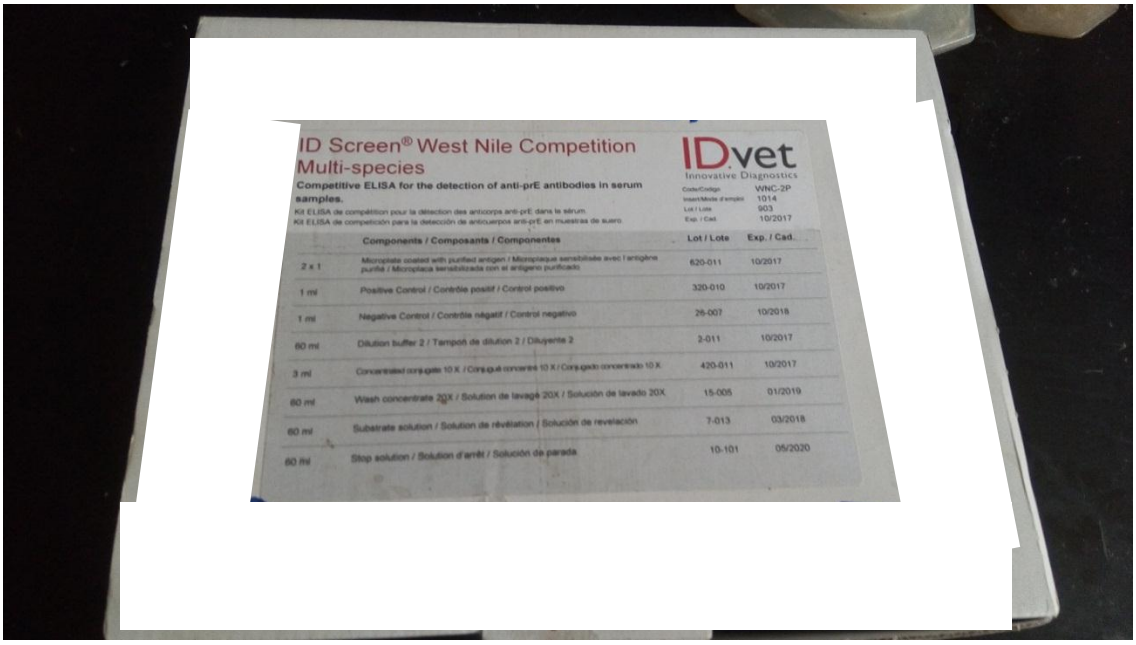


Plate I : ELISA kit used for the study

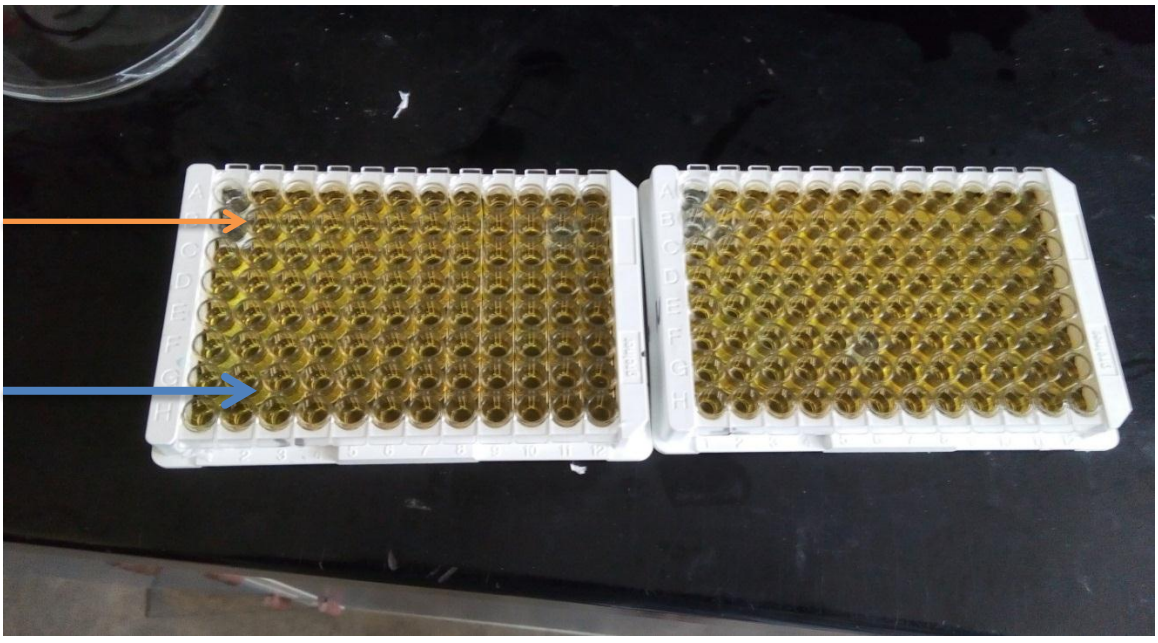


Plate 2: Showing ELISA test samples .Brown arrow shows (colorless well) positive sample to WNV antibody, while the blue arrow (Yellow well) indicates negative