OCCURRENCE OF MIDDLE EAST RESPIRATORY SYNDROME - CORONA VIRUS IN RANCHED CAMELS IN NAIVASHA, NAKURU COUNTY, KENYA

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A Thesis Submitted in Partial Fulfilment of the requirements for the award of Degree of Master of Science in Veterinary Epidemiology and Economics

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2019

DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University.

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DEDICATION

To my late father Mr. Sanni Sadiq Ozomata, my caring mother Hajia Rekiyat Ozomata, my wife Hajia (Mrs) Kuriyetu Ajoge, my children Miss Umul-Salma Abdullahi, Master Mohammed Thanni Abdullahi, Miss Amatullah Abdullahi, my siblings Ayishat Tijani, Nafisat Ozomata, Abdulmalik Ozomata, Nana Hawa Ozomata, Khadijat Sanni, Abdulganiyu Ozomata, Rabeeyat Ozomata, Hadiza Sanni, Muhammed Ataaba Ozomata, Abdulbasit Ozomata, Abdulmanan Ozomata, Umul-Khair Ozomata. Thank you for walking this journey with me, sharing in the highs, offering prayers, encouragement and above all bearing with me in my lapses in my duties to you all. All praise be to Almighty Allah for having you all.

ACKNOWLEDGEMENTS

Glory is to Almighty Allah, the Most Beneficent and the Most Merciful.

I would honestly like to acknowledge the efforts of my supervisors Professor Kitala M. Phillip, Dr. Thaiyah Andrew and Professor Folorunso O. Fasina for their patience, guidance and supervision. I would also like to acknowledge Dr. Stella Kiambi, Co-ordinator MERS-CoV Project at the Food and Agriculture Organization of the United Nations (FAO-UN). Her availability for consultation, guidance, provision of enabling environment and tasking made it possible for me to complete my work on time.

I truly appreciate and thank Dr. Thaiyah Andrew for believing in me enough to recommend me as a student attached to the MERS-CoV project of FAO-UN. I have experienced immense professional capacity building as a result of this singular opportunity.

I am grateful to the Central Veterinary Laboratory Kabete team. I sincerely thank Dr. Rinna Sitawa for her support through my project proposal stage, field sampling and availability for consultation. I thank Mr. James Mwangi for ensuring prompt testing of samples in the laboratory and training me in laboratory procedures. The laboratory experience garnered from you was a timely and useful capacity building. Other laboratory team with whom I worked with so well and gave themselves above what was expected of them are Dr. Eunice Omondi, Dorcas Omoga, Daniel Mbugua, and Wilson Mbaya. I sincerely appreciate your dedication and commitment.

I want to specially appreciate the FAO-UN team; Stephen Gikonyo, Joseph Matere, and Dr. Austin Bitek for their unalloyed technical support, perusal of my write-up and advising accordingly. Also, my appreciation goes to John Nguguna for his logistical support. My

gratitude goes to all of you for your help through study design, facilitation of the field and laboratory work and being available for consultation.

I also thank the management of Soysambu Camel Ranch in Naivasha and the herders for their cooperation throughout the sampling period.

I am hugely indebted to The United States Agency for International Development through the FAO Middle East Respiratory Syndrome Corona Virus (MERS-CoV) Project in Kenya for facilitating my work. Without their support, it would have been difficult for me to complete my project.

I wish to sincerely appreciate Barr. Shehu Wada, Ahmed Tijani Ajayi and Muhammed Kabiru Abdulmumin for their timely support.

Indeed, I have completed my work because many people invested their time and resource in me. I thank you all.

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LIST OF ABBREVIATIONS

ASAL – Arid and Semi- Arid Lands

DPP4 – Dipeptidyl peptidase-4

DVM- Doctor of Veterinary Medicine

EMC – Erasmus Medical Center

FAOSTAT – Food and Agriculture Organization Statistics

GDP – Gross Domestic Product

KNBS – Kenya National Bureau of Statistics

MERS - Middle East Respiratory Syndrome

MERS-CoV – Middle East Respiratory Syndrome Corona Virus

ORF1a – Open Reading Frame 1a

RNA – Ribonucleic acid

RVF – Rift Valley Fever

FAO-UN- Food and Agriculture Organization of the United Nations

URT – Upper Respiratory Tract

WHO – World Health Organization

βCoV – Betacoronavirus

ABSTRACT

Middle East Respiratory Syndrome is a zoonotic disease caused by Coronavirus (MERS-CoV). The infection gained prominence following the death of an infected patient with lower respiratory tract involvement in mid-2012; and MERSCoV was confirmed. The objectives of this study included; the estimation of the seroprevalence of MERS-CoV infection in Dromedary camels in Soysambu Ranch in Naivasha, Nakuru County, to determine the factors associated with MERS-CoV infection in camels in Soysambu ranch; and to determine the temporal pattern of exposure of MERS-CoV infection in camels in Soysambu Ranch. The study was conducted both as a cross-sectional and longitudinal. There was only one camel herd in the Conservency with a herd size of 121 camels, of these 50 camels were randomly selected and bled for serology. Data were collected via questionnaires administered to either the ranch owner or the manager of the ranch. The data collected included the general management of the ranch, camel sex, age, lactation status, pregnancy status, and use. The selected camels were bled via venopuncture and tested for presence of viral antibodies using an ELISA test. A total of 63 camels were selected for follow-up and bled at two-weekly intervals for a period of four months and antibodies determined. Association between testing positive to MERS-CoV antibodies and various factors were assessed using univariate logistics regression analysis and Multivariate analysis. Out of 50 camels tested in the cross-sectional study, 7 camels tested positive to antibodies of MERS-CoV virus equivalent to a seroprevalence of 14% (95% CI: 0.18, 0.10). Of the factors assessed, for their association with testing positive to antibodies to the virus, 4 were positively associated with testing positive including sex (OR=10, p=0.002), animal use (OR=1, p=0.0001) shows no association, pregnancy status (OR=38, p=0.0001), and lactation status (OR=12, p=0.0001). However, age was negatively associated with MERS-CoV antibodies with < 2years old being 10 times (OR=0.1, p=0.0002) less likely to test positive to antibodies to the virus relative to

the adult camels. However, in the final Multivariate logistic regression model on lactation status regained its significance (OR=3.9, p=0.003). Analysis of the antibodies to the virus in the followed –up study indicated a seasonal occurrence of the virus; the levels of antibodies increased from April end peaked in June 2016 coinciding with the peak rains in the conservancy. In conclusion, this study has demonstrated the exposure of camels to the MERS-CoV virus in the conservancy. However, despite the apparent presence of the virus, none of the residents of the conservancy reported any signs of infection with the virus which has been shown to be a zoonosis in other studies thus creating a need for further studies to understand the natural history of this virus in the conservancy and indeed in other camel-keeping areas of Kenya.

1.0: INTRODUCTION

1.1 INTRODUCTION

Middle East Respiratory Syndrome (MERS) is a zoonotic disease affecting the respiratory system and caused by a Coronavirus (MERS-CoV) (Alimuddin *et al.,* 2015). The virus was described as a novel virus sequel to the death of a sick person admitted in a hospital in June 2012 from a severe infection affecting the lower respiratory tract in Jeddah, Kingdom of Saudi Arabia. The ailment was confirmed and Middle East Respiratory Syndrome Corona Virus was isolated (Zak *et al., 2012*; Assiri *et al*., 2013).

The burden of MERS-CoV confirmed in the laboratory globally following the first isolated case in June 2012, is estimated at 1782 human cases and approximately 634 associated deaths (35.6% mortality) (WHO, 2016). The cases mostly started from the Middle East particularly in Saudi Arabia. According to WHO (2015), cases associated with travels to the Middle East have occurred in over 20 countries and of late, an outbreak was reported in South Korea (Benjamin *et al.,* 2015). The clinical outcomes of MERS-CoV vary and are diverse. According to Mailles *et al.* (2013) and Kraaij *et al.* (2014), fever and diarrhoea are some of the characteristics of the disease in humans. Other characteristics include muscle pains, laryngitis, cough, difficulty in breathing, occasional haemoptysis (Puzelli *et al.,* 2013) and vomiting (Christian *et al.,* 2013; Mailles *et al.,* 2013 and *Kraaij et al.,* 2014). Progressive lower respiratory tract distress and lymphopaenia have also been documented following a week of manifestation of infection of upper part of the respiratory tract (Al-Hameed *et al.,* 2015). It is pertinent to note that the greater part of human infection happen following contact with infected humans largely within health care facilities.

Sources of human MERS-CoV transmission are still poorly understood. Hemida *et al.* (2015) in a study carried out in Saudi Arabia, associated human transmission of the virus to Dromedary camels. Also studies by Memish *et al.* (2013), carried out also in Saudi Arabia, showed that 90% of adult dromedary camels were exposed to the virus. Similar observations were made in other Arabian and Africa countries that export dromedary camels to Saudi Arabia (Mackay and Arden, 2015). Clinically, Azhar *et al.* (2013) showed that MERS-CoV disease is not easily noticed in camels save for signs of nasal discharge.

Kenya remains one of the African countries with a substantial and growing camel population (FAOSTAT, 2015) and is a major component of the livestock sub-sector contributing 9% of the total meat production and 19% of total whole milk produced in the country (FAOSTAT, 2015). Based on 2014 statistics, there were approximately 2.9 million camels in Kenya (FAOSTAT, 2015). They are also used for transportation, safaris (Eco-tourism) and have significant cultural functions in the pastoral communities in Arid and the Semi-Arid Lands (ASALs).

Dromedary Camels have also been associated with transboundary, notifiable and emerging zoonotic diseases such as Brucellosis (Muhamed *et al.*, 2010), Rabies, Rift Valley Fever (RVF), Q-fever, Campylobacteriosis (Ulrich, 2014), and MERSCoV (Nahla and Ragab, 2014). Specifically, recent evidence has linked MERS-CoV, a novel viral zoonotic disease of the respiratory system in humans to camel production (Alimuddin *et al.,* 2015).

JUSTIFICATION

To date, there is inadequate data on the presence or lack thereof of MERS-CoV in camel keeping areas of Kenya although Corman *et al.* (2014) had provided serological evidence for

the virus in Kenya and linked human coronavirus to bats and dromedary camels (Corman *et al.,* 2015; 2016). Recent changes in camel production system from subsistence to commercial (dairy industry) (Noor *et al.,* 2012) and the huge increase in population changes in Kenya particularly (Schwartz, 2013) has necessitated closer associations between camels and their owners with consequential potential zoonoses (Ulrich, 2014). Understanding the dynamics of the changing management systems and the associated human-livestock-wildlife ecology is important to characterize future potentials for zoonoses and target critical intervention points. Most of the corroborating information associating camels to MERS-CoV is from cross-sectional studies which do not provide information on camel MERS-CoV infection dynamics. Thus, the current longitudinal study was designed to determine the epidemiology of MERS-CoV in ranched camels in Naivasha, Nakuru County, Kenya. This study was aimed to provide information on observed camel production system and ecology within the livestock-wildlife-human interface and dynamics and seropositivity of ranched dromedary camels for MERS-CoV infection in the study area.

1.2 STATEMENT OF THE PROBLEM

Following the initial human MERS-CoV infection in Saudi Arabia and its spread to other nations in Middle East (Jordan, Qatar, Oman, United Arab Emirate, Kuwait, Egypt and Lebanon), Europe (Germany, France, United Kingdom, Turkey, Netherlands, Greece and Italy), Africa (Algeria and Tunisia), Asia (Malaysia and Philippine) and North America, the possible risk of human pandemic has been a major public health concern. Globally, Kenya has the third highest population of dromedary camels and in addition, camels are exported to Middle East countries from Kenya. Dromedary camels are thought to be amongst reservoirs of MERS-CoV, a deadly zoonosis. In Kenya, Anne *et al.* (2016) confirmed that 2 out of 1010 (0.002%) identified sera from the archive in 2013 and 2014 of livestock handlers tested, had

serologic evidence of MERS-CoV exposure. Most of the participants had kept livestock chiefly donkeys, sheep, goats and cattle.

1.3 Objectives

1.3.1 Overall objective

The primary objective was to estimate sero-prevalence of MERS-CoV infection in selected ranched dromedary camels in Soysambu Ranch, Kenya.

1.3.2 Specific objectives

1. To estimate the seroprevalence of MERS-CoV infection in Dromedary camels in Soysambu Ranch in Naivasha, Nakuru County.
2. To determine the factors associated with MERS-CoV infection in camel in Soysambu ranch.
3. To determine the temporal pattern of exposure of MERS-CoV infection in camels in Soysambu Ranch.

2.0 LITERATURE REVIEW

2.1 AETIOLOGY

The MERS-CoV is a viral zoonotic disease spread from animals to humans. It is suggested to have originated from bats according to analysis of different virus genome, and transmitted to camels. The origin is not completely understood (WHO, 2015).

2.1.1 Taxonomy, Nomenclature and General Virology

The MERS-CoV is a respiratory zoonotic novel virus (Azhar *et al.,* 2014) and the transmission route to humans is not well ascertained, but camels are possible sources of infection. It is a novel coronavirus (de Groot *et al.,* 2013), the other names of the virus include betacoronavirus England 1, human betacoronavirus 2c England-Qatar, human betacoronavirus 2c EMC, human betacoronavirus 2c Jordan-N3 and human coronavirus EMC (de Groot *et al.,* 2013). Erasmus Medical Center (EMC) in Rotterdan, Netherlands, represents the first place, where the sequencing of complete viral genome of the virus and first laboratory confirmation was done.

Since 2012, MERS-CoV has led to sudden increase in human cases of terrible respiratory ailment in Middle East with incidental extension occurring in Africa, North America, Asia, and Europe (Zaki *et al.,* 2012) associated with a 35% mortality (Assiri *et al.,* 2013). According to Jasper *et al,* (2013), this virus might have originated from bats and passed different species barrier to infect humans and could be comparable with Severe Acute Respiratory Syndrome (SARS).

The virus *(Coronaviridae*) is in the order *Nidovirales* and the lineage C of the genus Betacoronavirus (βCoV) (Jasper *et al*., 2013). It is the sixth Coronavirus and first lineage C

βCoV to cause human infection. The amino acid sequencing identity of MERS-CoV is less than 90% to all other identified Coronavirus sequel to pairwise estimation of seven replicate domain evolutionary distances (Sander *et al.*, 2012). With guanine - cytosine material of 41%, it contains 5′-methyl-capped, polyadenylated, polycistronic RNA and a magnitude of 30 kb (Cotten *et al*., 2013). The MERS-CoV is an RNA virus and like other Coronaviruses is cocooned, positive-sense and single-stranded (van Boheemen *et al*., 2012). The arrangement of the genome 5′ -replicate-structural proteins (spike-envelop-membrane-nucleocapsid)-poly (A)-3 is useful for vaccination, diagnosis and therapeutics. The genome arrangement also differentiates MERS-CoV from lineage A βCoV which generally contain hemagglutinin-esterase (HE) gene which is characteristic (Frey *et al*., 2012).

2.2 EPIDEMIOLOGY OF MERS-CoV

Human MERS-CoV infection can be more severe as an opportunistic disease causing agent responsible for 40% mortality in reported cases (WHO, 2013). There is no proof yet to ascertain that infections acquired from animal origin caused more serious consequence compared to those observed following inter human infections (WHO, 2013). It has been shown that that the average incubation period for MERS is between 5 to 6 days, and ranges between 2-16 days with onset of illness from 13-14 days (Assiri *et al.*, 2013; Memish *et al.*, 2013; Ki, 2015). Elevated body temperature and gastrointestinal disorders may appear during the prodromal stage, thereafter the symptoms fade away, and are consequently succeeded by a syndrome characterised by severe systemic and respiratory maladies (Mailles *et al.*, 2013; Kraaij-Dirkzwager *et al.*, 2015).

2.2.1 Occurrence And Distribution

Since 2012, the Severe Acute Respiratory Syndrome (SARS) was rapidly stamped out, but the Middle East Respiratory Syndrome (MERS) epidemic persists (Woo *et al*., 2007) making it different. About four-hundred-and-forty (440) out of six-hundred-and-ninety-nine (669) (63%) laboratory-established cases of human MERS-CoV in Middle East, Europe and Africa, were in males with median age and range of 47 years and 9 months to 94 years, respectively (WHO, 2014). Human cases of MERS-CoV have been reported; mostly from Saudi Arabia and other countries like Jordan, Qatar, and the United Arab Emirate (Assiri *et al.*, 2013; Milne-price *et al.*, 2014). The continuous sporadic outbreak has been assumedly attributed to recurrent animal-human transmission from a probable particular reservoir which associates with human constantly in the area. The outbreak is usually not sustained except for household clusters and nosocomial outbreaks (Memish *et al*., 2013). Thus, determining the before and after state of the outbreak is difficult (Cauchemez *et al.,* 2016). According to WHO (2014), cases of human MERS-CoV, with total mortality of 35% has occurred in 21 countries. In other reviews, dromedary camels or infected humans have been linked to human infection via contact. It was documented by Assiri *et al.* (2013) that not all laboratory-confirmed cases had contact with camels, hence alternative sources of MERS-CoV infection exist.

Epidemiologically, primary infections of Middle East Respiratory Syndrome were suggested to have come from the Middle East and incidental cases outside Middle East were associated with imported primary infections from the Middle East. According to Assiri *et al.* (2013), subsequent to infection, clinical manifestation of MERS-CoV sets in after 12 days due to a short incubation period of about 5 days. The rate of secondary transmission from MERS patients to household (family members) in close contact is about 4% (Drosten *et al.*, 2014).

2.2.2 MERS-CoV in Different Species

It has been shown that Dromedary camel is a reservoir of MERS-CoV, but some other animals as hosts (Fig 2.1). Animals in red are those animals whose serum samples were positive to MERS-CoV antibodies occurring naturally in them while those in orange are animals that could act potentially as host following experimental susceptibility to MERS infection. Animals in red and orange such as Alpacas are capable of showing natural antibodies and could act as experimental host. Animals such as Bats (in black) have differently arranged RNA sequences of MERS virus-like virus.

Continuous arrows: intra- and inter-species transmission events.

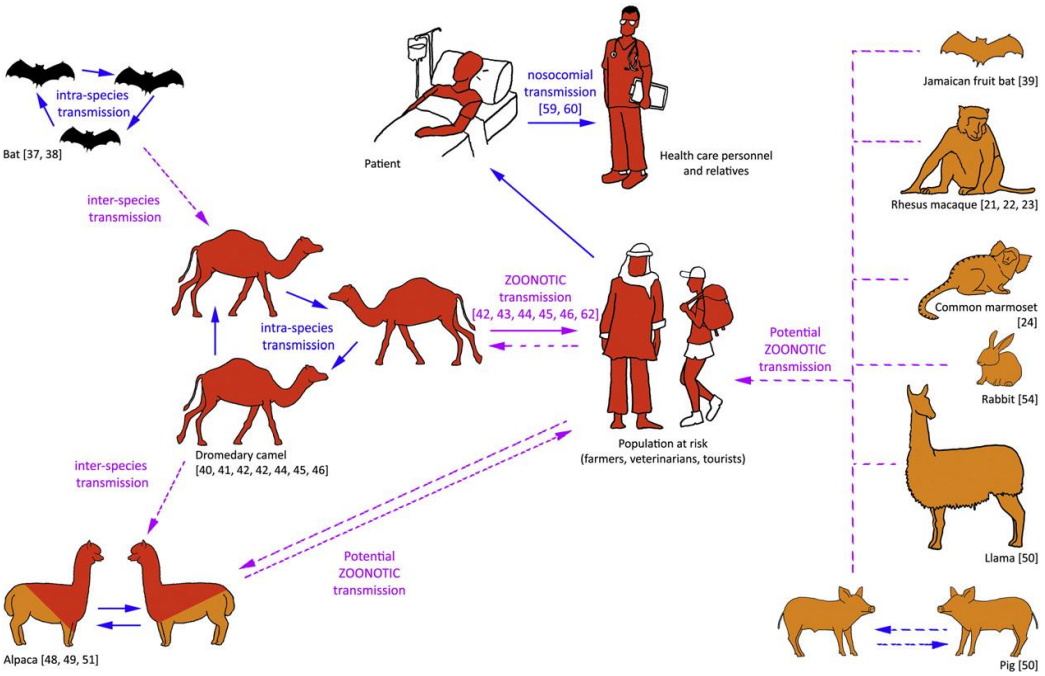

Figure. 2.1. Potential array of hosts for MERS-CoV (Vergara-Alert *et al.*, 2017).
Broken arrows: inter-species transmission

To date, cases of human MERS-CoV in Kenya hasn't been substantially evident (Anne *et al.*, 2016). This knowledge gap has led to a hypothesis on the difference in Middle East Respiratory Syndrome disease mode of transmission between the African continent and Arabian Peninsula and in the role of dromedaries as source of the disease.

2.2.3 Reservoirs of MERS-CoV and the role of domestic animals

A lot of species of animals have been established in MERS-CoV disease transmission to humans (Fig. 2.1). Bats are thought to be probable reservoirs for these viruses, even though ribonucleic acids of MERS-like corona viruses (but not MERS-CoV) have been identified in different bat families across the world (Reusken *et al*., 2013; Meyer *et al*., 2015). In recent times, Jamaican fruit bats (*Artibeus jamaicensis*) confirmed that MERS-CoV can replicate in bats following experimental infection (Reusken *et al.,* 2016).

Evidence has shown that MERS-CoV neutralizing antibodies exist in dromedaries (Reusken *et al*., 2013; Adney *et al.,* 2016). It has been shown that there is a correlation between MERS-CoV infection in camels and the infection in human (Haagmans *et al.,* 2013; Muller *et al*., 2014 and Farag *et al.,* 2015;). The 2015 human outbreak of this infection in Saudi Arabia was traceable to dromedaries; the molecular studies revealed that MERS-CoV was produced in this species as a result of recombination (Sabir *et al*., 2016). Furthermore, experimental evidences were provided to confirm the reservoir role of dromedary camels in the transfer of the causal virus (Adney *et al*., 2014). There is evidence that alpaca and llama who are members of the *camelidae* family, are also susceptible to MERS-CoV infection (Adney *et al*., 2016; Crameri *et al.,* 2016; Vergara-Alert *et al.,* 2017). Reusken *et al.* (2016) confirmed that during field studies in Qatar, alpacas are susceptible. Also, the causal virus has been demonstrated in domestic pigs, suggesting the possibility of MERS-CoV circulation in other unsuspected animal species (Vergara-Alert *et al*., 2017).

2.2.3.1 CAMELIDS

It has been established beyond reasonable doubt that Dromedaries are the principal source of MERS-CoV zoonosis (Mohd *et al.,* 2016). The initial evidence linking MERS-CoV to

dromedaries came from a serological study which investigated animals such as: dromedaries, cattle, sheep and goats including several other camelids. Antibodies specific to MERS-CoV were found only in dromedaries (Reusken *et al.,* 2013). Previous evidence has shown that prevalence of MERS-CoV was high among the young and the pregnant camels (Zumla *et al.,* 2015) and therefore the study focused on these high risk groups. The MERS-CoV had been linked to dromedaries when two human cases of MERS-CoV infection, diagnosed in October 2013, were traced to a Qatar farm (Haagmans *et al.,* 2013). All fourteen dromedaries on the incriminated farm were tested with RT-PCR in response to the 2 human cases. Swabs from the nostrils of eleven dromedaries tested positive to MERS-CoV. Sequencing of ORF1a fragment and a 4.2 kb concatenated fragment of nucleotide in three dromedary camel samples resembled the sequence from the 2 human cases earlier linked to the Qatar farm (Haagmans *et al.,* 2013).

A different study in Saudi Arabia showed a 43-year-old male who owned nine dromedaries and had direct contact with the camels diagnosed with MERS-CoV infection in November 2013 (Azhar *et al.,* 2014). Prior to exhibition of his symptoms, four (4) of his dromedaries were sick. Genetically similar MERS-CoV Viruses were grown in culture of cells from both dromedary and the patient in a laboratory (Azhar *et al.,* 2014). The previous two studies in addition to confirmation of MERS-CoV in dromedaries, has shown a potential cross-infection between humans and dromedaries especially through intimate contact (Haagmans *et al.,* 2013; Azhar *et al*., 2014).

In an experimental MERS-CoV inoculation through the nasal route of dromedaries, mild signs such as nasal discharges and infection of the urinary tract were noticed (Haagmans *et al*., 2016). Ribonucleic acid of MERS-CoV was detected in extra-pulmonary tissues and

respiratory tract swabs. Infectious MERS-CoV was in contrast, detected only in the infection of the lymph nodes of the urinary tract, trachea, large bronchus and tracheobronchus. There were no visible lesions observed in dromedaries, except for the inflammation of the respiratory tract. The propagation of MERS-CoV was observed only in the cells covering the epithelium of the urinary tract which was infected (Adney *et al*., 2014; Haagmans *et al*., 2016). Clinical pathology like those presented in camels was noticed in both llamas and alpacas following experimental infection with MERS-CoV which was inoculated through the nasal route. Clinical symptoms were not observed in the alpacas while mild symptoms (mucus discharge) were noticed in the llamas. The MERS-CoV antibody was detected in swabs from the nostrils, and in the upper respiratory tract and trachea of both animals. Microscopic lesions were not observed in both species other than rhinitis and metaplasia of the turbinate epithelia while the epithelium of urinary tract infection was noticed as predilection for viral replication (as in the dromedaries). The urinary tract infection was cleared of the virus 7-10 days post experimental infection following antibody response. (Adney *et al.,* 2016; Crameri *et al.,* 2016; Vergara-Alert *et al.,* 2017)

2.2.3.2 Non-Camelid Domestic Species

Other species of animals, specifically the domestic piglets presented mild production of mucus when MERSCoV was inoculated intranasally (Vergara-Alert *et al.,* 2017). Ribonucleic acid of the virus was detected from swabs of the nasal cavity, urinary tract, trachea and bronchus. No macroscopic lesions were seen in pigs, but mild rhinitis and virus replication was observed in the epithelial cells as a result of urinary tract infection. Furthermore, viral shedding was observed in their nasal swabs 1-10 days after infection. However, the infectious MERS-CoV was only detected 4 days after infection and its ribonucleic acid was detected in the urinary tract, trachea and bronchus (Vergara-Alert *et al.,*

2017) following the conduct of other investigations and screening in order to detect other domestic animal reservoirs in Saudi Arabia. The results of the serology of screened domestic animals conducted between 2010 and 2013; sera from cattle, sheep, goats, and chickens from different geographical locations were negative for MERS-CoV (Hemida *et al.,* 2013). Another study conducted in different location in the same country, screened sera samples from sheep and goats which tested negative for MERS-CoV antibodies (Alagailli *et al*., 2014). The conduct of a similar study in Jordan in 2013 none of the cattle, sheep and goats screened were found positive for MERS-CoV antibodies (Reusken *et al.,* 2013). Different animal species (catle, sheep, goats, horses, donkeys and mules) screened in European countries had no evidence of MERS-CoV antibodies as well (Reusken *et al.,* 2013; Meyer *et al.,* 2015). Thus, the study demonstrated that the virus was not circulating in these areas or that the species were not natural reservoir of the virus (Meyer *et al*., 2015).

Dromedaries were the only domestic animals which were reservoirs for MERS-CoV until a study in qatar proved this otherwise. Majority of Alpacas in a herd in close proximity with dromedaries suffered this infection and all tested infected alpacas were seropositive to MERS-CoV. Ninety percent (90%) of the dromedary camels in the same barn were seropositive for MERS-CoV (Reusken *et al*., 2016). This indicates the susceptibility of alpacas to natural MERS-CoV infection and the potential for a new MERSCoV animal reservoir. In another study conducted with 3 alpacas that were experimentally infected intranasally with the causal viruses, all of the animals got infected and were shedding the virus. They also infected other alpacas sharing the same barn. Observation revealed that the infected alpacas did not present fever which is similar to the dromedary camels. Also, the alpacas did not manifest visible nasal discharge during the course of infection, but had neutralizing antibodies to MERS-CoV (Adney *et al.,* 2016). The aforementioned studies

show that just like the dromedaries, alcapas can be infected by MERSCoV viruses and could serve as reservoir host.

2.3 MERS-CoV studies in Kenya

According to Sharon *et al.* (2015) MERS cases have not been reported in humans in Kenya. However, a study in Laikipia County revealed that MERS-CoV antibody prevalence was high (46.9%) in camels. Also Victor *et al.* (2014) in a study conducted in Kenya revealed that camels screened in varios regions in Kenya between 1992 to 2013 had MERS-CoV antibodies. High number of dromedary populations correlated with increased seropositivity and might be an index for predicting long-term MERS-CoV maintenance.

2.4 Risk factors of MERS-CoV infection

This infection has been postulated to have the potential risk of spreading globally (Memish *et al*., 2014). Intrusion of humans into animal natural habitats and different species of animals mixing frequently in densely populated areas like markets and holding stations, have facilitated emergence of coronavirus. Cultural and religious practices have also propagated the emergence of mutating coronavirus (Jasper *et al*., 2015). Other risk factors include, comorbidities and age (older people are at higher risk than the young) (Assiri *et al.,* 2013). Food-borne transmission has been figured out as a likely route of infection, because of the possibility of the consumption of unpasteurized dromedary milk and unprocessed meat, and the utilization of dromedary urine for therapeutic purpose. Also, zoonotic transmission from other species is a possibility.

2.5 Clinical presentation of MERS-CoV in humans and camels

The MERS-CoV presents mild to severe infection of the lower respiratory tract and extra pulmonary organs in humans with high fatality (Chan *et al*., 2012). Typical signs of the disease include cough, fever and shortness of breath, and diarrhoea in humans. Though not always present, pneumonia is a common finding (WHO, 2015). In camels, there is mild nasal discharge to asymptomatic presentation.

2.6 Diagnosis

To differentiate MERS-CoV infection from other causes of pulmonary diseases, pathognomonic clinical features are not sufficiently reliable, hence laboratory confirmation. To issue MERS-CoV confirmation from the laboratory, the most widely used methods are nucleic acid amplification assays (Muller *et al.*, 2015). These methods have short cycle using a generally accepted testing protocol earlier established in the epidemic. According to WHO (2015), the measure of a case confirmed in the laboratory include a positive RT-PCR for at least two precise variant targets on MERS-CoV RNA and genes.

Evidence of infection can also be provided via serological assay from precise neutralizing anti-MERS-CoV antibodies estimation in paired sera, collected at the acute and recuperating phases 2 weeks to 3 weeks apart. Serological diagnostic methods developed so far are yet to be ascertained for adoption (Muller *et al.,* 2015).

2.7 Control and prevention

Neither specific vaccine nor treatment is yet to be developed (Zumla *et al.,* 2015). Symptomatic treatment and ancillary service is the best management option for critical MERS cases (Bermingham *et al.,* 2012). Reduction of contacts of humans with camels and

other suspected animals, routine herd evaluation and isolation of infected camels, use of personal protective equipment by livestock handlers, implementation of policies prohibiting all intake of unpasteurized dromedary milk and reporting of early signs of respiratory related illness and avoidance of mixed livestock grazing, are considered important in the control of the disease (Zumla *et al.*, 2015).

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in a selected camel ranch having the required number of camels for the study within Soysambu conservancy (http://www.soysambuconservancy.org/field-studies.html) in Naivasha Sub-county, Nakuru County, Kenya. Soysambu Conservancy is contiguous to Lake Elementeita, a protected area due to its relevance in wild and migratory birds (geo-coordinates: S-0.522574 E36.165794 δ4.00m). Westward, it is bordered by Lake Nakuru National Park, to the north and south, bordered by Mengai and OI Doinyo Eburu extinct volcanic mountain, respectively. The conservancy consists of 48,000 acres of diverse ecological profiles. More than 50 species of mammals (wildlife and livestock inclusive, n ≈ 12,000), and approximately 450 species of birds reside within the conservancy. Table 3.1 shows the types and numbers of livestock in Naivasha sub-County and Figure 3.1 shows the map of Kenya, the study site and grazing routes.

The conservancy is populated by flora of short dry grass, which sprouts regularly in the beginning of the rains, rich acacia woodland vegetation and open savannah grasslands. It is part of the "Kenya Lake System in the Great Rift Valley" World Heritage Site, a Ramsar Convention Wetlands Site and a Bird Life International-Important Bird and Biodiversity Area. Naivasha lies North-West of Nairobi in Nakuru County of Kenya with a human population of about 181,966 people according to 2009 population census (KNBS, 2012). In addition, with Lake Naivasha, Mt. Longonot National park and Hell's Gate National Park, Naivasha is a popular tourist destination.

Table 3.1: Types and numbers of livestock in Naivasha Sub-County, 2017.

Species of livestock	Number
Sheep	240,746
Cattle	139, 501
Goats	115, 363
Donkeys	19,375
Camels	121

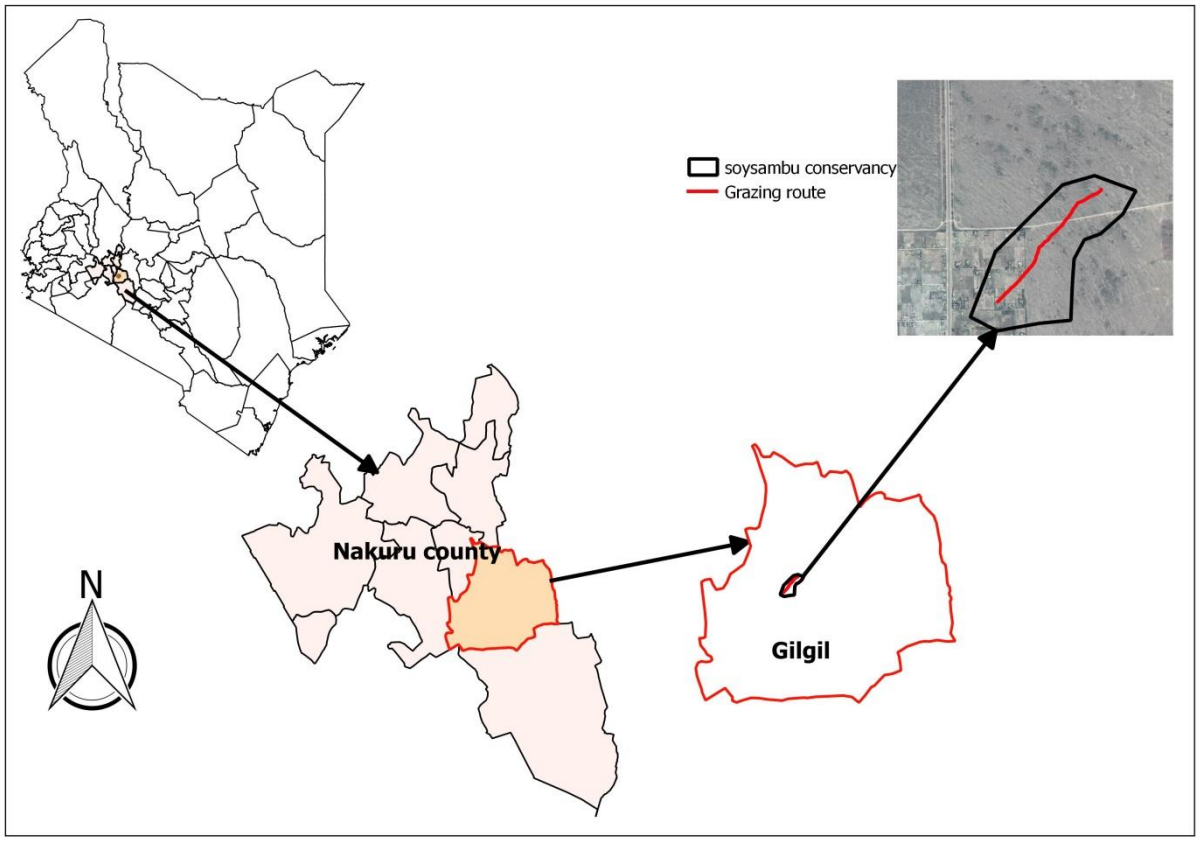

Figure 3.1: Map of Kenya showing Nakuru County, the study site and camel grazing route

3.2 Study design

3.2.1 Cross sectional study

An initial cross sectional study was carried out on randomly selected 50 camels from 121 camels in the herd for inclusion into the study in Soysambu conservancy. In January, 2017 a total of 50 blood samples were collected from 50 camels randomly selected for the cross-sectional studies. The cross-sectional study was to serve as a precursor for the longitudinal study, and was to determine seroprevalence of the virus and factors linked to seropositivity.

3.2.2 Longituidinal Study

In Soysambu Conservancy, samples were collected at two weeks intervals for a period of 4 months. The animals were ear-tagged for identification. During each visit, identified camels were bled for determination of antibodies to the virus. A total of 63 camels were followed-up. An adult camel was that above 2 years old and a calf was that at 2years and below. This design was to determine the temporal patterns of antibodies to MERS-CoV. Initially all the 63 camels were tested and found to be free of the antibodies to the virus. Any calf born into the cohort during follow-up was also included into the study.

3.3 Case Definition

A camel having an antibody titre of ≥ 1.1 against MERS-CoV was considered a sero-positive case.

3.4 Sampling

The study population was1 camel herd, grouped into males, females and the young (calves and weaners) within the conservancy. Age definition: <1-2years = calf, 2-3years = weaners, >3years = Adult (Zumla *et al.*, 2015).

3.4.1 Sample size determination

Sample size determination was according to the method in Dohoo *et al.* (2008): $n = Z^2_{\alpha}PQ/L^2$

Where 'n' is sample size required,

'Z' is value of Z that provides 95% confidence intervals (1.96)

'P' is approximated prevalence of MERSCoV in Kenya,

'Q' is (1-P),

'L' is allowable error or required precision.

According to Sharon *et al.* (2015) sero-prevalence of 46.9% of MERS-CoV in dromedaries was estimated in Laikipia Kenya, and therefore this value of P was adopted for the current study. Thus, $n = (1.96)^2 \times 0.469 \times 0.531) / 0.05^2$, $n = 0.9567/0.0025$, $n = 383.68$. Therefore, $n = 384$ approximately. A total of 63 camels including calves, and pregnant camels were selected from the herd purposively and weaners were randomly sampled for a follow up in 8 rounds of sampling to meet up with the above calculated sample size.

3.5 Data Collection

Some pretested interview guide (inventory) (Appendix 1) were utilized to collect data on the Conservancy. These were designed to obtain information about demographics and management practices of the camels in the ranche via personal interviews with the manager. The type of data collected included: herd structure; production system; routine management practices; health history; interaction with wildlife and description of the agro-ecological conditions. In addition to the interviews conducted, geo-coordinates (way points for the vector coordinates) for water, vaccination and milking points, as well as the linear fiction for grazing routes were collected using the Global Positioning System (GPS) in GPS Essentials® downloaded into a Samsung galaxy S7 edge. The software was used without internet to track the routes (linear fictions for grazing routes) and also obtain geo-cordinates.

Records on annual rainfall were obtained from the ranch administration office as secondary data and were extracted for the past 68 years. Additional data on the current prevailing eco-climatic conditions were collected through physical observation of the conservancy, the camel ranch, the people in the conservancy and its environments. Furthermore, data on the animal population censuses which are routinely carried out using sampling-resampling methods as well as aerial survey were obtained from the record of the Conservancy. Secondary data from literature was used to obtain information about camel production systems in Kenya. Additional data was collected through visual observation.

3.6 Sample collection

A sample of 8-10ml of blood was collected via venipuncture using 18 gauge needles. Camels were manually restrained by the herders (Plate 3.1). Blood samples were collected aseptically into two (2) vacutainer tubes, one with no anticoagulant and one with EDTA anticoagulant. The vacutainer tubes were labelled and transported on ice packs to Nakuru Veterinary Investigation Laboratory. At the laboratory the blood in the non-anticoagulant tube was centrifuged at 3,000 rpm for 5 minutes and serum aliquots were transferred into labelled cryovials. Whole blood from the coagulant tube was also decanted into cryovials and labelled. The samples in the cryovials were then transported to the Central Veterinary Laboratory in Kabete on ice pack for laboratory analysis. The samples were stored at -80°C until laboratory testing. Swabs from the nostrils were collected from the bled dromedaries by inserting cotton swabs in the nostrils and twirling them. The swabs were stored at -18°C in cryovials containing trizols® reagent virus for future use.

Plate 3.1: Blood sample collection from a restrained camel in Naivasha conservancy, Nakuru County

3.7 Laboratory tests

The laboratory tests were conducted at the Central Veterinary Laboratory, Kabete. The sera were tested for MERS-CoV antibodies using indirect Enzyme Linked Immuno Sorbent Assay (iELISA) with an Anti-MERS-CoV ELISA Camel (IgG) as instructed by the manufacturer. Antibodies were detected based on the recombinant MERS-CoV spike protein subunit 1 that specifically determines IgG (Muller *et al.,* 2015). Seroprevalence was determined from the numbers of samples that tested positive to MERS-CoV antibodies over the total number tested.

3.8 Data handling and analysis

The generated data was coded, filtered and input into Microsoft Excel (Microsoft Corporation Redmond, WA, USA) and exported to Stata® statistical software version 15 (Lake Way Drive, College Station, Texas, USA) for statistical analysis. Descriptive analyses including means and proportions were generated using the same package. The seroprevalence of MERS-CoV was calculated by dividing the number of positive sera by the total number of sera tested. Associations between MERS-CoV seropositivity and various factors were initially assessed in univariate analysis using logistic regression model at a P-value of <0.2.

3.9 Ethical approval

Ethical approval for collection of samples and conduct of interviews was obtained from the Directorate of Veterinary Services and the Faculty of Veterinary Medicine Ethical and Biosafety Committee (FVMEBC) to ensure integrity and quality of the research. Standard Operating Procedures were followed for tracking, bleeding and post-operative care of the animals to minimise stress. Consent was also sought from the management of Soysambu Conservancy.

4.0 RESULTS

4.1 Rainfall patterns in the Soysambu Conservancy

Over the last 68 years (1948 - 2016), the mean annual rainfall was 732.22 ± 21.03mm (CI95%: 690.26-774.19) with highest mean annual rainfall recorded in the years 1961, 1978, 2010 and 2012. Monthly variations in amount of rainfall occurred with periods between December and March recording the lowest mean monthly rainfall (30.09 - 48.20 mm) (Figure 4.1). The peak monthly rainfall occurred in the months of April (102.45±7.23 mm) and May (82.78±5.57 mm). Thus, rainfall occurred in all months of the year for the period under review.

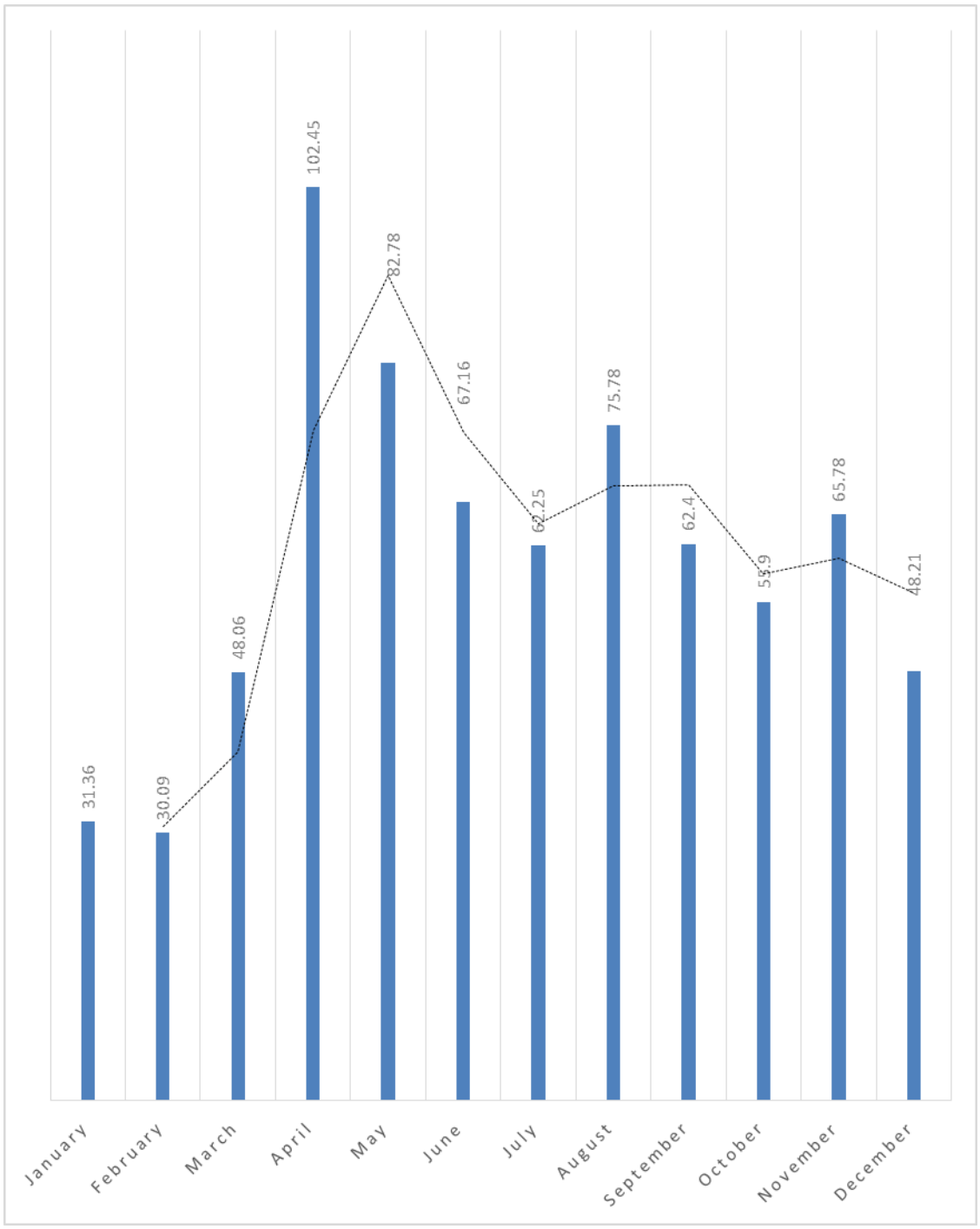

Figure 4.1: Average annual rainfall with monthly averages in the Soysambu conservancy based on aggregated data, 1948-2016

4.2 Wildlife-livestock interaction

The camel ranch is owned by an individual who rented the land from the conservancy management. Soysambu Conservancy had approximately 48,000 acres of diverse ecological

significance with more than 450 bird species and over 50 species of mammals (wildlife; n ≈ 12,460) including rare species like the Rothschild's Giraffe. Other livestock found in the Conservancy include ≈ 8700 cows, 2000 sheep, 1200 goats and 121 camels which share the ecosystem's resources. Ranching and wildlife conservation co-exist in this Conservancy (Plate 4.1). The camel herds reportedly relocated originally from Laikipia County and are managed chiefly for milk production and safaris but are also used for meat. At the time of the study, there were 121 camels in the study area. Water for the ranch was sourced from boreholes which were spread along the camel grazing routes. Wildlife had access to the same water sources, thus, there was a lot of interaction between livestock and the wildlife in this ranch (Plate 4.2).

Plate 4.1: Zebra and Thompson gazelles grazing together in the Soysambu Conservancy, 2016.

Plate 4.2: Zebra and cattle sharing a water trough at the Soysambu conservancy

4.3 Camel Herd structure and management practices

4.3.1 Herd Structure

Except for calves less than a year old, where the female: male sex ratio was 1:1, there were more female camels in the yearlings age-class and in the adult (Fig.4.2). The male are either sold as calves or weaners. Overall, adult camels comprised of 62% of the herd and calves and yearlings 31% and 7% respectively.

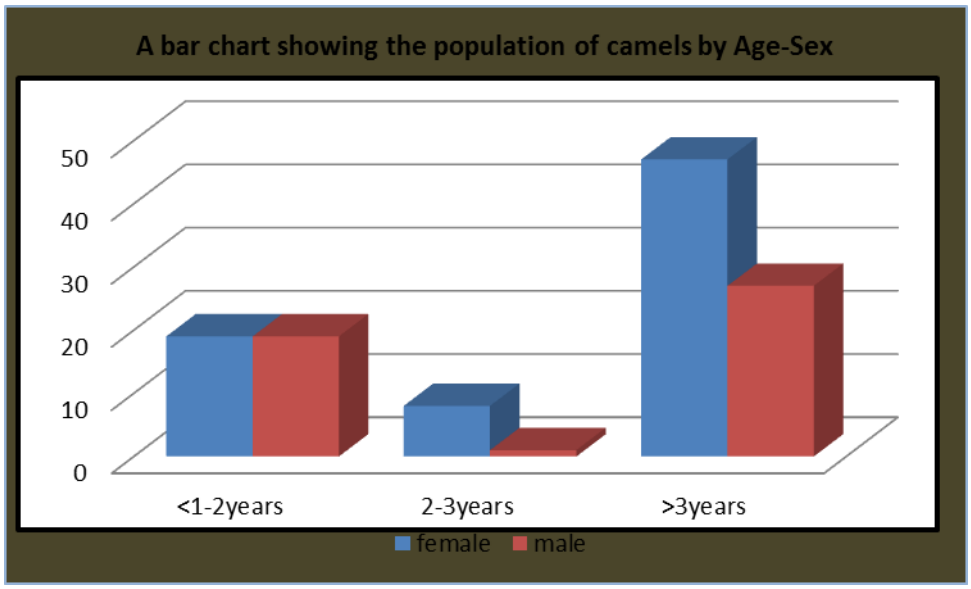

Figure 4.2: Age-Sex structure of a herd of 121 camels in the Soysambu Conservancy, Nakuru County, 2017

4.3.2 Feeding

A total of 8 workers were employed by the conservancy to take care of camels. One of them was a manager and the others served as herders and milkers. Feeding of the camels was by grazing along some designated grazing routes within the conservancy. Watering points were also provided along the grazing routes. During grazing, the camels mixed freely with other livestock as well as wildlife. Salt licks were also provided at some designated points within the conservancy to alleviate zinc and iron deficiency which is common in the study area.

4.3.3 Breeding

Bulls are used for breeding purposes in this conservancy. Bulls with desired traits were selected by the ranch manager. At the time of the study, 22 bulls were used for breeding. At the time of calving, the dams were separated from the grazing herd and confined within some temporary shelter. In most instances, the females were assisted to calf by the herders who wore no protective clothings. Although calving occurred throughout the year, the peak period was in the months of March and April which coincides with the start of the long rains. After calving, the dams and their calves were confined within the "boma", for a week before rejoining the rest of the herd.

4.3.4 Milking hygiene

Milking was done once daily and 10-15 litres were produced with an average of 1-2 litres per camel. Milking containers were usually cleaned and smoked prior to milking. The milkers who were almost always men, washed their hands with water and detergent. The milk was stored in plastic containers and transported to the market. The milking parlour was rarely cleaned exposing the camels to mastitis-causing organisms.

4.3.5 Carcass disposal

Carcasses of dead camels were never buried or burnt. Instead they were left in the wild to be fed by carnivores and carrion birds (Plate 4.4 and 4.5).

Plate 4.3: Decomposing camel carcass within Soysambu Conservancy

Plate 4.4: carcass disposed in an open space within Soysambu Conservancy

4.3.6 Disease Management

In this herd, the camels grazed on a restricted area of the conservancy with watering points and limited supplementation of minerals was provided due to mineral deficiencies in the soil. However, de-wormers, trypanocides and some antibiotics were sometimes used.

4.4 Seroprevalence of MERS-CoV

4.4.1 Cross-sectional study

Out of the 50 camel sera tested, 7 were positive to MERS-CoV antibodies giving a seroprevalence of 14% (95% CI: 0.18, 0.10). The entire 7 positive sera were from female camels, 6 of which were pregnant.

4.4.2 Longitudinal Study

Between 30th March, 2017 and 4th July 2017, blood samples were collected from the sample population. The total sample population included 16 calves, 22 weaners and 25 pregnant camels sampled every 2 weeks for a period of 4 months (8 rounds of sampling). A total of 545 blood samples were collected. Initially 63 camels were recruited into the cohort of camels to be followed-up for a period of 4 months. During the follow-up period, 12 calves were born and these were added to the study population. Two (2) camels were lost during the follow up, 1 of the camels was attacked and killed by an intruder from the nearby village while the other went wild into the forest and got lost. The MERS-CoV antibody profile is shown in Fig. 4.3. There was an increase in the levels of antibodies from the last week of April peaking sharply in the first week of June. Then there was a sharp drop in the third week of June. It is worth noting that this sharp increase in antibodies and therefore exposure to the virus coincided with peak rainfall.

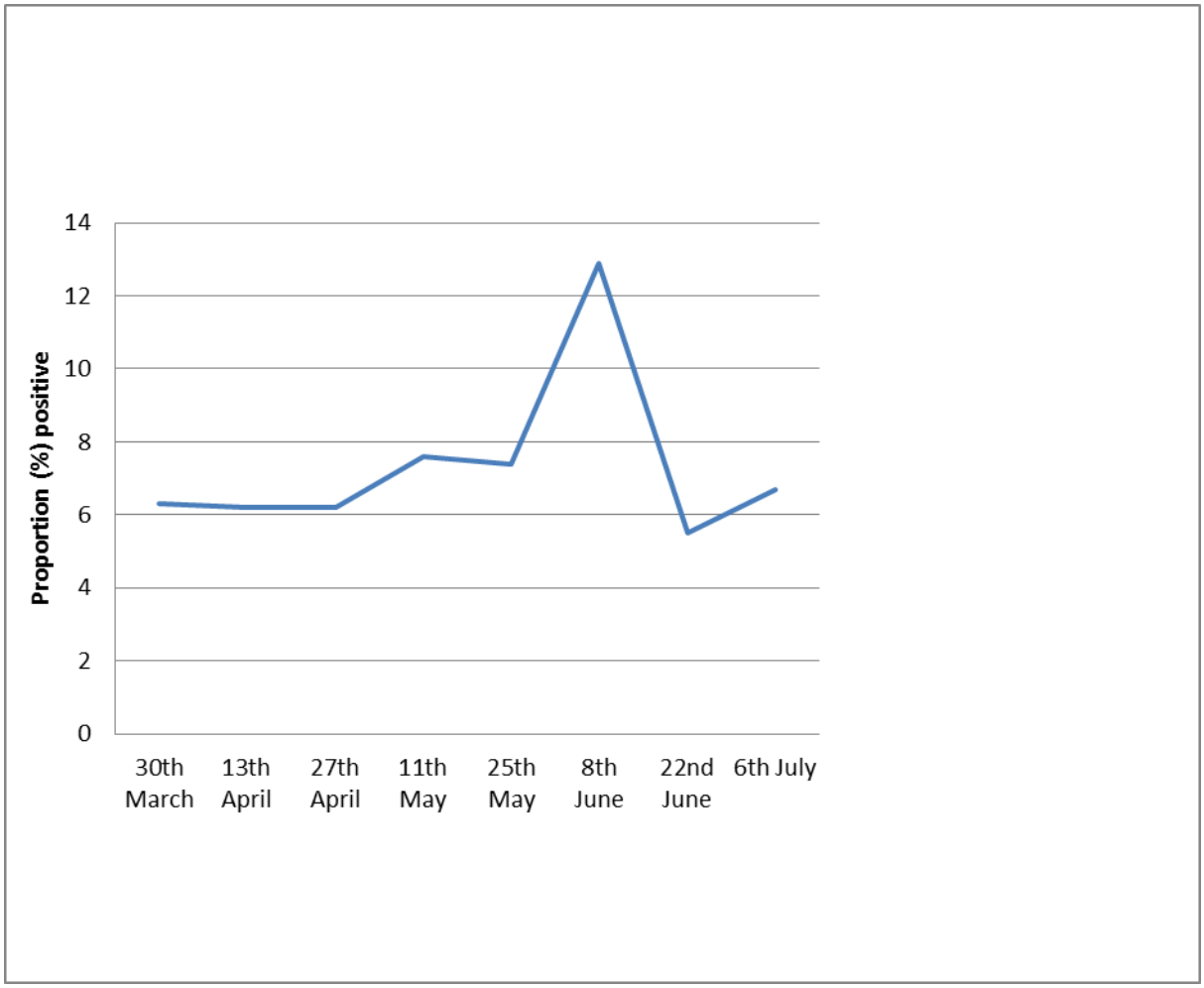

Figure 4.3: Antibody profile of MERS-CoV in 4 month follow up period of camels in Soysambu Conservancy, 2017

4.5 Risk factors for testing positive to MERS-CoV antibodies

4.5.1 Univariate analysis

In univariate analysis, five factors were associated with testing positive to antibodies including sex (OR=10, P=0.003), age (OR=0.1, P=0.002), pregnancy status (OR=38, P<0.0001) lactating status (OR=12, P<0.0001) and animal use (Table 4.1). Some of the factors had very strong associations, for example pregnancy status had an OR of 38 indicated that pregnant camels were 38 times more likely to test positive to the antibodies to MERS-CoV virus relative to non-pregnant camels. The other factors have the same interpretation

except age which had a negative association, i.e young camels (< 2years) were 10 times (OR=0.1) less likely to seroconvert relative to adult camels.

Of the five factors that were significant in the univariate logistic model, only pregnancy status retained significance, that is the final multivariate logistic model (OR=3.9, p=0.003) (Table 4.2). However, its OR dropped drastically from 11.67 in the univariate model to 3.9, which is a drop 67% indicating that it was greatly confounded by other factors unmeasured or measured (Dohoo et al., 2008). The loss of significance of the four factors in the final model was also an indication of the presence of confounders.

Table 4.1: Univariate logistic regression analysis of camel factors association with testing positive for MERS-CoV antibodies on ELISA

Variable	level	Number tested	Level Proportion (%)	Number positive	Odds ratio [95% CI]	*P*-value
Sex (n = 544)	Male	186	34	2	1.00	
	Female	358	66	38	10.11 [2.41; 42.39]	0.002
Age (n = 535)	Old (> 2 years)	327	61	38	1.00	
	Young (≤ 2 years)	208	39	2	0.10 [0.02; 0.44]	0.002
Breed (n = 544)	Pure Somali	24	4	1	1.00	
	Somali-Pakistan	520	96	39	1.86 [0.25; 14.18]	0.55
Animal use (n = 544)	Ecotourism	48	9	-	1.00	
	Milk and meat production	496	91	40	1.00	<0.0001
Pregnant status	Pregnant	224	58	38	38.00 [9.06; 159.38]	<0.0001
Lactating status	Non-pregnant	160	42	2	1.00	
	Lactating	24	6	9	11.67 [4.78; 28.45]	<0,0001
	Non-lactating	360	94	31	1.00	

Key: OR=Odds ratio

5.0: DISCUSSION

5.1 Discussion

The current study was part of a comprehensive project on disease ecology of camels in Kenya. The study was conducted in a single privately owned camel ranch where free-range production system was practiced. However, unlike in the other camel rearing areas of Kenya where nomadic pastoralism is practiced, camels in the ranch were confined. Such close confinement of animals would be conducive for the transmission of infections such as the MERS-CoV. Indeed, like for most infectious diseases, the size and extent of MERS-CoV infections may be influenced by the livestock production system practiced. Most parts of Kenya experience rainfall in two seasons in a year, the long rains which occur between March and May, and the short rains which occurs between November and December. Kenya experiences dry spells, sometimes severe droughts in the intervening months. The situation was different in Naivasha and its environs where a sixty-eight year (68) rainfall data shows that the area received rainfall throughout the year. This may be because of precipitation from nearby lakes Naivasha and Elementeita. Thus, the Soysambu Conservancy always had adequate pastures for both livestock and resident wildlife. Livestock-wildlife and indeed human-wildlife conflicts usually occurs when competing for scarce water and pastures. This appears not to have been the case with the three co-existing peacefully. However, disease transmission between the different species cannot be ruled-out.

Evidence of the existence of MERS-CoV virus in Soysambu Conservancy was produced in this study. Above 7% of the surveyed camels showed positive antibodies to the virus. These results are in agreement with the results of Sharon *et al.* (2015) who reported a seroprevalence of 46.9% in a study conducted in Laikipia.

Although the presence of antibodies to the virus was established, its reservoir remains unknown. No attempts were made in this study to isolate the virus in the seropositive camels. There is a need to attempt virus isolation and further study to identify the virus reservoir(s).

Despite the apparent presence of the virus in the conservancy, no ranch worker or any of the residents in the conservancy reported having suffered signs consistent with MERS-CoV virus infection. Similarly, Sharon *et al.* (2015) in their study in Laikipia did not report any signs on human consistent with MERS-CoV. There is a need for further studies to fully understand the natural history of the virus including the circumstances conducive to human infection. The zoonotic potential of this virus was not established in this study.

The result of the longitudinal study appears to suggest a seasonality of occurrence of the MERS-CoV virus. The antibody profile appeared to coincide with the peak rains in the conservancy probably suggesting a vector involvement in the epidemiology of the virus. A build-up of vectors concomitant with increased rains has been reported for most vector-borne diseases such as Rift Valley fever. However, the follow-up period needs to be increased from 4 months to a year to pick clear seasonality patterns.

The camel production system in this study present with two major products and services which are milk and camel safaris (Eco-tourism) as exist elsewhere in Kenya. The unwholesome practices by the milking men and milk collectors as evident in this study pose a serious zoonotic threat to milk handlers and consumers to diseases such as brucellosis and MERS CoV. Reusken *et al.* (2014) had earlier raised a serious concern on the need for measures to mitigate against putative foodborne transmission of MERS-CoV in Qatar. They detected viral RNA of MERS-CoV in milk of 5 camels out of 7 camels that were actively shedding MERSCoV in their nasal secretion. In addition, poor milking hygiene serves as a barrier to the growth of peri-urban camel production system (PUCPS) since the quality are

unlikely to meet the standard for urban consumers in Kenya (Issack *et al.,* 2013). The udders of cows in the study area were not washed before milking and this comes with the potential to introduce disease pathogens in raw milk thereby ultimately reducing the keeping quality (Mogessie and Fekadu, 1993). Furthermore, the disposal of carcasses in the study area makes the environment a high-pathogen burden environment that attracts wildlife and carrion eaters.

It is possible that MERS-CoV infection may exist in the conservancy among other zoonotic threats. Wildlife plays a major role in the epidemiology and maintenance of a number of infectious diseases of livestock and poses challenges that policies have not addressed (Benka Valerie 2012). In addition, major information gaps exist in this field. For example, MERS-CoV remains a threat with pandemic potential to public health but to date the dynamics of the virus is not yet completely understood between the camel, wildlife and humans. Elhadi *et al.* (2015) have demonstrated that lactating camels in Somali and Borana households dominated the herds to satisfy milk demand as was the case in the current study. Although the traditional camel herds in East Africa range from 10–100 heads, in this study the herd had >120 camels due to the fact that it was moving towards commercialization. Whereas camels are said to be seasonal breeders, calving in Kenya is all-year round (Ihuthia, 2010; Mahmoud, 2010) although this is positively skewed to the wet season. The focus group discussions provided explanation for this observation: bulls are unwilling to mate during starvation period associated with the dry season and the herders allow controlled mating in wet season when the bulls are more active. Finally, in the peri-urban camel production systems, producers mostly purchase foundation stocks from livestock markets (Noor, 2013).

To gain significant understanding and have a good knowledge of the epidemiology of emerging zoonoses like MERS-CoV, sero-surveillance is very important particularly in a situation where virology and genetic analyses still present with some doubt. This work has attempted to determine the sero-prevalence and temporal intensity of MERS-CoV in

Soysambu camel ranch as a means to understand the dynamics of virus circulation within the camel herd population. The overall mean seroprevalence estimated from this study show low levels (14%) of seroconversion to MERS-CoV in the camel ranch. This is instructive as it revealed some exposure of the camel to previous or ongoing viral activity within the herd. Antibodies were found in pregnant camels, lactating camels and less likely calves. Matured and weaned male camels were all negative throughout the sampling period. The seropositivity amongst the pregnant and lactating camels, were higher than the seropositivity in calves but this result should be taken with caution since the number of positive calves may not be truly representative of the group. Hemida *et al.* (2014), Alagaili *et al*. (2013) and Wernery *et al*. (2015) have all concluded that calves are more susceptible to MERS-CoV than matured non-pregnant camels. While these findings are agreeable, it is plausible that the sedentary nature in the ranch in this study contributed to lower exposure of the calves and changed the dynamics of virus activity in this study. However, the seropositivity in the calves suggests that there was an on-going infection within the ranch. In addition, it implies that irrespective of Eco-tourism (Safari) and interactions with other camels, Soysambu camel ranch remains exposed to MERS-CoV and other coronaviruses. Deem *et al.* (2015) had earlier reached similar conclusion in ranched camels in Laikipia, Kenya. Using some critical evaluation criteria to assess the health of the camels by at least four veterinarians, the camels in this study were apparently healthy with no obvious respiratory signs. According to Hemida *et al*. (2014), MERS-CoV and other coronavirus infections in camels usually present in asymptomatic form. This finding of this study is consistent with this assertion. Hence, it is plausible that high level of MERS-CoV infection in camel herds may go unnoticed with significant zoonotic implication to humans.

In past studies (Hemida *et al.,* 2013; Alagilli *et al*., 2014), younger camels had lower seropositivity compared to older adult camels. The finding of this study suggests that younger

camels under two years are ten times less likely to be positive compared with those greater than 2 years (OR = 0.1; *P* = 0.002). Also calves are at higher risk of getting infected than weaned camels. Whereas this work has evaluated seropositivity to coronavirus antibodies in camels, it is possible that certain positive animals may have been missed due to the assay's specificity and sensitivity. At least, some camels were initially on the borderline but became positive on subsequent testing rounds in this study. Gossner *et al*. (2014) had suggested possible undetected MERS-CoV outbreaks in camels previously and therefore; it becomes necessary to constantly re-evaluate the test protocols for this emerging zoonosis. While eco-tourism primarily using the males in this herd does not result in seropositivity, it is yet to be understood fully the reason for this observation as these males mix with herds from higher seroprevalence Counties (Nyandarua, Laikipia, Marsabit and Turkana) but were negative. Perhaps, mixing of herds may not be the only factor that supports seropositivity. It is also plausible that for infection to occur there should be close and prolonged contacts. The resulting effect of MERS-CoV transmission to humans during eco-tourism (Safari) is not evaluated in this study.

In conclusion, this study has demonstrated the presence of antibodies to the MERS-CoV virus in the camel population of Soysambu Ranch indicating either a current or previous exposure to the virus. Although the possibility of the existence of the virus is evident, none of the residents of the ranch including the workers who are at a higher risk of infectionon had reported suffering signs consistent of MERS-CoV infection. There is a need for further studies to investigate the natural history of this virus particularly its zoonotic potential.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were drawn from the study:

- The overall seroprevalence of MERS-CoV virus in camels in Soysambu Ranch was 14% (95% CI). The seroprevalence was significantly (P<0.05) more in adult camels (61%) than in weaners and calves (39%). The exposure to the virus was more in pregnant (58%) and milking camels (6%).
- The following factors were positively associated with testing positive to MERS-CoV virus antibodies in univariate analysis. Sex (OR=10, P=0.002), Animal use (OR=1, P=0.00001), Pregnancy status (OR=38, P=0.0001), lactating status (OR=12, P=0.0001). However, the association was negative for age with calves <2 years being 10 times (OR=0.1) less likely to test positive to the virus relative to adult camels.
- In the multivariate logistic regression, the odds of camels testing positive for antibodies of MERSCoV were 3.88 times higher for lactating camels compared to non-lactating camels. All the five factors significant in the univariate analysis lost their significance in the final multivariate logisticanalysis except lactation status (OR=3.8, p=0.003).

6.2 Recommendations

- Given the zoonotic potential of the MERS-CoV virus, there is a need to educate the milkers on the ranch on hygienic measures during milking and generally handling camels particularly those presenting respiratory tract signs.
- More investigations are needed to have a better understanding of the natural history of the virus on this ranch and indeed in other camel-keeping areas of Kenya. Soysambu conservancy presents the best example of applying ‘One Health’ approach focusing on the environment, host (livestock, wildlife, and human) and the virus.

- There is a need to replicate the current study in large camel keeping areas of Kenya such as in Turkana, Marsabit, Samburu, Wajir and Garissa to establish the status of the MERS-CoV virus in these areas where nomadic pastoralism is practiced.
- Of particular importance would be to explore the potential role of vectors in the epidemiology of the MERS-CoV virus.

REFERENCES

Adney D.R., Bielefeldt-Ohmann H., Hartwig A.E., Bowen R.A., (2016), Infection, replication, and transmission of Middle East respiratory syndrome coronavirus in alpacas, *Emerging Infectious Diseases,* **22 (6):** 1031–1037.

Adney D.R., van Doremalen N., Brown V.R., Bushmaker T., Scott D., de Wit E., (2014) Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels, *Emerging Infectious Diseases*. **20(12):** 1999–2005.

Al- Hameed F., Wahla A.S., Siddiqui S., Ghabashi A., Al-Shomrani M. and Al-Thaqafi, (2015). Characteristics and outcomes of Middle East Respiratory Syndrome Coronavirus Patients Admitted on intensive Care Unit in Jeddah, Saudi Arabia. *Journal of Intensive Care Medicine,* **31(4):** 289

Alagaili A.N., Briese T., Mishra N., Kapoor V., Sameroff S.C., Burbelo P.D (2014), Middle East Respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *American Society for Microbiology,* **5 (2):** e00884-14 doi: 10.1128/mBio.00884-14

Anne M.L., Benjamin M., Joerg J., Marcel A.M., Erik L., Ian N., Bernard B., Christian D. and Vietor M.C., (2016); MERS-CoV antibodies in humans, Africa, 2013-2014. *Emerging Infectious Diseases*. **22 (6):** 1086-1089.

Assiri A., Al-Tawfiq J.A., Al-Rabeeah A.A., Al-Rabiah F.A., Al-Hajjar S., Al-Barrak A., Flemban H., Al-Nassir W.N., (2013) Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East Respiratory syndrome coronavirus diseases from Saudi Arabia: a descriptive study. *Lancet Infectious Diseases,* **13(9):** 752-61.

Assiri A., McGeer A., Perl T.M., Price C.S., Al Rabeeah A.A., Cummings D.A., (2013) Hospital outbreak of Middle East respiratory syndrome coronavirus. *New England Journal of Medicine,* **369:**407–16.

Awassa M.A. and Fekadu B., (1993): Effect of container smoking and udder cleaning on the microflora and keeping quality of raw milk from a dairy farm,Department of Basic Sciences and Department of Animal Production and Rangeland Management. Awassa College of Agriculture. Addis Ababa University, PO Box 5. Awassa, Ethiopia; *Tropical Science*. **33:** 368-376

Azhar E.I., Ph.D., Sherif A. El-Kafrawy, Ph.D., Suha A. Farraj, M.Sc., Ahmed M. Hassan, M.Sc., Muneera S. Al-Saeed, B.Sc., Anwar M. Hashem, Ph.D and Tariq A. Madani, M.D (2014); Evidence for Camel–to-human transmission of MERS Coronavirus. *New England Journal of Medicine* (2014); **370:**2499-2505, DOI: 10.1056/NEJMoa1401505

Bekele T., Zeleke M. and Baars R.M.T (2002): Livestock production science **76(1-2):**37-44

Benjamin J.C., Minah P., Vicky J., Peng W., Gabriel M.L. and Joseph T.W. (2015), Preliminary epidemiologic assessment of MERS-CoV outbreak in south korea; *Euro Surveillance* **20 (25):** 21163,

Benka V., (2012); Human-Wildlife Conflict, Interspecies Disease, and justice in a Wildlife Rich Region in Kenya; *http://hdl.handle.net/2027.42/96190*

Bernard F., Hassani M., Sageed A.H. and El Rouili (2014): Camel milk Value chain in Nothern Saudi Arabia: *Emirate Journal for Food and Agric*. **26 (4):** 359-365. Doi:10.9755/ejfa.v26i4.17278.

Bialek S.R., Allen D., Alvarado-Ramy F., (2014) First confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in the United States, updated information on the epidemiology of MERS-CoV infection, and guidance for the public, clinicians, and public health authorities—May 2014. *MMWR Morb Mortal Wkly Rep* **63:**431–6.

Cauchemez S., Pierre N., Anne C., Thibaut J., Garske T., Hannah C., Sean M., Harriet L.M., Henrik S., Collins C., Isabel R.B., Steven R., Shaun T., Homoud A., Rafat A., Khalid A., Abdulhafiz T., Ricardo J.A., Cummings D.A.T., Maria D.V.K., Donnelly C.A., Justin L., Christophe F., Al-Barrak A., Ferguson N.M., (2016). Unravelling the drivers of MERSCoV transmission, Proceedings of the national academy of sciences 113 **(32),** 9081-9086**.**

Christian D., Michael S., Victor M., Wulf H., Gregor S., Stefan S., (2013) Clinical features and virological analysis of a case of Middle East Respiratory Syndrome Coronavirus infection. *Lancet Infectious Diseases,* **13 (9):** 745-751,

Chu D.K., Oladipo J.O., Perera R.A., Kuranga S.A., Chan S.M., Poon L.L., (2015); Middle East respiratory Syndrome Coronavirus (MERS-CoV) in dromedary camels in Nigeria, 2015: *Euro Surveillance,* **20:**30086.

Corman V.M., Jores J., Meyer B., Younan M., Liljander A., Said M.Y., (2014) Antibodies against MERS coronavirus in dromedary camels, Kenya, 1992–2013, *Emerging Infectious Diseases* **20(8):** 1319–1322.

Cotten M., Watson S.J., Kellam P., Al-Rabeeah A. Makhdoom H.Q., Assiri A., Al-Tawfiq J.A., Rafat F.A., Madani H., Fahad A.A., Al Hajjar S., Wafa N.A., Ali A., Hesham F., Hannan H.B., Sarah A., Anne L.P., Astrid G., Racheal B.R., Andrew R., Alimuddin I.Z., Ziad A.M., (2013) Transmission and evolution of the middle east respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. The Lancet **382** (9909), 1993-2002.

Crameri G., Durr P.A., Klein R., Foord A., Yu M., Riddell S., (2016) Experimental infection and response to rechallenge of alpacas with Middle East respiratory syndrome coronavirus, *Emerging Infectious Diseases*. **22(6):** 1071–1074.

De Groot R.J., Susan C.B., Ralph S.B., Caroline S.B., Christian D., Luis E., Ron A.M.F., Monica G., Alexander E.G., Z iad .A.M., Stanley P., Leo L.M.P., Eric J.S., Gwen M.S., Patrick .C.Y.W., Ali M.Z., Maria Z., John Z., (2013): Middle East

Respiratory Syndrome Coronavirus (MERS-CoV): announcement of the Coronavirus study group. Journal of virology **87** (14), 7790.

Deem S.L., Fevre E.M., Kinnaird M., Browne A.S., Muloi D., Godeke G.J., *et al.*, (2015) Serological evidence of MERS-CoV in Dromedary camels (*Camelus dromedary*) in Laikipia County, Kenya. *PLoS ONE* **10(10):** e0140125. Doi:10.1371/journal.pone.0140125

Drosten C., (2013); Is MERS another SARS? *Lancet Infectious Diseases.* **13:**727-728.

FAOSTAT, 2015

FAOSTAT, 2016. http://faostat3.fao.org/download

Farag E.A., Reusken C.B., Haagmans B.L., Mohran K.A., Stalin R.V., Pas S.D., (2015) High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014, *Infectious Ecology and Epidemiology*. **5 (1):** 28305.

Haagmans B.L., Al Dhahiry S.H., Reusken C.B., Raj V.S., Galiano M., Myers R., (2014). Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation, *Lancet Infectious Disease.* **14(2):** 140–145.

Haagmans B.L., Al Dhahiry S.H.S., Reusken C.B.E.M., Raj V.S., Galiano M., Myers R., (2013) Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infectious Diseases;* **14:**140–5. doi:10.1016/S1473-3099(13)70690-X.

Haagmans B.L., van den Brand J.M., Raj, V.S.A., Wohlsein V.P., Smits S.L., (2016). An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels, Science **351 (6268):** 77–81.

Hemida M.G, Al-Naeem A., Perera R.A.P.M., Chin A.W.A., Poon L.L.M., Peiris M. (2015); Lack of Middle East Reaspiratory Syndrome Coronavirus transmission from infected camels. *Emerging Infectious Diseases,* **21:** 4.

Hemida M.G., Perera R.A., Wang P., Alhammadi M.A., Siu L.Y., Li M., (2013) Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. Euro Surveillance **18(50):**20659. doi:10.2807/1560-7917.ES2013.18.50.20659.

Ian M.M and Katherine E.A., (2015); MERS Coronavirus: Diagnostics, Epidemiology and Transmission: *Virology Journal*. **12:** 222.

Ihuthia P.M., 2010: An analysis of Camel Calf growth and survival under pastoral camel production system in Samburu: http://erepository.uonbi.ac.ke:8080/handle/123456789/4187

Isaack M.N., Abdi Y.G., Muhammad T. and Bockline O.B., (2013): Assessment of camel and camel milk marketing practices in an emerging peri-urban production system in Isiolo County, Kenya. Pastoralism: Research, Policy and Practice, **3:** 28

Isaack M.N., Bockline O.B. and Abdi Y.G., (2012): Analysis of an emerging peri-urban camel production in Isiolo county Northern Kenya. *Journal of Camelid Science,* **5:** 41-61

Jasper F.W.C., Kelvin K.W.T., Herman T.D.Y., Kwok Y.Y., (2013); Interspecies transmission and emergence of novel viruses: lessons from bats and birds; Trends in microbiology **21(10):** 544-555.

Jones J., 2015 Middle East Respiratory Syndrome Coronavirus in Camels: an overview for Sub-Saharan and North Africa. Evidence on demand, UK.2015.

Kenya dairy board, Available at: http://www.kdb.co.ke/contact/livestock-population.html

Kenya National Bureau for Statistics, 2016. ISBN: **9966**- 767-54.

Ki M., (2015), MERS outbreak in Korea: hospital-to-hospital transmission. *Epidemiology Health.* 2015; **37:** e2015033.

Kigomo, N. B., (2001). State of Forest Genetic Resources in Kenya. The sub- regional workshop FAO/IPGRI/ICRAF on the conservation, management, sustainable utilization and enhancement of forest genetic resources in Sahelian and North-Sudanian Africa (Ouagadougou, Burkina Faso, 22-24 September 1998). Forest Genetic Resources Working Papers. Available at: *ftp://ftp.fao.org/docrep/fao/004/AB396E/AB396E00.pdf.*

Kinnaird M.F. and O'Brien T.G., (2012); Effects of private-land use, livestock management and human tolerance on diversity, distribution and abundance of large African mammals. *Conservation Biology* **26:**1026-1039

Kraaij D.M., Timen A., Dirksen K., Gelnick L., Leyten E., Groeneveld P., (2014). Middle East respiratory syndrome coronavirus (MERS-CoV) infections in two returning travellers in the Netherlands, May 2014. *Euro Surveillance;* **19:**20817-7.

Mailles A., Blanckaert K., Chaud P., Van der S. W., Lina B., Caro V., Campese C., Guery B., Prouvost H., Lemaire X., Paty M.C., Haeghebaert S., Antoine D., Ettahar N., Noel H., Behillil S., Hendricx S., Manuguerra J.C., Enouf V., La Rouche G., Semaille C., Coignard B., Levy Bruh D., Weber F., Saura C., Che D., 2013. First cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections in France, investigations and implications for the prevention of human to human transmission, France, May *Euro Surveillance.* **18:**24.

Memish Z.A., Zumla A.I., Al-Hakeem R.F., Al-Rabeeah A.A., Stephen G.M., (2013); Family cluster of Middle East Respiratory Syndrome Coronavirus infections *New England Journal Medicine*. **368:** 2487-2494.

Meyer B., García B.I., Wernery U., Wernery R., Sieberg A., Müller M.A., (2015) Serologic assessment of possibility for MERS-CoV infection in equids. *Emerging Infectious Diseases* **21(1):** 181–2. doi:10.3201/eid2101.141342.

Mohamed N.S., Stephen M.B., and Nammalwar S., (2010): Brucellosis: A re-emerging zoonosis. *Veterinary microbiology* **140(3-4):** 392-398

Mohd H.A., Al- Tawfiq J.A., Memish Z.A., (2016). Middle East Respiratory Syndrome Coronavirus (MERSCoV) origin and animal reservoir Virology journal 13 (1), 87.

Muller M.A., Corman V.M., Jores J., Meyer B., Younan M., Anne L., Bosch B.J., Lattwein E., Hilali M., Musa B.E., Bornstein S., Drosten C., (2014). MERS coronavirus neutralizing antibodies in camels, eastern Africa, 1983-1997. Emerging infectious disease 20 (**12**), 2093.

Müller M.A., Meyer B., Corman V.M., Al-Masri M., Turkestani A., Ritz D., (2015) Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study, *Lancet Infectious Diseases* **15(5):** 559–564.

Musinga, M., (2008) the camel milk industry in Kenya. Resource mobilization center, Nairobi. See *www.snvworld.org/en/regions/world/publication?filter=~camel%2520milk*

Nahla K.R.I., (2014). Surveillance of communicable diseases in era of emerging viral zoonotic infections; lessons from H1N1 and MERS-CoV: *Austin Journal of Public health epidemiology*. **1(1):**1005.

Patrick M.M., Raphael W.W., Laban M., Paul K., (2015); livestock herd structures and dynamics in Garissa County, Kenya; Pastoralism, **5:**26

Puzelli S., Azzi A., Santini M.G., Di M.A., Facchini M., Castrucci M.R., (2013) Investigation of an imported case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in Florence, Italy, May to June 2013. *Euro Surveillance.* **18(34):**1

Raj V.S., Farag E.A., Reusken C.B., Lamers M.M., Pas S.D., Voermans J., (2014) Isolation of MERS coronavirus froma dromedary camel, Qatar, 2014, *Emerging Infectious Disease.* **20 (8):** 1339–1342.

Reusken C.B., Ababneh M., Raj V.S., Meyer B., Eljarah A., Abutarbush S., (2013) Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. *Euro Surveillance*. **18 (50):** doi: 10.2807/1560-7917.ES2013.18.50.20662

Reusken C.B., Haagmans B.L., Muller M.A., Gutierrez C., Godeke G.J., Meyer B., (2013) Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: A comparative serological study. *Lancet Infectious Diseases* **13:**859–66. doi:10.1016/S1473-3099(13)70164-6.

Reusken C.B.E.M., Schilp C., Raj V.S., De Bruin E., Kohl R.H.G., Farag E.A.B.A., (2016) MERS-CoV infection of alpaca in a region where MERS-CoV is endemic [letter]. *Emerging Infectious Diseases*. doi:10.3201/eid2206.152113.

Reusken., 2014, *Eurosurveillance* Volume 19, issue 23 (article)

Sabir J.S., Lam T.T., Ahmed M.M., Li L., Shen Y., Abo-Aba S.E., (2016) Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia, *Science* **351 (6268):** 81–84.

Sander V.B., Miranda.G., Chris L., Theo M.B., Stalin V.R., Ali M.Z., Albert D.M.E.O., Bart L.H., Alexander E.G., Eric J.S., Ron A.M.F., 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans MBio 3 (6), e00473-12.

Sharon L.D., Eric M.F., Margaret K., Springer B.A., Dishon M., Gert-Jan G., Marion K., Chantal B.R., (2015), Serological Evidence of MERS-CoV Antibodies in Dromedary Camels (Camelus dromedaries) in Laikipia County, Kenya; *PLOS ONE* **12(5):** Available at: https://doi.org/10.1371/journal.pone.0140125

Shauna M.P., Kerri L.M. and Vincent J.M., (2014); The emergence of the Middle East Respiratory Syndrome coronavirus, Pathogens and Disease, **71(2):**121–1361, Available at: https://doi.org/10.1111/2049-632X.12166

Simon C., Pierre N., Anne C., Thibaut J., Tini G., Hannah C., Sean M., Harriet L.M., Henrik S., Caitlin C., Isabel R-B., Steven R., Shaun T., Homoud A., Rafat A., Khalid A., Abdulhafiz T., Ricardo J.A., Derek A.T.C, Maria D.V.K., Christl A. **D., Justin L., Christophe F., Ali Al-B., and Neil M. F., (2016)** Unraveling the drivers of MERS-CoV transmission; Proceedings of the National Academy of Sciences of the United States of America **113(32):** 9081-9086. doi: 10.1073/pnas.1519235113.

Soren A., Kobinger G. P., Soule G., Ulrich W., (2014), Middle East Respiratory Syndrome Coronavirus Antibody Reactors Among Camels in Dubai, United Arab Emirates, in 2005. *Transboundary and Emerging Diseases*, **61(2):** 105-108. DOI: 10.1111/tbed.12212

The WHO MERS-CoV Research Group. State of Knowledge and Data Gaps of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Humans. PLoS Curr. (2013); Edition 1.

Ulrich W., (2014): Zoonosis in the Arabian peninsula Saudi Medical Journal; **35(12):** 1455-1462

Vergara-Alert J., van den Brand J.M., Widagdo W., Muñoz M., Raj S., (2017) Livestock susceptibility to infection with Middle East respiratory syndrome coronavirus, *Emerging Infectious Disease.* **23(2):** 15, http://dx.doi.org/10.3201/eid2302.161239

Victor M.C., Joerg J., Benjamin M., Mario Y., Anne L., Mohammed Y.S., Ilona G., Erik L., Berend-Jan B., Jan F.D., Set B., Christian D. and Marcel A.M., (2014); Antibodies against MERS Coronavirus in Dromedary Camels, Kenya, 1992–2013. *Emerging Infectious Disease*, **20 (8):** 1319-1322. doi: 10.3201/eid2008.140596

Zak A.M., van B.S, Bestebroer T.M., Osterhaus A.D., Fouchier R.A., (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *New England Journal of Medicine*;**367:**1814-1820.

Zumla A., David S.H., and Stanley P., (2015); Middle East Respiratory Syndrome; *The Lancet Infectious Diseases*, **386 (9997):** 995-1007.

APPENDIX 1: House hold (Inventory) To be completed at the conservancy]

CROSS SECTIONAL SURVEY OF MERS CoV in Soysambu conservancy, Nakuru County

1. Name of interviewer (First and last name):

   _____

2. Date of interview: _____
3. Site Name: **Soysambu ranch**
4. GPS coordinates (read from the smart phone):
   a. Eastings_____
   b. Northings _____
5. Primary Administrative (County Name): **Nakuru**
6. County code: 032
7. Sub-county name: **Gilgil**
8. Sub-location/Village: **Jolai 1**
9. Site Contact Person:
   a. Name: _____
   b. Function/role: _____
   c. phone number (free text) _____
10. Site type (select one):

Primary production holding
ground

Feedlot/fattening

Other (Specify):

If site type is primary production, then:

11. Type of primary production site (select one):
    Grazing area
    Watering point
    Ranch
    Farm
    Other (Specify): _____
12. How many camels are on site? _____
13. Management system:
    Intensive
    Extensive

Semi-intensive

14. Species other than camels present on site (select all that apply):

Sheep	Dogs	Horses
Goats	Cats	Cattle
Poultry	Pigs	Donkeys

15. What are primary camel use(s) (select all that apply):

Meat	Prestige
Milk	Draft
Ecotourism/safari	Breeding
Racing	Cash/income

16. Do your camels interact (grazing together, drinking water together etc.) with camels from other pastoralists or from other ranches?
Yes
No

17. If Yes above, how frequent is this interaction/contact?
Daily
Dry seasons
Wet seasons
During routine vaccinations or medical camps
Other (specify) _____

18. Watering: Where do your camels drink water from? (tick the **most** common source)

Always from a private source (on farm/ ranch)

Always from shared communal source

Sometimes from either of the two

Other (specify) _____

19. How your camels are mainly confined at night?
Confined separately from the other species
Kept together with other species
Other (specify) _____

20. Which wild animal species are present in the vicinity of your livestock? Select all that apply

None
Bats
Primates (e.g. monkeys)
Wild pigs
Warthogs
Wilder beast
Giraffe
Rats and other rodents
Antelopes/ gazelles
Zebras
Wild birds
Wild carnivores

Other (specify): _____

21. Have you experienced any camel disease/condition in your herd in the last 6 months?

YES /NO

22. What other treatments have been given to the camel herd during the last one year

(type, please describe):

_____

23. Any other relevant information to note on the site?

_____

_____

_____