MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS- SERO-PREVALENCE IN CAMELS, KNOWLEDGE OF CAMEL HANDLERS, HYGIENE AND SLAUGHTER PRACTICES AT ATHI-RIVER SLAUGHTERHOUSE, MACHAKOS COUNTY, KENYA

ESTHER MUTHONI KAMAU

MASTER OF SCIENCE

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Middle East Respiratory Syndrome Coronavirus- Sero-prevalence in Camels, Knowledge of Camel Handlers, Hygiene and Slaughter Practices at Athi-River Slaughterhouse, Machakos County, Kenya

Esther Muthoni Kamau

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Signature: .....Date: .....

Esther Muthoni Kamau

This thesis has been submitted for examination with our approval as University Supervisors

Signature: ..... Date: .....

Prof. Juliette R. Ongus, PhD
JKUAT, Kenya

Signature.....Date: .....

Prof. George K. Gitau, PhD
UON, Kenya

DEDICATION

I thank the Almighty God for protection, good health and for enabling me to complete this work. This thesis is dedicated to my late father and my mother for the care and encouragement she continues to give our family. My brother, sisters, nephews and nieces and most importantly my daughter Salome have given me a lot of encouragement during my studies and have supported and assisted me throughout this work. To my friends who encourage me always.

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ABBREVIATIONS AND ACRONYMS

ARDS	Acute respiratory distress syndrome
Beta-CoV	Beta corona virus
CDC	Centres for Disease Control and Prevention
Dromedary	One humped camel
ECDC	European centre for disease prevention and control
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron Microscopy
EMC	Erasmus Medical Centre
HCoV	Human Corona virus
HPA	Health Protection Agency, UK Novel coronavirus investigation team
IgG	Immunoglobulin G
KEMRI	Kenya Medical Research Institute
MERS	Middle East Respiratory Syndrome
mRNA	Messenger Ribonucleic acid
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
rELISA	recombinant MERS- CoV spike protein subunit 1–based ELISA
rRT-PCR	Reverse transcription real-time Polymerase Chain Reaction
SARS	Severe acute respiratory syndrome
WHO	World health organization

ABSTRACT

The dromedary camel is a reservoir and source for zoonotic transmission of Middle East Respiratory syndrome coronavirus with similarity of camel and human-derived sequences. Middle East Respiratory Syndrome, first identified in 2012 is caused by the MERS coronavirus (MERS-CoV). Between 2012 and June 2018, there were over 2200 confirmed human cases. There is possible occupational exposure among camel handlers with slaughter-house workers being at high risk. Affected camels are asymptomatic with prevalence being higher in mature camels. Sero-prevalence of MERS-CoV in a study carried out in 2012 in Kenya was reported at 29.5%. The objectives of this study were to determine the sero-prevalence of MERS-CoV in camels at the Athi-river slaughterhouse, determine knowledge regarding MERS among camel handlers and identify bio-safety practices during slaughter. The study was cross-sectional; the study used systematic random sampling to select camels from which 372 blood samples were collected. An indirect immune-fluorescent Enzyme linked immune-sorbent assay (IgG ELISA) was performed to detect anti-MERS-CoV antibodies. Structured questionnaires were administered to collect data on knowledge of MERS among camel handlers. A check list was used to collect data on slaughter practices. Proportions were calculated and associations between anti-MERS-CoV antibody sero-positivity and age group, sex, and origin of camels were assessed using Chi-square tests. Anti-MERS-CoV antibody sero-prevalence obtained overall was 77.4% (95% CI: 72.83-81.57). Prevalence in camels aged < 5 years (n=4) was 50%, 81.6% in those aged ≥ 5–8 years (n=98), 76.1% in those >8-11 years (n=255), and 80% in those over 11 years (n=15). Prevalence in males camels (n=176) was 76% (95% CI 74-86%) while in females (n=196), it was 78% (95% CI 78-88%). Based on origin of camels: Northern Kenya (77.5%, 95% CI: 70.4-83.57), coastal region (77.7%, 95% CI: 71.2-83.4), and Rift Valley region (70%, 95% CI: 34.8-93.3). There was no statistical difference in prevalence based on age group (□2 (2, N=372) =0.835 p=0.659), sex (□2 (1, N=372) =0.195 p=0.659), or origin of the camels (□2 (2, N=372) =0.326 p=0.851). Among 22 persons (5 herders and 17 slaughter-house workers), 18 had worked with camels for over 3 years. Sixteen (73%) were unaware of MERS-CoV. All reported washing hands after handling camels while 3/22 drank raw camel milk. Nineteen were aware of zoonotic diseases and common ways of transmission being: eating improperly cooked meat (90%), drinking raw milk (68%), and slaughter processes (50%). On bio-safety measures: - among 17 slaughter-house workers, 82% wore gumboots and 65% wore overalls/dustcoats with none using gloves or facemasks. All handlers interviewed lacked information on interaction of camels across borders but reported frequent interaction during grazing and transportation. High MERS-CoV sero-prevalence observed was consistent with other studies in Africa among adult camels. Increase in sero-prevalence over time could be due to continued exposure to the virus. Workers at this slaughter-house lacked knowledge about MERS-CoV but were aware of zoonotic diseases and their transmission. This could serve as an entry point to create awareness on MERS. Use of personal protective clothing to prevent direct contact with discharges and aerosols was lacking. There is need to enhance hygiene and bio-safety practices among camel handlers mainly slaughter-house workers to reduce opportunities for potential virus transmission. These results will contribute towards an effective integrated human-animal MERS-CoV control strategy in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background

Middle East respiratory syndrome coronavirus (MERS-CoV) was recognized as a cause of severe human respiratory disease in 2012 (Dudas *et al.*,2018). It causes a severe pneumonia-like acute, life-threatening respiratory illness (ECDC, 2014). The first patient, a 60-year-old Saudi man in Jeddah died of severe pneumonia and multiple organ failure (Alkot *et al*., 2016). Major symptoms of the syndrome in humans are a high fever (over 38°C), cough, respiratory symptoms, gastro-intestinal symptoms and occasional kidney failure (WHO, 2014). Between 2012 and July 2018, over 2200 cases had been reported in humans globally. Up to 35% of the cases were fatal and were mainly from countries of the Arabian Peninsula (WHO, 2018). The origins and geographic source of the virus are still unclear (Alnakli, Z., 2016). Close contact with patients in community and hospital settings has been found to propagate spread of the virus (Alkot *et al.*, 2016). The World Health Organization (WHO) has reported increase in cases of human-to-human transmission since March, 2014 (WHO, 2015).

Camels have been found to be reservoirs for the MERS-Co virus and a source for zoonotic transmission due to the high prevalence of MERS-CoV antibodies in dromedary camels in various regions including the Middle East and Africa (Chu *et al.*, 2014). Phylogenetic comparison of camel-derived and human-derived MERS-CoV sequences in the Middle-east show a match suggesting zoonotic transmission (Alagaili *et al.*,2014).

Research evidence shows that a virus similar to or the MERS-CoV circulated in dromedary camels in Africa and the Arabian Peninsula long before 2012 (based on when the index case was detected) including in regions where there were no human cases (Funk et al., 2016). MERS-CoV was detected in camels in Saudi Arabia as early as 1992 and in the United Arab Emirates as early as 2003 (Yusof *et al.*, 2015). In

Kenya, dromedary camels have had antibodies to MERS-CoV as early as 1992 (Corman *et al*., 2014).

Risk factors associated with zoonotic transmission of MERS-CoV identify the respiratory route as a transmission pathway of the virus from camels to humans, with possibility of oral transmission (Esam *et al*., 2014). MERS-CoV ribonucleic acid (RNA) has been detected in nasal, oral, and faecal specimens from camels. The virus was identified in aerosol samples in a shed that housed a camel confirmed to have the virus and which infected its owner (Azhar *et al.*, 2014). The conjunctiva and respiratory secretions of infected camels have been found to have high viral loads and it is from these sites that the MERS-Co virus is thought to be transmitted to humans (Esam *et al.*, 2014). Camels have been reported to be infected simultaneously with one or more MERS-CoV (Breise *et al*., 2014). Infection in humans may also occur through consumption of unpasteurized camel milk since the MERS-CoV can survive for long periods in fresh camel milk at ambient temperatures of between 4^0C and 22^0C (Van Doremalen *et al*., 2014). Consumption of raw milk is common in communities keeping camels in Kenya and could potentially expose them to MERS and other zoonotic diseases (Edelstein and Heymann, 2015).

In Kenya, persons with possible occupational exposure to camels include the camel traders, the slaughterhouse personnel, and herders of camels. Studies done on slaughterhouse workers have demonstrated that they are at a high risk of MERS-CoV exposure compared to the general population (Muller *et al.,* 2015). High viral loads found in camels in slaughter-houses pose a possible risk for human exposure due to high viral circulation (Farag *et al*., 2015). Handling of live camels, treatment of sick camels, waste removal and cleaning of camel pens have been significantly associated with increased risk of MERS-CoV infection among camel handlers (Khudhair *et al*., 2019).

Knowledge of MERS varies depending on population dynamics and location. In the Middle East, especially in Saudi Arabia where half of the global cases have been found, health care workers and the general population are aware of MERS (Alnakli,

2016). Health care personnel who take care of MERS patients have been found to be at a high risk of exposure. A study carried out on knowledge, attitudes and practices of health care workers towards MERS in Saudi Arabia reported knowledge of MERS but inadequate use of personal protective equipment, and poor infection prevention and control measures (Alsahafi and Cheng, 2016). A study carried out in 2018 by Alkot *et al* in Saudi Arabia reflected on the importance of health education as a cornerstone element in improving knowledge, attitudes and practices towards MERS-CoV infection (Alkot *et al*., 2016). Currently, there are no reported studies on knowledge, attitudes and practices among groups at high risk of exposure to MERS in Kenya. Farmers, especially in pastoral communities are mainly aware of common zoonotic diseases and their modes of spread. A study carried out among pastoralists who are cattle herders in Kajiado, Kenya showed that farmers were aware of zoonotic diseases, mainly anthrax and brucellosis, and were generally aware of potential pathways of exposure (Onono *et al*., 2019).

This study sought to determine sero-prevalence of MERS-CoV antibodies in dromedaries in Kenya where previous exposure had been demonstrated and to assess knowledge and practices of slaughter-house workers and camel traders regarding MERS. The data generated will inform health and veterinary professionals and assist in formulating surveillance programs for detecting MERS-CoV infection in high-risk human populations and regions with high camel populations. Information obtained from the knowledge and practices survey will assist in developing targeted messages for camel handlers mainly slaughterhouse workers.

1.2 Statement of the problem

Middle East Respiratory Syndrome (MERS) is one of the emerging viral diseases having been detected in human beings in 2012 (Dudas *et al.*, 2018). The detection of MERS occurred after another coronavirus disease outbreak, the severe acute respiratory syndrome (SARS). The SARS outbreak occurred in China in 2003 and spread to four other countries causing over 8000 infections and approximately 800 deaths (Al-Tawfiq *et al*., 2014). Middle East Respiratory Syndrome poses a serious

public health threat to humans due to its ability to cause serious disease especially among humans with comorbities. The actual number of human beings who have been infected with MERS-CoV to date may never be quantified. This is due to the asymptomatic nature of MERS in most people and the fact that it mimics other respiratory illnesses with which it can be easily confused (Meyer *et al.*, 2014). Despite this, the number of laboratory confirmed MERS-CoV human cases reported to WHO have significantly increased since March 2014. The reports indicate high numbers of hospital acquired cases, including outbreaks of MERS and an increase in fatalities (WHO, 2014). The WHO has also reported an increase in number of patients who acquire MERS from other sources other than through contact with infected people since early 2014 (WHO, 2014). Some patients reported to have been in contact with camels which have been confirmed to be the reservoir and the primary source of infection to humans while they remain asymptomatic (Sikkema *et al*., 2019). The disease in humans is of great public health implication due to losses from high medical expenses, productivity losses and mortality rate of over 30% among the cases. From September 2012 to July 2018, the WHO has reported over 2200 laboratory confirmed cases of MERS-CoV globally (WHO, 2018).

Kenya has a high camel population and the MERS-CoV antibodies have been detected in Kenya from archived camel sera since 1992. This implies that the MERS coronavirus has been circulating in camels in Kenya for a long time leading to possible human exposure. Camel herders, traders and slaughterhouse workers are groups that are at high risk of exposure to the MERS virus and other zoonotic infections due to increased contact with camels. Due to the fact that MERS is a newly identified infection, these groups may not be aware of the disease and may be exposed to the virus during handling and slaughter practices especially because the camels harbouring the virus do not show any symptoms of clinical disease.

1.3 Study justification

Kenya is a country with a large camel population of over 3 million (Kagunyu and Wanjohi, 2014). Camel rearing is common in semi-arid areas and plays an important

economic role by providing employment, transport of goods and production of milk and meat. Camel meat and milk consumption and demand have increased in Kenya leading to increased production (Muloi *et al*., 2018). Increase in camel population translates to higher contact and exposure of camel handlers to camels which have been identified as the reservoir and source of infection for the MERS-CoV. Some camels found in Kenya could be from neighboring countries like Somali and Ethiopia leading to higher circulation and introduction of variations of the virus to different camel populations.

Studies done in the Middle East and in Africa in countries like Nigeria, Egypt and Ethiopia show a high prevalence of MERS-CoV antibodies in camels (Chu *et al*., 2014). An investigation in Egypt, using real time polymerase chain reaction (RT-PCR) showed 3.6% (4 of 110) apparently healthy dromedary camels in a slaughterhouse to be infected with the MERS-CoV (Mackay and Arden, 2015). High sero-prevalence of more than 50% was noted in Kenya mainly in camels from north-eastern provinces since 1992 (Corman *et al*., 2014). However, studies done in Kenya used sera that had been archived for a long time (thirty years) and were limited by the fact that they and that did not test for cross-reactivity between MERS-CoV and the closely associated bovine corona virus which also infects camels (Corman *et al*., 2014). There is therefore a need to carry out a current sero-prevalence study using a test that is specific for the MERS-CoV antibodies.

Though no active human cases have been identified in Kenya, two people in Tana-river County have been found to be positive for IgG antibodies against MERS-CoV meaning that they at one time were infected with the virus (Liljander *et al.*, 2016). It is therefore possible that people in contact with camels are exposed to the virus which may precipitate to clinical disease with severe consequences.

Coronaviruses have been found to have a potential to mutate and adapt to new hosts which may propagate rapid spread causing an epidemic or pandemic. MERS has been proven to produce pockets of outbreaks among community set-ups and in hospital facilities. Rapid spread of the infection among patients in close contact and among

health personnel tending to MERS patients have been reported (Myoung-don *et al.*, 2016). Direct exposure to dromedary camels and other risk factors like diabetes mellitus, heart disease, and smoking have been associated with higher risk of MERS-CoV illness (Alraddadi *et al*., 2016). Camel workers especially at slaughter houses have been identified to be at a higher risk of exposure to MERS due to direct contact with camels and exposure to aerosols and body fluids during slaughter (Alshukairi *et.al.*, 2018). A better understanding of the sero-prevalence of MERS-CoV infection in camels in Kenya is important given that camels are the reservoirs for the MERS coronavirus and act as a possible source for human exposure and infection

Since herders, traders and slaughterhouse workers are at high risk of exposure, studies need to be carried out which address their knowledge of MERS and risk of occupational exposure. Studies carried out on knowledge, attitudes and practices of zoonotic diseases in Kenya have targeted common zoonotic infections. There was no study identified that had specific reference to knowledge and practices towards MERS among any of the high-risk groups in Kenya. This study aimed at identifying knowledge and measures undertaken by slaughterhouse workers which could prevent exposure to the MERS-CoV. Data on the knowledge and practices study will be used to develop targeted messages to these high risk groups using an integrated one-health approach.

1.4 Research questions

1. What is the sero-prevalence of MERS-CoV in camels presented for slaughter at Athi- river slaughterhouse?
2. What are the knowledge and practices regarding MERS among camel herders, traders and slaughterhouse workers at the Athi-river slaughterhouse?

1.5 Objectives

1.5.1 General objective

To determine the prevalence of MERS-CoV in camels at the Athi-river slaughterhouse and identify the knowledge and practices regarding MERS among herders, traders and slaughterhouse workers at the Athi-river slaughterhouse.

1.5.2 Specific objectives

1. To determine the sero-prevalence of MERS-CoV IgG antibodies in camels presented for slaughter at Athi-river slaughterhouse.
2. To identify the knowledge and practices regarding MERS among camel herders, traders and slaughterhouse workers at the Athi-river slaughterhouse during the study period.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and importance of MERS-CoV

The causal agent for the Middle East Respiratory Syndrome was first identified by an Egyptian virologist; Prof Ali Mohamed Zaki in 2012 (Dudas *et al*., 2018). The MERS-CoV was detected from cell culture of a 60-year-old male (the index case) from Saudi Arabia who succumbed to a viral respiratory illness (Dudas *et al*., 2018). Virus samples from this first case were also confirmed to have the virus by a leading coronavirus researcher, Ron Fouchier, at the Erasmus Medical Centre (EMC) in Rotterdam, Netherlands (Lau *et al*., 2017). The UK Health Protection Agency confirmed the second laboratory-proven case and named the virus the "London1_novel CoV 2012" (Mackay and Arden., 2015). In May 2013, to provide uniformity about MERS, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses in conformity with the WHO adopted the official designation, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Dires and Dawo, 2015).

Single cases, clusters of cases and outbreaks have been reported in numerous areas. All cases that have been identified since 2012 are associated with countries in the Middle East (Muller *et al*., 2015). Many cases of hospital, community acquired infections and transmission through contact have been detected in several areas in the Arabian Peninsula, Europe, the United States, Asia and Africa (Oboho *et al*., 2015). In Asia, Malaysia and Philippines have reported cases from people who travelled from the Middle East while in Africa cases have been found in Tunisia and Egypt (Abroug *et al*., 2014). The United States of America had also reported 2 imported cases (WHO, 2014). Movement in search of treatment across the globe has led to cases of secondary transmission with the virus (Azhar *et al*., 2019). Cases found in Europe were of patients who travelled from other countries to seek treatment (Dires and Dawo, 2015). Zoonotic diseases such as the MERS can spread rapidly among humans in community and hospital settings with the possibility of reaching epidemic or pandemic

proportions (Drosten *et al*., 2014). Super spreaders have been associated with rapid spread of disease in hospital and community settings. In 2015 in Korea, in a single outbreak in which 133 cases were confirmed, the MERS-CoV was spread by one traveller who returned to Korea after travelling to the Middle East (Myoung-don *et al.*, 2018). Spread of human disease has now become more rapid due to increase in international travel, trade interconnections, travel in search of treatment and tourism. A disease with a potential for a pandemic can cause disease and massive loss of human life, increase in medical expenses and serious implications on the economies nationally, regionally or worldwide.

2.2 Aetiology and taxonomy of Middle East respiratory syndrome coronavirus

Middle East Respiratory Syndrome is caused by the newly emerged MERS coronavirus (Dudas *et al*., 2018). The MERS coronavirus is an enveloped, single-stranded positive-sense ribonucleic acid (RNA) species of the genus *Betacoronavirus* (Ommeh *et al*., 2018).

The family *Coronaviridae* has 2 sub-families *Coronavirinae* and *Torovirinae*. MERS-CoV is a *Betacoronavirus* of the sub-family *Coronavirinae* in the family *Coronaviridae* and the order *Nidovirales (*Ahmed and Abdel-Moneim, 2014*).* The subfamily *Coronavirinae* is further subdivided into four genus sub-groupings; those of the genus *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, which infect mainly domestic birds and *Deltacoronavirus*. Betacoronaviruses have 4 lineages, A, B, C and D (Han and Yu, 2016). In humans, the Beta coronaviruses that have been shown to cause disease in humans are those of the A, B and C lineages. From lineage A, OC43 and HKU1, from lineage B, SARS-CoV, and MERS-CoV of the C lineage infect humans. Of the *betacoronaviruses* in the C lineage, SARS-CoV and MERS-CoV have been found to be infectious to people and to be of animal origin (Ahmed and Abdel-Moneim, 2014). Table 2.1 below shows the taxonomy of the MERS coronavirus.

Table 2.1: Taxonomy of MERS Coronavirus

Category	Name
Realm	Riboviria
Kingdom	Orthornavirae
Phylum	Pisuviricota
Class	Pisoniviricetes
Order	Nidovirales
Family	Coronaviridae
Sub-family	Coronavirinae
Genus	Betacoronavirus
Species	Middle East Respiratory Syndrome Coronavirus

2.3 Morphology and classification of Coronaviruses

Coronaviruses are named after the Latin word "*corona"*, meaning crown or halo, which describes the structure of the virions when viewed under electron microscopy. The virions are spherical or pleomorphic enveloped particles containing single-stranded (positive-sense) RNA. The ribonucleic acid is contained in a capsid comprised of matrix protein which is associated with a nucleoprotein. The envelope bears club-shaped glycoprotein projections which are known as the viral spike (S) peplomers (Schoeman and Fielding, 2019). These peplomers are responsible for attachment to the host cell and aid the host in responding to external stimuli. The genomic size of corona viruses ranges from approximately 26 to 32 kilobases (Schoeman and Fielding, 2019). Figure 2.1 below shows the structure of the virions under electron microscopy.

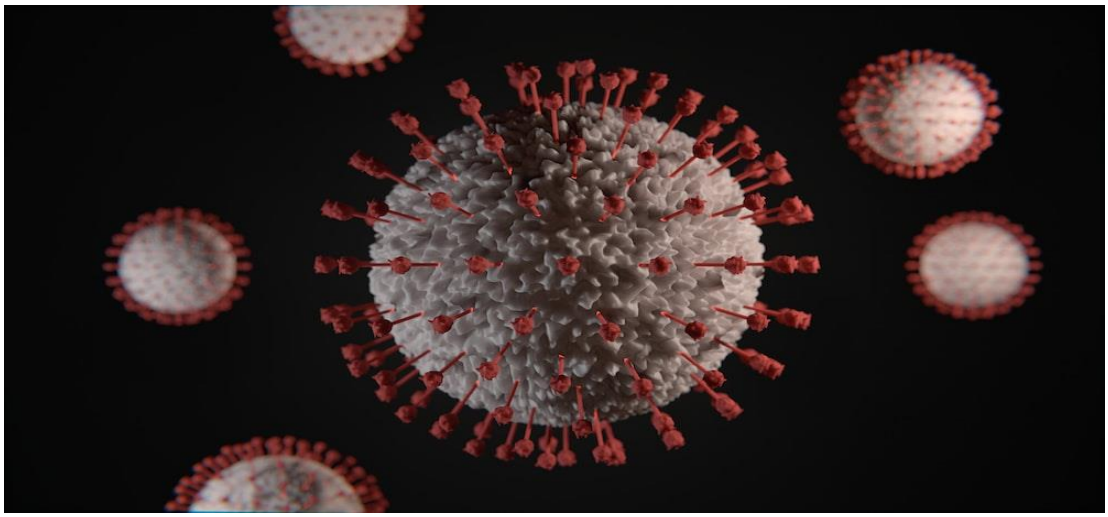

Figure 2.1: Image of Corona virus

Source:https://images.unsplash.com/photo-1583423230902-b653abc541eb?ixlib=rb-

2.4 Origin of MERS coronavirus

Studies on identification of the animal reservoir for MERS have mainly focused on bats due to isolation of the MERS-CoV or a similar virus in insect eating bats and in one-humped camels (Mohd *et al*., 2016). Bats have been known to harbour various coronaviruses, including human CoVs, from where the viruses are thought to spill-over to intermediate animal hosts (Meyer *et al.*, 2014). Bat cell lines have been found to contain a MERS-CoV receptor dipeptidyl peptidase 4 and have been propagated in in-vitro studies (Meyer *et al.*, 2014). There was a complete match between the virus isolated from the index case and specimens taken from an Egyptian tomb bat in his neighbourhood (Milne-Price *et al*., 2014). Though the MERS-Co virus has been identified in bats, they are not a likely source of continuous zoonotic transmission of the virus due to limited contact between humans and insectivorous bats (Meyer *et al.*, 2014:WHO, 2014). MERS is thought to have spilled over from bats to camels which now act as a source of zoonotic transmission (Han and Yu, 2016). There are numerous reports of people who have acquired infection from camels which have been identified as a reservoir and the origin of animal to human transmission (Han and Yu, 2016).

Specimens from camels, especially respiratory and faecal specimens have been found to contain MERS Viral RNA (Breise *et al.*, 2014). In November 2013, DNA samples from a man who took care of a sick camel and became ill with MERS were similar to those taken from the camel thus showing evidence of zoonotic transmission (Esam *et al.*, 2014). Viruses isolated from respiratory samples of the one-humped camels in Saudi Arabia were shown by phylogenetic analysis to be completely identical to published human MERS-CoV sequences (Breise *et al*., 2014).

2.5 Transmission of MERS-CoV in humans

The WHO and OIE have documented three ways of MERS spread in humans. These include: nosocomial infections, close human to human contact in households being the most common way and community acquired infections where exposure source includes contact with animals mainly dromedaries, or exposure in the surrounding vicinity (OIE, 2014).

Spread among humans was first reported in France, Tunisia and England in 2013 (Al-Tawfiq *et al*., 2014). Majority of human cases were reported among hospital workers and contacts of affected persons. Outbreaks in health care facilities have also been reported in some areas following close contact with affected cases (Oboho *et al*., 2015). Transmission between humans has been reported to run a short span with few tertiary cases reported (Memish, 2016).

The transmission pathway from camels to humans is thought to occur from the respiratory route, mainly from nasal and conjunctival discharges due to high virus titres in these two locations. Consumption of raw camel milk, which is a common practise, is another possible way of transmission of the virus. The MERS-CoV has been found to survive in fresh milk in in-vitro studies for extended periods under normal temperatures (Van Doremalen *et al*., 2014). Consumption of raw camel milk is a common practise among many cultures in Kenya and the Arabic countries. There is a significant chance of contracting the infection from camel urine which is occasionally used for medicinal purposes in the Arabic culture (Hemida *et al*., 2015).

Other possible ways of virus transmission include direct contact with camels, contact with or consumption of raw camel products like meat and milk including contact with fomites or contaminated food (WHO, 2014: Gossner *et al*, 2016). In Kenya, persons who have occupational exposure to the camels include the traders who transport the camels to the slaughterhouse, the slaughterhouse personnel due to contact with aerosols and discharges, those who take care of the camels at the holding pens prior to slaughter as well as herders of camels.

2.6 Clinical signs and pathogenesis of MERS-CoV in humans

In humans, MERS has been associated with respiratory illness. Symptoms of MERS-CoV can range from asymptomatic, mild respiratory to severe acute respiratory disease. It has been described as a flu-like disease with a respiratory syndrome which presents with a pneumonia. Common clinical signs observed in humans are chills, fever, shortness of breath and cough with pneumonia. The illness can progress to respiratory failure where patients may require ventilation support (White, 2014). Gastro-intestinal disease and kidney failure have been reported in several cases. These signs resemble those caused by the SARS-CoV except for kidney failure.

An autopsy carried out on a person who died of MERS-CoV identified respiratory syncytial cells and type 2 alveolar pneumocytes as the predilection sites of the virus (Goldstein and Weiss, 2017). In the lower lobes of the lungs, the infection causes massive alveolar damage leading to a viral interstitial pneumonia. Coronaviruses also possess mechanisms which delay or inhibit the host immune response. People who are in contact with camels, smokers, older males, and patients with underlying medical conditions such as chronic lung disease, cardiovascular disease and diabetes tend to be at a higher risk of developing a more severe disease (Hui *et al*., 2018).

2.7 Other coronaviruses in humans

The first identification of human coronaviruses (HCoV) was done in the 1960s from specimens of nasal swabs taken from humans with mild respiratory illness of short incubation periods like the common cold. Approximately one third of common colds are caused by two human coronaviruses-HCoV-OC43 and HCoV-229E while the

HCoV-HKU1 and the HCoV-NL63 are also known to cause significant disease (ECDC, 2014). Immunity against coronaviruses is usually short lived and there is no cross protection among strains of coronaviruses. Coronavirus colds are contagious due to their ability to mutate and have on rare occasions been associated diarrhoea in children.

Human coronaviruses are mainly spread though airborne transmission from infected droplets during coughing and sneezing. Close contact with an infected person, contact with body fluids and infected fomites and the faecal-oral route have also been identified as ways of virus spread (ECDC, 2014). The severe acute respiratory syndrome (SARS) coronavirus, a beta coronavirus caused approximately 8000 infections with over 800 deaths in 2003 in Asia. The SARS affects both the respiratory and gastrointestinal systems (Thanigaimalai, 2016). The SARS CoV is suspected to have been a result of recombination between mammalian and avian strains with the S gene representing a mammalian (group 1)–avian origin mosaic identified through viral studies (Su *et al*., 2016).

2.8 MERS –CoV and other coronaviruses in camels

Most camel infections with MERS-CoV have been associated with mild respiratory illness with occasional nasal discharge, but mainly the virus causes an insignificant illness which is self-limiting (Dires and Dawo, 2015: Sikkema *et al*., 2019). Camels have been found to harbor concurrent infections of more than one MERS coronavirus (Breise *et al.*, 2014). In Egypt, a study of 110 healthy camels in a slaughterhouse showed 4 (3.6%) to be infected with the MERS-CoV using a real time polymerase chain reaction (RT-PCR) assay. Sera collected from fifty-two of the camels showed presence of anti-MERS-CoV antibodies (Chu *et al*., 2014). In a study carried out in a slaughterhouse in Qatar where respiratory and rectal swabs were collected from a random group of 105 camels, 59% were found to be shedding the MERS-CoV but showed no clinical signs at the time of slaughter (Mohd *et al*., 2016).

Dromedary camels have been found to differ in their vulnerability to MERS-CoV infection based on age group. A study carried out among camels by Alagaili *et al* in the Kingdom of Saudi Arabia in 2014 showed sero-positivity to be significantly higher

(95%) among adult camels compared to juvenile dromedary camels (55 %). The difference was attributed to subsequent infection and increased likelihood of exposure over time (Alagaili *et al*., 2014). In Kenya, a study carried out on archived sera showed a higher sero-positivity (7%) in mature camels compared to younger ones (Corman *et al.*, 2015). Detection of MERS viral RNA in the rectal and nasal swabs of young camels was found to be more frequent than in older camels (Alagaili *et al*., 2014). A longitudinal study carried out in a camel herd, identified age of infection of calves with MERS-CoV to be when maternal antibodies wane at 5–6 months with production of antibodies occurring at the age of approximately one year (Alagaili *et al*., 2014). MERS sero-prevalence in adult camels in most areas has been shown to be significantly high with some areas reporting over 90%. In a study carried out in Kenya, camels from Northern Kenya were found to have higher sero-prevalence than camels from the rift valley region (Corman *et al*.,2014). Other coronaviruses like the bovine coronavirus also infect camels (Amer, 2018).

2.9 Coronaviruses in other animal species

Few studies have been done on MERS coronavirus in other domestic animal species. Studies on domestic animals indicate that those reared in close contact with camels risk being infected with the MERS coronavirus (Kandeil *et al*., 2019). Coronaviruses are known to cause a range of diseases in farm animals and pets, where great economic losses occur in the farming industry. They resemble human coronavirus in morphology and chemical structure. The avian infectious bronchitis virus, a coronavirus causes high mortality in poultry stocks (Dhama *et al.*, 2014). Coronaviruses can infect rodents, birds, ungulates domestic animals and herbivores. The porcine epidemic diarrhoea CoV (PEDV) first appeared in Europe and Asia in the 1970s and 1980s, causes mortality in piglets and is now endemic in swine (Lee, C., 2015). Other coronaviruses known to infect farm animals include porcine coronavirus (transmissible gastroenteritis coronavirus) and bovine coronavirus, turkey coronavirus, the feline Coronavirus (which occurs in two forms), ferret enteric coronavirus, canine coronavirus (which occurs in 2 forms) and mouse hepatitis virus (Halstead, 2014).

The ability of coronaviruses to mutate and produce new strains that are more virulent and potent is high. The bovine CoV acquired new influenza C-like hemagglutinin genes via recombination. Targeted recombination between feline and mouse S proteins enables feline CoV to infect mice. Coronaviruses have been noted to have zoonotic potential and can jump from one reservoir species to various species (Myoung-don et al., 2018).

2.10 Diagnosis of MERS-CoV

Patients who present with pneumonia and a history of travel to or residence in the Arabian Peninsula should be tested for MERS. Contacts of cases of MERS who develop respiratory illness within a fortnight should also be considered. A picture of pneumonitis on radiological examination with the appropriate travel/contact history including a history of exposure to camels should be considered when testing patients (CDC. 2014).

Nasopharyngeal and oropharyngeal swabs, sputum, serum, and stool/rectal swabs from suspected MERS-CoV cases are specimens which can be used for laboratory testing. To reduce chances of exposure to the MERS-CoV, the CDC recommends following of infection control precautions when collecting specimens including following of appropriate transport regulations (CDC. 2014).

2.10.1 Clinical signs of MERS-CoV

Middle East Respiratory Syndrome in humans presents with different clinical syndromes. These range from disease with no clinical symptoms to a severe acute respiratory illness with pneumonia which precipitates in acute hypoxemic respiratory failure. It has the presentation of a common cold with symptoms such as sore throat, fever, cough, sore throat, running nose, breathing difficulties and muscle pain (CDC, 2014). There may be gastrointestinal symptoms with diarrhoea, vomiting, abdominal pain, chest pain, malaise and headache, renal failure, extra-pulmonary organ dysfunction and changes in the circulatory system. The incubation period of MERS-CoV ranges between 2 and 14 days. MERS produces severe disease in those with a pre-existing illness. These include conditions like chronic obstructive pulmonary

disease, heart and lung conditions, cancer, kidney diseases those on immune-suppressive treatments and the elderly (CDC, 2014).

2.10.2 Culture of MERS-CoV

The MERS-CoV strain HCoV-EMC is propagated in-vitro in cell culture using LLC-MK2 and Vero cells (Hajjar *et al*., 2014). A pooled cell suspension prepared from the kidney tissue of adult rhesus monkeys (*Macaca mulatto*), was discovered in the 1950's to produce the LLC-MK2 cell lines which are used to produce antigens required for the detection of antibodies in humans via immune-fluorescence methods. The cells exhibit epithelial morphology and produce the protease plasminogen activator that typically initiates the process of fibrinolysis by converting plasminogen to plasmin. The HCoV-EMC strain also replicates in fully differentiated human airway bronchial epithelium cultures grown at the air-liquid interface (Jonsdottir and Dijkman, 2015).

2.10.3 Serology of MERS-CoV

Serological tests detect presence of antibodies developed during an immune response and are performed on blood and serum samples. They include serum neutralization tests and protein micro assays. Following exposure, the first antibody to appear is IgM (Immunoglobulin M), which is followed by a much higher titer of IgG (Immunoglobulin G) (Dires and Dawo, 2015).

Serology testing for MERS-CoV includes three separate tests: ELISA (enzyme-linked immunosorbent assay) is a test used to screen and identify presence and concentration of specific antibodies that bind to a viral protein. Immuno-fluorescent assay (IFA) confirms presence of attached antibodies that are specific to virus-infected cells fixed on a glass slide. These antibodies are identified by adding a secondary antibody labelled with a compound that gives fluorescence of an apple-green colour when viewed under a fluorescent microscope (Dires and Dawo, 2015). This secondary antibody binds to any antibodies present in the blood sample and have attached to the virus-infected cells. The neutralizing antibody assay (micro-neutralization assay), a

third more definitive test is considered the gold standard used to measure neutralizing antibodies and for detection of specific antibodies in serum samples (CDC , 2017). The EUROIMMUN assay is a commercial ELISA test that uses a recombinant MERS-CoV spike protein subunit 1-based ELISA and has been proven to show accurate results at 100% sensitivity and 100% specificity.

Coronaviruses have been known to cross-react and similar species of coronaviruses have been known to infect a wide range of mammalian hosts making analysis by serological means very challenging (Meyer *et al.*, 2014). Cross reactivity of antibodies directed against some of the major antigens of different CoVs has been found to occur in standard serologic assays. Challenges in laboratory diagnosis occur because camels have been found to be infected with coronavirus which affect bovine species. For human and camel testing, it is imperative to use tests that are specific for the MERS-CoV antibodies to avoid cross-reaction. The EUROIMMUN assay contains recombinant MERS-CoV spike protein which prevents cross-reaction with the bovine coronavirus (EUROIMMUN, 2015).

2.10.4 Molecular techniques for the diagnosis of MERS-CoV

These techniques establish presence of active infection in people suspected to be infected with the virus based on their symptomatic presentations and history of travel to places where MERS has been reported or contact with camels (CDC, 2014). Camels are also tested for presence of virus using molecular techniques though they may not show signs of illness.

Molecular techniques are based on performing reverse transcription Real time Polymerase chain reaction (rRT-PCR) on nasal, conjunctival and rectal swabs or on sputum and tracheal discharges. Polymerase chain reaction (PCR) tests aim to detect genetic material specific to the virus. Sequencing for MERS-CoV virus is then performed on samples found to be positive. Full sequencing of the virus is done to identify viral genetic material (CDC, 2014).

For PCR testing in humans, the WHO recommends obtaining samples from the lower respiratory tract via broncho-alveolar lavage (BAL), sputum sample

or tracheal aspirate as these have the highest viral loads (WHO, 2018). For camels, nasal, conjunctival, oral and rectal swabs are used.

2.10.5 Other methods for the laboratory detection of MERS-CoV

Radiology can be used alongside other laboratory methods and usually precedes other confirmatory tests. Chest X- rays of MERS-CoV patients show patchy infiltrates on both sides of the lungs. A viral pneumonitis with acute respiratory distress syndrome (ARDS) which tends to affect mainly the lower lobes of the lungs is observed. Computerized Tomography (CT scan) shows interstitial infiltrates. Histological examination shows that samples from MERS cases show a reduction in number of white blood cells, in particular lymphopenia. Liver function tests tend to show elevated levels of the enzymes creatinine kinase, alanine aminotransferase and lactate dehydrogenase (Das *et al*., 2017).

2.11Treatment of MERS-CoV infections

According to the WHO, there are no specific treatments for patients who become ill with MERS-CoV infection (WHO, 2014). Supportive medical care to help relieve the symptoms, complications and side effects is recommended with mechanical ventilation and intensive care usually required for severe cases.

Experimental studies showed that when rhesus macaques given interferon-α2b and an antiviral drug ribavirin were exposed to MERS, they developed less pneumonia than control animals (Breise *et al*., 2014). The interferon therapy has been used in humans with diverse results (Dawson *et al.*, 2019). The marmoset model, ritonavir/lopinavir and interferon α1b (either alone or combined) has in some cases shown success (Chan *et al.*, 2014).

In camels which are the reservoir for the MERS-CoV, the disease is either asymptomatic or presents as a self-limiting illness that does not require medical treatment.

2.12 Control of MERS-CoV infections

Control is mainly centred on prevention of infection among hospital and household contacts and hygiene measures when handling food and animals. Infection prevention and control measures are critical to prevent the possible spread of MERS-CoV in healthcare facilities (WHO, 2014). Droplet precautions should be observed when providing care to all patients with symptoms of acute respiratory infection together with contact precautions and eye protection. Health care providers should adhere to recommended personal protective equipment (gown, gloves, respirator, and eye protection) and avoid direct contact with infectious materials and secretions (CDC, 2014). Slaughterhouse workers and other camel handlers are advised to use personal protective equipment when handling camels (WHO, 2017)

Travel advisory to reduce the risk of exposure to MERS-CoV amongst travellers' including those going on Hajj pilgrimages is crucial (Conzade et al., 2018). This is especially important for those with increased chance of illness due pre-existing chronic conditions (WHO, 2014). Travellers who develop acute respiratory illness with fever within 14 days after returning from travel should seek immediate medical attention. General hygiene measures such as regular hand-washing after handling animals, avoiding contact with sick animals, and following food hygiene practices reduce risk of infection (Dires and Dawo, 2015).

Research into development of vaccines and evaluation for their efficacy in animal models is underway (Du *et al*., 2016). Scientists have developed a strain of the MERS-CoV that could be used as a vaccine due to its capability of infecting a cell and replicating its genetic material, while they deprive the virus the ability to infect other organs to cause illness (Yong *et al*., 2019)

Most of the recommended measures for preventing MERS spread among camel populations may not be feasible due to camel rearing practices and also since camels do not present with clinical symptoms. Controlling the virus spread can be achieved through separating juvenile camels which are at the stage of viral shedding from

adults, controlled regulation of camel movement, isolation of infected camels which may not be noticeable and regular herd screening (Omrani *et al*., 2015). Risk for spread from camel-to- camel or from camel-to-human is elevated by over-crowding, mixing of camels from different sources, mixing during grazing, transport and in animal markets (Yusof et al., 2017). Culling of camels to stop spread of the MERS coronavirus is not a recommended practice (Nowotny *et al*., 2014).

2.13 Slaughter practices and Knowledge regarding MERS

Recommendations for people involved in slaughter activities include long sleeved coat and long pants, closed rubber boots, use of protective rubber gloves, head caps, safety glasses, shields and masks to protect against aerosols, chemical and fluid splashes (Compsource, 2018). Middle East Respiratory Syndrome being a newly emerging infectious disease is not well known and understood. In countries that have had cases, the general population is aware of MERS but there are several misconceptions about the disease, risk factors, and control measures (Al-Mohrej *et al*., 2016). In a study carried out in Saudi Arabia which has the majority of cases to date, people did not seem to know that one could get the disease and not manifest any clinical signs. They also did not know that infected milk or meat could be a potential source of infection. Majority of people interviewed thought that the risk of MERS is higher among children what is usually the case with most infectious diseases due to low immune status in children (Bawazir, 2016). This has not been found to be the case in MERS with older people with co-morbidities being at highest risk. A study carried out by Zahrah Alnakli in the Republic of Saudi Arabia on knowledge, attitudes and practices regarding MERS on the general population showed that MERS was well known and females tended to have a better knowledge of risk and prevention than males (Alnakli, 2016).

A study carried out in Kenya among pastoralists on knowledge, attitudes and practices regarding zoonoses, potential pathways of exposure were well known. This included consumption of unpasteurized milk, handling infected discharges and aborted foetuses

without protective measures and consumption of raw meat and raw blood (Onono *et al.*, 2019).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study site

The study was conducted at Athi River slaughterhouse, which is located in Athi River town; Machakos County in Eastern Kenya. Machakos County has an area of 693 km^2 (268 sq. m) and is located to the west of Nairobi, Kenya's capital city at latitude of 1° 27' 27.81" S and longitude of 36° 58' 42.6108" E.

Athi River slaughterhouse receives camels from the Eastern and North Eastern counties of Garissa, Isiolo, Mandera, Wajir and Marsabit where two thirds of Kenyan camels are reared. It also receives camels from the Rift Valley regions of Baringo, Samburu and Turkana as well as from the coastal counties of Taita Taveta and Tana River. About 100-170 camels are slaughtered per month (approximately 4 daily) and the meat supplied largely to Eastleigh area in Nairobi.

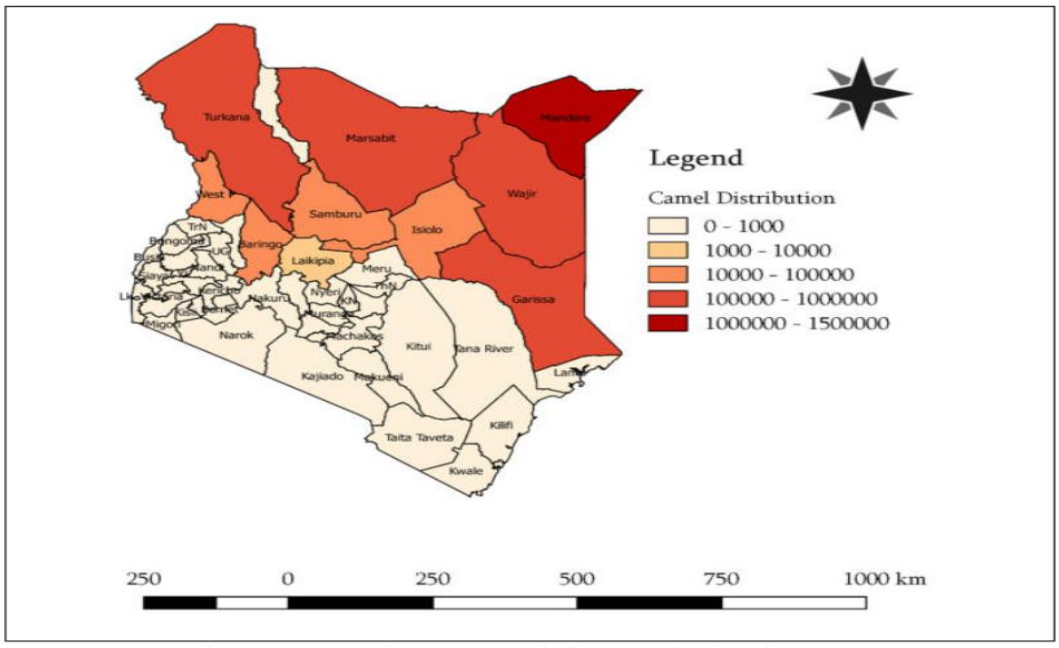

Figure 3.1: Map of Camel Population Distribution in Kenya

(Isako and Kimindu, 2019)

3.2 Study design

This study was a cross sectional study. This study design was used to determine the prevalence of MERS-CoV in camels originating from different regions of Kenya brought to slaughter at the Athi-river slaughter house.

Since workers in a slaughterhouse are few and are a closed population, all workers working at the slaughterhouse who participated in slaughter during the study period and all herders or traders who supplied camels during the study period were interviewed as a convenience sample for the knowledge and practices survey.

3.3 Study Population

Camel study population consisted of camels from Northern Kenya (Marsabit, Isiolo, Wajir, and Garissa), Rift valley region (Baringo), and Coastal Counties (Taita Taveta and Tana River), which were presented for slaughter at the slaughterhouse between May and July, 2015. The camels were those destined to be slaughtered for consumption, according to technical recommendations of the Meat Control Act of Kenya, 1995.

All seventeen workers at the slaughterhouse participating in the slaughtering process and handling of camels and 5 camel traders consented to be interviewed on knowledge and practices regarding MERS.

3.3.1 Inclusion criteria

(i) All camels presented to Athi River slaughter house for slaughter

(ii) All persons handling camel (herders, traders and slaughterhouse workers) who consented to participate in the questionnaire survey

3.3.2 Exclusion criteria

(i) Camels that were difficult to restrain

3.4 Sample size determination

Using the Cochran formula, the camel sample size was determined as follows: (Charan and Biswas, 2014)

$$n = \frac{Z^2\ p(1-p)}{e^2}$$

Where Z value for 95% confidence level is 1.96, p is the prevalence found in a previous study at 29.5% (Corman *et al*., 2014) and *e* is the precision (margin of error) at 5%.

$$\frac{1.96^2 \text{ X } 0.295\ (1\text{-}0.295)}{0.05^2} \qquad n = 320 \text{ camels}$$

Four Elisa kits were procured from EUROIMMUN, Germany and each could test 93 samples, a calibrator, positive and negative control. In order to maximize on the kit, 372 samples were collected. All 22 persons encountered in the slaughterhouse handling camels agreed to participate in the study by signing consent forms and were interviewed for the knowledge and practices survey.

3.5 Selection of camels for the study

A systematic random sampling technique was used to select camels for sample collection. On each sampling day every first camel to be brought to the entry of the slaughter house was sampled followed by every subsequent 2nd camel. In case a camel proved difficult to restrain, the one that followed it was sampled followed by every subsequent second camel after that. As required by most serological tests that specimens from an animal are taken on two different occasions, this was not possible in this study as camels sampled immediately proceeded to slaughter.

3.6 Blood sample collection

Blood samples were collected from the camels at the entrance to the slaughterhouse at the holding area. Phlebotomy was performed by veterinary staffs that were

adequately trained on sample collection. Blood specimens were collected from the jugular vein. The animals were restrained with the head elevated on one side to expose the jugular groove. The jugular vein was raised by applying pressure at the base of the jugular groove. Approximately 10mL of whole blood was collected from the jugular vein using sterile technique (disinfecting puncture site with 70% alcohol) into a red-capped plain vacuum plastic tube (Vacutainer®) with a clot activator. The blood sample was allowed to clot at room temperature, transported at ambient temperature (2-8° C in a cool box) to the Central Veterinary laboratories, Kabete. Samples were centrifuged and the serum collected from the Vacutainer using a disposable plastic Pasteur pipette. They were then separated into 2 equal aliquots into Eppendorf tubes.

3.7 Sample storage

At the Central Veterinary laboratory in Kabete, all samples in Eppendorf tubes were labelled and stored in cryoboxes in the –20°C freezer in readiness for testing.

3.8 Laboratory detection of MERS-CoV IgG antibodies

An Indirect immuno-flouroscent Enzyme-linked Immunosorbent assay (IgG ELISA) from EUROIMMUN, Germany was used for the detection of MERS-CoV antibodies (Appendix 6). The laboratory kit was among the first commercial kits to be produced worldwide and has been found to be suitable for screening and for conducting epidemiological studies. The quality of the MERS-CoV antigen used in the test had been tested to ensure that no cross-reactivity with the bovine coronavirus which also affects camels occurred. The test kit was reported to be reliable at high sensitivity (100%) and high specificity (100%) for MERs-CoV antibodies (EUROIMMUN, 2015).

3.8.1 Anti-MERS-CoV IgG Elisa test procedure

Samples were diluted 1:101 in sample buffer and mixed using a vortex mixer. For incubation,One hundred (100) µl of the calibrator, the positive and negative controls or the diluted samples was transferred into individual microplate wells. The test plates

were covered with protective foil and incubated for 30 minutes at +37°C ± 1°C. The protective foil was removed and the wells emptied and washed 3 times using 300 µl of working strength wash buffer. The micro plates were tapped on absorbent paper facing downwards to remove residual wash buffers. Enzyme conjugate (peroxidase-labelled anti-camel IgG) 100 µl was then pipetted into each of the microplate wells which were covered with protective foil and incubated for 30 minutes at +37°C ± 1°C. The protective foil was then removed and the wells emptied and wash performed. Chromogen/substrate solution (100 µl) was pipetted into each of the microplate wells which were incubated for 15 minutes at room temperature (+18°C to +25°C). Stop solution (100 µl) was then pipetted into each of the microplate wells in the same order and at the same speed that the chromogen/substrate solution was introduced.

3.8.2 Measurement of optical density

Photometric measurement of the color intensity was done using an Elisa reader at a wavelength of 450 nm. Prior to measurement, the microplate was slightly shaken to ensure a homogeneous distribution of the solutions. The Elisa results were then printed out from a printer attached to the Elisa reader.

3.8.3 Calculation and interpretation of the results

The results were evaluated semi-quantitatively by calculating a ratio of the extinction value of the control or sample over the extinction value of the calibrator. The ratio was calculated according to the following formula:
Optical density ratio = Extinction value of sample/extinction value of calibrator
EUROIMMUN recommended interpreting results as follows:
OD Ratio <0.8: negative
OD Ratio: 0.8 to <1.1: borderline
OD Ratio; >1.1: positive

3.9 Data collection

All blood specimens collected from the camels were recorded on the laboratory tracking sheet where the variables recorded were the date of sample collection, unique animal identifier, approximate age, sex, and place of origin of the camels. The age of the camels was determined using their dentition (Bello *et al*., 2014). Camels that still had milk teeth were recorded as below 5 years of age. Camels that had a full set of permanent teeth that had not started wearing out were recorded as between 5 and 8 years of age. Camels which showed wearing out of the first set of incisors were recorded as between 8 and 11 years. Camels showing wearing out of canine teeth were recorded as above 11 years. Place of origin was recorded as that given by the camel handlers and was divided into 3 categories, Northern Kenya, coastal region and rift valley region. A pre-tested structured questionnaire (Appendix 1 and 2) was administered to collect information from the camel handlers (meat inspectors, slaughterhouse workers, herders and traders). Information collected was regarding whether the camels brought to slaughter had interacted with camels from outside the country which could introduce new variants of the virus to the camel population, and mixing of camels during transport, grazing and watering. Information was collected on knowledge and practices regarding Middle East Respiratory Syndrome and knowledge of zoonotic diseases and their mode of transmission. A check list was used to collect information through observation on the slaughter and hygiene practices.

3.10 Data management, analysis and presentation

3.10.1 Data Storage

Data were stored in a password protected computer to enhance security and in an external hard drive under lock and key and backed up in Google drive. All hard copies were stored in a drawer under lock and key.

3.10.2 Data Management

All data was entered, cleaned and analysed using EPI Info 7 (CDC, Atlanta, GA, USA) and Ms Excel 2007 (Microsoft, Seattle, WA, USA). MERS-CoV sero-positivity was calculated from the results obtained on analysis of the camel samples. Univariate analysis was performed where proportions' were calculated for categorical variables (age, sex and place of origin of the camels). Chi-square tests were conducted to compare camel age, place of origin and sex with MERS-CoV sero-positivity: $P < 0.05$ was considered significant. Proportions were calculated from the data obtained from the questionnaires on knowledge and practices regarding MERS and on safety measures undertaken during slaughter.

3.11 Ethical Approvals and Considerations

Protocol approval was sought and obtained from Board of postgraduate studies of Jomo Kenyatta University of Agriculture and Technology (JKUAT) and ethical clearance was sought and obtained from Kenya Medical research institute (KEMRI) Ethical Review Committee (SSC No. 2963). This study was part of a larger study on sero-prevalence and circulating genotypes of Middle East respiratory syndrome coronavirus conducted through the Centres for disease control and prevention (CDC) on Middle East respiratory syndrome coronavirus (Appendix 7). I, Esther Kamau did the sero-prevalence study for my Masters' thesis. Permission to do the study was sought from the Director of Veterinary services and the Machakos County Veterinary authorities.

The aims and procedures of the study were explained to participants who were required to give consent prior to participation in the study. The collected serum samples were used for purposes of this study only. Confidentiality of personal and laboratory information was observed and maintained.

CHAPTER FOUR

RESULTS

Between May and July 2015, 372 specimens were collected from 372 camels for a period of 10 weeks. Of the camels sampled, 196 were female while 176 were male. One percent (4/372) of the camels were below five years of age and were brought to slaughter due to injuries. Twenty six percent (98/372) were between 5 and 8 years of age, 69% (255/372) were between 8 and 11 years, and 4% (15) were over 11 years old. Camels supplied to the slaughter-house during the study period were from 7 counties. One hundred and sixty-nine (169/372) camels originated from Northern Kenya (Marsabit, Wajir, Garissa and Isiolo), one hundred and ninety-three (193/372) from the coastal areas of Tana River and Taita Taveta Counties with ten from the Rift valley County of Baringo (10/372). There were no camels received from the counties of Samburu and Turkana during the study period.

Two hundred and eighty-eight (77.4% 95% CI: 72.83-81.57) of the camels had an optical density ratio of over 1.1 which was the cut-off for the positive samples. Twenty-two (6%) had borderline results (optical density ratio between 0.8 and 1.1) while 62 (17%) had negative results (optical density ratio less than 0.8) (Appendix 9). Due to financial constraints and lack of another available test locally, it was not feasible to carry out another serological test on the borderline samples. All border-line samples were regarded as negative samples during the analysis to avoid over-estimation of the prevalence obtained.

4.1 Prevalence of MERS-CoV antibodies by age group of the camels

Among 4 camels which were below 5 years of age brought to the slaughterhouse due to injuries, two had positive results, one showed border-line results while one was negative for MERS-CoV antibodies. Among those aged between 5 and 8 years, 80 were positive, 13 were negative and 5 had borderline results. In the 8-11 age groups, 194 were positive, 45 were negative and 16 had borderline results. In the camels aged

above 11 years, 12 had positive results and 3 had negative results.

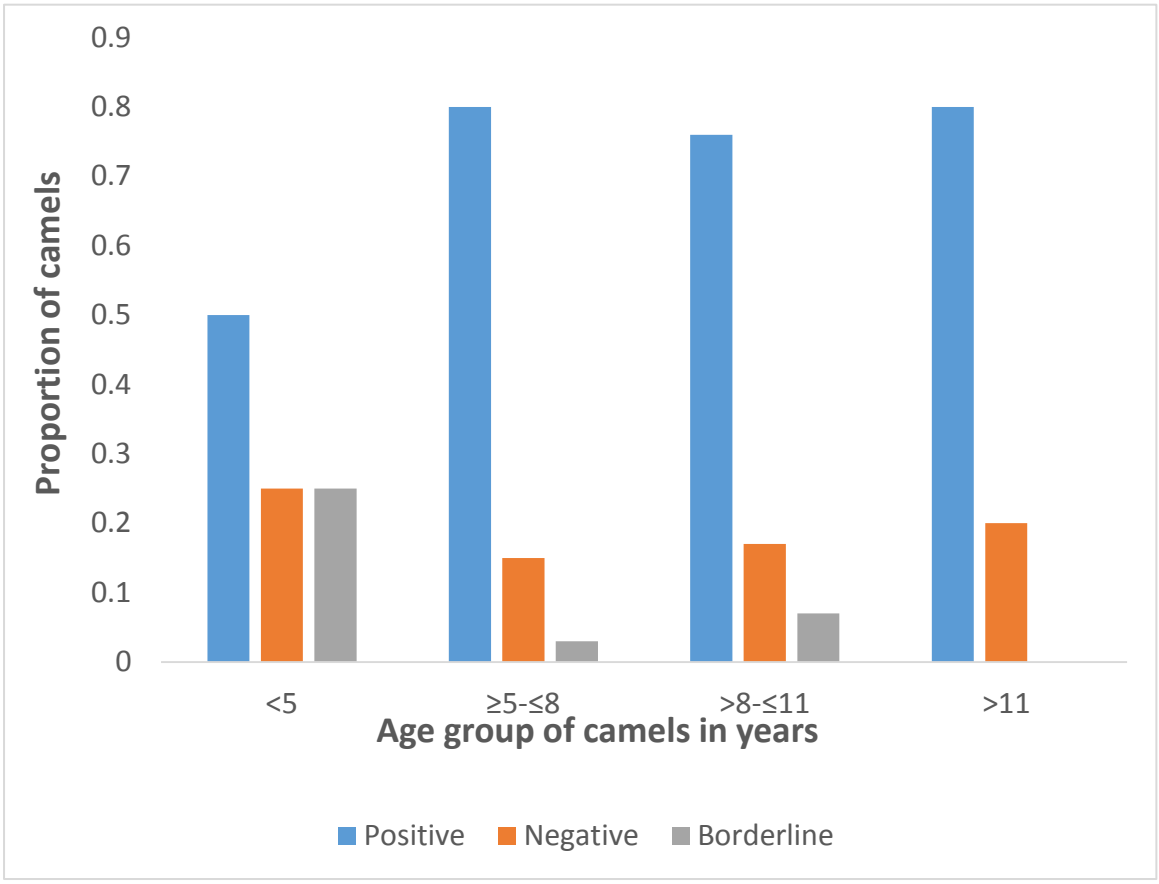

Figure 4.1: Sero-status of camels according to their age groups

4.2 Sero-prevalence of MERS-CoV according to sex of the camels

Among the camels sampled, 196 (53%) were female, while 176 (47%) were male. Sero-prevalence in females was 78.1% (153/196), (95% CI: 71.6-83.6), which was slightly higher than that in male camels at 76.1% (134/176) (95% CI: 69.14-82.23).

4.3 Sero-prevalence according to place of origin

Camels brought to the slaughter-house during the study period were from 3 regions:-

1. Northern Kenya -Marsabit, Wajir, Garissa and Isiolo
2. Coastal region -Tana River and Taita taveta

3. Rift valley region-Baringo

Table 4.1: Status and distribution of camels by region of origin

Status/origin	Northern Kenya (n=169)	Coastal region (n=193)	Rift Valley (n=10)	Total
Positive	131 (77.5%) (95% CI:70.4-83.57)	150 (77.7%) (95% CI: 71.2-83.4)	7 (70%) (95% CI: 34.8-93.3)	**288 (77.4%)**
Negative	38 (22.5%)	43 (22.3%)	3 (30%)	**84 (22.6%)**
Total	**169**	**193**	**10**	**372**

4.4 Results of Chi-square tests to compare MERS Sero-positivity with age, sex and origin

In order to compare sero-positivity with age group of the camels, the animals were divided into 3 groups, those below 8 years of age which were 102, those with age of 8 to 11 years (255) and those above 11 years (15). Chi square tests performed to compare sero-positivity with age group did not show any significant statistical difference sex □2 (2, N=372) =0.835 p=0.659.

Prevalence in males was 76.1% (95% C.I 69.1%-82.2%) while in females it was 78.1% (95% C.I 71.6-83.8). Chi square tests to compare sero-positivity with sex did not show any statistical difference □2 (1, N=372) =0.195 p=0.659.

To assess statistical difference by place of origin, sero-prevalence was compared among camels from Northern, coastal and Rift valley regions. Chi square tests to compare sero-positivity with place of origin did not show any significant statistical difference (□2 (2, N=372) =0.326 p=0.851).

Conclusion: There was no statistical difference observed based on age, sex and place of origin of the camels sampled.

4.5 Risk factors associated with exposure to MERS-CoV in camels

Five herders/traders 4 from northern Kenya and one from Baringo (Rift valley) region and 17 slaughterhouse workers were interviewed. All reported that camels brought for slaughter were from within the country and did not have any knowledge of contact of the camels with others camels from outside the country. They reported that camels brought to slaughter had frequent contact with other herds during grazing, watering and transportation.

4.6 Knowledge and practices regarding MERS and zoonotic diseases among herders, traders and slaughterhouse workers

Twenty-two people who included 5 herders/traders, 14 slaughter house workers, 2 meat inspectors and 1 County supervisor were interviewed. There were no people encountered who declined to participate in the study. Majority were aged 31-50 (46%) and 10 (46%) had a Secondary school education. Of the sixteen who participated in slaughter, 13 had worked in the slaughter house for more than 3 years. Five herders and traders reported having participated in camel herding and trade for over 3 years. On knowledge of MERS-CoV, sixteen (73%) out of 22 were unaware of MERS-CoV in camels or humans, and 20 (90%) were aware that disease can be transmitted from animals to human with most being aware of common ways by which disease can be transmitted from animals to human.

Table 4.2: Knowledge and practices regarding MERS and zoonotic diseases among slaughterhouse workers, herders and traders

	Categorical variable	Frequency (n)=22	Percentage (%)
Age in years	18-30	9	41
	31-50	10	46
	Over 50	3	14
Level of education	Religious education	2	9
	Primary	6	27
	Secondary	10	46
	College	4	18
Nature of work	Herder/trader	5	23
	Slaughter	14	64
	Meat inspection	2	9
	Supervision	1	5
No. of years worked	1-3	4	18
	Over 3 years	18	82
Hand washing after handling	Yes	22	100
	No	0	0
Heard about MERS	Yes	6	27
	No	16	73
Drank raw camel milk	Yes	3	14
	No	19	86
Disease can be transmitted from animals to man	Yes	20	91
	No	0	0
	Don't know	2	9
Knowledge of ways of zoonotic disease transmission	Drinking raw milk	15	68
	Slaughtering animals	11	50
	Close contact with animals*	4	18
	Eating improperly cooked	20	91
	Contact with discharges	7	32

* Close contact was defined as participating in activities such as milking, providing care and treatment to the animals, and assisting in calving.

On assessment of personal protective clothing used while carrying out slaughter practices, a checklist was used to collect information. We observed 1 supervisor, 2 meat inspectors and 14 slaughterhouse workers.

Table 4.3: Use of protective wear among slaughterhouse workers

Personal protective wear	No. (n=17)	Percentage (%)
Lab-coats/overalls	13	76
Gumboots	15	88
Hand gloves	0	0
Face masks	0	0
No use of personal protective equipment	3	18

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The results of this study depict a high sero-prevalence of MERS-CoV antibodies in camels presenting for slaughter at the Athi-river slaughterhouse from various regions of Kenya (77%). This indicates that MERS-CoV is widespread in camels in Kenya and the prevalence in the camel populations could be increasing due to continued exposure. This fact can be further elaborated by the fact that MERS-CoV sero-prevalence from camel sera from various regions in Kenya that was archived for over 30 years and tested in 2012 reported a prevalence of 30% compared to 77% obtained in this study (Corman *et al*., 2014). These results compare with other tests done among sera collected in Marsabit County in 2014 in which sero-prevalence was found to be 90% (Munyua *et al*., 2017). It also compares with studies done in other African countries in which sero-prevalence in Ethiopia and Nigeria exceeded 90% (Reusken *et al.*, 2014).

Sero-positivity has been shown to be significantly higher among adult camels compared to juvenile ones with the difference being attributed to subsequent infection and increased likelihood of exposure over time (Alagaili *et al*., 2014). In Kenya, a study carried out on archived sera showed a higher sero-positivity (7%) in mature camels compared to younger ones which may suggest that majority of the camels had developed antibodies from infection earlier in life (Corman *et al.*, 2014). Since young camels are not typically brought to slaughter, this study recorded only 4 camels below the age of 5 years. Sero-prevalence in this age group was 50%, compared to 77.7% in camels over 5 years of age. Due to this small number, we were not able to make conclusions based on age differences. Sero-prevalence based on the sex of the camels did not show any significant statistical difference in this study. Since there were almost equal numbers of male and female camels, one may conclude that factors associated with exposure among the different sexes are similar. Similar sero-prevalence among

male and female camels has been found to be the similar in other studies carried out among dromedaries in Africa (Kandeil *et al*., 2019).

Sero-prevalence may also differ according to the place of origin of the camels. In a previous study carried out in Kenya, sero-prevalence was reported to be higher in camels from Northern and North-eastern regions compared to the Rift valley region (Corman *et al.*, 2014). This study showed prevalence in camels from all regions sampled (Northern, coastal and rift valley) to be at or above 70%. There were few (10) camels brought to slaughter from the Rift valley region (Baringo County), but nevertheless, there was no statistically significant difference according to place of origin.

Sero-prevalence of MERS-CoV may vary based on laboratory tests performed (WHO, 2014). Sero-prevalence obtained in this study was higher than that obtained in a previous study in Kenya which reported an overall sero-prevalence of 30% using a recombinant MERS-CoV spike protein subunit 1-based ELISA (rELISA) with varying prevalence from different regions of Kenya (Corman *et al*., 2014). The MERS-CoV EUROIMMUN assay used in this study was sourced from EUROIMMUN, Germany and has been shown to have 100% sensitivity and 100% specificity. The assay also uses a recombinant MERS-CoV spike protein subunit 1-based ELISA which prevents cross-reaction with the bovine coronavirus (which also affects camels). Its validity had been proven when compared with other assays by production of accurate results when carried out on diverse camel herds (EUROIMMUN, 2015). Due to financial constraints and lack of availability of another test locally, we did not carry out other serological tests on the border-line samples which accounted for 6% of the total samples collected. We therefore recorded border-line samples as negative for MERS-CoV to avoid over-estimation of the actual sero-prevalence.

MERS-CoV sero-prevalence estimates in camels could differ based on the population of camels tested, and camel production systems. Camels have frequent contact in market places and during grazing, watering, and transportation, with camels from various herds being commonly transported together. Camel population mixing influences the age of exposure to the MERS-CoV as those in the virus shedding phase

could easily transmit the virus to those not already exposed. This has been found to occur quite naturally due to the fact that infected camels are either aymptomatic or show signs of mild respiratory infections. The asymptomatic nature of MERS in camels makes it difficult to detect clinically camels at the virus shedding phase. A study carried out in Africa, reported high prevalence among camels in market places and in slaughter houses compared to camels in barns in homesteads (Kandeil *et al*., 2019). The MERS-CoV sero-prevalence that we found in this study exceeded that reported in camels in Laikipia County in 2015 (46.9%), where camels sampled were from nine closed herds (Deem *et al*., 2015). The difference could be attributed to interaction of camel populations from different areas in different husbandry practices leading to circulation and introduction of the MERS-CoV virus to naive populations. The five herders interviewed in this study reported that diverse camel herds frequently mix during transportation and grazing activities.

Knowledge on MERS-CoV among the 22 camel handlers was found to be low and those who were aware were informed by our team during the initial planning stages. This lack of awareness could lead to be possible exposure during camel handling and slaughter processes from discharges and aerosols. Slaughterhouse workers' awareness about other zoonotic diseases and how they are transmitted was high. Good hygiene was demonstrated by washing of hands after handling camels. Use of personal protective clothing was observed to be low and was limited to use of gumboots and overalls which suggested that they were mainly protecting themselves from dirt. There was no use of gloves and face masks which was a significant finding in this study due to zoonotic transmission of MERS through aerosols and body discharges. Several herders reported drinking of raw camel milk, which is a common practise among camel keeping communities in Kenya. This practise may increase overall risk of exposure to zoonotic diseases including MERS which can be transmitted through milk. Though the population of those interviewed was low, this study provided useful qualitative information on camel handling and slaughter practices. This could work as a good entry point for creating awareness about MERS among camel handlers to enable them to better protect themselves from exposure to MERS and other zoonotic infections (Bawazir *et al*., 2018).

5.2 Conclusions

From this study, the following conclusions are made:-

- Prevalence of MERS-C0V among camels presenting for slaughter at Athi-river slaughter-house is high (77%)
- Prevalence of MERS did not significantly differ by age, sex and place of origin of the camels
- Prevalence of MERS has increased over time (since the last study done in 2012) as more camels get exposed. This could pose a risk to human populations living with or handling camels as MERS is a zoonotic disease
- Camel herders, traders and slaughter-house workers are not aware of MERS-CoV
- There was poor use of personal protective equiment among slaughter-house workers limited to use of gumboots and overalls. Use of hand gloves and face masks (which are crucial in protection from aerosols and discharges) was lacking
- Some herders reported drank raw camel milk which could expose them to the MERS virus among other zoonotic diseases.

5.3 Recommendations

1. There is a need to sensitize people working with camels (herders, traders and slaughterhouse workers) about MERS and its zoonotic nature. Since most of the people interviewed had some knowledge of zoonotic diseases, one could leverage on this to inform and create awareness.
2. There is need to evaluate bio-safety practices among people working with camels to enhance hygiene and bio-safety practices. Use of hand gloves and face masks should be a requirement for those participating in slaughter practices and those in close contact with camels. This reduces opportunity for potential virus transmission since, studies have indicated that slaughterhouse

workers could be at a higher risk of exposure to MERS-CoV (Muller *et al*., 2015).

3. Education on ways of MERS transmission through aerosols and discharges to communities in camel keeping areas and education of benefits of boiling or pasteurization of milk could help to inform on safety measures to undertake to reduce risk.

5.4 Recommendations for further studies

- Evaluation of the exposure status among persons working with camels would give more information on development of surveillance and prevention guidelines
- More studies on younger populations of dromedaries would assist in determining the dynamics of virus transmission to the younger animals and at what ages the virus transmission is likely to occur. Monitoring of camel herds longitudinally could establish mode of virus transmission.
- Identification and characterization of the MERS-CoV in Kenya can help prevent outbreaks and identify genetic differences with MERS-CoV virus causing outbreaks in other countries. Understanding the epidemiology of MERS-CoV in Kenya will contribute to more rapid detection and control of MERS-CoV in Kenya, thereby enhancing global health security.

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APPENDICES

Appendix I: Anti-MERS-CoV ELISA Camel (IgG) kit instructions

(EUROIMMUN- Germany)

Principle of the test: The ELISA test kit provides a semi-quantitative in vitro assay for antibodies of class IgG against MERS coronavirus in serum or plasma of camels. The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified S1 antigen of MERS coronavirus (MERS-CoV S1). In the first reaction step, diluted samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-camel IgG (enzyme conjugate) catalyzing a colour reaction.

Sensitivity and specificity: To confirm assay sensitivity, 151 sera from camels collected in Dubai were analyzed and results were compared to in-house assays of the Institute of Virology, University of Bonn, Germany. To assess assay specificity, 20 sera from camels collected in Germany with negative predictive value and, additionally, 13 camel sera from the UAE negative for MERS-CoV antibodies but positive for bovine coronavirus antibodies in a recombinant IFA were tested. Sensitivity and specificity of the EUROIMMUN Anti-
MERS-CoV ELISA Camel (IgG) both amounted to 100 %.

Technical Data:

Antigen Purified S1 antigen of MERS coronavirus (MERS-CoV S1).

Evaluation Semi-quantitative evaluation using ratio values (extinction value of the control/sample over the extinction value of the calibrator).

Interpretation EUROIMMUN recommends interpreting results as follows:

Ratio < 0.8: negative

Ratio ≥ 0.8 to < 1.1: borderline

Ratio ≥ 1.1: positive

Sample dilution Serum or plasma; 1: 101 in sample buffer.

Reagents Ready to use

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable for 4 months if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

The thermostat adjusted ELISA incubator must be set **at 37°C ± 1°C**.

- **Coated wells:** Ready for use. Tear open the re-sealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrator and controls:** Ready for use. The reagents must be mixed well before use.
- **Enzyme conjugate:** Ready for use. Enzyme conjugate must be mixed well before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with de-ionized or distilled water (1 part reagent plus 9 parts distilled water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.

The working strength wash buffer is stable for 1 week when stored at +2°C to +8°C and handled properly.

Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

Stop solution: Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Samples, calibrator, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: All materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the samples

Samples: Camelide serum or plasma.

Stability: Samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Samples are diluted 1:101 in sample buffer.

For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrator and controls are prediluted and ready for use, do not dilute them.

Sample incubation: (1st step)

Transfer 100 µl of the calibrator, positive and negative controls or diluted samples into the individual microplate wells according to the pipetting protocol.

For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.

Incubate for 30 minutes at +37°C ± 1°C.

Washing: Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.

Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-camel IgG) into each of the microplate wells. The wells have to be covered with the protective foil when incubating manually. Incubate for **30 minutes at +37°C ± 1°C**.

Washing: Remove the protective foil and empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes at room temperature** (+18°C to +25°C) (protect from direct sunlight).

Stopping the reaction: Pipette 100 µl of stop solution into each of the microplate wells in the same order and the same speed as the chromogen/substrate solution was introduced.

Measurement: Photometric measurement of the colour intensity should be made at a Wavelength of 450 nm

Appendix II: Animal sample collection form (n=372)

Form No…….

Title: Sero-prevalence of Middle East Respiratory Syndrome Coronavirus in Camels at Athi River Slaughterhouse

ANIMAL OWNER.....

ANIMAL IDENTITY NUMBER ……

Age of camel ≤ 5 years ☐ 5 -8years ☐ 8-11 years ☐ Over 11 years ☐

Sex of Camel Male ☐ Female ☐

Origin of camel (County) ……..……

Any symptoms of disease observed in camels Yes ☐ No ☐

Samples collected (Check all that apply)

Blood ☐

Laboratory results (to be filled after laboratory results are available)

Blood

Sample No ……

Elisa Results (Optical density ratio)……

Appendix III: Questionnaire for herders, traders and slaughter-house workers (n=22)

Title: Sero-prevalence of Middle East Respiratory Syndrome Coronavirus in Camels at Athi River Slaughterhouse

Name of the interviewee (herder/trader/SH worker) .....

Date of interview: .....

General and demographic information

For the interviewer:

1. Who is the respondent?
   Trader ☐ Herder/owner ☐ SH worker ☐
2. How old are you?
   18- 30 ☐ 31–50 ☐ Over 50 ☐
3. What is the highest level of education you have completed?
   No school ☐ Primary ☐ Secondary ☐ College ☐ Religious ☐
4. What is your primary occupation?
   Camel trader ☐ Camel owner/herder ☐ other (specify) ☐
5. How long have you engaged in the camel trade/worked in the slaughter-house?
   Less than 1 year ☐ 1-3 years ☐ > 3 years ☐

   History and possible exposure factors in camels
6. Did this group of camels come into contact with other camels during grazing/ watering/transportation in the last three months? Yes ☐ No ☐
7. Have you ever bought/raised/slaughtered camels that have come from outside the country? Yes ☐ No ☐
8. If yes to Q 6 above, which country did they come from……
9. Do you wash your hands after handling camels/camel meat/ slaughter?
   Yes ☐ No ☐
10. Do you usually consume camel milk? Yes ☐ No ☐
11. If yes to Q 13 above, do you usually boil camel milk before consumption? Yes ☐ No ☐

MERS knowledge and awareness

12. Have you ever heard of Middle East Respiratory syndrome? (If No skip to question 14) Yes ☐ No ☐
13. If yes, what was the source of information?
    Radio ☐ TV ☐ Newspaper ☐ Religious leader ☐ other (specify)…….....
14. Do you think disease can be transmitted from animals to humans?
    Yes ☐ No ☐
15. If yes to Q 14 above, how is disease transmitted from animals to humans? (Check all mentioned.)
    Drinking raw milk ☐ Slaughtering animals ☐ milking animals ☐
    eating uncooked meat ☐ Contact with discharges during calving ☐
    Contact with surfaces contaminated with secretions, faeces and urine
    Others (specify): …… ☐

For Slaughter-house workers only

Check list on use of personal protective equipment during slaughter practices

1. Is interviewee wearing personal protective equipment when slaughtering/handling animals? Yes ☐ No ☐
2. If yes to 6 above, which of the following personal protection equipment is used

    Lab coat/overall ☐ rubber boots/gumboots ☐ Hand gloves ☐
    Face mask ☐ Eye shield ☐
3. Washing of hands after handling camels/camel meat/ slaughter?
    Yes ☐ No ☐

Appendix IV: Consent form for herders, traders and slaughter-house workers

Title of study: Sero-prevalence of Middle East Respiratory Syndrome Coronavirus in Camels at Athi River Slaughterhouse

Introduction:

Investigators from MALF (Ministry of Agriculture, livestock and fisheries) in collaboration with CDC/KEMRI would like to learn more about a new infection that affects camels and people. MERS-CoV is a disease that can be passed from animals (camels) to humans that is of public health importance. It could pass from animals to humans when people get exposed to infected livestock and their products which may act as a source of infection.

Purpose of study:

Since some germs can be passed from camels to humans, we would like to test your camel for any signs of these germs. To do this, we would like to collect blood samples from part or all your camels and test them for some of the germs that may possibly cause illness among humans and animals. We would also like to ask you some questions about how the animals are managed. This process will take from 30 minutes to one hour, depending on the number of camels.

Alternatives

The alternative to choosing to be in the study is to decline to be in the study.

Why You Have Been Chosen

We are interviewing persons who work with camels here at the slaughter house or are involved with camels in any way.

Handling of specimens (For animal herders and traders only)

We will test the samples collected from your animals at the Central Veterinary laboratory in Kabete, Kenya. We would also like to ask if we can store these samples to do more tests at a later time. However, additional ethical approval will be sort from KEMRI prior to any tests in future on these specimens.

Risks:

Handling and restraining animals for sample collection can be slightly stressful for the animals and for people who are participating. Every care will be taken to minimize this stress. Drawing blood can cause brief pain to the animals, and may result in brief bleeding. Sampling the animals may take some time, as will answering the questions about the animals.

Benefits:

This study may lead to a better understanding of knowledge, attitude and practices regarding MERS CoV infection. The results of this study will be communicated and disseminated to the people concerned for them to take action on the recommendations that will come out from the study results. This will include necessary control measures if need be.

Confidentiality:

Any information obtained from you will be kept confidential and used solely for purposes of this research only. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, but your identity will not be disclosed.

Compensation:

If you accept to take part in this study, there will be no payment for participation.

Voluntary participation

Deciding whether or not to be in the study today is your choice. You can choose not to join, or to drop out at any stage. Should any more questions arise or if you feel like you or your animals might have been harmed by being in the study, please contact Esther Kamau- Ministry of Agriculture, Livestock and Fisheries- 0722917041. Should you have any questions about your rights as research participants, please contact the secretary, KEMRI/NERC (tel. 0202722541 or 0722205901 or 0733400003). You will receive a copy of this signed consent form to take away with you

Approval of the study:

This study was approved by:

Kenya Medical Research Institute (KEMRI) Ethics & Research Committee

P.O. BOX 54840 - 00200

Nairobi, Kenya

And

Board of Post graduate studies

Jomo Kenyatta University of Agriculture and Technology

P.O. Box 62,000, Juja, Kenya

The consent form has been explained to me and I agree that I/and my animals can take part in the study. I have been told that I am free to choose not to take part in this study at any time and that saying "NO" will have no effect on me. I agree to allow samples from my animals to be stored at KEMRI and CVL for possible future testing in Kenya and abroad. This testing will not include genetic testing.

Owner of camels/ slaughterhouse worker

Name: Signature/Thumb print: date□□/□□/□□

Witness Name: Signature: date□□/□□/□□

Interviewer Name: Signature: date□□/□□/□□

Study Staff member obtaining this consent

Name: Signature: date□□/□□/□□

Appendix V: Translated consent form for herders and traders/ fomu ya ridhaa kwa wachungaji na wafanyi biashara

Title of study:

Seroprevalence of Middle East respiratory syndrome corona virus in camels at Athi-river slaughter house

Mwanzo:

Sisi ni maofisa kutoka vizara ya mifugo na KEMRI na tungetaka kuchunguza ugonjwa ambao unadhuru binadamu na ngamia. Huu ugonjwa wa MERS (homa ya ngamia) ni ugonjwa ambao unatoka kwa ngamia na unaweza sababisha madhara kwa watu kutoka kwa ngamia mgonjwa au bidhaa zinazotoka kwa ngamia walio na maradhi.

Kusudi

Kwa vile ugonjwa unaweza toka kwa ngamia ukapita kwa binadamu, tungependa kumpima ngamia wako ili tuone kama ako na virusi vya homa ya ngamia.

Ili tuweze kufanya hivyo tutachukua sampuli za damu. Tungetaka pia kukuuliza maswali machache ya vile unavyotunza ngamia wako ambayo yanaweza kuchukua muda wa nusu saa.

Mbadala

Ikiwa hautaweza kuturuhusu kufanya utafiti huu, waweza ukakataa kujiunga na hii kazi bila masharti yoyote.

Tumekuchagua kwa sababu tunahitaji watu ambao wanafanya kazi hapa kwa kichinjio au ambao wanahusika na ngamia.

Sampuli zitakazotolewa zitapelekwa kwenye maaraba Kabete. Tungependa kuomba ruhusa kwako ili tuweze kuhifadhi hizo sampuli zaidi. Wakati tutakavyohitaji kuzitumia kwa utafiti mwingine, tutaomba ruhusa huko KEMRI.

Hatari

Kushika ngamia na kutoa damu kwaweza kusababisha uchungu kwa ngamia na pia damu kigo kumwagika. Pia kwaweza kumwumiza mtu mwenye ameshilka ngamia. Kuchukua sampuli kutachukua muda na pia kujibu maswali.

Faida

Hakutakuwa na faida yoyote kwako ukishiriki, lakini matokeo yatatolewa kwa watu ili waweze kufuata maagizo yatakayotoka kwa matokeo yetu.

Usiri

Maoni na majibu ambayo yatatoka kwako yatawekwa katika hali ya usiri. Matokeo ya utafiti huu yaweza yakachapishwa kwenye magazeti ya siansi lakini bila kukutaja wewe binafsi.

Fidia

Ukikubali kushiriki kwenye utafiti huu, hakutakuwa na malipo yoyote.

Ushiriki wa hiari

Ushiriki wako kwenye utafiti huu ni wa hiari. Ukiamua kutoshiriki au kutoka kabla hatujakamilisha, hakuna madhara yoyote kwako. Ukiwa na maswali yeyote kuhusu wewe, familia yako au wanyama wako jinsi wameumizwa, piga simu kwa Esther Kamau- Ministry of Agriculture, Livestock and Fisheries- 0722917041. Ikiwa una maswali yoyote kuhusu haki zako ongea na karani, KEMRI/NERC (tel. 0202722541 or 0722205901 or 0733400003).

Fomu ya ridhaa imeelezwa kwangu na nimekubali kuwa mimi/wanyama wangu twaweza kushirikishwa kwenye utafiti. Nimeambiw naweza kuchagua kutoshiriki bila madhara yoyote

Mwenye ngamia: Jina ..... Sahihi ..... Tarehe .....

Shahidi: Jina ..... Sahihi ..... Tarehe .....

Interviewer:Jina ..... Sahihi ..... Tarehe .....

Appendix VI: KEMRI ethical appro`val

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205401, 0733-400033. Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

May 27, 2015

TO: PENINAH MUNYUA,
PRINCIPAL INVESTIGATOR

THROUGH: DR. STEPHEN MUNGA,
THE DIRECTOR, CGHR,
KISUMU

Dear Madam,

RE: SSC PROTOCOL NO. 2963 (*RESUBMITTED INITIAL SUBMISSION*): SEROPREVALENCE AND CIRCULATING GENOTYPES OF MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS IN CAMELS AT ATHI RIVER SLAUGHTERHOUSE-(*VERSION 1.3 DATED 15TH APRIL, 2015*)

Reference is made to your letter dated 7th May, 2015. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on May 8, 2015.

This is to inform you that the Committee notes that the issues raised during the 237th SERU meeting of the SERU held on 18th March, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **27th May, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **May 26, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **April 14, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix VII: Approval from Director of Veterinary Services

REPUBLIC OF KENYA

MINISTRY OF AGRICULTURE, LIVESTOCK & FISHERIES
STATE DEPARTMENT OF LIVESTOCK
Office of the Director of Veterinary Services

Telephone: 020 – 8043441
E-mail: veterinarydep@gmail.com

Veterinary Research Laboratories
Private Bag, Kangemi 00625
Nairobi

When replying, please quote:
Ref: KAB/VEEU/DSE.INV./VOL.1/7
All correspondences should be addressed to:
The Director of Veterinary Services

Date: 30th January, 2015

TO WHOM IT MAY CONCERN

RE: APPROVAL TO CONDUCT SURVEILLANCE FOR MIDDLE EAST RESPIRATORYCORONA VIRUS (MERS-COV)

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a corona virus that is distantly related to Severe Acute Respiratory Coronavirus (SARS), an acute lower respiratory diseases syndrome (ARDS) characterized by fever and dyspnea. It was first identified in 2012 in the Middle East and outbreaks of the infection have been reported in over 17 countries last year. Camels have been suspected to be a reservoir for the MERS-CoV virus and a possible source for human infection on the basis of presence of MERS-CoV neutralizing antibodies and and high prevalence of MERS CoV antibodies in dromedary camels in a number of countries.

The Ministry of Agriculture, Livestock and Fisheries in collaboration with the KEMRI/CDC are planning to conduct surveillance for MERS-CoV in camels presented for slaughter at Athi river slaughter house in Machakos County. The aim of the study is to assess exposure and infection status of the local camel population to MERS-CoV which will provide useful information to the Ministry on situation of MERS-CoV in the country.

I have therefore given approval for the conduct of the study.

Yours Sincerely,

Dr. Dulu Thomas Daido
***For*: DIRECTOR OF VETERINARY SERVICES**

Appendix VIII: Anti MERS-CoV IgG Elisa laboratory results

Status	Optical density Ratio	Number	Percentage (%)
Negative	<0.8	63	17
Borderline	0.8-1.1	22	6
Positive	>1.1 to 2.0	40	10.7
	>2.0 to 3.0	101	27.1
	>3.0 to 4.0	101	27.1
	>4.0 to 5.0	34	9.0
	>5.0	12	3.2

Appendix IX: Publication of MERS knowledge and practices in Zoonoses and Public Health

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S H O R T C O M M U N I C AT I O N

Knowledge and practices regarding Middle East Respiratory Syndrome Coronavirus among camel handlers in a Slaughterhouse, Kenya, 2015

Abstract

Dromedary camels are implicated as reservoirs for the zoonotic transmission of Middle East Respiratory Syndrome coronavirus (MERS- CoV) with the respiratory route thought to be the main mode of transmission. Knowledge and practices regarding MERS among herders, traders and slaughterhouse workers were assessed at Athi- River slaughterhouse, Kenya. Questionnaires were administered, and a check list was used to collect information on hygiene practices among slaughterhouse workers. Of 22 persons, all washed hands after handling camels, 82% wore gumboots, and 65% wore overalls/dustcoats. None of the workers wore gloves or facemasks during slaughter processes. Fourteen percent reported drinking raw camel milk; 90% were aware of zoonotic diseases with most reporting common ways of transmission as: eating improperly cooked meat (90%), drinking raw milk (68%) and slaughter processes (50%). Sixteen (73%) were unaware of MERS- CoV. Use of personal protective clothing to prevent direct contact with discharges and aerosols was lacking. Although few people working with camels were interviewed, those met at this centralized slaughterhouse lacked knowledge about MERS- CoV but were aware of zoonotic diseases and their transmission. These findings highlight need to disseminate information about MERS- CoV and enhance hygiene and biosafety practices among camel slaughterhouse workers to reduce opportunities for potential virus transmission.

KEYWORDS

biosafety, camel, Middle East respiratory syndrome, slaughterhouse

Impacts

• Our results documented low knowledge of MERS among herders, traders and slaughterhouse workers.

• Abattoir workers were unaware of hand and face protection as ways of protecting themselves from exposure to MERS.

• Herders and slaughterhouse workers were aware of zoonotic diseases which could potentially reduce opportunity for potential virus transmission