SEROSURVEY FOR MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS ANTIBODY IN DROMEDARY CAMELS AND PATIENTS AT A SECONDARY CARE HOSPITAL, ILLELA, SOKOTO STATE, NIGERIA.

BY

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Middle East Respiratory Syndrome (MERS), also known as camel flu (Richard, 2015), is a viral respiratory infection caused by the MERS coronavirus (MERS-CoV) (WHO, 2015a). Symptoms may range from mild to severe (Zumla *et al*., 2015) and these include fever, cough, diarrhoea and shortness of breath (WHO, 2015a). However, the clinical manifestation in man range from asymptomatic disease to severe pneumonia leading to acute respiratory distress syndrome (ARDS) (Assiri, 2013a), Kidney failure, disseminated intravascular coagulation (DIC), and pericarditis have also been reported (WHO, 2013a). The disease is typically more severe in individuals with other health problems (Zumla *et al*., 2015). MERS-CoV is a single-stranded RNA virus belonging to the genus Betacoronavirus, family Coronaviridae and distinct from Severe Acute Respiratory Syndrome (SARS) coronavirus and the common-cold coronavirus (Saey, 2013). Its genomes are phylogenetically classified into two clades, Clades A and B. Early cases of MERS were of clade A clusters (EMC/2012 and Jordan-N3/2012) while new cases are genetically different in general belonging to clade B (Cotton *et al*., 2013). The first identified case occurred in 2012 in Saudi Arabia and most cases have occurred in the Arabian Peninsula (WHO, 2015a).

Since the discovery of MERS-CoV in 2012, accumulating serologic and molecular evidence demonstrates that the virus in dromedaries is genetically very similar to MERS-CoV in humans and points to the conclusion that dromedary camels are reservoirs for human infection (Reusken *et al*., 2014). There are two known ways of transmission of MERS-CoV; animals to humans and humans to humans. In the case of animals to humans, it is not yet fully understood how people become infected with MERS-CoV, which is a zoonotic virus. However, it is believed that humans can be infected through direct or indirect contact with infected dromedary camels (Martins, 2015; Memish *et al*., 2013; Graham *et al*., 2013). Research shows a high seroprevalence of above 80% MERS-CoV antibodies in dromedary camels in African countries such as

Egypt, Ethiopia, Nigeria and Sudan, and of 30 to 54% in Tunisia (Reusken *et al*., 2014). However, zoonotic human disease has so far been reported only from countries in the Middle-East. The reason for the absence of MERS zoonotic disease report/occurrence in Africa is unclear (Chu *et al*., 2015).

Data suggest a direct zoonotic risk for MERS-CoV infection among persons in contact with camels, where MERS-CoV neutralizing antibodies in healthy persons who had daily occupational contact with dromedaries was detected but not in persons without such contact in Qatar (Chantal *et al*., 2015). While the WHO has not declared MERS as a public health emergency, it has stated that the rise in cases and deaths signifies a "wake-up call" (Reusken *et al*., 2014). A report published by the Centers for Disease Control and Prevention (CDC USA) in August 2014, found evidence of the MERS coronavirus in African camels, including Nigeria (Reusken *et al*., 2014). Livestock dependent people in the horn of Africa region who suffer from malnutrition are potentially at risk of contracting the disease (Eric and Joerg, 2015). Outbreaks of MERS in other parts of the world like South Korea have been linked to individuals originally acquiring infections from chains of transmission originating in the Middle East (Eric and Joerg, 2015). As of July 2015, MERS-CoV cases have been reported in over 21 countries, including Saudi Arabia, Jordan, Qatar, Egypt, the United Arab Emirates, Kuwait, Turkey, Oman, Algeria, Bangladesh, Indonesia (none were confirmed), Austria (WHO, 2014a), the United Kingdom, South Korea (WHO, 2015a; Myung, 2015), the United States (Roos, 2014a), Mainland China, Thailand, and the Philippines (WHO, 2015a).

As at 2015 there is still no specific vaccine or treatment for MERS-CoV infection (WHO, 2015a) and as of 13 November 2015, there were 1,618 laboratory confirmed cases of MERS reported to the World Health Organization (WHO), associated with 579 deaths (WHO, 2015b). About 40% of those who become infected die from the disease (Zumla *et al*., 2015). The disease usually affects patients who are immunocompromised or suffering from other conditions like diabetes, lung and liver infections. The outbreak of MERS in some Asian countries has been a source of worry to global health bodies and stakeholders (Martins, 2015). A

former Regional Virologist at the WHO and the President, National Academy of Science, Professor Oyewale Tomori raised alarm on the need for the Nigerian government to be proactive against MERS-CoV in order to prevent its spread to the country (Martins, 2015).

1.2 Statement of Research Problem

Camels are an extremely important source of livelihood, nutrition and income in Sokoto State as well as some parts of Africa and they are especially common in arid and semi-arid areas of the continent (Bello *et al*., 2012). There is an increasing demand for camel meat as it has become popular in Sokoto State. An average of 10 camels (minimum of 5) is slaughtered at Sokoto Central Abattoir on a daily basis and a total of 3172 camels were slaughtered between November, 2010 and May, 2011 (Bello *et al*., 2012). Also there is active importation of camels through the study area and other border town and villages. There is maximum interaction between the camels, other species of animals, camel handlers and individuals within Illela International Market; this close interaction may predispose to spread of infection.

Despite the fact that camels are in abundance in the study area, there is dearth of information on the occurrence of MERS-CoV infection. Needful to recognize that MERS-CoV is a zoonosis which has a high case fatality rate, at around 37% in the Middle East (Eric and Joerg, 2015) and 40% in Saudi Arabia where the disease originated from (Zumla *et al*., 2015).

Most of the camels found in the study area migrate across the borders which are vast and largely unmanned. The infection has been established in the Middle East and most parts of Africa from which most of these camels originate (Reusken *et al*., 2014; Kamau *et al*., 2015). The lack of effective surveillance at the border posts and international market may lead to introduction of the infectious agent to Nigeria through Illela. There are more than 35 illegal routes linking Illela with Konni town in Niger Republic and this cut across

several villages, which to a large extent enable them to escape being tracked at the border post (Mohammed, 2013).

1.3 Justification of the Study

Determining the seroprevalence of MERS-CoV in Illela will provide a baseline data to support the establishment of surveillance system for the infection, particularly within areas of Nigeria that border with North Africa and subsequently Middle Eastern countries which are the epicenter of the infection.

The study will provide information for monitoring and early detection of MERS-CoV in camels which serve as sentinels for early disease detection and possible response in other species of animals and human population.

The close interaction between humans with camels tends to predispose to several infectious diseases (Chantel *et al*., 2015; WHO, 2015). Handlers of camels virtually live and make contact with them daily with no precautions towards preventing zoonotic diseases. Other susceptible humans may as well interact with infected humans at the market, social gathering, hospitals and other functions thereby increasing the number of secondary cases from a primary case.

So far to the best of our knowledge, there is no available literature on MERS-CoV infection in camel and man from Illela. However, there have been few published works on MERS-CoV in camels within Nigeria; Chu *et al*. (2015) detected MERS-CoV RNA in 14 (11%) of 132 nasal swabs and antibody in 126 (96%) of 131 serum samples both collected from dromedary camels in Kano State. In another study by Reusken *et al*. (2014), high seroconversion (94%) to MERS-CoV from stored samples was reported in Nigerian camels (Kano, n=245; Sokoto, n=51; Borno, n=51; and Adamawa, n=11; N=358).

Data obtained from this study will thus elucidate more on the presence or absence of the infection and its prevalence in the study area.

1.4 Aim of the Study

The aim of the study was to detect MERS-CoV antibodies using an indirect ELISA in dromedary camels and patients in a secondary care human hospital in Illela, Sokoto State, Nigeria.

1.5 Objectives of the Study

The objectives of the study were to:

I. determine the seroprevalence of MERS-CoV in dromedary camels in Illela, Sokoto State.

II. determine the seroprevalence of MERS-CoV in some patients in a secondary care human hospital in Illela, Sokoto State.

III. identify possible risk factors associated with MERS-CoV infection among camels, their handlers and patients in the secondary care human hospital using information obtained from data form survey in Illela, Sokoto State.

1.6 Research Questions

i. What is the seroprevalence of MERS-CoV in dromedary camels in Illela?

ii. What is the seroprevalence of MERS-CoV in General hospital, Illela?

iii. Are there risk factors that predispose camels and humans to MERS-CoV infection in Illela?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The MERS-CoV

The virus MERS-CoV is a new member of the beta group of coronavirus (betacoronavirus), lineage C. Betacoronaviruses are one of four genera of coronaviruses of the subfamily Coronavirinae in the family Coronaviridae, of the order Nidovirales. They are enveloped, positive-sense, single-stranded RNA viruses of zoonotic origin (ECDC, 2013). Coronaviruses infect and cause disease in a broad array of animals and humans. The virus particles range from 70 to 120nm in diameter and are surrounded by characteristic spike-shaped glycoproteins. The ca.180 kDa spike glycoprotein mediates host cell attachment and entry, utilizing virus- and host-specific cell receptors. The receptor-binding domain (RBD) of the spike glycoprotein is poorly conserved among viruses and, as a result, host receptor usage varies between viral genera and species (Graham *et al*., 2013). This variability contributes to the broad host range of coronaviruses, as the viruses can breach cell type, tissue and host species barriers with relative ease. Both MERS-CoV and SARS coronavirus (SARS-CoV) recognize exopeptidases as receptors for docking and entry, and other human coronaviruses use aminopeptidases or carbohydrates (Graham *et al*., 2013).

Three real-time reverse-transcription polymerase chain reaction (rRT-PCR) assays for routine detection of MERS-CoV have been developed and their details published (Corman *et al*., 2012a). Currently described tests are an assay targeting upstream of the E protein gene (upE) (Corman *et al*., 2012a) and assays targeting the open reading frame 1b (ORF 1b) (Corman *et al*., 2012b). The assay for the upE is considered highly sensitive and is recommended for screening, with the ORF 1a assay considered of equal sensitivity. The ORF 1b assay is considered less sensitive than the ORF 1a assay (WHO, 2013a). Early reports compared MERS-CoV virus to SARS (Zaki *et al*., 2012), and it has been referred to as Saudi Arabia's SARS-like virus (Doucleff, 2012). The virus was initially designated Human Coronavirus- Erasmus Medical Center (HCoV-EMC) (Zaki *et al*., 2012) but after global consensus comparing the phylogenetic trees it was renamed

MERS-CoV (De Groot *et al*., 2013). Betacoronaviruses are one of four genera of coronaviruses of the subfamily *Coronavirinae* in the family *Coronaviridae*, of the order *Nidovirales*.

The name Coronavirus is derived from the Latin "corona" meaning crown or halo, referring to their image under electron microscopy of crown-like spikes on the surface similar to the solar corona (Figure 2.1). This morphology is created by the viral spike (S) peplomers, which are proteins that populate the surface of the virus and determine host tropism. The order Nidovirales is named for the Latin nidus, which means nest. It refers to this order's production of a 3' co-terminal nested set of subgenomic mRNA's during infection (Woo *et al*., 2010). The coronavirus genera are each composed of varying viral lineages with the betacoronavirus genus containing four such lineages (A, B, C and D) commonly recognized (ECDC, 2013); Lineage A includes HCoV-OC43 and HCoV-HKU1 (various species), Lineage B includes SARS-CoV (various species), Lineage C includes Tylonycteris bat coronavirus HKU4 (BtCoV-HKU4), Pipistrellus bat coronavirus HKU5 (BtCoV-HKU5), and MERS-CoV (various species) and Lineage D includes Rousettus bat coronavirus HKU9 (BtCoV-HKU9).

Coronaviruses contain the largest single-stranded, positive-sense RNA genomes currently known, which range from 25.5 to nearly 32 kb in length. Whereas all coronaviruses encode strain-specific accessory genes in ORFs downstream of ORF1 (the replicase–transcriptase), the order of essential structural genes is remarkably well-conserved: all coronaviruses encode the replicase–transcriptase (gene 1), spike, envelope, membrane and nucleocapsid proteins (Graham *et al*., 2013). Crystal structures of both SARS-CoV and MERS-CoV RBDs complexed with their corresponding receptors have been solved (Graham *et al*., 2013). Post-entry events are dependent on one or more cellular proteases, such as cathepsins, human airway trypsin-like protease (HAT) or transmembrane protease serine 2 (TMPRSS2), which cleave the spike protein and initiate a variety of conformational changes that are important for membrane fusion and entry (Graham *et al*., 2013).

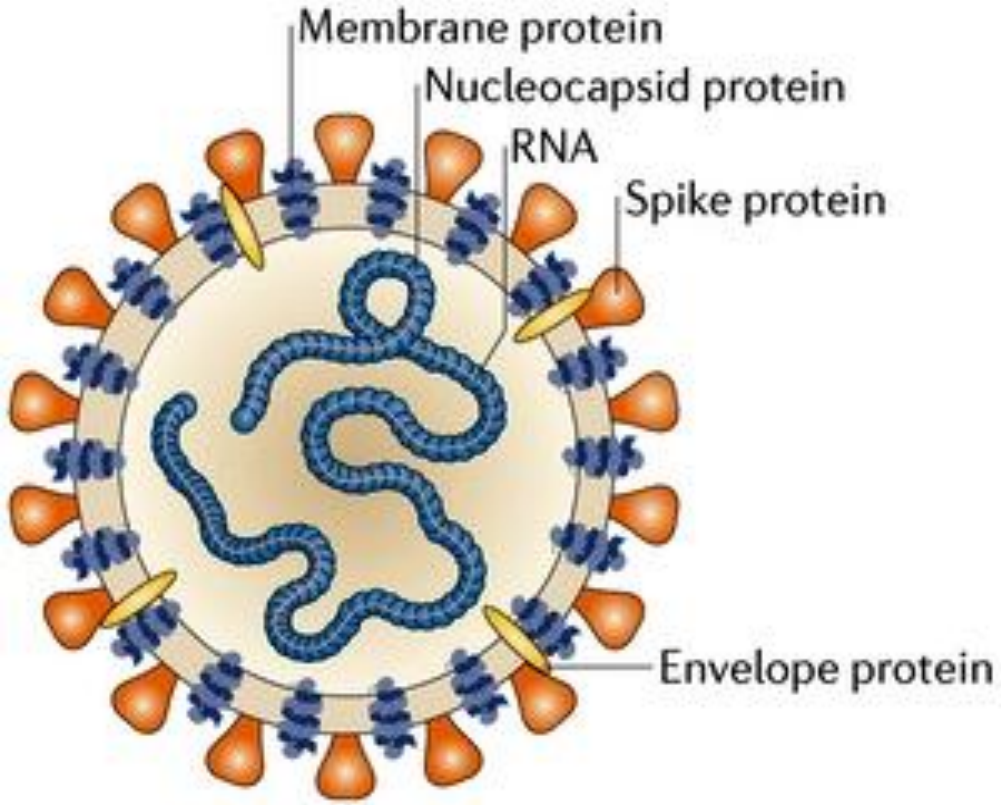


Figure 2.1: Structure of Coronavirus.

Adapted from Nature Reviews Microbiology (Graham *et al*., 2013).

2.2 Pathogenesis

The pathogenesis of MERS-CoV infection is not well understood (Kenneth *et al*., 2016). The virus has a strong tropism for nonciliated bronchial epithelial cells, and it has been shown to effectively evade the innate immune responses and antagonize interferon (IFN) production in these cells. This tropism is unique in that most respiratory viruses target ciliated cells (Kindler *et al*., 2013; Raj *et al*., 2013). Due to the clinical similarity between MERS-CoV and SARS-CoV, it was proposed that they may use the same cellular receptor; the exopeptidase, angiotensin converting enzyme 2 (ACE2) (Jia *et al*., 2005). However, it was later discovered that neutralization of ACE2 by recombinant antibodies does not prevent MERS-CoV infection (Muller *et al*., 2012). Expression of human and bat dipeptyl peptidase 4 (DPP4) in non-susceptible cells enables infection by MERS-CoV (Kenneth *et al*., 2016). Further research identified DPP4 also known as CD26 as a functional cellular receptor for MERS-CoV (Raj *et al*., 2013). Unlike other known coronavirus receptors, the enzymatic activity of DPP4 is not required for infection. As would be expected, the amino acid sequence of DPP4 is highly conserved across species and is expressed in the human bronchial epithelium and kidneys (Raj *et al*., 2013; Declan, 2013). Bat DPP4 genes appear to have been subject to a high degree of adaptive evolution as a response to coronavirus infections, so the lineage leading to MERS-CoV may have circulated in bat populations for a long period of time before being transmitted to people (Cui *et al*., 2013).

2.3 Epidemiology

Camels are ancient animals well known in the history of human civilization and have been domesticated for transportation, meat, clothing and milk over 4000 years ago (Wilson, 1984). The meat is of good quality especially in areas where other meat animals find it difficult to thrive (Kadim *et al*., 2008) and the milk quality is of comparable quality to cattle and it provides milk for longer duration compared to other similarly domesticated animals (Mehala *et al*., 1995). More than 60% of the global populations of dromedary camels are distributed in African countries. Some of these countries are important camel

exporters to the Arabian Peninsula (Faye and Bonnet, 2012). For many years' African camels have carried antibodies to MERS-CoV and the risk of it spreading to humans is still low, but more research is necessary to understand the disease better (Eric and Joerg, 2015). Though the vast majority of cases have so far occurred in the kingdom of Saudi Arabia, there are other 25 countries where cases of MERS have been reported. They include: Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, the United Arab Emirates, and Yemen (Middle East); Austria, France, Germany, Greece, Italy, Netherlands, Turkey, and the United Kingdom (UK) (Europe); Algeria, Tunisia and Egypt (Africa); China, Malaysia, Republic of Korea and the Philippines and Thailand (Asia); and the United States of America (Americas) (Martins, 2015).

A study carried out in Saudi Arabia between 2010 and 2013, in which the incidence of MERS was evaluated in 310 dromedary camels, revealed high titers of neutralizing antibodies to MERS-CoV in the blood serum of these animals (n=280, 90%) (Hemida *et al*., 2013). A further study sequenced MERS-CoV from nasal swabs of dromedary camels in Saudi Arabia and found they had sequences identical to previously sequenced human isolates. Some individual camels were also found to have more than one genomic variant in their nasopharynx (Breise, 2014). There is also a report of a Saudi Arabian man who became ill seven days after applying topical medicine to the noses of several sick camels and later he and one of the camels were found to have identical strains of MERS-CoV (Azhar *et al*., 2014; Gallagher, 2014).

The MERS-CoV is the first betacoronavirus belonging to lineage C that is known to infect humans (Memish *et al*., 2013). The first person to be infected with MERS, in June 2012, had a "seven-day history of fever, cough, expectoration, and shortness of breath" (Zaki *et al*., 2012). One review of 47 laboratory confirmed cases in Saudi Arabia gave the most common presenting symptoms as fever in 98%, cough in 83%, shortness of breath in 72% and myalgia in 32% of people. There were also frequent gastrointestinal symptoms with diarrhea in 26%, vomiting in 21%, abdominal pain in 17% of people and 72% of people required mechanical ventilation. There were also 3.3 males for every female (Assiri, 2013a).

Studies in Kenya shows that, despite its recent identification as a human pathogen, MERS-CoV has been circulating for many years over wide geographical areas (Eric and Joerg, 2015). Camel sera collected as far back as 1983 in Kenya shows high rates of seroconversion to the virus. This means that the animals have been infected, probably by a transient respiratory disease, and recovered (Eric and Joerg, 2015). MERS in camels, it seems, is much like being infected by the common cold. Some populations of camels in Kenya (having the third largest camel population in East Africa) tested recently show seroconversion rates of 47% (Eric and Joerg, 2015)

The deadly respiratory virus that has spread from Saudi Arabia around the world was found in camels in Nigeria, Tunisia and Ethiopia, showing the pathogen is more widespread than previously known (Bennett, 2014). The virus (MERS-CoV), was found in almost all blood samples taken from 358 dromedary camels in Nigeria and 188 camels in Ethiopia (Bennett, 2014). In Tunisia, MERS-CoV was found in 54 percent of adult camels, and in all of the animals from one southern province. The findings add to previous studies that have found the virus in camels in Spain's Canary Islands and Egypt, as well as in several nations on the Arabian Peninsula, and suggest that there may be undiagnosed human cases in Africa according to research reports from the Netherlands (Bennett, 2014). This result may also suggest the virus was circulating well before 2012 when the first human case was identified (Bennett, 2014).

In April 2014, MERS was also implicated in an outbreak in Saudi Arabia, where MERS had infected 688 people and 282 MERS-related deaths had been reported since 2012 (McNeil Jr., 2014). Eighteen more cases were reported in early May 2014 (McDowall, 2014). In June 2014, Saudi Arabia announced 113 previously unreported cases of MERS, revising the death toll to 282. A hospital-related outbreak in Riyadh in the summer of 2015 increased fears of an epidemic occurring during the annual Hajj pilgrimage that begins in late September. After a period of few cases, cases began increasing in the middle of the summer (McKenna,

2015). The CDC placed the travel health alert to level 2, which calls for taking enhanced precautions (CDC, 2015).

On 2 May 2014, the CDC confirmed the first diagnosis of MERS in Indiana, the United States of America. The man diagnosed was a health care worker who had been in Saudi Arabia a week earlier, and was reported to be in good condition (McKay, 2014; Stobbe, 2014). A second patient who also traveled from Saudi Arabia was reported in Orlando, Florida on 12 May 2014 (CDC, 2014; Santich, 2014). On 14 May 2014, officials in the Netherlands reported the first case (Chicago Tribune, 2014). In May 17, 2014, a man from Illinois who was a business associate of the first U.S. case (he had met and shook hands with the Indiana health care worker) tested positive for the MERS coronavirus, but has not, as of yet, displayed symptoms (others are probably also, at least temporarily if not permanently, non-symptomatic carriers). The CDC's Dr. David Swerdlow, who was leading the agency's response, said the man, who feels well and has not yet sought and does not yet need medical care, has not been deemed an official case yet and prevention guidelines have not changed. Laboratory tests showed evidence of past infection in his blood (Aleccia, 2014).

In May 2015, the first case in South Korea was confirmed in a man who had visited Saudi Arabia, United Arab Emirates and Bahrain (Szabo, 2015). Another man from South Korea, who was travelling to China, was diagnosed as the first case in China. So far, no Chinese citizen has been found infected (Xinhua News Agency, 2015). As of 27 June 2015, 19 people in South Korea have died from this outbreak, with 184 confirmed cases of infection (Kim, 2015). There have been at least 6,508 quarantined in South Korea (Park and Pomfret, 2015; Jeyup, 2015).

In April 2014, MERS emerged in the Philippines with a suspected case of a home-bound overseas Filipino worker. Several suspected cases involving individuals who were on the same flight as the initial suspected

case were being tracked but are believed to have dispersed throughout the country. Another suspected MERS-involved death in Sultan Kudarat province caused the Department of Health (DOH) to put out an alert (Del Callar, 2014; Fernandez, 2014). On July 6, 2015 the DOH confirmed the second case of MERS in the Philippines when a 36-year-old male foreigner from the Middle East was tested positive (Bonalos, 2015).

On 27 July 2015, the Accident and Emergency Department at Manchester Royal Infirmary closed after two patients were suspected to be infected with MERS-CoV virus (Gallagher, 2015). The facility was reopened later that evening, and it was later confirmed by Public Health England that the two patients tested negative for the disease (Gallagher, 2015).

2.4 Host Distribution

Early research suggested that MERS-CoV in camels is related to the MERS-CoV found in the Egyptian tomb bat in Africa and Middle East (Doucleff, 2012). In September 2012, Ron Fouchier speculated that MERS-CoV might have originated in bats (Doucleff, 2012). Work by Ian Lipkin showed that MERS-CoV virus isolated from a bat looked to be a match to the MERS-CoV virus found in humans (Abedine, 2013; Doucleff, 2012; Beth, 2013). Betacoronaviruses were detected in Nycteris bats in Ghana and Pipistrellus bats in Europe that are phylogenetically related to the MERS-CoV virus (Augustina *et al*., 2013). However the major natural reservoir where humans get the virus infection remained unknown until the 9th August 2013, a report in The Lancet Infectious Diseases Journal showed that 50 out of 50 (100%) blood serum from Omani camels and 15 of 105 (14%) from Spanish camels had protein-specific antibodies against the MERS-CoV spike protein. Blood serum from European sheep, goats, cattle and other camelids had no such antibodies (Reusken *et al*., 2013).

Soon after on 5th September 2013, a seroepidemiological study by Perera *et al*. (2013) investigated 1, 343 human and 625 animal sera, high level of MERS-CoV specific antibody was found in 108 out of 110 Egyptian dromedary camels but none in humans and other animals such as goats, cows or sheep were obtained. These are the first and significant scientific reports that indicated the role of "dromedary camels" as a reservoir of MERS-CoV. Recent work links camels to the virus. An ahead-of-print dispatch for the journal 'Emerging Infectious Diseases' records research showing the coronavirus infection in dromedary camel calves and adults, 99.9% matching to the genomes of human clade B MERS-CoV (Hemida *et al*., 2014). At least one person who has fallen sick with MERS was known to have come into contact with camels or recently drank camel milk (Roos, 2014b).

Countries like Saudi Arabia and the United Arab Emirates produce and consume large amounts of camel meat (Kai, 2013). The possibility exists that African or Australian bats harbor the virus and transmit it to camels. Imported camels from these regions might have carried the virus to the Middle East (Kai, 2013). In 2013 MERS-CoV was identified in three members of a dromedary camel herd held in a Qatar barn, which was linked to two confirmed human cases who have since recovered. The presence of MERS-CoV in the camels even though asymptomatic was confirmed by the National Institute of Public Health and Environment of the Ministry of Health and the Erasmus Medical Center, a WHO Collaborating Center in the Netherlands. None of the camels showed any sign of disease when the samples were collected. The Qatar Supreme Council of Health advised in November 2013 that, people with underlying health conditions, such as heart disease, diabetes, kidney disease, respiratory disease, the immunosuppressed, and the elderly, avoid any close animal contacts when visiting farms and markets, and to practice good hygiene, such as washing hands (Michael, 2013).

A further study on dromedary camels from Saudi Arabia published in December 2013 revealed the presence of MERS-CoV in 90% of the evaluated dromedary camels (310), suggesting that dromedary camels not only

could be the main reservoir of MERS-CoV, but also the animal source of MERS (Hemida *et al*., 2013). According to the 27 March 2014 MERS-CoV summary update, studies support that camels serve as the primary source of the MERS-CoV infecting humans, while bats may be the ultimate reservoir of the virus. Evidence includes the frequency with which the virus has been found in camels to which human cases have been exposed, serological data have shown widespread transmission in camels and the similarity of the camel CoV to the human CoV (Chu, 2014).

2.5 Types of Camel

Camels are mammals with long legs, a big-lipped snout and a humped back. There are two types of camels worldwide. The dromedary camel (*Camelus dromedarius*) with one hump is found in Africa and the Middle East; and the bactrian camel (*Camelus bactrianus, Camelus ferus*) with two humps, found in Asia (Eric And Joerg, 2015; Alina and Nina, 2014). Camels' humps consist of stored fat, which they can metabolize when food and water is scarce. No matter the type, camels are usually found in the desert, prairie or steppe (Alina and Nina, 2014). Though many people think that camels only live in hot climates, they do well in temperature ranges from 20°F to 120°F (-7°C to 49°C) (Alina and Nina, 2014).

2.6 Mode of Transmission

The MERS-CoV grows readily in several bat-derived cell lines (Muller *et al*., 2012). Following experimental inoculation, MERS-CoV has also been shown to cause widespread but asymptomatic infection of Jamaican fruit bats, supporting the hypothesis that bats may be ancestral reservoirs for MERS-CoV (Munster *et al*., 2016). Although bats may be a reservoir of MERS-CoV, it is unlikely that they are the immediate source for most human cases because human contact with bats is uncommon (Ali *et al*., 2013).

2.6.1 Camel-to-human transmission

Dromedary camels appear to be the primary animal host for MERS-CoV (Kenneth *et al*., 2016). The strongest evidence of camel-to-human transmission of MERS-CoV comes from a study in Saudi Arabia in which MERS-CoV was isolated from a man with fatal infection and from one of his camels; full-genome sequencing demonstrated that the viruses isolated from the man and his camel were identical (Azhar *et al*., 2014). Other phylogenetic analyses comparing portions of the MERS-CoV genome obtained from camels to MERS-CoV obtained from humans with epidemiologic links to the camels have demonstrated that the viruses were similar (Alagaili *et al*., 2014; Chu *et al*., 2014; Haagmans *et al*., 2014; Memish *et al*., 2014). Serologic studies have also suggested that camels are an important source of MERS-CoV (Alagaili *et al*., 2014; Chu *et al*., 2014; Haagmans *et al*., 2014)

2.6.2 Human-human transmission

Case clusters in the United Kingdom, Tunisia, and Italy, and in healthcare facilities in Saudi Arabia, the United Arab Emirates, Iran, France, and South Korea strongly suggest that human-to-human transmission occurs (Assiri, 2013b; Guery *et al*., 2013; WHO, 2013c; Abroug *et al*., 2014; Drosten *et al*., 2015; Park *et al*., 2015a; WHO, 2015b). The number of contacts infected by individuals with confirmed infections, however, appears to be limited (Drosten *et al*., 2014). An exception to this is the outbreak in South Korea in May and June 2015, where many secondary and some tertiary cases occurred; a total of 186 cases were reported (Cowling *et al*., 2015; Da Guan *et al*., 2015; KCDC, 2015; Park *et al*., 2015a; Park *et al*., 2015b). In this outbreak, 83 percent of transmission events were epidemiologically linked to five superspreaders, all of whom had pneumonia at presentation; these individuals were each in contact with hundreds of people (KCDC, 2015). The outbreak in South Korea is the first MERS outbreak in which superspreader events have been identified. The severe acute respiratory syndrome (SARS) outbreak in Hong Kong in 2003 was also associated with superspreaders. Superspreaders are individuals who are responsible for a disproportionately large number of transmission events (Stein, 2011).

Secondary cases have tended to be milder than primary cases, and many secondary cases have been reported to be asymptomatic (Drosten *et al*., 2014; WHO, 2014b). Possible modes of transmission include droplet and contact transmission (CDC, 2013). More than half of all laboratory-confirmed secondary cases have been associated with healthcare settings (WHO, 2014c). The majority of cases in the spring of 2014 in Saudi Arabia were acquired through human-to-human transmission in healthcare settings, likely due at least in part to systemic weaknesses in infection control (WHO, 2015b; Oboho *et al*., 2014; WHO, 2015c). One study of a hospital-based outbreak of MERS had an estimated incubation period of 5.5 days (95% confidence interval 1.9 to 14.7 days) (Assiri, 2013b).

2.7 Diagnostic Tests

In general, laboratory tests for MERS-CoV virus fall into two categories:

I. Serology tests, which look for previous infection by detecting antibodies to MERS-CoV. Serology tests are for surveillance or investigational purposes and not for diagnostic purposes.
II. Molecular tests, which look for evidence of active infection.

2.7.1 Serology tests

Serology testing is used to detect previous infection (antibodies to MERS-CoV) in individuals who may have been exposed to the virus. The presence of antibodies to MERS-CoV indicates that an individual had been previously infected with the virus and developed an immune response (CDC, 2016).

The CDC has a two-phase approach for serology testing, using one screening test and two confirmatory tests to detect antibodies to MERS-CoV; ELISA, or enzyme-linked immunosorbent assay, is a screening test used to detect the presence and concentration of specific antibodies that bind to a viral protein. The ELISAs usually produce results within a few hours. If a clinical sample is determined to be antibody-positive by ELISA, CDC then uses the immunofluorescence (IFA) assay and/or microneutralization assay to confirm

the positive result. IFA assay is a confirmatory test in which specific antibodies, if present in the person's blood, attach to virus-infected cells fixed on a glass slide. These attached antibodies are detected by adding a secondary antibody labeled with a compound that makes them glow an apple-green color when viewed under a special microscope. This secondary antibody will bind to any antibodies which are present in the blood and have attached to the virus-infected cells. Like the ELISA results, IFA results can also be obtained in a few hours. If a clinical sample is positive by both ELISA and IFA, the specimen is determined to be positive. If a clinical sample is positive by ELISA but indeterminate or negative by IFA, CDC then performs additional confirmatory testing.

The microneutralization assay is a highly specific confirmatory test used to measure neutralizing antibodies, or antibodies that can neutralize virus. This method is considered the gold standard for detection of specific antibodies in serum samples. However, compared with the ELISA and IFA, the microneutralization assay is labor-intensive and time-consuming, requiring at least 5 days before results are available. If a clinical sample is positive by ELISA, indeterminate by IFA, and positive by microneutralization, the specimen is determined to be positive. If a clinical sample is positive by ELISA, indeterminate or negative by IFA, and negative by microneutralization, the sample is determined to be negative. In the end, a final determination of a positive serology result requires a positive ELISA test and confirmation by IFA or microneutralization assay.

For public's safety, initial serology results before completion might be reported for proper and immediate response in suspected MERS-CoV outbreaks. However, serology tests as an entity is for surveillance and investigational purposes and not for diagnosis.

2.7.2 Molecular tests

Molecular tests are used to diagnose active infection (presence of MERS-CoV) in people who are thought to be infected with MERS-CoV based on their clinical symptoms and having links to places where MERS has been reported (CDC, 2016). Real-time reverse-transcription polymerase chain reaction (rRT-PCR) assays are molecular tests that can be used to detect viral RNA in clinical samples. The CDC's current case definition for laboratory confirmation of MERS-CoV infection requires either a positive rRT-PCR result for at least two specific genomic targets, or a single positive target with sequencing of a second target.

Most State laboratories in the United States are approved to test for MERS-CoV by using the rRT-PCR assay developed by CDC. This test is done under authority of an Emergency Use Authorization (EUA) because there are no FDA-cleared/approved tests available for this purpose in the United States. The success of rRT-PCR testing depends on several factors, including the experience and expertise of laboratory personnel, laboratory environment (e.g., avoidance of contamination), and the type and condition of specimens being tested. For this rRT-PCR assay, CDC recommends collecting multiple specimens, including lower (bronchalveolar lavage, sputum and tracheal aspirates) and upper (e.g., nasopharyngeal and oropharyngeal swabs) respiratory samples, serum and stool specimens.

The CDC considers a patient under investigation to be negative for active MERS-CoV infection following one negative rRT-PCR test on the recommended specimens. Since a single negative result does not completely rule out MERS-CoV infection, in some circumstances additional specimens may be tested. It is considered by CDC that a known MERS patient is negative for active MERS-CoV infection following two consecutive negative rRT-PCR tests on all specimens.

2.8 Treatment, Prevention and Public Health

Since there's no vaccine or treatment for the infection yet, a number of antiviral agents are currently being studied (WHO, 2015a). The WHO recommends that those who come in contact with camels wash their hands frequently and do not touch sick camels. They also recommend that camel products be appropriately cooked. Among those who are infected treatments that help with the symptoms may be given (WHO, 2015d). According to WHO, the interim case definition is that a confirmed case is identified in a person with a positive laboratory test by "molecular diagnostics including either a positive PCR on at least two specific genomic targets or a single positive target with sequencing on a second (WHO, 2013b). A probable case given by WHO (2013b) is; A person with a fever, respiratory infection, and evidence of pneumonia or ARD syndrome and testing for MERS-CoV is unavailable or negative on a single inadequate specimen and the person has a direct link with a confirmed case, a person with acute febrile respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or ARDS) and an inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation) and a resident of or traveler to Middle Eastern countries where MERS-CoV virus is believed to be circulating in the 14 days before onset of illness and a person with an acute febrile respiratory illness of any severity and an inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation) and the person have a direct epidemiologic-link with a confirmed MERS-CoV case.

2.9 Transboundary Diseases

2.9.1 Transboundary animal diseases

Many countries use the term exotic or foreign animal disease to designate those diseases that would have a disastrous consequence if they were to enter their territory, either because of the direct losses to the domestic population suffering the disease or required counter-epizootic measures, loss in trade, or possibly the potential zoonotic spill over (Lubroth and de Balogh, 2009). From a United Nations point of view the preferred term is transboundary animal diseases (TADs) as nothing is per se exotic or foreign in the global

theatre. TADs are defined by Food and Agricultural Organization (FAO) as those diseases that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries. Such definition should include emerging infectious diseases (EIDs), most of which will likely be zoonoses, but of uncertain impact (Lubroth and de Balogh, 2009). Furthermore, their potential consequences are of such a magnitude that their occurrence may also have a significant detrimental effect on national economies (FAO, 2016).

Transboundary animal diseases according to FAO (2016) have the potential to: threaten food security through serious loss of animal protein and/or loss of draught animal power for cropping, increase poverty levels particularly in poor communities that have a high incidence dependence on livestock farming for sustenance, cause major production losses for livestock products such as meat; milk and other dairy products; wool and other fibers and skins and hides, thereby reducing farm incomes. They may also restrict opportunities for upgrading the production potential of local livestock industries by making it difficult to utilise exotic high producing breeds which tend to be very susceptible to the transboundary disease, add significantly to the cost of livestock production through the necessity to apply costly disease control measures, seriously disrupt or inhibit trade in livestock and livestock products either within a country or internationally. Their occurrence may thereby cause major losses in national export income in significant livestock-producing countries, cause public health consequences in the case of those transboundary animal diseases which can be transmitted to humans (i.e. zoonoses), cause environmental consequences through die-offs in wildlife populations in some cases and cause pain and suffering for affected animals.

2.9.2 Transboundary zoonoses

Transboundary zoonotic diseases can maintain a dynamic focus and have pathogens circulating in geographic regions encircling multiple geopolitical boundaries. Global change is intensifying transboundary

problems, including the spatial variation of the risk and incidence of zoonotic diseases. The complexity of these challenges can be greater in areas where rivers delineate international boundaries and encompass transitions between ecozones (Esteve-Gassent *et al*., 2014). Some of the transboundary zoonoses include;

i. Anthrax
ii. Bovine Spongiform Encephalopathy
iii. Brucellosis
iv. Ebola virus
v. Highly Pathogenic Avian Influenza
vi. Marburg haemorrhagic fever
vii. Nipah virus
viii. Q fever
ix. Rabies
x. Tularemia
xi. Rift valley fever
xii. and recently MERS-CoV

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Illela Local Government Area (LGA) of Sokoto State, Nigeria. Illela shares a border with the Republic of Niger to the north, Gwadabawa LGA to the south, Gada LGA to the east and Tangaza LGA to the west. However, Gada and Tangaza LGAs also share border with Republic of Niger each to their north (Figures 3.1 and 3.2). It has an area of 1,246 km² and a population of 150,489 as at 2006 census (Mohammed, 2013). Its geographical coordinates are; 12°11′0″ N and 4°45′0″ E. Sokoto State has five international border towns in Illela, Gudu, Tangaza, Sabon Birni and Gada local government areas of the State. However, the Illela international border post is the most strategic and about 120 kilometres away from the Sokoto metropolis. The Illela border is a gateway to Niger Republic, Benin Republic and other countries in North and West Africa (Mohammed, 2013). Nigeria shares border with four countries; Niger Republic to the North, Cameroon to the South, Chad to the East and Benin Republic to the West.

The people of Illela LGA mostly speak Hausa, however other tribes within Nigeria and Republic of Niger are also found there. They major in trade as goods are constantly moved in and out of Illela international border. Majority of the inhabitants are Muslims and share culture with Konni town clans in Republic of Niger as it was once in Sokoto State.

3.2 Study Design

A cross sectional study with convenient sampling technique was employed to the survey for MERS-CoV in dromedary camels (at Illela international border and Illela international market) and some patients in Illela General Hospital, Sokoto State.

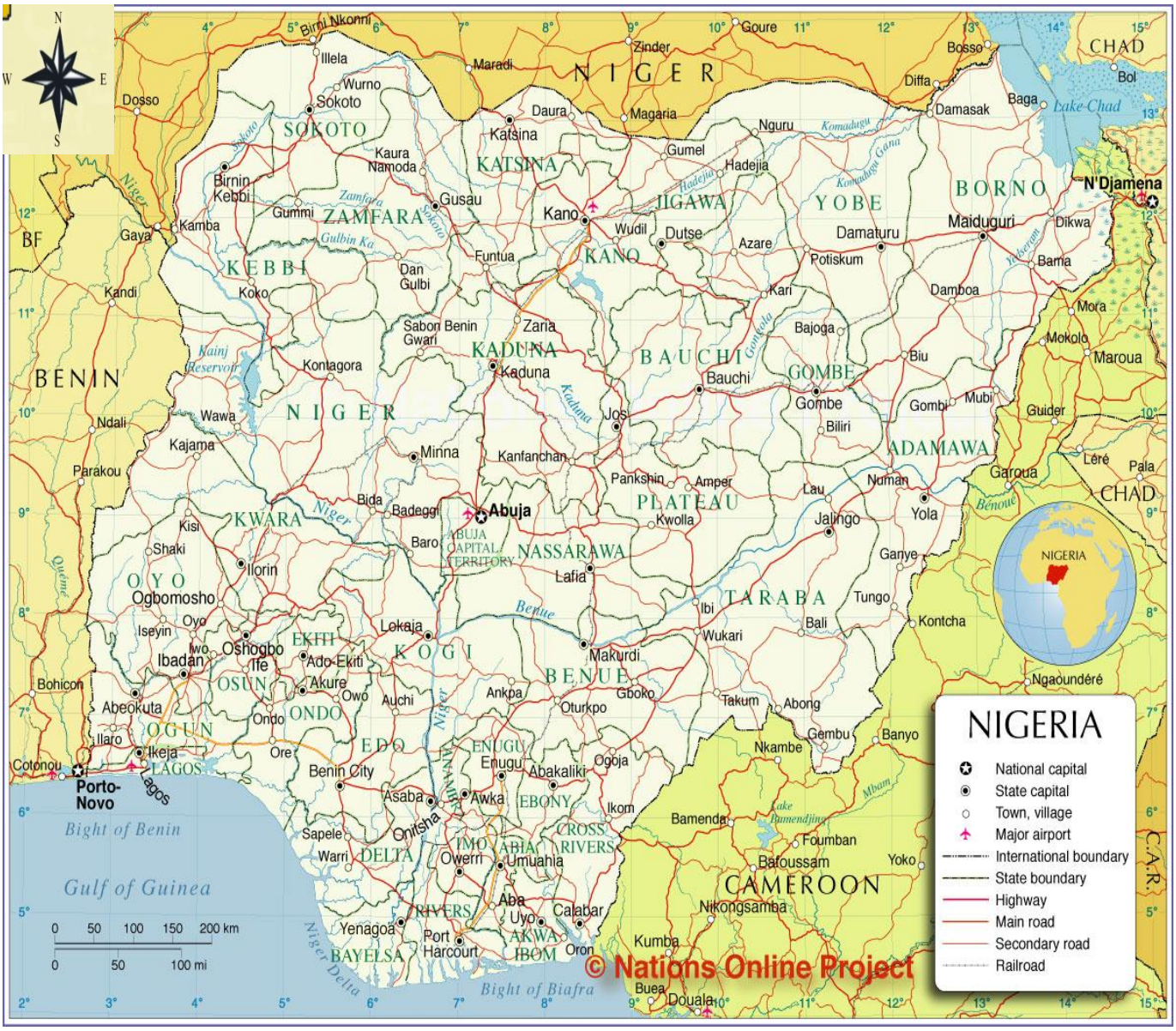


Figure 3.1: Map of Nigeria and bordering countries.

Source: http://www.nationsonline.org/maps/nigeria_map.jpg

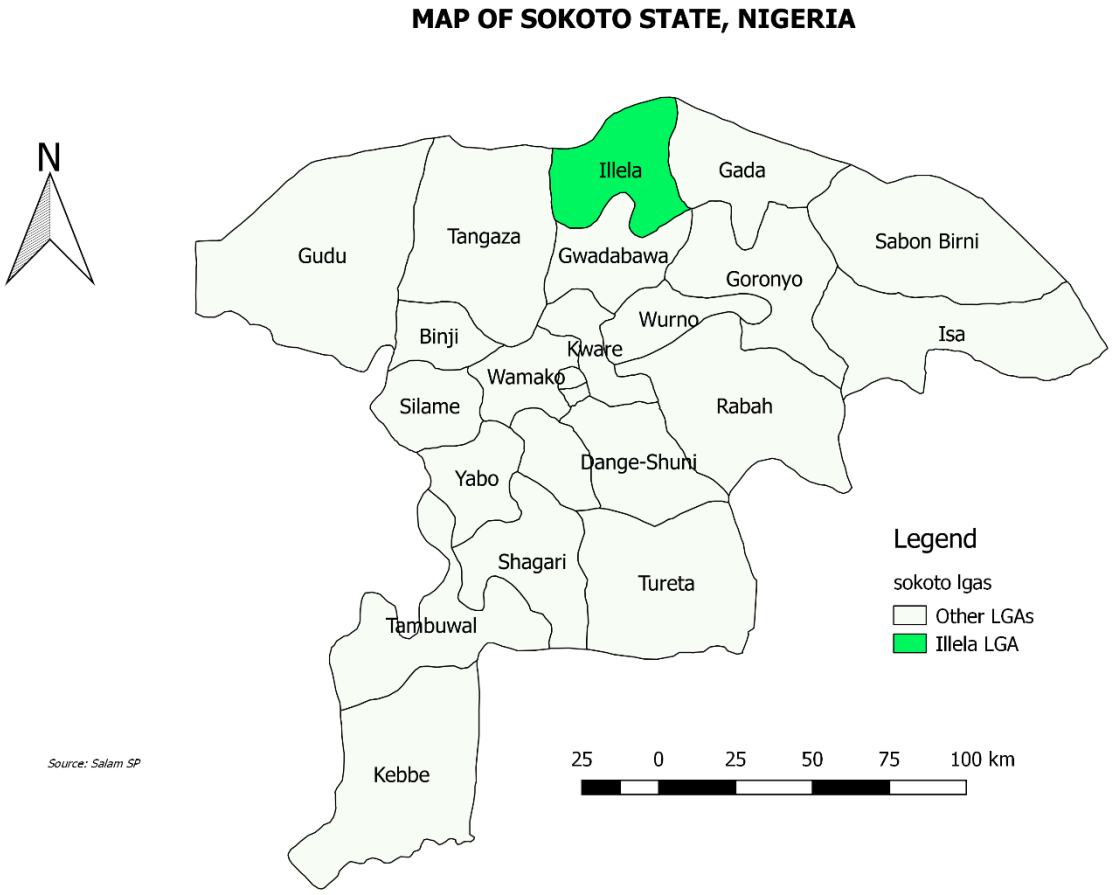


Figure 3.2: Map of Sokoto State showing Illela Local Government Area.

3.3 Sampling Method

A non-randomized sampling method (convenience sampling) was employed to select the camel batches and also human patients in the hospital. Camels from each batch were sampled based on the consent of the handlers and each identified to avoid repetition. The owner of each sampled batch decides the number of camels he would allow to be sampled.

3.4 Study Population

Herds of dromedary camels crossing into Nigeria at Illela international border post, herds of dromedary camels traded at Illela international market (Kara), consented camel handlers and patients in Illela General Hospital constituted the sampling population for the research.

3.5 Inclusion Criteria

The criteria used to select camels, camel handlers and patients in Illela General Hospital in this study included:

i. Consent from camel owners, handlers and patients in Illela General Hospital
ii. Availability of a reasonable number (≥ 66) of camels
iii. All age groups, sexes, apparently healthy and sick camels will be sampled
iv. Patients sampled in Illela General Hospital must house any animal.

3.6 Exclusion Criteria

The criteria used to exclude camels (n=74), camel handlers (n=40) and patients (n=39) in Illela General Hospital in this study included:

i. Batches of camels without consent.
ii. Patients in the hospital suspected to have diseases like HIV and Hepatitis.
iii. Pediatric and Geriatric cases were not sampled from the hospital.

3.7 Ethical Consideration and Consent

Ethical clearance was obtained before the study from the Ministry of Health, Sokoto State (Appendix I). After proper restrain by the camel handlers, 5-10mls of blood was collected from each camel and 2-3mls from humans.

3.8 Data Collection

A data form was made available to all consenting camel handlers and patients in Illela General Hospital. This was used to obtain information on the sources and population of camels and associated risk factors (sex, age, occupation, practice and the presence of camels in patients' houses) (Appendix II). Face to face interview method was employed while the interviewer filled the forms appropriately after which 74, 40 and 39 data forms were filled for camels, camel handlers and patients in the hospital respectively.

3.9 Sample Size Determination

The sample size for the dromedaries was determined by the formula described by Thrusfield, (2007).

$$n = \frac{z^2pq}{d^2}$$

Where:

n = Minimum sample size

z = Appropriate value for the standard normal deviation set at 95% confidence interval (1.96)

p = Prevalence (camels) 96% (Chu *et al*., 2015)

q = Complementary probability (1-p)

d = Level of significance 5%

This implies;

$$n = \frac{1.96^2\ x\ (prevalence\ x\ 1 - prevalence)}{(0.05)^2}$$

$$n = \frac{1.96^2 \, x \, (0.96 \, x \, 1 - 0.96)}{(0.05)^2}$$

n = 59.01.

With 10% non-response rate (Araoye, 2004), minimum sample size = 59.01/0.9

n = 66. However, 74 camel samples were collected to improve the chances of detection.

For humans: Sampling was purposive. Thirty-nine samples from outpatients were collected in Illela General Hospital.

3.10 Sample Collection and Processing

Samples were collected (Appendix V) and processed in the months of November and December 2016. The site for blood collection (jugular and cephalic/basilic vein for camels and humans respectively) was well swabbed using methylated spirit before blood collection with the aid of sterile needles and syringes (18G 10ml syringes and 21G 5ml syringes for camels and humans respectively). Blood was discharged into plain labeled sample bottles and allowed to clot by placing on a rack for 3-5 hours. The samples were stored on ice packs in an insulated box carrier while on transit to Usman Danfodio University, Sokoto Central laboratory in city campus where the sera were separated from the plasma by centrifugation at 2500RPM for 5min and gently decanted into complementary labeled serum vials (1ml). The sera were then transported to Zaria on ice packs in an insulated box, frozen for 6days at -20°C until it was time to run the ELISA test in the public health laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

3.11 Sample Analysis

The Anti-MERS S1 Igs (IgA/IgG/IgM) ELISA works based on the binding of antibodies in samples to the purified MERS antigen immobilized on the microwells. Bound antibody is detected by anti-IgG or IgM-

HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue) developed. Stop Solution is added to terminate the reaction, and absorbance (yellow color) is then measured using an ELISA reader at 450nm, which is directly proportional to the amount of antibody present in the sample. The presence of antibody (IgA/IgG/IgM) in samples is determined relative to anti-MERS S1 Ig's Calibrators and Controls.

Using the ELISA technique (ADI® Camel/Human Anti-MERS-S1 IgG Indirect kit), the MERS-CoV antibody titre of camels and human patients in Illela, Sokoto State of Nigeria was determined as described below:

3.11.1 Enzyme Linked Immuno-Sorbent Assay procedure (ELISA)

All steps were performed at room temperature, following the manufacturer's instructions. After each reagent (sample diluent, AntiMERS IgG-HRP conjugate and TMB substrate) is added, the plate was tapped gently to mix the well contents prior to beginning incubation.

Samples were diluted at 1:10 (20ul sample into 180ul of sample diluent).

About 100ul of diluted samples, calibrators and positive control each was added to pre-determined wells. The plate was tapped gently to mix reagents and incubated for 60 minutes after which wells were washed 4 times and pat dried on fresh paper towels. The next step involved 100ul of Anti-Camelid/Human IgG HRP added to each well and incubated for 30 minutes, this time the wells were washed 5 times after incubation. This was followed by 100ul TMB Substrate added to each well. The liquid in the wells turned blue and was followed with incubation for 15 minutes in a drawer. The last step involved adding 100ul of Stop Solution (diluted sulphuric acid) to each well. The wells were tapped gently to mix after which the liquid in the wells was observed to turn yellow (Appendix VIII).

Microplate ELISA reader (Appendix IV) of 450nm wavelength was used to read the Optical density (OD) and absorbance of the entire plate using a single wavelength within 5 minutes after Stop Solution addition. Samples were then compared to the 1U/ml Calibrator = Positive/Negative Cut-off which was used to calculate (Appendix V) the threshold index that numerically discriminates Positives from Negatives: Each Sample OD was divided by the 1U/ml calibrator OD and values obtained above the 1U/ml value were a measure of positive antibody activity; below was negative for antibody. A 'Cut-off' line was drawn to indicate a threshold distinguishing between Positive/Negative.

3.12 Data Analyses

The data obtained from serology and data forms were entered into a spreadsheet and analyzed using SPSS (version 20.0) and Microsoft Excel 2010.

Counts and frequencies for variables were presented using graphs and tables, and then summarized using percentages.

Prevalence of MERS-CoV in camels and patients in the secondary care hospital were calculated using the formula below;

$$P = \frac{Number\ Positive}{Number\ Sampled}$$

Sampling points, sources and destinations of camels were represented by spot maps. STATA® 14 was used to calculate 95% Confidence Interval for prevalence respectively. Association between MERS-CoV and categorical variables were analyzed using Chi-square (χ^2) test. Where significant association was observed, odds ratio were calculated to determine the magnitude and direction of the association. Values of $P \leq 0.05$ were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Information Analyses on Camels

Dromedary camels were sampled at Illela international border and market, in which 32 (43%) of sampled camels were at the border post while 42 (57%) were at the international market. At both sampling points, 40 (54%) of these camels were transported on hoof while 34 (46%) by vehicle.

The sampled camels were sourced from Mali (n=4, 5.4%), Algeria (n=6, 8.1%), Gada local government (n=8, 10.8%) and Niger Republic (n=56, 75.7%) (Figure 4.1). Figure 4.2 illustrates the number of sampled camel(s) that were to be transported to various destinations from the sampling points; Benue 1 (1.4%), Ilorin 1 (1.4%), Illela abattoir 1 (1.4%), Gwadabawa 2 (2.7%), Lagos 3 (4.1%), Gusau 5 (6.8%), Birnin Kebbi 7 (9.5%), Sokoto town 25 (33.8%) and Illela international market 29 (39.2%), and there were 27 batches of camels sampled in which the number of camels/batch were in the frequencies; 1 (1), 3 (8), 5 (8), 6 (5), 7 (8), 8 (4), 9 (1), 11 (3), 12 (1), 13 (1), 17 (3), 19 (2), 20 (1), 23 (14) and 32 (14) (Figure 4.3).

Species co-transported with the various batches of sampled camels (Figure 4.4) in combination were; cattle and sheep 1 (1.4%), goats and sheep 1 (1.4%), goats, sheep and dogs 2 (2.7%), cattle alone 4 (5.4%), donkeys alone 4 (5.4%), sheep alone 5 (6.8%), cattle and donkeys 14 (18.9%) and those batches that were strictly camels were 43 (58.1%). Among the sampled camels, adult males were 52 in number, adult females 10, young males 8 and young females 4 (Figure 4.5).

The positive control (validation) and all the samples had a threshold index value greater than the cut-off which discriminates them as positives (Figure 4.6).

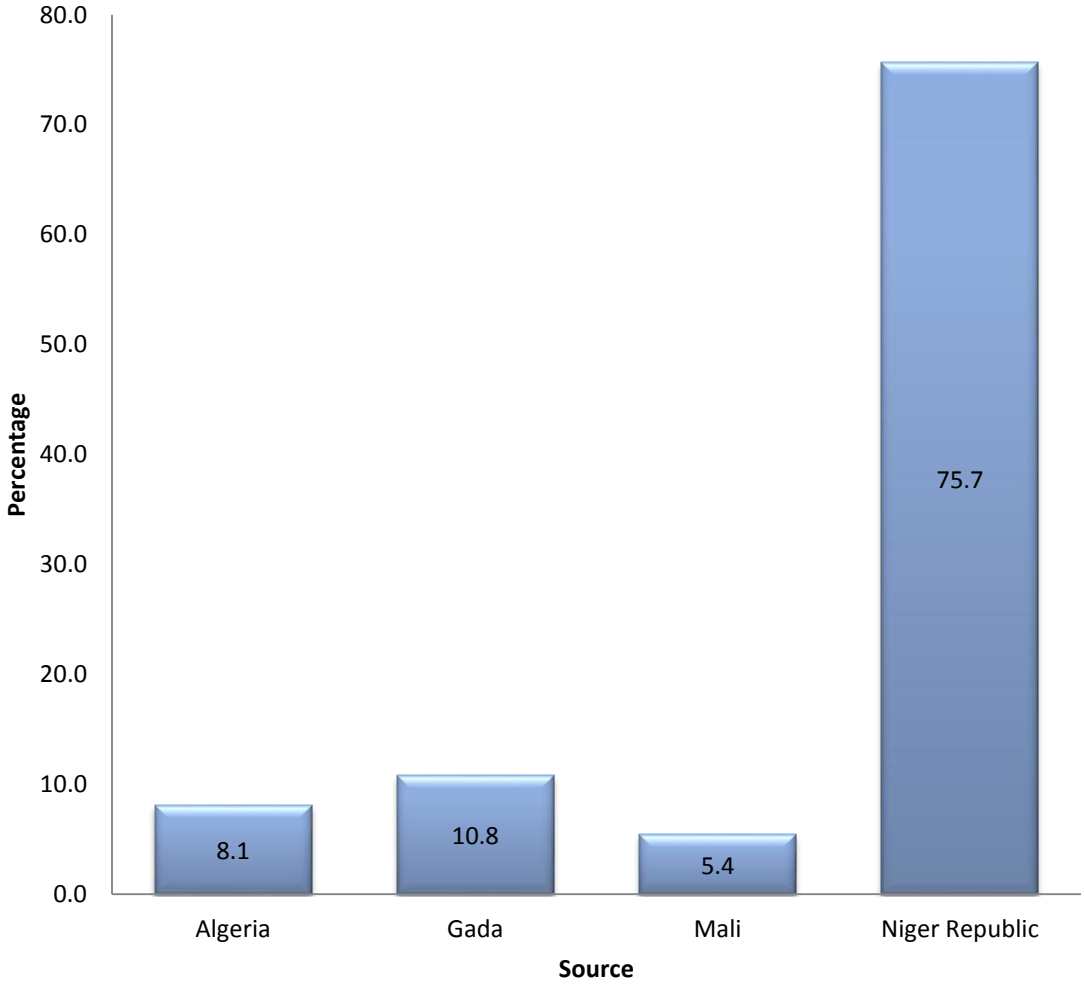


Figure 4.1: Sources of camels sampled in Illela, Sokoto State.

Note: Gada is a local government area in Sokoto State, Nigeria

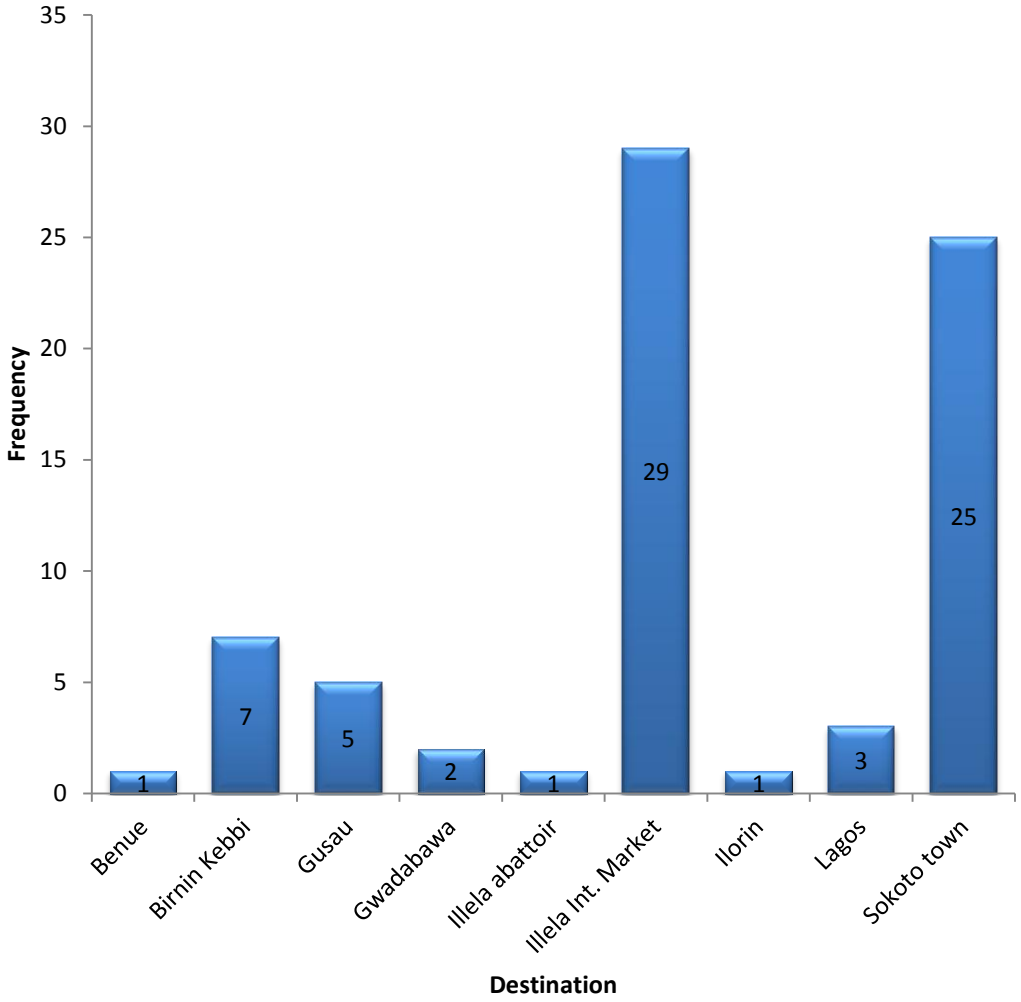


Figure 4.2: Final destinations of camels sampled as expressed by handlers in Illela, Sokoto State.

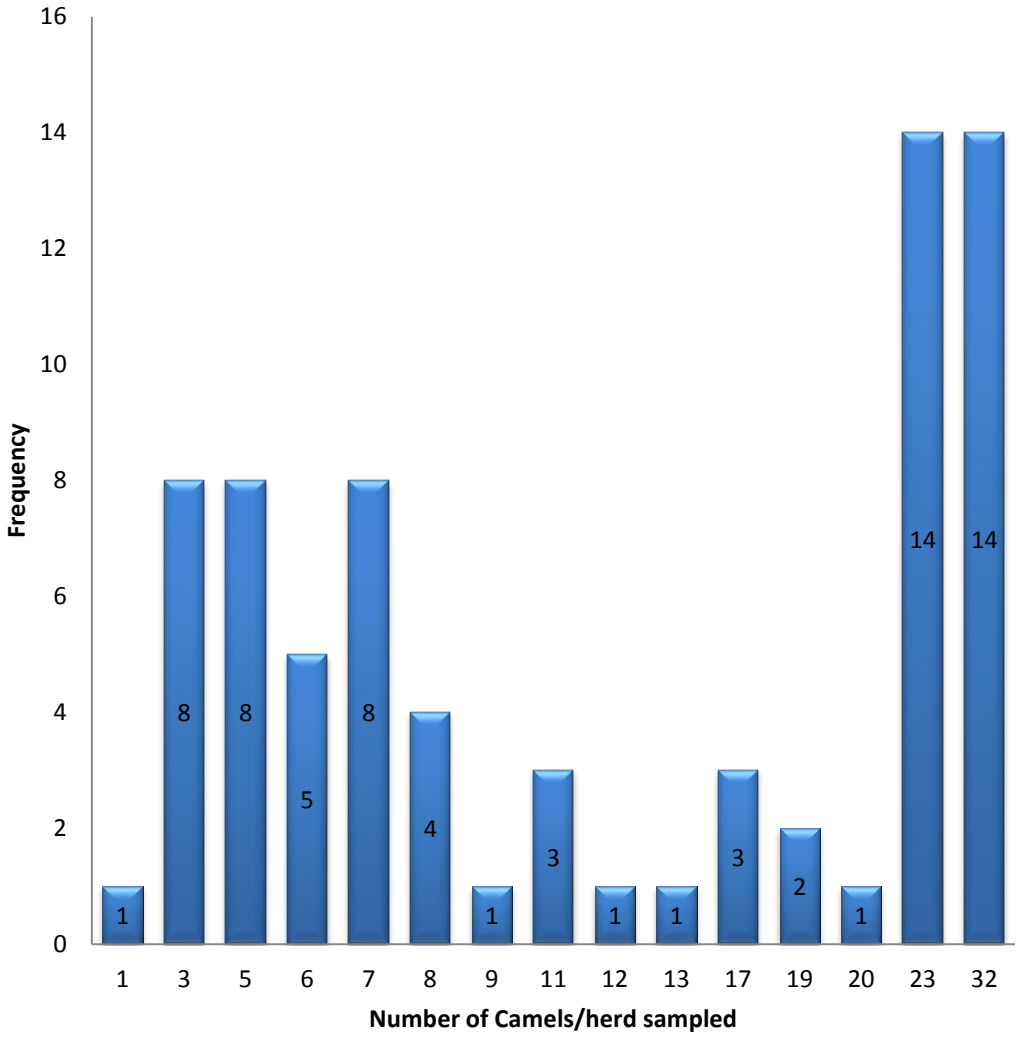


Figure 4.3: Frequency of camels/batch sampled during the study period in Illela, Sokoto State.

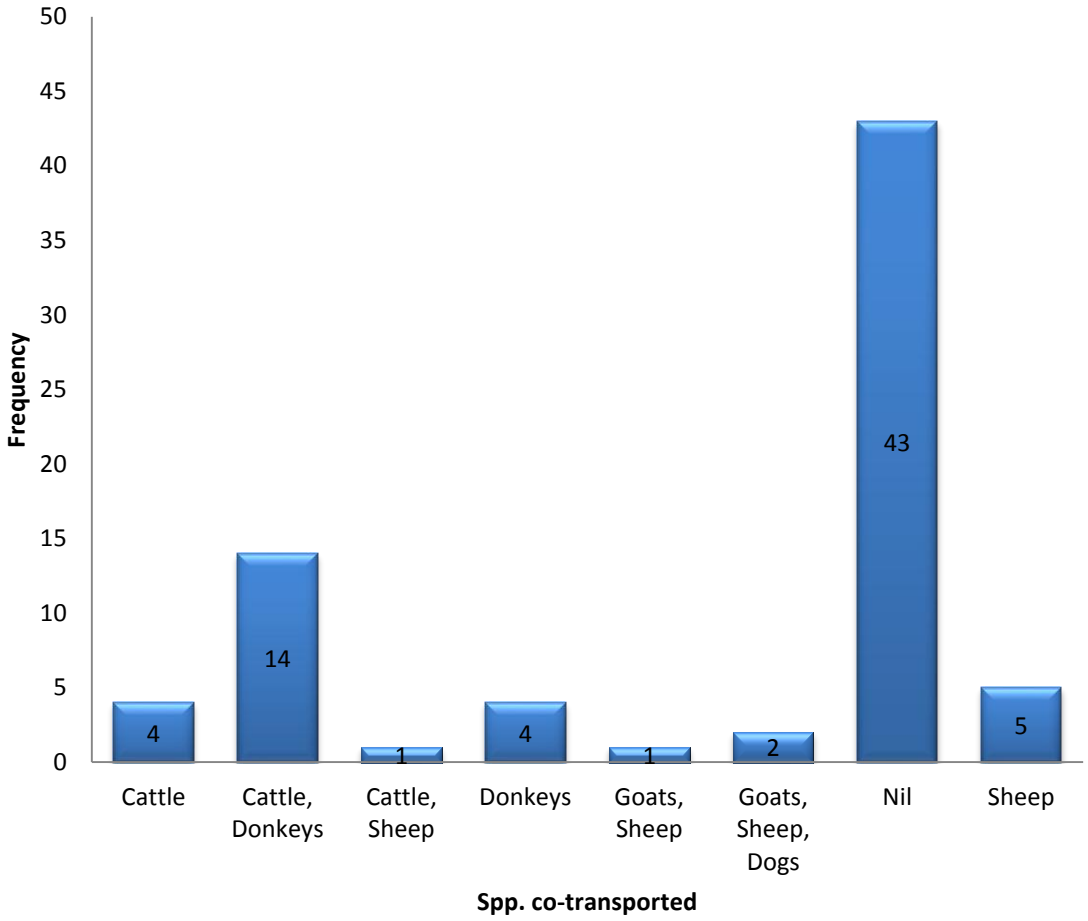


Figure 4.4: The different species of animals transported with camels during the study in Illela, Sokoto State.

Note: Nil = Camels only

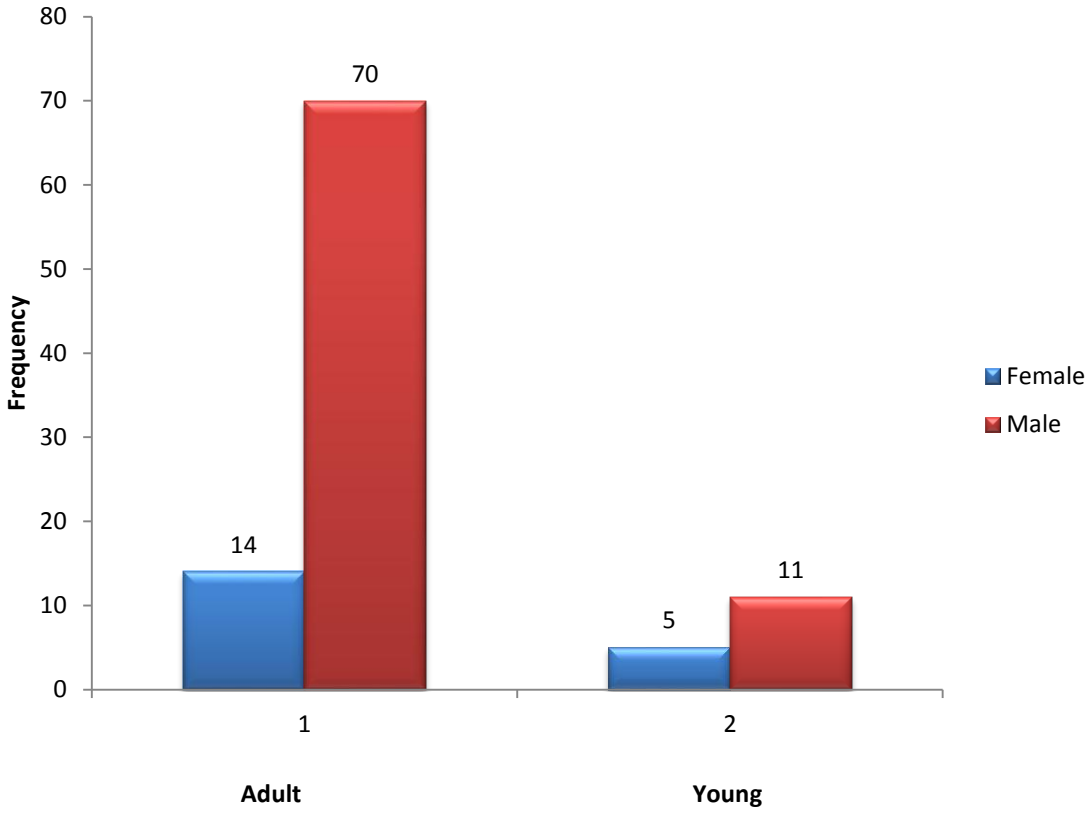


Figure 4.5: Population of camels sampled by their sex and age at Illela International border and market, Sokoto State.

Note: Adult = > 1year old, Young = < 1year old

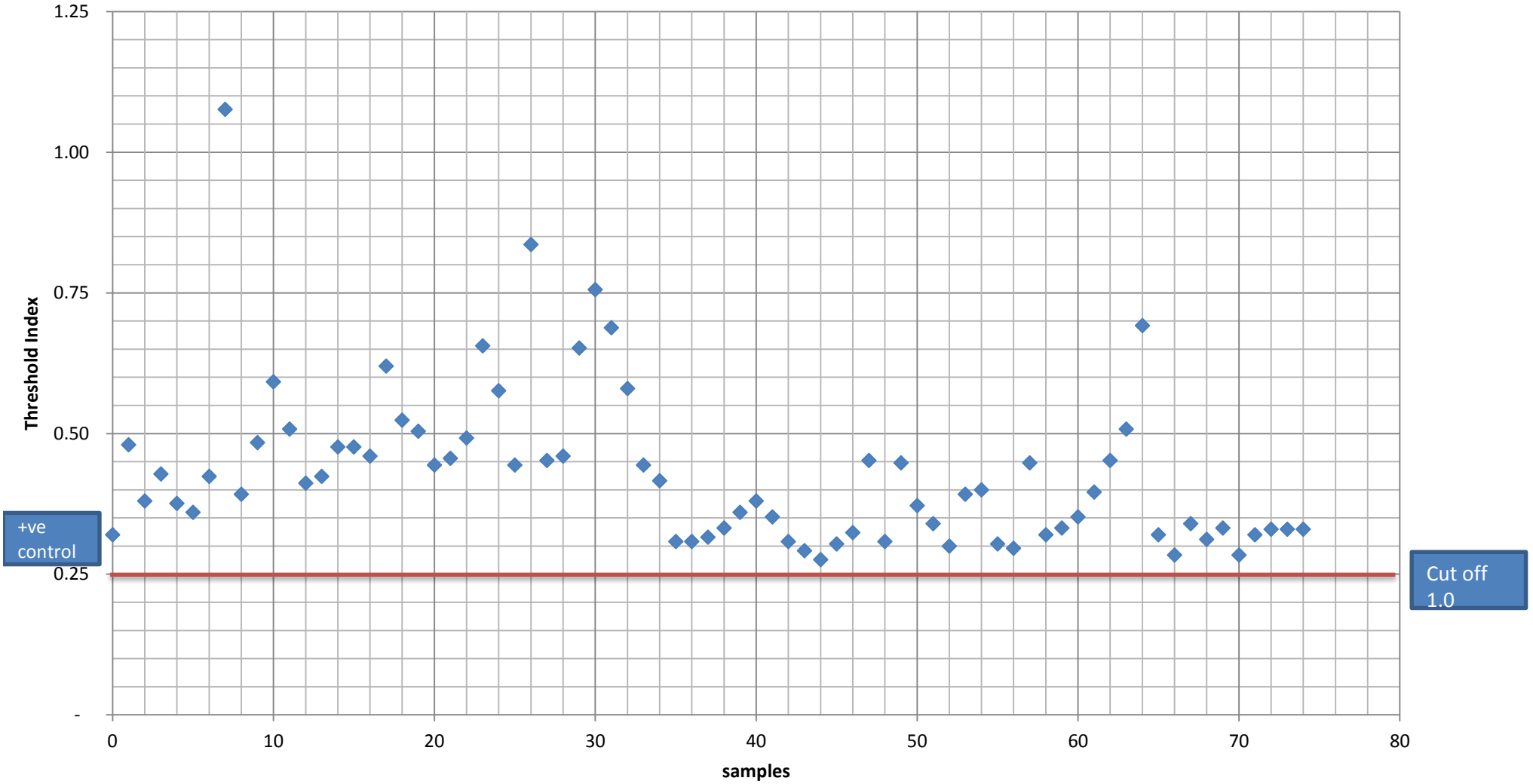


Figure 4.6: Threshold index for positive control and camel samples from Illela, Sokoto State.

4.1.1 Prevalence of MERS-CoV in sampled camels

All the camels tested were positive for MERS-CoV antibody. Each sample was calculated in relation to 1U/ml calibrator which had an optical density (OD) of 0.25 (Appendix V) and served as the cut-off as indicated by the manufacturer (ADI®) (Figure 4.6). All camel sera tested had threshold values greater than 0.25 and therefore were positive (Figure 4.6).

4.1.2 Spot maps presentation of sampling point, source and destination of camels obtained during the study in Illela, Sokoto State.

Plate I, II and III illustrates the Sampling points (Illela international border and market) (yellow spots), Sources (Algeria, Mali, Republic of Niger and Gada) (green spots) and destinations (Benue, Kebbi, Zamfara, Gwadabawa, Kwara, Lagos, Sokoto town, Illela abattoir and Illela market) (red spots) of sampled camels.

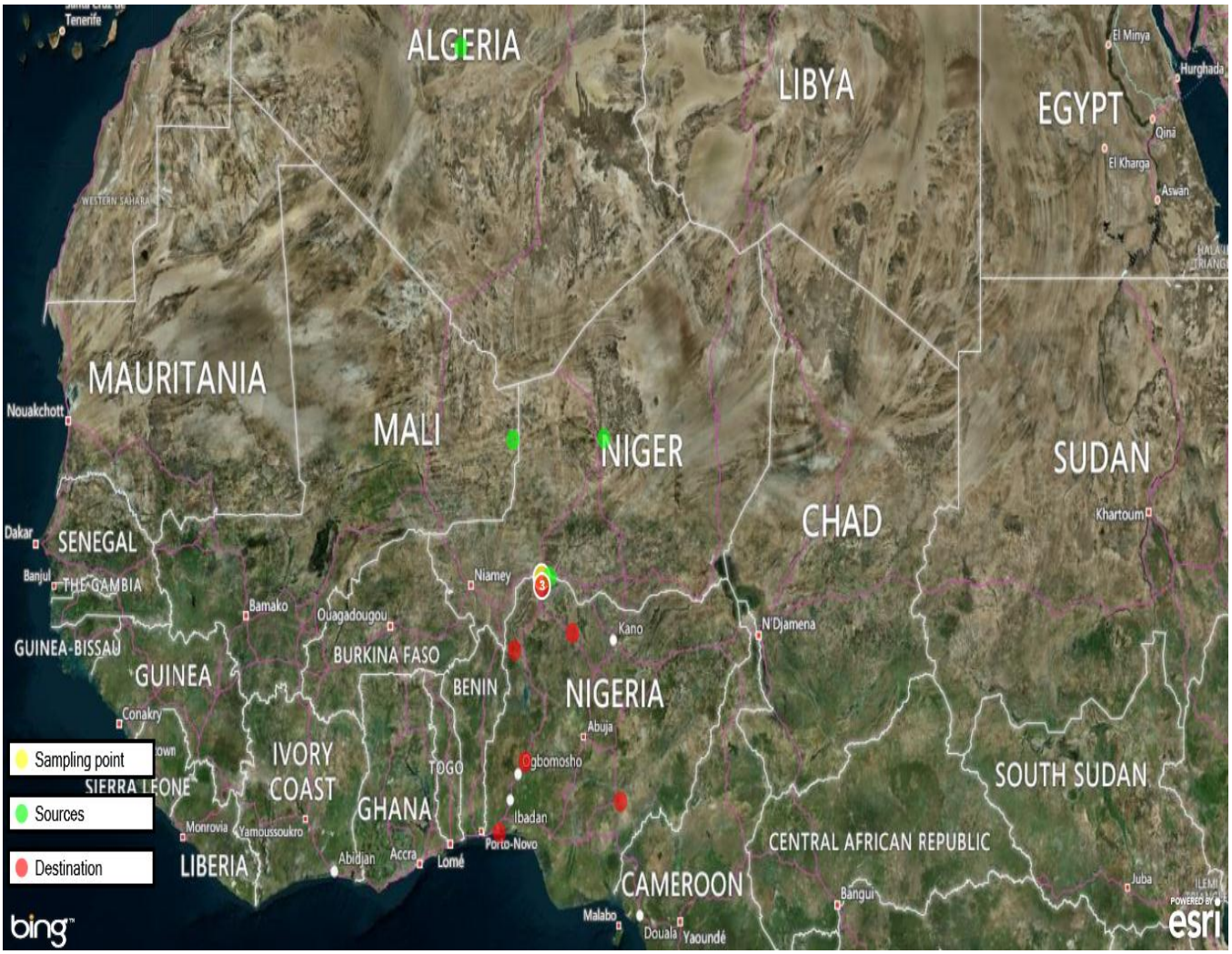


Plate I: An Overview of the Sampling points, Sources and Destinations of Camels Sampled in Illela, Sokoto State.



Plate II: An Overview of the Sampling Points and Destinations of Camels Sampled in Illela, Sokoto State.

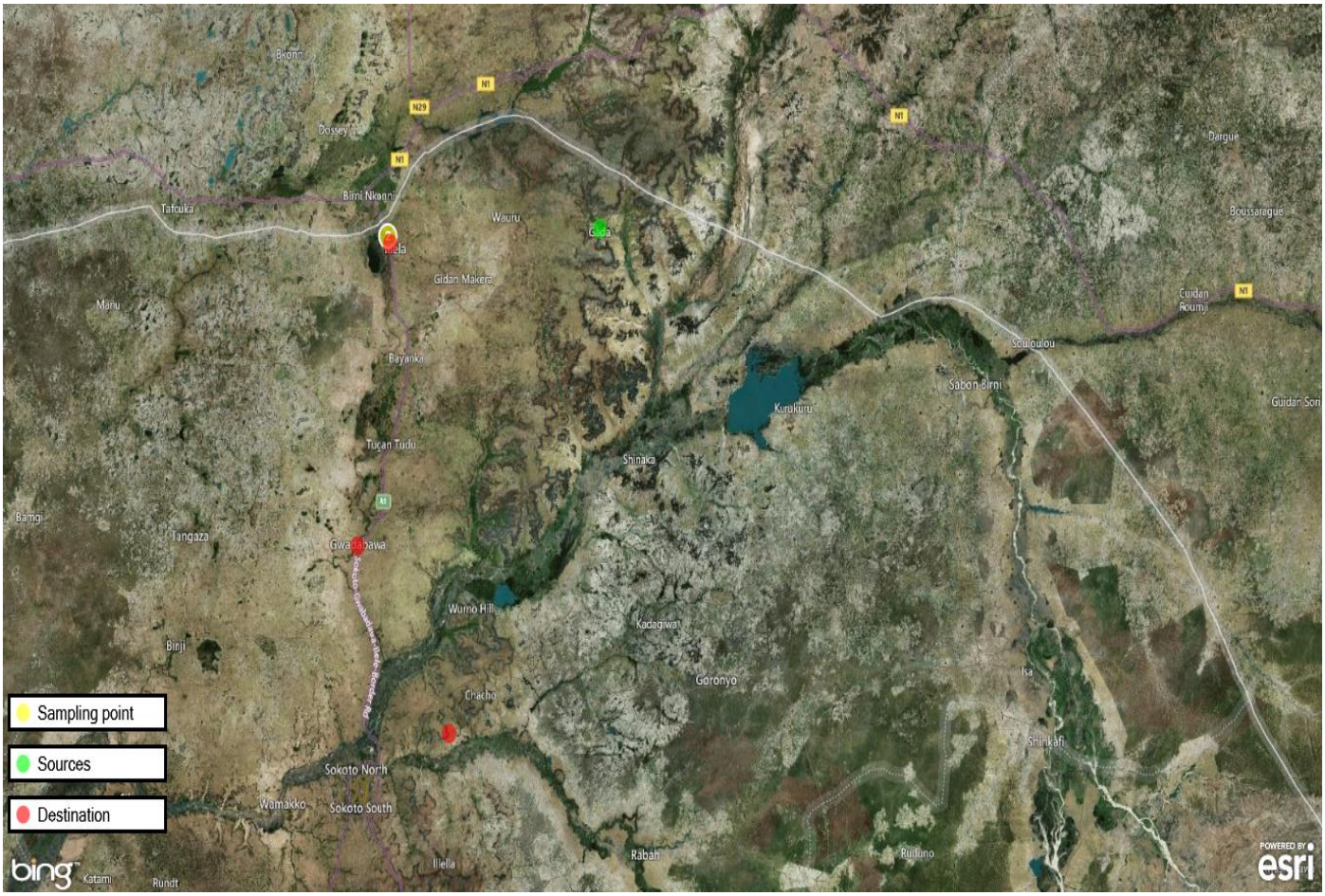


Plate III: A zoomed portion of the Sampling points, Source and Destinations (within Sokoto State) of camels sampled in Illela.

4.2 Analyses of Camel Handlers from Data Form

The response from the camel handlers was obtained concurrently with the camel data in November, 2016 (Appendix I).

4.2.1 Characteristics of handlers accompanying sampled camels

All the camel handlers from sampled camels were males (100%) and their categorized ages from the highest to the lowest frequency were 31-40 years (48%), 20-30 years (33%), >40 years (12%) and <19 years (7%) (Figure 4.7). Camel handlers/batch of sampled camels were in the frequencies; 2 (15) (highest), 1 (8), 3 (7), 7 (6), 6 (2) and 5 (2) (Figure 4.8). The handlers had different intensity of commitment to their trade in which 26 (65%) were full time handlers while 14 (35%) were part time handlers.

Data on trade practices was collected and 2 (5%) of the handlers had been practicing camel trade for <1year, 12 (30%) had been in the practice for >10years while 26 (65%) had been in the practice for 1-10years (Table 4.1). Handlers that rather treat their camels by self were 40 (100%) and same percentage would do nothing to their hands after handling camels. The respiratory sign observed by handlers in their sampled camels was coughing (n=4; 10%) while 36 (90%) noticed no respiratory signs in the past 2weeks before the study. Likewise, observed gastrointestinal sign by handlers in their camels was 13 (32.5%) for diarrhoea while 27 (67.5%) did not notice any gastrointestinal signs in the past 2weeks before the study (Table 4.1).

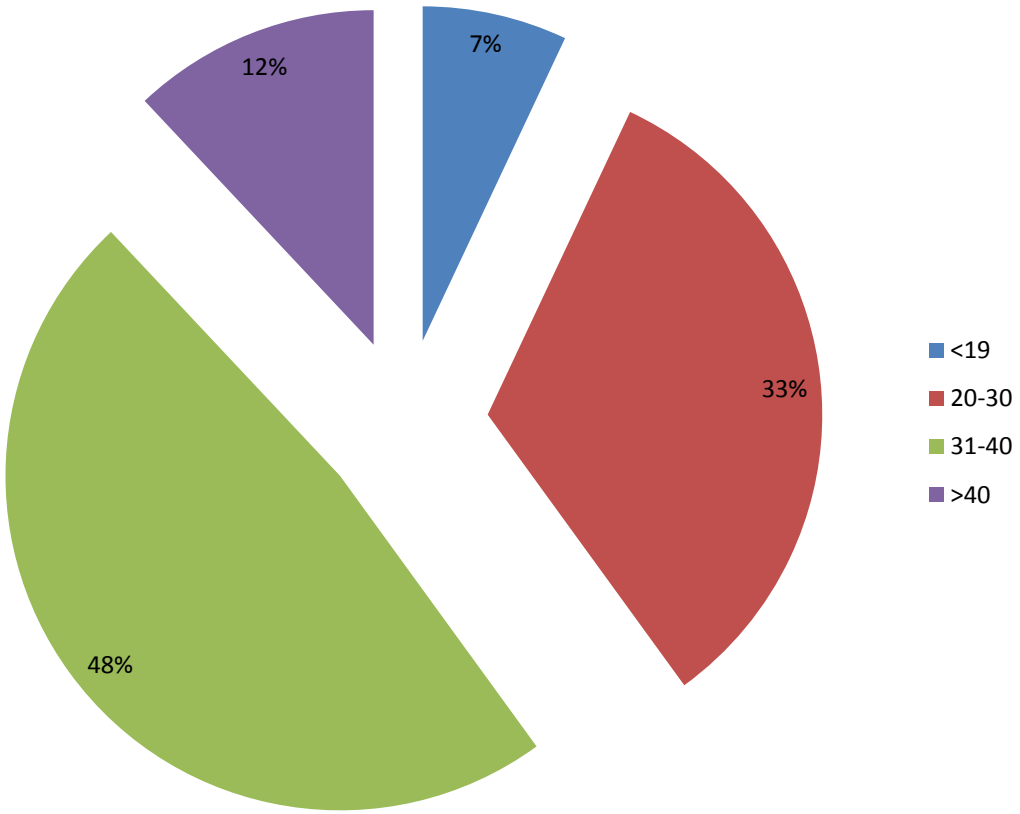


Figure 4.7: Categorized ages of camel handlers in the period of this study at Illela, Sokoto State.

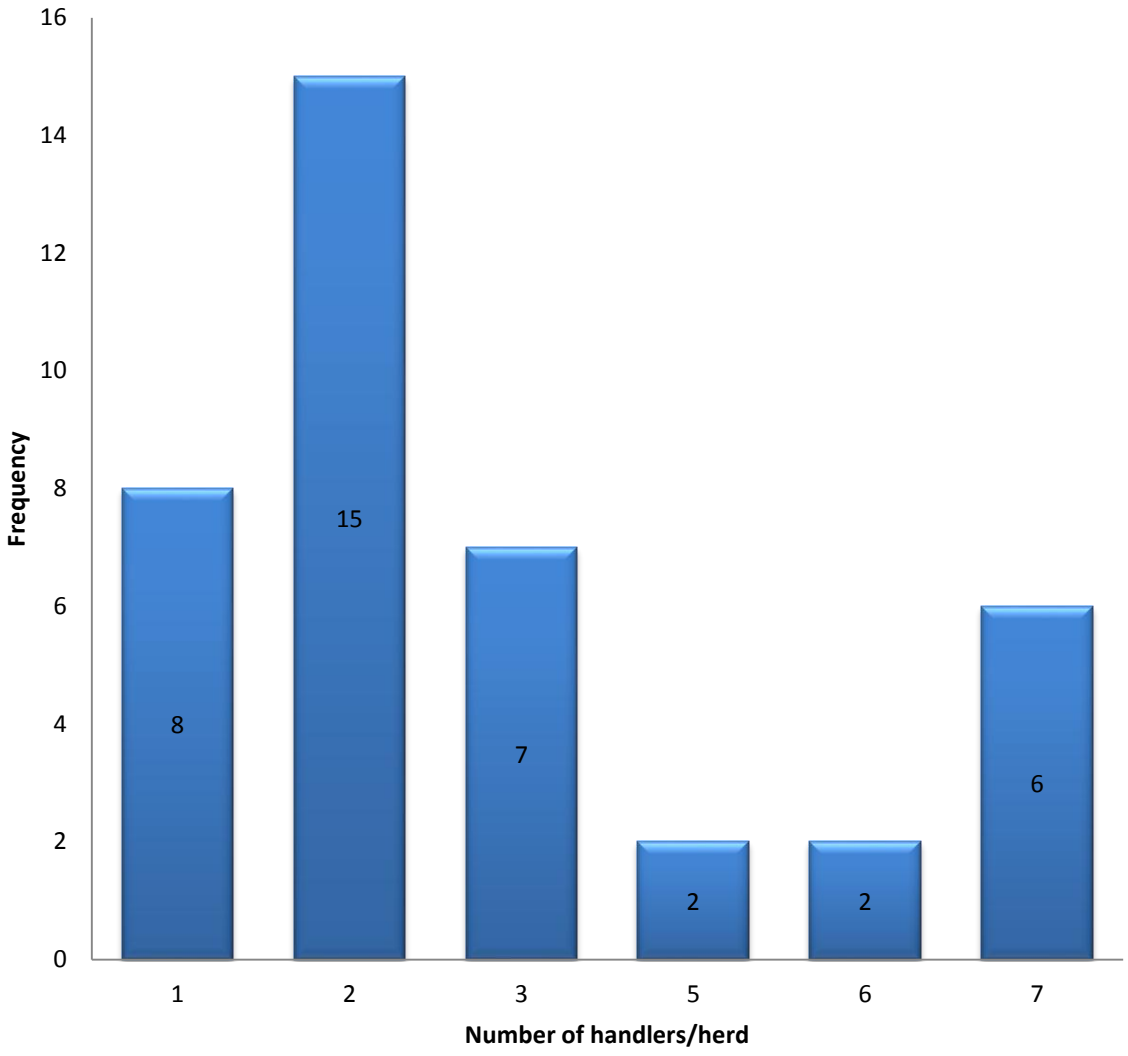


Figure 4.8: Camel handlers/batch of camels during the study at Illela, Sokoto State.

Table 4.1: Assessment of Camel Handlers Practices and Knowledge of Respiratory and Gastrointestinal Signs of Disease in their camel-herd in Illela, Sokoto State

Practice/Knowledge	Outcome	Percentage (%)
How long have you been practicing this trade?	<1yr	5
	1-10yrs	65
	>10yrs	30
How do you treat sick camels?	Self	100
What do you do after handling camels?	Nothing	100
Respiratory signs observed in the past 2weeks	Coughing	10
Gastrointestinal signs observed in the past 2weeks	Diarrhoea	32.5

4.3 Result of Patients Sampled in Illela General Hospital, Sokoto State.

A total of 39 patients were sampled in November, 2016 and they were coded G1 – G39 (Appendix V).

4.3.1 Demographic characteristics from data form of respondents in Illela General Hospital

Twenty-six (67%) respondents were males while 13 (33%) were females. Based on age categories, 48.72% were between the ages of 20-30yrs, 33.3% were between 31-40yrs, 15.38% were above 40yrs of age and only 1 (2.56%) was less than 19yrs.

Animal contacts with outpatient respondents at Illela varied based on species of animals (Figure 4.9): Camels alone (1), Camels and Cattle (1), Camels, Cattle and Donkeys (1), Camels and Dogs (1), Camels and Donkeys (5), Camels and Sheep (1), Cattle alone (3), Cattle and Chickens (1), Cattle and Donkeys (1), Cattle, Goats and Chickens (1), Cattle, Goats and Sheep (1), Cattle and Sheep (1), Chickens alone (3), Donkeys alone (5), Goats alone (3), Goats and Chickens (1), Goats and Sheep (3), Sheep (2) and Sheep and Chickens (2). Therefore, frequencies of species of animals found in sampled patient's household were; Donkeys (13), Cattle (11), Goats (11), Sheep (11), Camels (10), Chickens (9) and Dogs (1) (Figure 4.10).

Distribution of the MERS-CoV ELISA antibody resulted in 3 categories; the Indeterminate/Invalid 1 (3%), Negative 9 (23%) and Positive 29 (74%) group (Figure 4.11), these groups in relation to the animals they housed had Camels 8, 1 and 1, Donkeys 10, 3 and 0, Cattle 6, 5 and 0, Goats 8, 3, and 0, Sheep 9, 2 and 0, Dogs 1, 0 and 0 and Chickens 6, 3 and 0 for positive, negative and invalid respectively in each species (Figure 4.12).

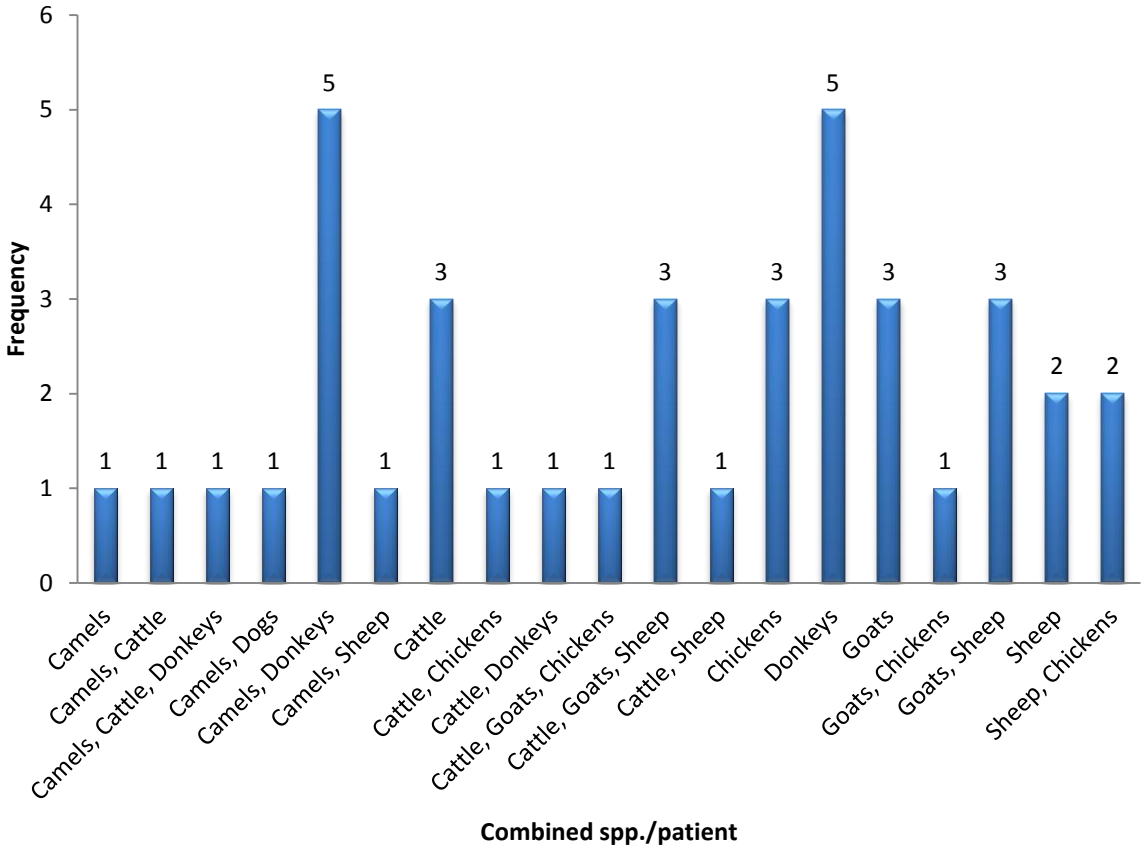


Figure 4.9: Species of animals in contact with patients sampled during the study in Illela General Hospital, Sokoto State.

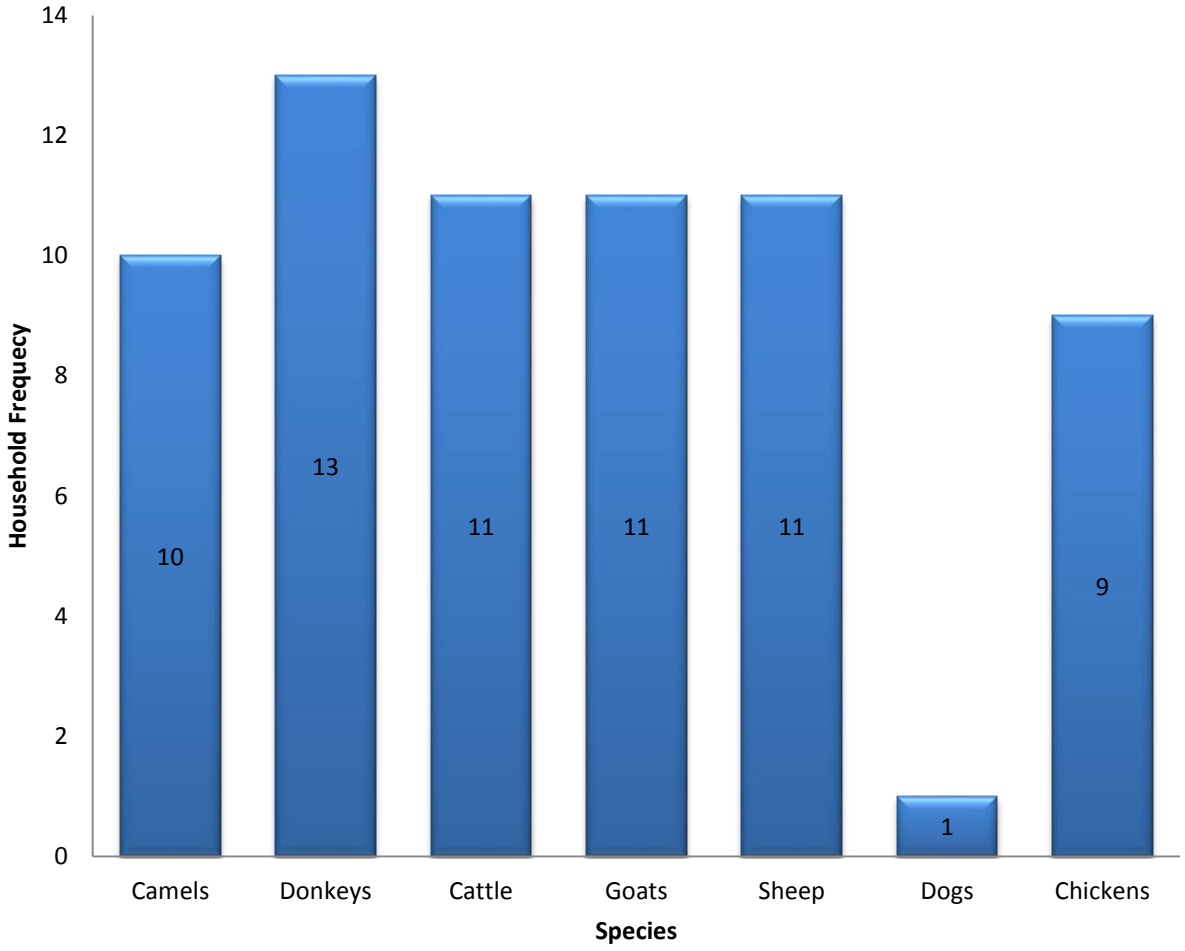


Figure 4.10: Distribution of animal species in patients' sampled household at Illela General Hospital.

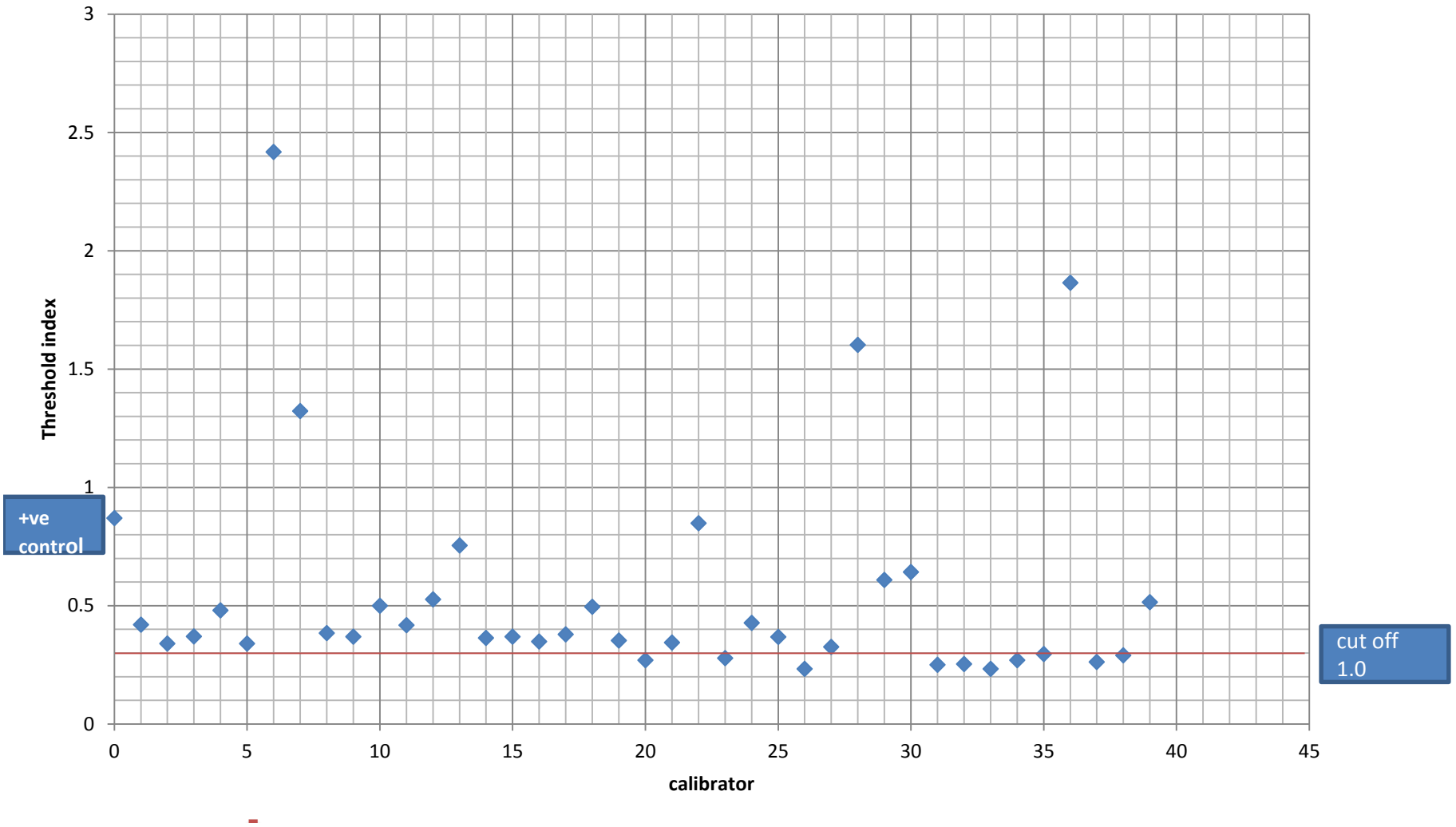


Figure 4.11: Threshold index for positive control and patients' sera from Illela General Hospital, Sokoto State.

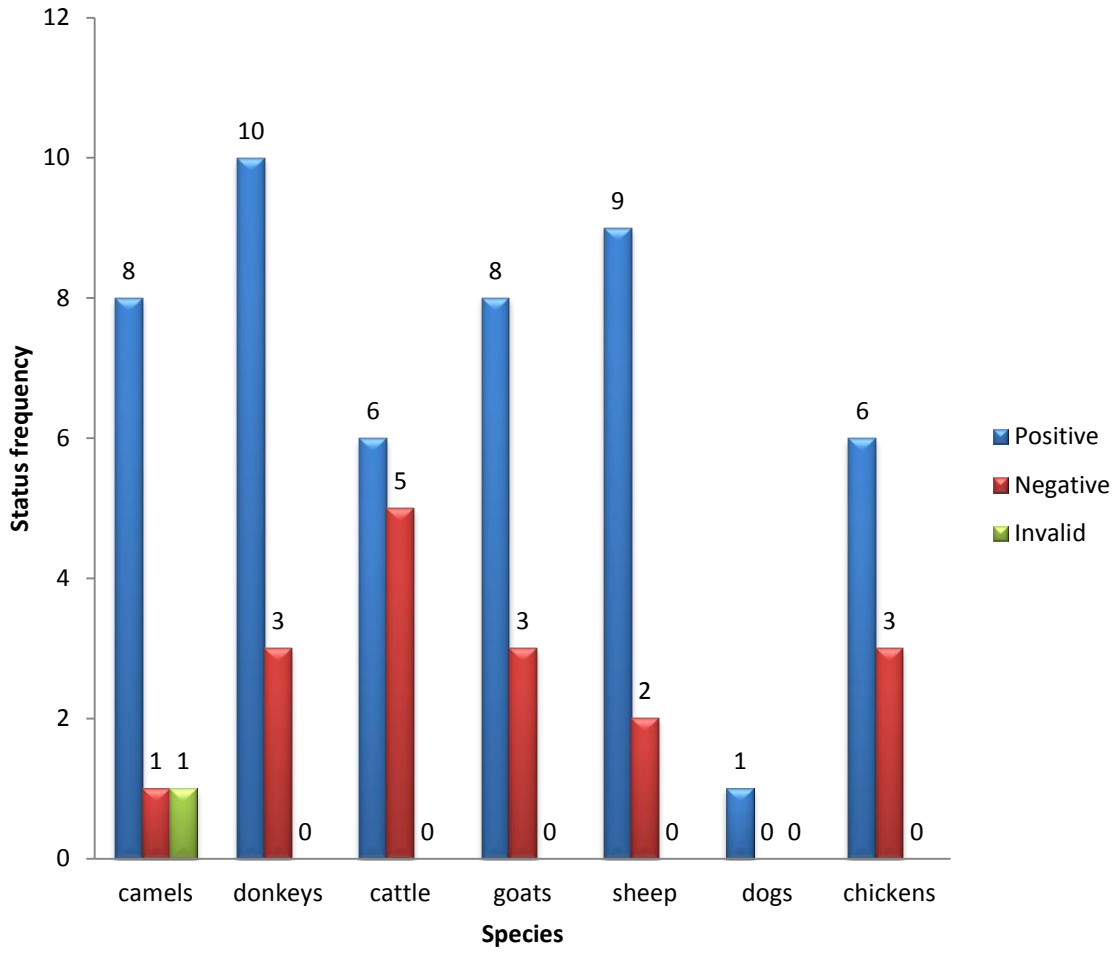


Figure 4.12: Antibody status of sampled patients in relation to the animal species they house.

4.3.2 Assessment of MERS-CoV in sampled patients at Illela General Hospital, Sokoto State

The prevalence of MERS-CoV (74.4%) in sampled patients at the selected secondary care hospital had a confidence interval of 0.5787261 – 0.8696232 at 95% confidence limit using STATA® 14. This was significant.

4.3.3 Assessment of distribution by status of the respondents in Illela General Hospital

The distribution of status by occupation of sampled patients had Civil servants (1, 3 and 0), Farmers (4, 0 and 0), Housewives (7, 2 and 0), Paramilitary (1, 0 and 0), Students (7, 2 and 0) and Traders (9, 2 and 1) for positive, negative and invalid group respectively. The categorized ages of the patients in relation to their status were <19 (1, 0 and 0), 20-30 (14, 4 and 1), 31-40 (9, 4 and 0) and >40 (5, 1 and 0) for positive, negative and invalid group respectively. Male patients were (19, 6 and 1) while the females (10, 3 and 0) for positive, negative and invalid groups respectively (Table 4.2).

Table 4.2: Demographic distribution of ELISA positive patients from Illela General Hospital, Sokoto State, Nigeria

Demographic Variable	Total No. sampled	Invalid	Negative	Positive
Occupation				
Civil Servant	4	0	3	1
Farmer	4	0	0	4
Housewife	9	0	2	7
Paramilitary	1	0	0	1
Student	9	0	2	7
Trader	12	1	2	9
Age				
<19	1	0	0	1
20-30	19	1	4	14
31-40	13	0	4	9
>40	6	0	1	5
Sex				
Female	13	0	3	10
Male	26	1	6	19

The different categories of age groups of patients in the hospital were compared with the ELISA test results, odds ratio showed the strength of association and were obtained as follows; > 40years with 20-30years (OR=2.969; 95% CI 0.136, 64.962) and > 40years with 31-40years (OR=2.374; 95% CI 0.226, 24.967). Hence, compared to > 40years, there was no significant difference. From the assessment of occupation of patients, Traders with Civil servants (OR=0.078; 95% CI 0.004, 1.428), Traders with Housewives (OR=0.979; 95% CI 0.090, 10.675) and Traders with Students (OR=0.510; 95% CI 0.044, 5.948). There was no significant difference compared with Traders (Table 4.3). Patients' antibody status whose collective percentage does not add up to 100% had invalid (Indeterminate) counts and Age (<19yrs), Occupation (Farmer and Paramilitary) had '0' values, hence OR could not be analyzed for such data (Table 4.3).

Table 4.3: Assessment of Status based on some demographic variables of the Patient in Illela General Hospital

Demographic Variables	Positive (%)	Negative (%)	df	p-value	OR (95% CI on OR)
Age			3	0.855	
20-30	14(73.7)	4(21.1)			2.969 (0.136 – 64.962)
31-40	9(69.2)	4(30.8)			2.374 (0.226 – 24.967)
>40	5(83.3)	1(16.7)			
Occupation			1	0.615	
Civil Servant	1(25)	3(75)			0.078 (0.004 – 1.428)
Housewife	7(77.8)	2(22.2)			0.979 (0.090-10.675)
Student	7(77.8)	2(22.2)			0.510(0.044 – 5.948)
Trader	9(75)	2(16.7)			

CHAPTER FIVE

5.0 DISCUSSION

All camels sampled were positive for MERS-CoV antibody. It could be that all the camels sampled have come in contact with an infected host sometime in their life. This result might also suggest a cross reactivity between MERS-CoV and other coronaviruses as earlier observed by Perera *et al*. (2013). The high seroprevalence obtained from dromedaries during this study may be due to the porous border at Illela where no screening is undertaken for MERS-CoV, no proper diagnostic facilities and no animal health trained personnel Illela International Market to ascertain the health status of traded animals. This result is in agreement with the study conducted in Kano abattoir where daily, the seroprevalence of MERS-CoV ranged from 82% to 100% giving an overall seroprevalence of 96% (Chu *et al*., 2015). It also agrees with the result of Reusken *et al*. (2014), who obtained a high (94%) seroprevalence in stored serum samples collected in Adamawa, Borno, Kano and Sokoto States originally for the purpose of peste-des-petits ruminants (PPR) virus survey. Bennett *et al*. (2014), sampled 358 dromedary camels in Nigeria and almost all were positive for MERS-CoV antibody. In view of past infections, close persistent contact resulting in MERS-CoV transmission between animals and humans that are susceptible will increase the number of secondary cases (Martins, 2015; Gompf *et al*., 2016).

In other studies, outside Nigeria; high seropositivity rate was obtained in Morocco camels (100%), Ethiopia camels (99.4%) and Burkina Faso camels (84.6%) (Miguel *et al*., 2015). Bennett, (2014), documented 100% sero-prevalence of MERS-CoV antibody in camels from a southern province of Tunisia. As early as 2013, Saudi Arabia reported 90% seroprevalence in their camels after the index case (Hemida *et al*., 2013). Also another serological survey carried out on Omani camels had protein-specific antibodies (100%) against the MERS-CoV spike protein (Reusken *et al*., 2013).

Majority (74%) of the hospital patients sampled were positive for MERS-CoV antibody (Figure 4.11). This high percentage may be due to areas where patients are found crowded like the emergency department, inpatient areas and outpatient areas which increased the number of secondary cases from a primary infection. This result might also be explained considering MERS as an emerging zoonoses; hence, serological tests are not being conducted in hospitals to separate suspected individuals, prevent patient crowding and inconsistent use of infection control precautions (Hastings *et al*., 2014), this would encourage spread of MERS-CoV should there be introduction of an infected individual. A hospital related outbreak was reported in Riyadh (CDC, 2015) and more than half of all MERS secondary cases have been associated with health-care settings (WHO, 2014c; WHO, 2015a; Assiri, 2013a).

Prevalence in human patients did not significantly differ by age (P= 0.855), sex (P= 0.949) and occupation (P= 0.615) in this study and this is in agreement with a study in camels from Kenya where similar observations were made (Kamau *et al*., 2015). However, the result was 100% (n=4) positive for farmers as may reflect a relationship with occupational diseases when these farmers use animals for traction (Chantel *et al*., 2015). The` high prevalence in humans which is statistically significant (95% CI: 0.5787261-0.8696232) can possibly depict an ongoing non-pathogenic (non-virulent) strain of the virus circulating in the study area (WHO, 2015d).

The virus does not appear to pass easily from person to person unless there is close contact such as providing clinical care to an infected patient while not applying strict hygiene measures. This has been seen among family members, patients, and health care workers. The majority of cases have resulted from human-to-human transmission in health care settings. Infected persons with no symptoms have been found because they were tested for MERS-CoV during follow-up studies of contacts of people with MERS infection (Aleccia, 2014; WHO, 2015d). However, there have been no human cases of MERS diagnosed in Sokoto State and Africa at large. This could be because of limited clinical or epidemiological

surveillance for the virus where infections have gone unrecognized (Eric and Joerg, 2015). It is not known if the MERS-CoV in Nigeria has similar zoonotic potential like viruses currently circulating in the Arabian Peninsula. Other explanations for the lack of zoonotic MERS in Africa include differences in patterns of exposure to infected animals or animal products or differences in human susceptibility to MERS-CoV (Chu *et al*., 2015).

Nigeria has an extensive land border with Niger Republic where it extends from Kamba LGA in Kebbi State through Sokoto, Katsina, Jigawa, Yobe States and ends at Damasak town in Borno State. Most of the camels sampled in this study were brought from Niger Republic; this may be due to the extensive and porous border land shared between Nigeria and Niger Republic. The later serves as the route through which camels from Algeria and Mali are brought into Nigeria. This is in agreement with Chu *et al*. (2015), in a study in Kano abattoir where they reported Niger Republic and Mali among sources of slaughtered camels. Camels brought from Gada LGA were probably also sourced from Niger Republic since it shares a porous border with this country to its North. Convergence of camels in the market from the border and other sources if infected with MERS-CoV can be a source of spread to other camels and traders.

There were few female camels (n=14, 19%) sampled in this study because they were fewer in trade probably owing to reproductive reasons of breeding more offspring. Camel calving season occurs in the harmattan (between late October and late February); this may have also influenced the female population on transit in this study since the sampling occurred in November. The camel breeding season is also said to be a time when there is increased risk to humans of spill-over infections due to new infections among the handlers (Mackay and Arden, 2015). Most traded females for slaughter are either too old or females that have reproductive issues (Kamau *et al*., 2015).

All the camel handlers that responded to our data forms were males mostly between the ages of 31 and 40yrs (48%). The stressful nature of camel trade could have been discouraging for females to adopt. This age category could be because they have settled down over the years to make camel trade their only business. However, ages ≤19yrs (7%, n = 3) were the least compared to ages >40yrs (12%, n = 5) and 20 to 30yrs (33%, n = 13). This could be due to the danger camel handling pose as they are very aggressive and could kill humans when wrongly handled. Camel handling thus requires maturity and experience. A larger proportion of the respondents were solely camel traders (65%) while 35% were into it as a part time trade alongside other businesses. Camel trade is seasonal and so when not fully operational; some handlers may find alternative means to feed themselves.

Most respondents handling camels have been in the practice for 10yrs (65%, n = 26). This may be an indicator that the handlers and their immediate community may be at risk of MERS-CoV exposure since 100% of the sampled camels were positive for the MERS-CoV ELISA antibody test. Subsequently, the handlers in turn could serve as vehicles for MERS transmission resulting in MERS-CoV in the community hence secondary spread. The common practice among camel handlers of treating their camels by themselves (100%, n = 40) without any consideration to personal hygienic practices like immediate thorough washing of hands with soap and water may predispose to zoonotic disease transfer. Hence public enlightenment campaign might be necessary to create awareness on possible danger of such practices and prevail disease prevention and control since transmission is chiefly via contact with infected camels. It is believed that humans can be infected through direct or indirect contact with infected dromedary camels (Martins, 2015). Gompf *et al*. (2016), are of the opinion that those who handle camels are at higher risk of MERS-CoV infection than people who do not have regular close contacts with camels. Therefore, practice of good hygiene such as hand washing is advocated for people who come in contact with camels (Michael, 2013; WHO, 2015d).

From the assessment of the 40 camel handlers, most had not noticed any respiratory (90%, n = 36) and gastrointestinal (67.5%, n = 27) signs on their camels within the past two weeks prior to this study. This could be due to the infection being asymptomatic in most camels (Assiri, 2013a; Michael, 2013). Also, secondary MERS cases have tended to be milder than primary cases and most secondary cases have been reported to be asymptomatic (WHO, 2014a; Drosten *et al*., 2014)

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

In this cross sectional study conducted in Illela Sokoto State, Nigeria between October, 2016 and January, 2017 to survey the antibody profile of camels and humans against MERS-CoV, a seroprevalence of 100% (n=74) for dromedary camels and 74% (n=29) for patients in a secondary care hospital was obtained;

All the camels screened were positive for MERS-CoV antibody regardless their source;

The practice of treating camels by handlers and lack of personal hygiene after contact with camels may predispose these handlers to MERS-CoV infection;

Some patients screened in the secondary care hospital were positive for MERS-CoV antibody across all occupation regardless of the animals they housed. Furthermore, farmers (n=4) and paramilitary (n=1) were 100% positive each;

Age (P= 0.855), sex (P= 0.949) and occupation (P= 0.615) were not determinants for the presence of MERS-CoV antibody in sampled human patients;

Camels coming into Nigeria through Illela border and at Illela international market are mostly from Niger Republic (75.7%, n=56).

6.2 RECOMMENDATIONS

1. Awareness of Middle East Respiratory Syndrome coronavirus should be raised by stakeholders to the public. This will reduce the effect of morbidity in case of an outbreak.
2. Regular hygiene and sanitation campaigns should be carried out by stakeholders on camel handlers to reduce their chances of being exposed to the infection.
3. Further research and molecular study is needed to understand the molecular epidemiological trend of MERS-CoV by phylogenetic tree analysis. This would give an idea to the similarity between MERS-CoV strain in camels and humans within the study area.
4. Diagnostic tests in health facilities should include that of MERS-CoV especially when patients present clinical symptoms such as fever, myalgia, respiratory signs and gastrointestinal signs.
5. Nigerian borders should be re-enforced with equipment and personnel that will screen in-coming animals of various zoonotic infections and those of economic importance.
6. Surveillance mechanism in Nigeria should be strengthened through One World One Health (OWOH) concept. This will allow for the early detection of emerging and re-emerging zoonotic infections, hence effect proper prevention and control.

Limitation

Few human samples were collected from patients in the hospital due to time constraints imposed by the hospital management and caution on infections such as HIV and hepatitis.

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APPENDICES

APPENDIX I: Letter of Ethical Clearance

MINISTRY OF HEALTH

Block 16, Shehu Kangiwa Secretariat
P.M.B. 2113, Sokoto State – Nigeria
Tel: 060-232856, 232425,2321

SMH/1580/V.IV 23/6/2016

SALAM, Samson Polycarp,
Department of Veterinary Public Health and
Preventive Medicine,
Ahmadu Bello University, Zaria
Kaduna State.

ETHICAL CLEARANCE FOR RESEARCH ON "TRANSBORDER SURVEY OF MIDDLE EAST RESPIRATORY SYNDROMEIN DROMEDARY CAMELS AND THEIR HERDSMEN, IN SOKOTO STATE, NIGERIA (SKHREC/020)016)

I am directed to refer to your application on the above and to inform you that, the proposal submitted was reviewed by State Health Research Ethics Committee and found the protocol and other documents related to the survey satisfactory.

In the light of the above, I am further directed to convey the approval of the committee for the conduct of the above survey. It is however, expected that the results of the survey will be send to the committee for documentation as soon as it is concluded.

Accept the congratulations of the committee on behalf of the Honourable Commissioner, please.

DADI ADARE (DALLATUN GOBIR)
Director: H.P.R.S
For: Honourable Commissioner

APPENDIX II: Data Form

AHMADU BELLO UNIVERSITY ZARIA, KADUNA STATE.

FACULTY OF VETERINARY MEDICINE. DEPARTMENT OF PUBLIC HEALTH AND PREVENTIVE MEDICINE.

MACARTHUR FOUNDATION: VETERINARY EPIDEMIOLOGY.

Introduction: I am a postgraduate student in the above named Faculty with admission number; P13VTPH8036. I'm carrying out a survey on Middle East Respiratory Syndrome (MERS) coronavirus in dromedary camels and human patients in Illela of Sokoto State. I hope to get your cooperation. Thank you.

Instruction: Kindly fill in each space as provided accordingly. The information obtained will be used strictly for academic purpose and absolute confidentiality will be ensured.

A: CAMEL OWNER DATA

Camel Code: _____

Camel Data

1. Date _____
2. Location_____
3. Latitude/Longitude _____
4. Mode of transportation_____
5. Source of Camels _____
6. Destination of Camels_____
7. Number of Camels transported in the batch _____
8. Sex M _____F_____
9. Age Young _____ Adult _____
10. Species co-transported _____

Handler

Code: _____

SECTION A: DEMOGRAPHIC DATA

1. Sex: a. Male [ ] b. Female [ ]
2. Age (Years): a. ≤19 [ ] b. 20-30 [ ] c. 31-40 [ ] d. >40 [ ]
3. Number of handlers with the batch: _____
4. Occupation: a. Full-time camel handler [ ] b. Part-time camel handler [ ] c. Others [ ]

SECTION B: PRACTICE

1. How long have you been practicing this trade? a. <1year [ ] b. 1-10years [ ] c. >10years [ ].
2. How do you treat sick camels? a. Visit a clinic [ ] b. By self [ ] c. Others [ ]
3. What do you do after handling camels? a. Wash hands [ ] b. Remove gloves [ ] c. Bath d. Do nothing[ ]
4. Which of these respiratory symptoms have you noticed in your batch in the past 2weeks? a. Coughing [ ] b. Shortness of breath [ ] c. None [ ]
5. Which of these gastrointestinal symptoms have you noticed in your batch in the past 2weeks? a. Vomiting/Emesis [ ] b. Diarrhoea [ ] c. Abdominal pains [ ] d. None [ ]

B: PATIENTS DATA IN ILLELA GENERAL HOSPITAL, SOKOTO STATE.

Patient code: _____

DEMOGRAPHIC DATA

1. Sex: a. Male [ ] b. Female [ ]
2. Age (Years): a. ≤19 [ ] b. 20-30 [ ] c. 31-40 [ ] d. >40 [ ]
3. Occupation: a. Full-time camel handler [ ] b. Part-time camel handler [ ] c. Others [ ]
4. What animals do you house or come in contact with regularly?

APPENDIX III: Informed Consent Form on the Study

Survey of Middle East Respiratory Syndrome Coronavirus in Dromedary Camels and Patients in a Secondary Care Hospital in Illela, Sokoto State, Nigeria.

Introduction of the researcher

Good day Sir, I am Dr. Salam Samson Polycarp from the Department of Veterinary Public Health and Preventive Medicine (Center of Excellence in Veterinary Epidemiology MacArthur Foundation) of Ahmadu Bello University, Zaria.

I am currently carrying out this research as part of the requirement for the award of Masters in Veterinary Epidemiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria which will qualify me to become a specialist in Epidemiology.

Sponsor of research

MacArthur Foundation

Purpose of the research

This research is intended to know the prevalence of MERS-CoV in Nigeria. The aim of the study is to detect MERS-CoV in dromedary camels and their herdsmen, in Sokoto State Nigeria.

Procedure of the research

A data form will be given out to consented Herdsmen to fill while collecting their nasal swab. Blood sample will be collected from their camels.

Expected duration of research and of participants' involvement

This research is intended to last for 6 months. In May and June sampling will be carried out with laboratory analysis. In July data collected will be analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Thesis writing will commence in August/September, Internal defense in November while External defense and dissemination of information to stakeholders in December.

Benefits

At the end of this research, we should be able to know if camels coming into Nigeria from Niger Republic have been exposed to MERS-CoV, We would also know if the herdsmen carry strains of this virus then try to provide meaningful explanation as to why there hasn't been a human case yet in Nigeria.

Voluntary participation/withdrawal from the study

You are not under any obligation to participate in this study. Your decision to take part is completely voluntary. You have the right to freely withdraw from this study at any stage.

Risks

There will be no invasive procedure carried out on humans; as a matter of fact the nasal swap can be collected by the herdsmen themselves before storage. However blood samples collected from camels and nasal swabs from humans will be analyzed in the laboratory.

Cost

Agreeing to participate in the study is at no financial cost to the potential participant even though incentives might be given.

Confidentiality

All data collected in this study will be given code numbers and no names will be recorded. This cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study.

APPENDIX IV: Images of Field Work

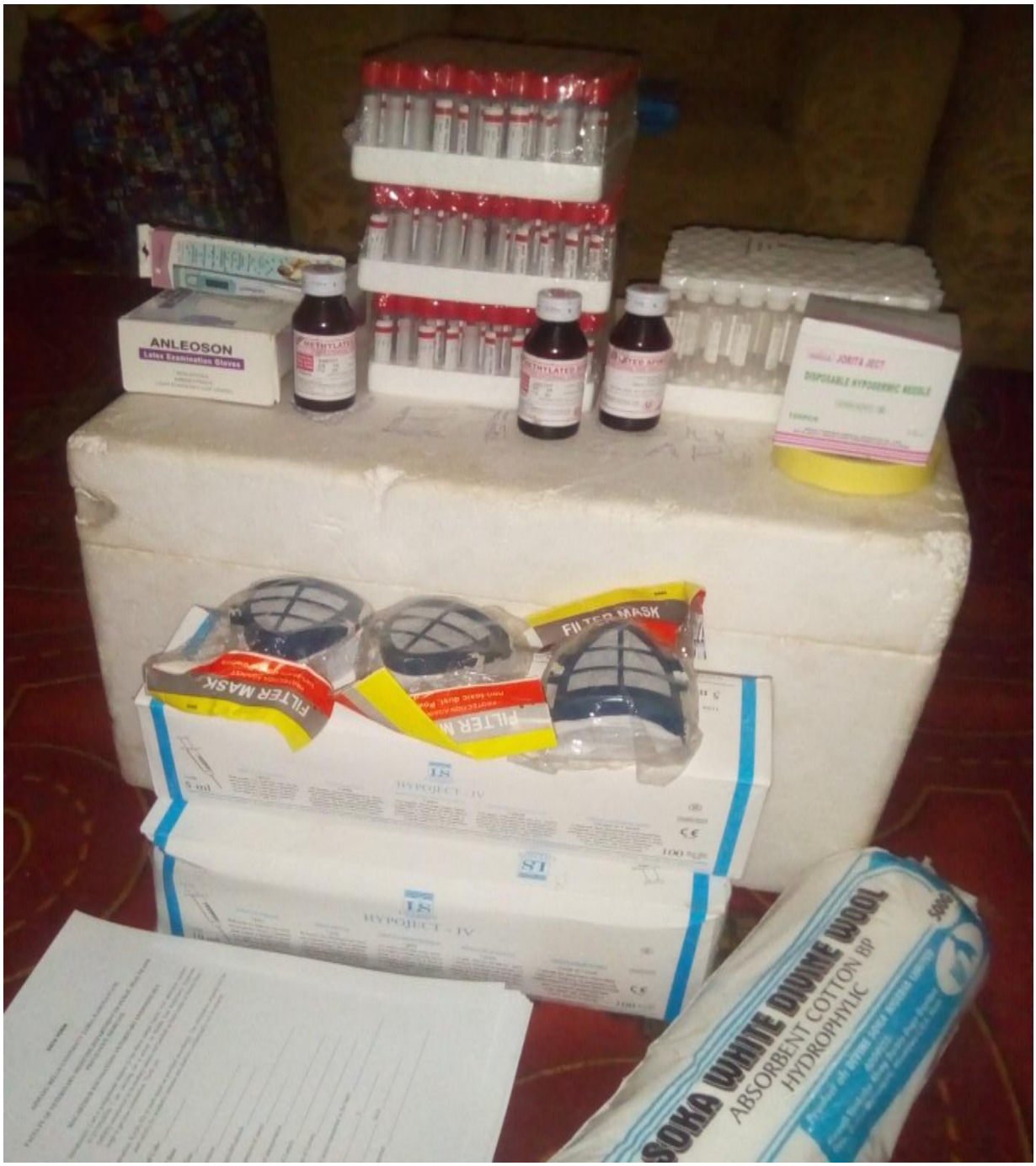


Image I: Materials for the field work



Image III: Batches of camel at Illela international market



Image IV: Camel Blood Sample collection at Illela, Sokoto State



Image V: The MERS CoV ELISA test kits from Alpha Diagnostic International

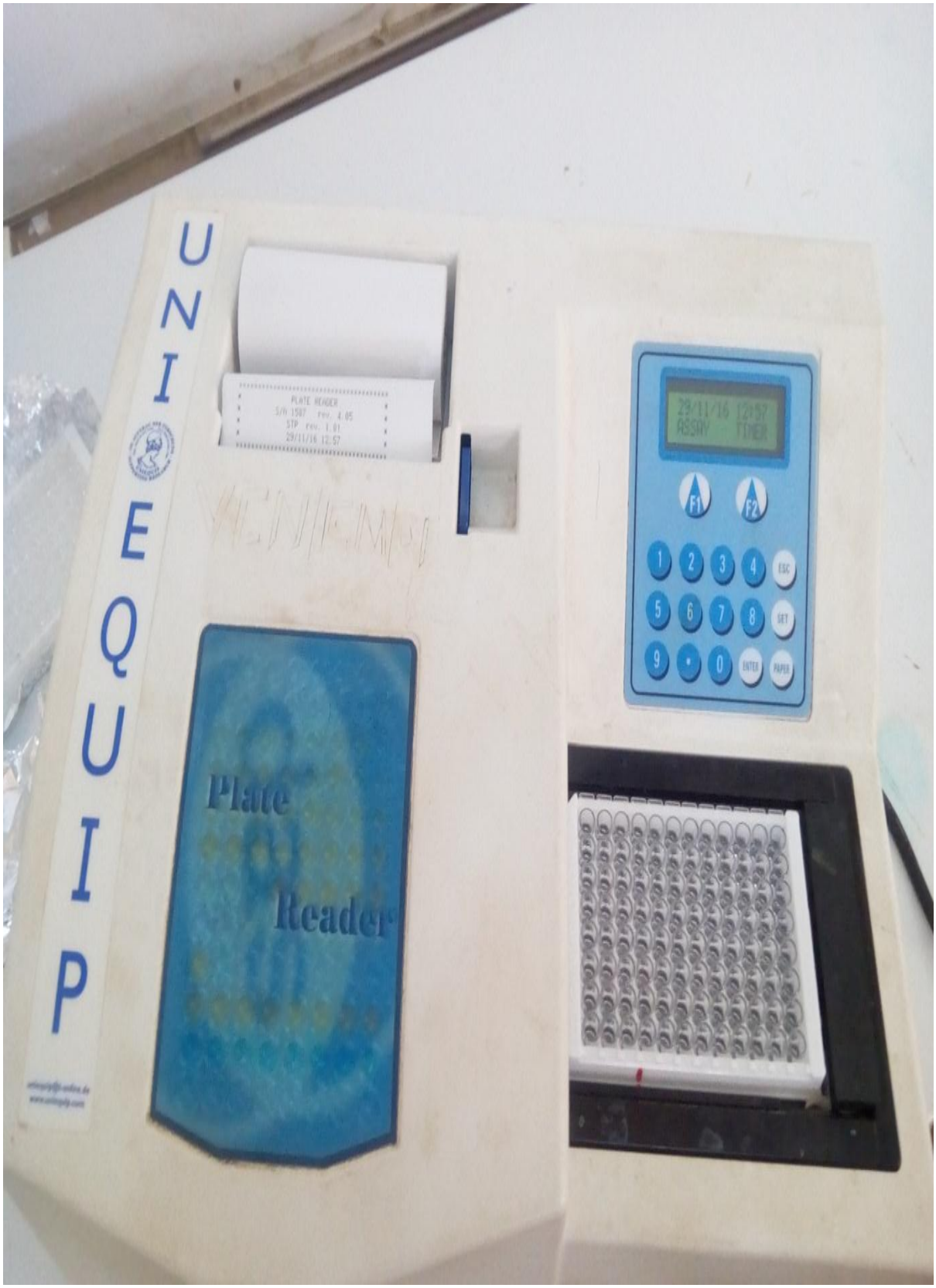
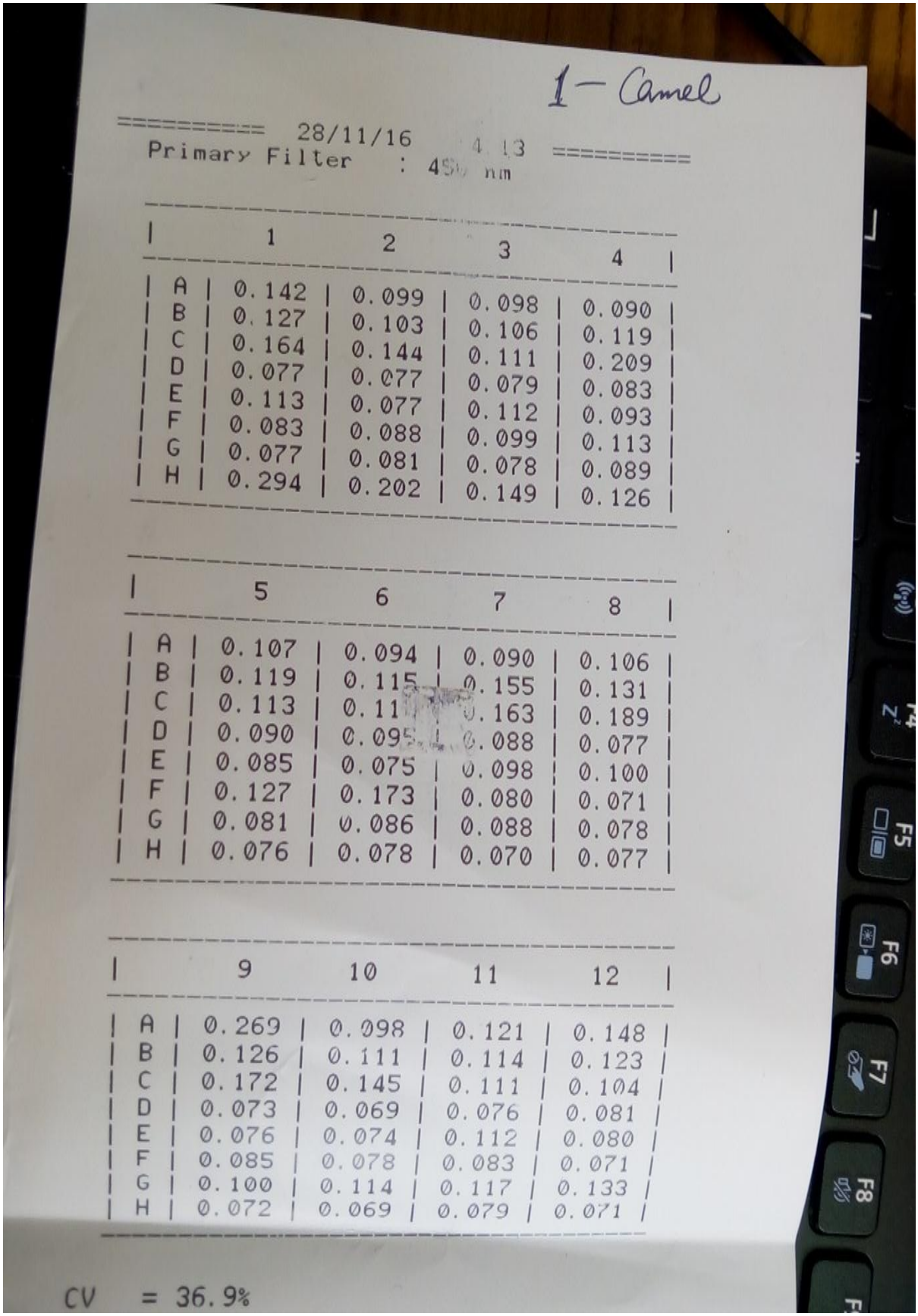


Image VI: The ELISA reader (450 nm) taking the OD reading of the sample wells



1 – Camel

========== 28/11/16 4:13 ==========
Primary Filter : 450 nm

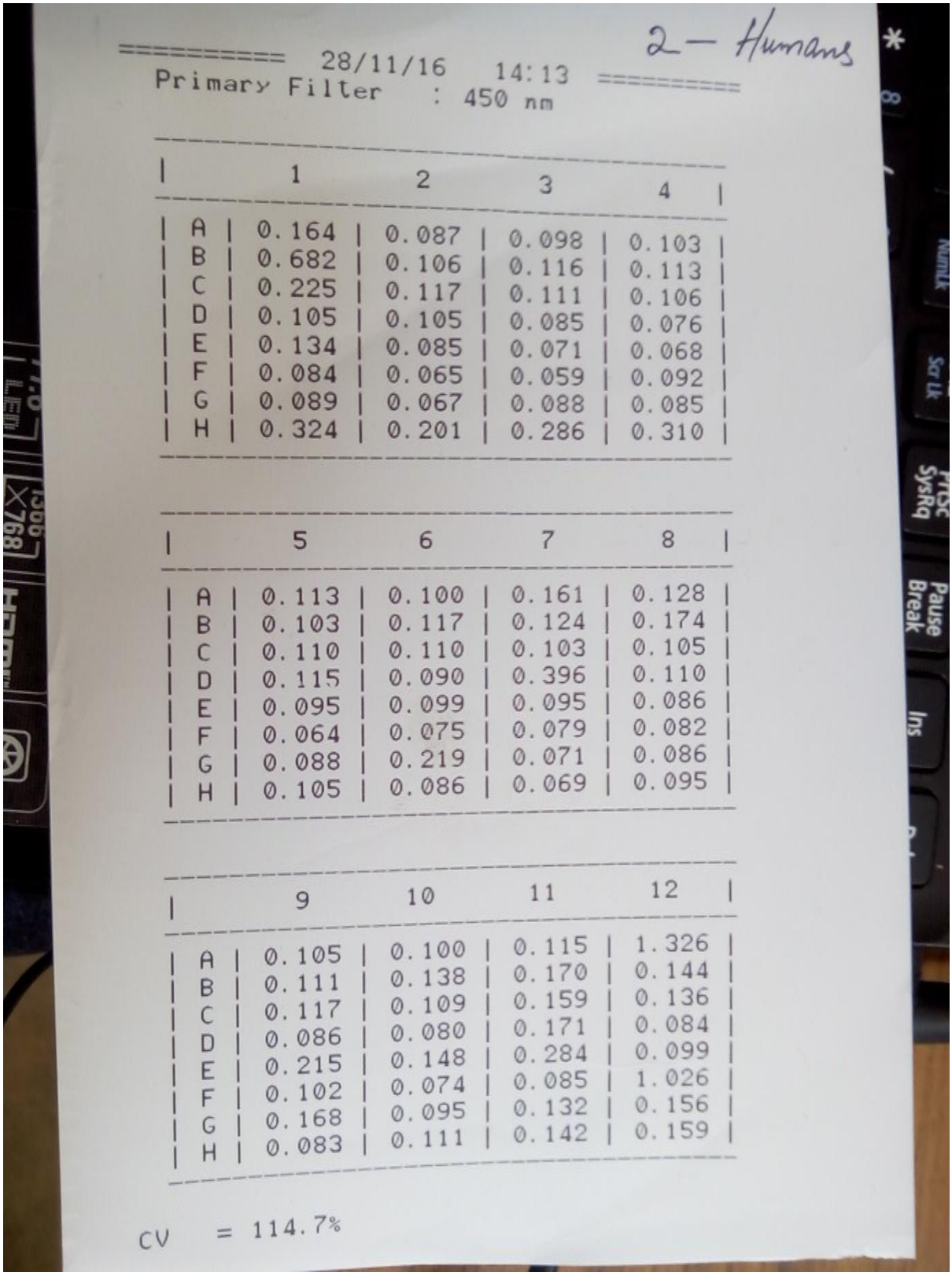
	1	2	3	4
A	0.142	0.099	0.098	0.090
B	0.127	0.103	0.106	0.119
C	0.164	0.144	0.111	0.209
D	0.077	0.077	0.079	0.083
E	0.113	0.077	0.112	0.093
F	0.083	0.088	0.099	0.113
G	0.077	0.081	0.078	0.089
H	0.294	0.202	0.149	0.126

	5	6	7	8
A	0.107	0.094	0.090	0.106
B	0.119	0.115	0.155	0.131
C	0.113	0.11	0.163	0.189
D	0.090	0.095	0.088	0.077
E	0.085	0.075	0.098	0.100
F	0.127	0.173	0.080	0.071
G	0.081	0.086	0.088	0.078
H	0.076	0.078	0.070	0.077

	9	10	11	12
A	0.269	0.098	0.121	0.148
B	0.126	0.111	0.114	0.123
C	0.172	0.145	0.111	0.104
D	0.073	0.069	0.076	0.081
E	0.076	0.074	0.112	0.080
F	0.085	0.078	0.083	0.071
G	0.100	0.114	0.117	0.133
H	0.072	0.069	0.079	0.071

CV = 36.9%

Image VII: ELISA MERS-CoV antibody Optical Density result for camel serum samples



2 – Humans

28/11/16 14:13

Primary Filter : 450 nm

	1	2	3	4
A	0.164	0.087	0.098	0.103
B	0.682	0.106	0.116	0.113
C	0.225	0.117	0.111	0.106
D	0.105	0.105	0.085	0.076
E	0.134	0.085	0.071	0.068
F	0.084	0.065	0.059	0.092
G	0.089	0.067	0.088	0.085
H	0.324	0.201	0.286	0.310

	5	6	7	8
A	0.113	0.100	0.161	0.128
B	0.103	0.117	0.124	0.174
C	0.110	0.110	0.103	0.105
D	0.115	0.090	0.396	0.110
E	0.095	0.099	0.095	0.086
F	0.064	0.075	0.079	0.082
G	0.088	0.219	0.071	0.086
H	0.105	0.086	0.069	0.095

	9	10	11	12
A	0.105	0.100	0.115	1.326
B	0.111	0.138	0.170	0.144
C	0.117	0.109	0.159	0.136
D	0.086	0.080	0.171	0.084
E	0.215	0.148	0.284	0.099
F	0.102	0.074	0.085	1.026
G	0.168	0.095	0.132	0.156
H	0.083	0.111	0.142	0.159

CV = 114.7%

Image VIII: ELISA MERS-CoV antibody Optical Density result for human serum samples

APPENDIX V: Threshold Index Values for Camels and Human Patients

On the 6th of November 2016, 44 camels were sampled and coded; B1C1, B1C2, B1C3, B1C4, B1C5, B1C6, B1C7, B1C8, B1C9, B1C10, B1C11, B1C12, B1C13, B1C14, B2C1, B2C2, B2C3, B3C1, B3C2, B3C3, B4C1, B5C1, B6C1, B6C2, B6C3, B7C1, B7C2, B7C3, B8C1, B8C2, B9C1, B9C2, B9C3, B10C1, B10C2, B10C3, B11C1, B11C2, B11C3, B11C4, B12C1, B13C1, B13C2 and B13C3.

On the 13th of November 2016, 30 camels were sampled and coded; B14C1, B15C1, B16C1, B16C2, B16C3, B17C1, B18C1, B19C1, B20C1, B20C2, B21C1, B22C1, B23C1, B24C1, B25C1, B25C2, B25C3, B25C4, B25C5, B25C6, B25C7, B25C8, B25C9, B25C10, B25C11, B25C12, B25C13, B25C14, B26C1 and B27C1.

On the 6th of November 2016, 19 handlers responded to the data forms. They were coded; B1H1, B1H2, B1H3, B2H1, B2H2, B3H1, B4H1, B4H2, B5H1, B6H1, B7H1, B8H1, B9H1, B9H2, B10H1, B11H1, B12H1, B13H1 and B13H2.

On the 13th of November 2016, 21 handlers responded to the data forms. They were coded; B14H1, B15H1, B16H1, B16H2, B17H1, B17H2, B18H1, B19H1, B20H1, B21H1, B21H2, B22H1, B23H1, B23H2, B24H1, B25H1, B25H2, B25H3, B26H1, B27H1 and B27H2.

Table 1: Collection of sample from patients

Date of Sample Collection	Patient Code
9/11/2016	G1 - G8
10/11/2016	G9 - G14
11/11/2016	G15 - G19
12/11/2016	G20 - G31
13/11/2016	G32 - G39

Table 2: Net OD values for human calibrators and positive control

Calibrator	**Well**	**Mean OD value(U/ml)**
1	H3 and H4	0.30
2.5	H5 and H6	0.10
5	H7 and H8	0.08
10	H9 and H10	0.10
Positive control	H1 and H2	0.26

Table 3: Threshold index for human samples

Threshold index for positive control = mean net OD for +ve control ÷ mean net OD for 1U/ml = 0.87

Sample	well	Average	Threshold Index
1	0.164	0.13	0.42
	0.087		
2	0.098	0.10	0.34
	0.103		
3	0.103	0.11	0.37
	0.117		
4	0.161	0.14	0.48
	0.128		
5	0.105	0.10	0.34
	0.1		
6	0.115	0.72	2.42
	1.326		
7	0.682	0.39	1.32
	0.106		
8	0.116	0.11	0.38
	0.113		
9	0.103	0.11	0.37
	0.117		
10	0.124	0.15	0.50
	0.174		
11	0.111	0.12	0.42
	0.138		
12	0.17	0.16	0.53
	0.144		
13	0.225	0.23	0.76
	0.225		
14	0.111	0.11	0.36
	0.106		
15	0.11	0.11	0.37
	0.11		
16	0.103	0.10	0.35
	0.105		
17	0.117	0.11	0.38
	0.109		
18	0.159	0.15	0.49
	0.136		
19	0.105	0.11	0.35
	0.105		
20	0.085	0.08	0.27
	0.076		

Sample	well	Average	Threshold Index
21	0.115		
	0.09	0.10	0.34
22	0.396		
	0.11	0.25	0.85
23	0.086		
	0.08	0.08	0.28
24	0.171		
	0.084	0.13	0.43
25	0.134		
	0.085	0.11	0.37
26	0.071		
	0.068	0.07	0.23
27	0.095		
	0.099	0.10	0.33
28	0.095		
	0.86	0.48	1.60
29	0.215		
	0.148	0.18	0.61
30	0.284		
	0.099	0.19	0.64
31	0.084		
	0.065	0.07	0.25
32	0.059		
	0.092	0.08	0.25
33	0.064		
	0.075	0.07	0.23
34	0.079		
	0.082	0.08	0.27
35	0.102		
	0.074	0.09	0.30
36	0.085		
	1.026	0.56	1.86
37	0.089		
	0.067	0.08	0.26
38	0.088		
	0.085	0.09	0.29
39	0.088		
	0.219	0.15	0.52

Table 4: Net OD values of camel calibrators and positive control

Calibrator	**Well**	**Mean OD value(U/ml)**
1	H1 and H2	0.25
2.5	H3 and H4	0.14
5	H5 and H6	0.08
10	H7 and H8	0.07
Positive control	H11 and H12	0.08

Table 5: Threshold index for camel samples

Threshold index for positive control = mean net OD for +ve control ÷ mean net OD for 1U/ml = **0.32**

Sample	well	Average/Net OD	Threshold Index
1	0.142 0.099	0.1205	0.4820
2	0.098 0.09	0.094	0.3760
3	0.107	0.107	0.4280
4	0.094	0.094	0.3760
5	0.090	0.09	0.3600
6	0.106	0.106	0.4240
7	0.269	0.269	1.0760
8	0.098	0.098	0.3920
9	0.121	0.121	0.4840
10	0.148	0.148	0.5920

11	0.127	0.127	0.5080
12	0.103	0.103	0.4120
13	0.106	0.106	0.4240
14	0.119	0.119	0.4760
15	0.119	0.119	0.4760
16	0.115	0.115	0.4600
17	0.155	0.155	0.6200
18	0.131	0.131	0.5240
19	0.126	0.126	0.5040
20	0.111	0.111	0.4440
21	0.114	0.114	0.4560
22	0.123	0.123	0.4920
23	0.164	0.164	0.6560
24	0.144	0.144	0.5760
25	0.111	0.111	0.4440
26	0.209	0.209	0.8360
27	0.113	0.113	0.4520
28	0.115	0.115	0.4600
29	0.163	0.163	0.6520
30	0.189	0.189	0.7560
31	0.172	0.172	0.6880
32	0.145	0.145	0.5800
33	0.111	0.111	0.4440
34	0.104	0.104	0.4160
35	0.077	0.077	0.3080

36	0.077	0.077	0.3080
37	0.079	0.079	0.3160
38	0.083	0.083	0.3320
39	0.090	0.090	0.3600
40	0.095	0.095	0.3800
41	0.088	0.088	0.3520

Sample	well	Average	Threshold Index
42	0.077	0.077	0.31
43	0.073	0.073	0.29
44	0.069	0.069	0.28
45	0.076	0.076	0.30
46	0.081	0.081	0.32
47	0.113	0.113	0.45
48	0.077	0.077	0.31
49	0.112	0.112	0.45
50	0.093	0.093	0.37
51	0.085	0.085	0.34
52	0.075	0.075	0.30
53	0.098	0.098	0.39
54	0.100	0.1	0.40
55	0.076	0.076	0.30
56	0.074	0.074	0.30
57	0.112	0.112	0.45
58	0.080	0.08	0.32

59	0.083	0.083	0.33
60	0.088	0.088	0.35
61	0.099	0.099	0.40
62	0.113	0.113	0.45
63	0.127	0.127	0.51
64	0.173	0.173	0.69
65	0.080	0.08	0.32
66	0.071	0.071	0.28
67	0.085	0.085	0.34
68	0.078	0.078	0.31
69	0.083	0.083	0.33
70	0.071	0.071	0.28
71	0.077 0.081	0.079	0.32
72	0.078 0.089	0.0835	0.33
73	0.081 0.086	0.0835	0.33
74	0.088 0.078	0.083	0.33

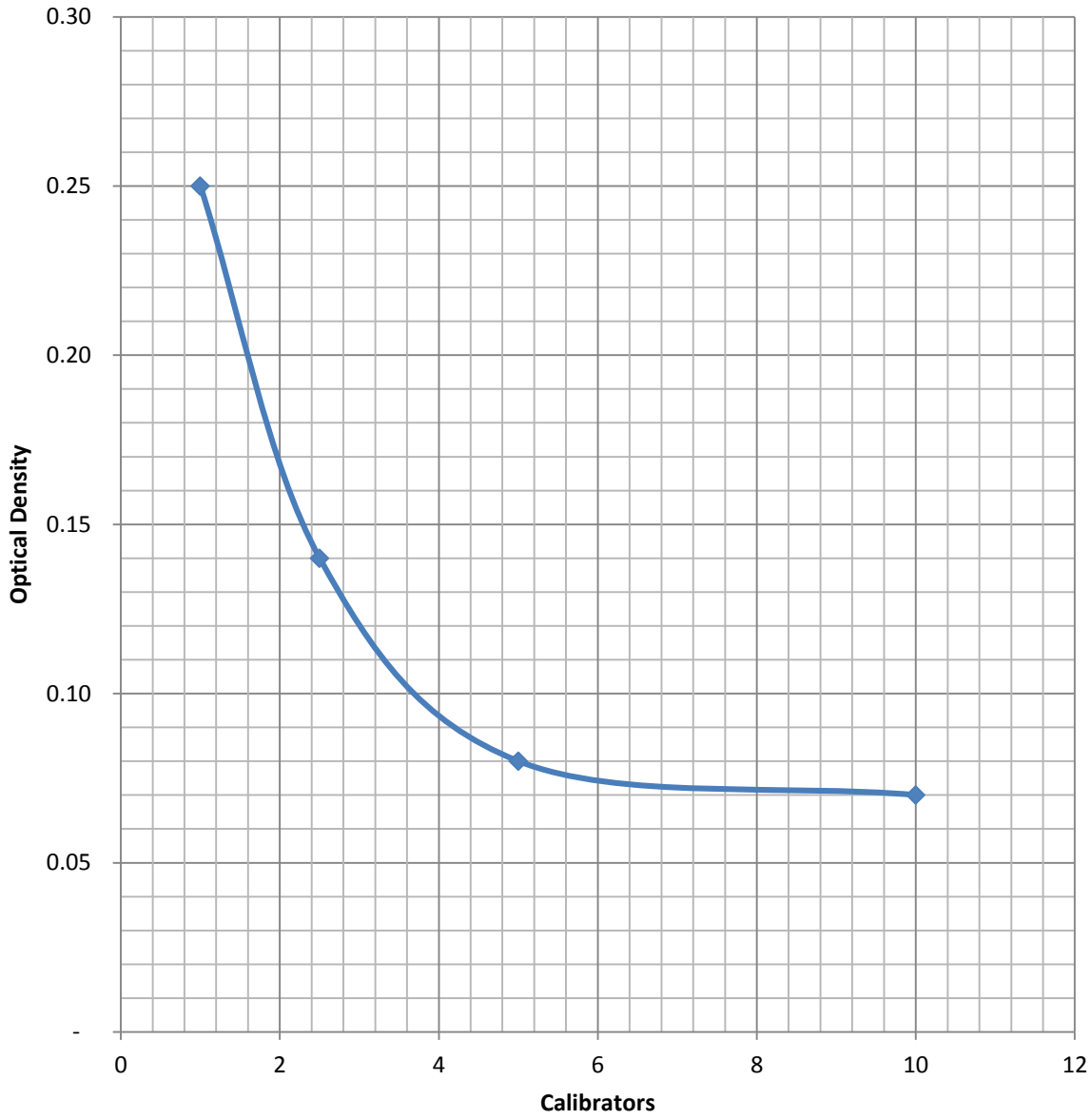


Figure 1: Graph showing obtained OD values for calibrators of camel ELISA

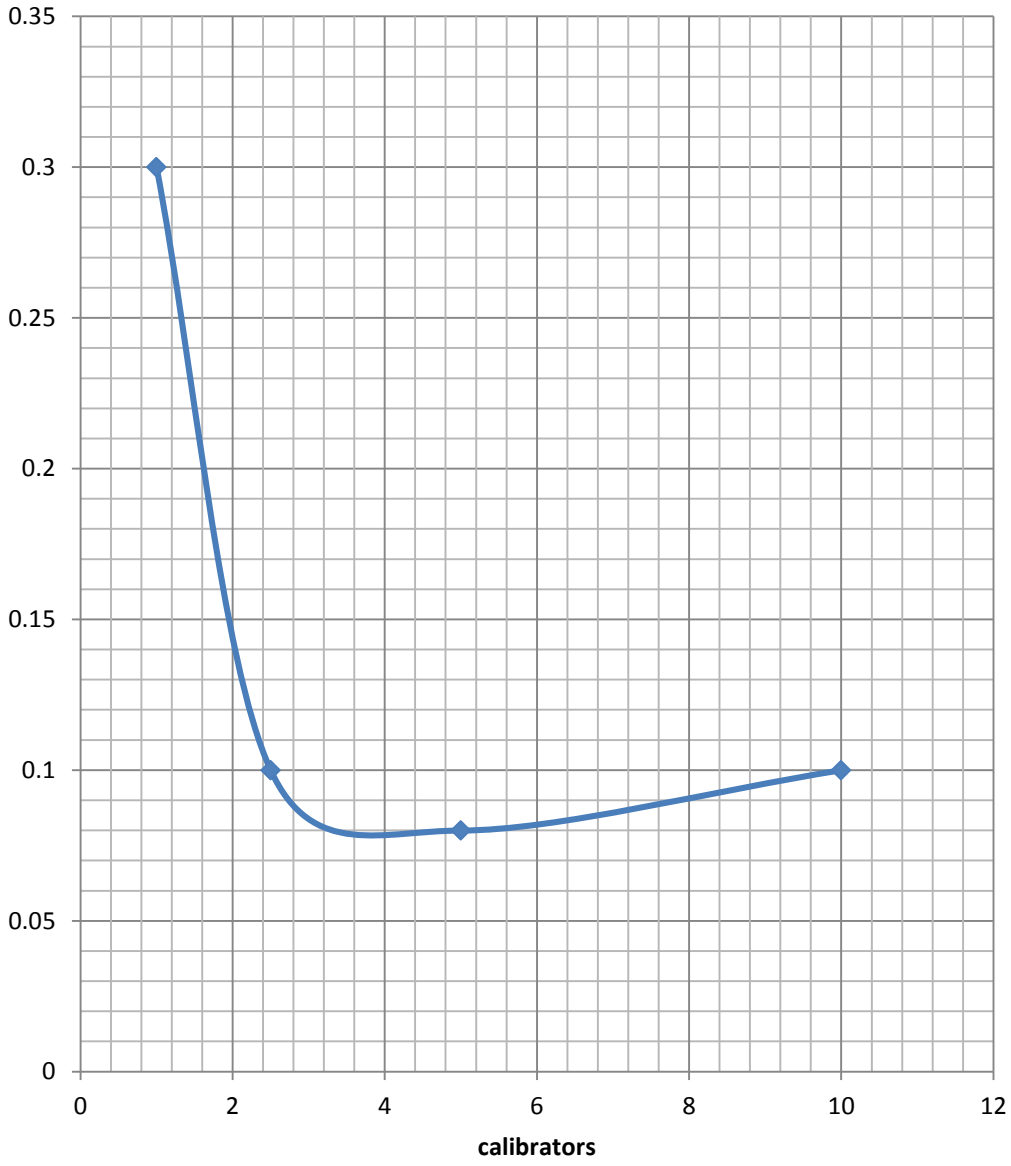


Figure 2: Graph showing obtained OD values for calibrators of human ELISA