Thesis Ref. No. ----------

CROSS SECTIONAL STUDY OF MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS IN SELECTED KEBELES OF YABELLO DISTRICT OF BORENA ZONE, OROMIA REGION, ETHIOPIA

MVSc. Thesis

ADDIS ABABA UNIVERSITYCOLLEGE OF VETERINARY MEDICINE AND AGRICULTUREDEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH

BY:

AYELECH MULUNEH MENGESHA

Bishoftu, Ethiopia

June, 2019

CROSSECTIONAL STUDY OF MIDDLE EAST RESPIRATORY SYNDROME CORONA VIRUS IN CAMELS OF OROMIA REGION,BORENA ZONE IN SELECTED KEBELES OF YABELLO DISTRICT, ETHIOPIA

MVSc. Thesis

A Thesis Submitted to College of Veterinary Medicine and Agriculture, Addis Ababa University, in partial fulfillment of the requirements for the degree of Masters of Veterinary Science in Veterinary Microbiology.

By

Ayelech Muluneh Mengesha

June, 2019

Bishofti, Ethiopia

Addis Ababa Universty

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As MVSc research advisors, we here by certify that we have read and evaluated this thesis prepared under our guidance by Ayelech Muluneh Mengesha

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DEDICATION

This thesis manuscript is dedicated to my husband Dereje Tilahun and our children Emmanuel, Halelujah and Kaleb who have provided me with emotional support, pacience and couragement.

STATEMENT OF AUTHOR

First, I declare that this thesis is my authentic work and that all sources of materials used for this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MVSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and it is deposited at the University /College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic certificate, diploma or degree.

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LIST OF ABBREVIATIONS

BSL	Bio Safety Level
BSC	Bio Saftey Cabinate
CDC	Center for Disease Control and prevention
DNA	Deoxyribose Nucleic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organisation
GHA	Great Horn of Africa
HCoV	Human Corona Virus
HKU	Hong Kong Universty
ICTV	International Comitte of Taxnomy of Viruses
KSA	Kingdom of Saudi Arabia
Kb	Kilo Base
MERS-CoV	Middle East Respiratory Syndrom- Corona Virus
MN	Micro Neutralisation test
nm	Nano meter
OIE	International Office de Epizooties
PCR	Polymerase Chain Reaction
RdRP	RNA dependent RNA Polymarase
RNA	Ribose Nucleic Acid
RT-qPCR	Reverse Transcriptase real time Polymerase Chain Reaction
PPNT	Pseudo Particle Neutralisation Test
SARS-CoV	Sever Acute Respiratory Syndrome Corona virus
UAE	United Arab Emirat
W H O	World Health Organisation

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ABSTRACT

Middle East respiratory syndrome is a respiratory and an enteric disease caused by a recently discovered virus called MERS-CoV. It is a zoonotic virus in which camels are implicated as the major source of infection in humans. A cross-sectional study was conducted from February, 2018 to April, 2019, with the objectives of detection and characterization of MERS-CoV and determining the seroprevalence and its association with risk factors. The study animals were camels found in Borena zone, in three kebeles, namely, Areri, Dikale and Harwoyu of Yabello district. A total of 1050 nasal swab and 525 sera samples were collected at a rate of 150 nasal swab and 75 sera at a time. The nasal swab were tested for the presence of specific viral RNA using qRT-PCR at Hong Kong University and NAHDIC.The sera samples were tested for the presence IgG antibody using indirect ELISA at NAHDIC, Ethiopia. Additionally 75 of 525 serums were retested by ppNT in Hong Kong University. Based on ELISA result, the overall seroprevalence of MERS-CoV in the study area was 74% (390/525, 95%CI 70 to78).Association of risk factors with seroprevalence was identified. The seroprevalence found were 87% in adult, 48% in young's and 50% in juvenile camels, the variation was statistically significant, adjusted OR; 7.5 CI= 4.5 - 12.4.Also small size herd had 80% seroprevalence than medium 78.8% and large herd size 67.8%, the variation was statistically significant, adjusted OR; 3.44;CI=1.76 to 6.75.High seroprevalence was recorded in Dikale 76% than Harwoyu74% and Areri 72%. ,the variation was statistically significant,adjusted OR=2.3;CI=1.27to4.35.Gravid females had high seroprevalence 91% than lactating 83% and dry females 67%,the variation was statistically significant,adjusted OR= 1.5;CI=1.045 to 2.917.No statistical significance difference was observed among sexs and sampling seasons.In comparison of the two serology methodologies, 69% of the test result agreed, and showed higher seroprevalence of MERS-CoV antibody in all kebeles of Yabello district camels which reflects the evidence of prior MERS-CoV infection. All 1050 nasal swab samples showed negative result. Zero detection might suggest absence of active circulation of the virus at the time of sampling and the need of designing longitudinal based active surveillance by concidering the calving season of the camels and the short viral shedding window (2-5days). Early detection and imposing control intervention reduces risk of transmission of the virus to the pastoralist and consumers of camel products in the country.

Key words: MERS-CoV, camels, seroprevalence, Yabello, Ethiopia.

1. INTRODUCTION

Middle East respiratory syndrome (MERS) is a highly fatal respiratory tract disease in humans caused by anewly emerged virus (MERS-CoV) that was first detected in 2012 in the Kingdom of Saudi Arabia (KSA)in an individual who died with an acute respiratory distress syndrome (Zaki *et al*., 2012;VanBoheemen *et al*., 2012;). In 2013 similar novel corona virus infection in human reported from Jordan and from other Middle East countries (Hijawi *et al*., 2013).

Since its identification in the Kingdom of Saudi Arabia (Zaki *et al*., 2012) and Jordan (Hijawi *et al*., 2013), Middle East Respiratory Syndrome (MERS) has become a global public health threat. Typical of an emerging zoonosis, MERS-CoV has an animal reservoir (dromedary camels) in which the virus causes little to no disease (Mohd *et al*., 2016).

In August 2013, dromedary camels *(Camelus dromedarius*) which is found in large numbers in many African countries and the middle east, were implicated for the first time as a possible source of infection in humans on the basis that MERS-CoV neutralizing antibodies were found in dromedaries from Oman and the Canary Islands of Spain (Reusken *et al*., 2013a). Later, the presence of MERS-CoV antibodies in camels have been reported in Jordan (Reusken *et al*., 2013b), in Egypt (Perera *et al*., 2013; Chu *et al*., 2014), in the United Arab Emirates (Meyer *et al*., 2014; Woo *et al*., 2014), and in Saudi Arabia (Hemida *et al*., 2014b; Alagaili *et al*., 2014).

In October 2013, analysis of an outbreak associated with one barn in Qatar found dromedaries and humans to be infected with nearly identical strains of MERS-CoV (Haagmans *et al*., 2014).In the following years it was indicated that MERS-CoV is endemic in dromedaries in many Africa countries including Ethiopia, and a number of reports showed sero prevalence of camels MERS CoV reported to be 93% to 97% in Ethiopia (Reusken *et al.* 2014a), 60.8% in Kenya (Deem *et al*., 2015) and 30% to 54% in Tunisia in youngs and adult respectivelly (Reusken *et al*.,2014a); 92.3% in Egypt (Daniel *et al*.,2014); 93% in Nigeria (Reusken *et al.*, 2014a). However; zoonotic

infections have not been reported from Africa (Reusken *et al*., 2016a; Chu *et al*., 2014; Chu *et al*., 2015; Miguel *et al*., 2017).

Globally, from the time the virus first identified to May, 2019, 2434 laboratory confirmed human cases with case fatality rate of 36%, (876 deaths) have been reported due to MERS. Since the last global update published on April 17 to 22 May 2019, with in 25 days, thirteen new human cases have been reported in Saudi Arabia including six fatalities (FAO, May 2019), indicating the ongoing circulation of the virus with high fatality rate and continue to be the threat for public health in the Middle East and the rest of the world.

In the Middle East, MERS CoV repeatedly transmitted to the human population via direct or indirect contact with infected dromedary camels especially in Saudi Arabia. Camels infected with MERS-CoV may not show any signs of infection. It is, therefore, not possible to know whether an animal in a farm, market, race track or slaughterhouse is excreting MERS-CoV that can potentially infect humans. However, infected animals may shed MERS-CoV through nasal and eye discharge, feces, and potentially in their milk and urine. The virus may also be found in the organs and meat of an infected animal (WHO, 2014).

Human Populations in close contact with dromedaries (e.g. farmers, abattoir workers, shepherds, dromedary owners and family members) and health care workers caring for MERS-CoV patients are believed to be at higher risk of infection(Farag *et al*.,2019).

In Ethiopia, dromedary camels represent a subset of major livestock resources with a population estimated at 1, 209,321 (CSA, 2016/17).The total camel population of the study area Borena zone and Yabello district were estimated to be about 232,589 and 44,042, respectively (Yabello district Agriculture office, April/2015).

The first MERS-CoV serology report from Yabello district camels by Reusken *et al.* (2014a) revealed 93-97% seroprevalence in camels, another study by Fekadu *et al*. (2016), reported seroprevalence of camels of Yabello district was 86% with 0% RNA detection .In another study in yabello district there was a report of sero positivity 85 % and 0% RNA detection (Miguel et

al., 2017). Recently another study by Wallelign *et al.* (2018), reported 56% seroprevalence of MERS CoV and 0% MERS CoV RNA detection in yabello district camel population.

Several serological evidences indicated MERS-CoV might be endemic in Yabello area camels(Reusken *et al.,* 2014;Fekadu *et al.,* 2016;Miguel *et al.,* 2017 and Walelign *et al.,* 2018), however, characterization of MERS-CoV from Yabello area camels were not yet acheived.Hence investigating, detection and characterization of the virus and continuous updates of its sero prevalence and identifying associated risk factors in order to initiate early warning and response systems in camel rearing areas of the country is important and should be continued.

Therefore the objectives of the study are:

- To determine the seroprevalence of MERS- CoV in camels of Yabello district of Borena zone
- To determinee the risk factors associated with the seroprevalence of MERS-CoV in camels
- To detect and characterize MERS-CoV RNA in camels of Yabello district of Borena zone

2. LITERATURE REVIEW

2.1 Definition of MERS- CoV

MERS-CoV is a newly emerged coronavirus causes sever and fatal respiratory diseas in humans, it was first identified in Saudi Arabia in 2012 (Zaki *et al*.,2012).It belongs to genus Corona viruses that cause diseases ranging from common cold to Severe Acute Respiratory Syndrome (SARS) in human(Dorsten *et al*., 2003;Woo *et al*., 2012a).Dromedary camels are a major reservoir host for MERS-CoV and are source of infection to humans (Assiri *et al*., 2013;Adney *et al*.,2014; AlAbdalat *et al*.,2014;AlTawfiq *et al* .,2014;Memish *et al*., 2014).

MERS- CoVs are group of viruses belonging to the *Nidovirales* order, *Coronaviridae* family and belonging to the sub family *Corona virinae* under the genus lineage *C beta* corona virus. MERS-CoV is an enveloped positive-sense single-stranded RNA virus (Vanboheemen *et al*., 2012). Its single-stranded RNA genome has a size of approximately 30 kb and a GC content of 41% and contains 5′-methyl-capped, poly adenylated,polycistronic RNA(Vanboheemen *et al* .,2012;cotton *et al.,* 2013;Woo *et al* .,2012b). MERS-CoV is the first lineage C CoV and the sixth CoV known to cause human infection.

2.1.1 Nomenclature of MERS-CoV

According to VanBoheemen *et al*. (2012) Calculation of pair wise evolutionary distances for seven replicas domains showed that MERS-CoV had an amino acid sequence identity of less than 90% to all other known Coronaviruses at the time when MERS-CoV was discovered. Before the virus was formally named MERS-CoV by the Corona virus Study Group, it was also known by other names, including “novel coronavirus,” “human coronavirus EMC,” “human beta coronavirus 2c EMC,” “human beta coronavirus 2c England-Qatar,” “human beta coronavirus 2C Jordan-N3,” and “beta coronavirus England 1,” which represented the places where the first complete viral genome was sequenced (Erasmus MedicalCenter, Rotterdam, the Netherlands) or where the first laboratory-confirmed cases were identified or managed (Jordan, Qatar,and

England) (Zaki *et al*., 2012;DeGroot *et al*.,2013;Bermingham *et al*., 2012;Pollack *et al* .,2013;Cotton *et al*., 2013a). It was designated as novel lineage C CoV based on the International Committee on Taxonomy of Viruses (ICTV) (VanBoheemen *et al*., 2012).

2.1.2 Genomic organization of coronavirus

Generally Corona viruses encode five structural proteins in their genomes. These are the Spike (S), Membrane (M), Envelope (E) glycoprotein Hem agglutinin Esterase (HE) and Nucleocapsid (N) protein. All envelope proteins and N protein is present in all virions but HE is only present in some beta corona viruses (Lissenberg *et al*., 2005). In addition to that, it is thought the virus particles are huddled together owing to interaction between these proteins (De Haan *et al*., 2005; Masters, 2006).The name "coronavirus" is derived from the Latin corona, meaning crown or halo, and refers to the characteristic appearance of virions under electron microscopy (Figure 2) with a fringe of large, bulbous surface projections creating an image reminiscent of a royal crown or of the solar corona. This morphology is created by the viral spike (S) peplomers, which are proteins that populate the surface of the virus and determine host tropism (Graham *et al*., 2013**).**

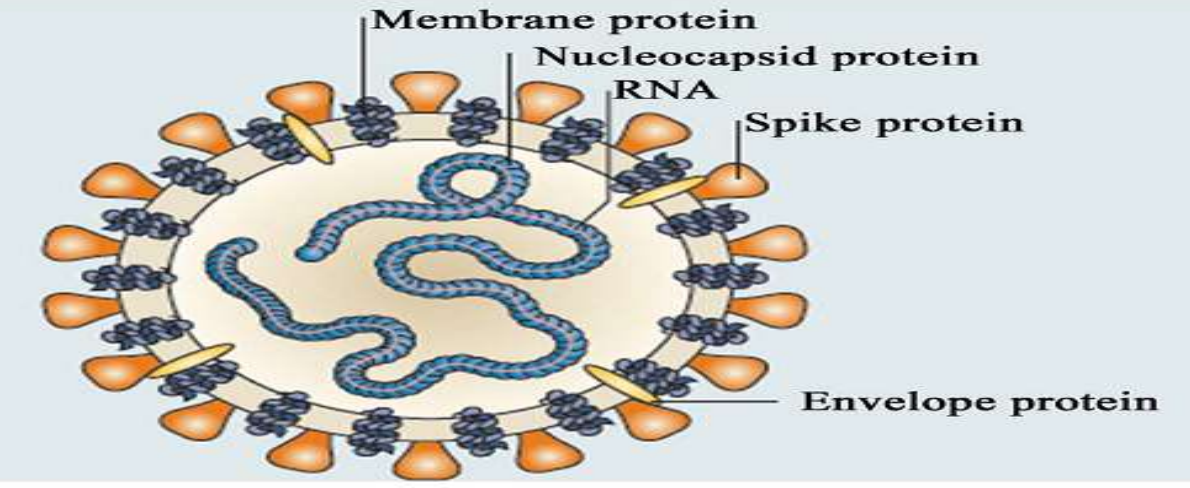

Figure 1: Genomic Structure of Corona viruses Source: Graham *et al*., (2013).

The membrane-anchored trimeric S protein of MERS CoV is a major immunogenic antigen involved in the virus attachment and entry into host cells and has an essential role in determining virus virulence, protective immunity, tissue tropism, and host range (Qian *et al*., 2013).

2.1.3 Virus infection, replication and transcription of MERS CoV

The initial attachment of the virion to the host cell is initiated by interactions between the **S** protein and its receptor (DPP4), The S-protein and receptor interaction is the primary determinant for a coronavirus to infect a host species, and also governs the tissue tropism of the virus. MERS-CoV binds to dipeptidyl-peptidase4 (DPP4) to gain entry into human cells since no infection occurs in its absence (Raj *et al*., 2013; Li *et al*., 2003).

In humans, the virus has a strong tropism for nonciliated bronchial epithelial cells, and it has been shown to effectively evade the innate immune responses and antagonize interferon (IFN) production in these cells. This tropism is unique in that most respiratory viruses target ciliated cells; the amino acid sequence of DPP4 is highly conserved across species and is expressed in the human bronchial epithelium and kidneys (Chan *et al*., 2014).The replication of corona viruses occurs in host cell cytoplasm. Once it gets access to the body the virus primarily bind to the receptor (DPP4) on the cell surface via the spike (S) protein. When S protein is bound to the receptor, a conformational structure occurs in the structure and the process of entry into cells begins (Bosch *et al*., 2003). This process with endocytosis is dependant of pH through the receptor (Blau and Holmes,2001;Chu *et al* .,2006;Eifart *et al*.,2007;Wang *et al* .,2008). After entering the cytoplasm, the virus particle releases the RNA genome. This genome is a single-stranded, non-segmented RNA virus with the largest known RNA genome, which is approximately 26-32 kb (Schochetman *et al*.,1977; Wege *et al*.,1978;Lomniczi,1997; Lai *et al*.,1984;Masters and Perlman,2013) up on entering the virus particle is un coated and the RNA genom is deposited in the cytoplasm.The coronavirus genom has 5`methylated and a 3`polyadenylated tail,this allows the RNA to attach Ribosom for direct translation.Coronavirus also have protein replicase encoded in its genom which allows the RNA viral genom to be transcribed in to new RNA copies using the host cell machinery(Figure 2).After all the progeny virus is generated and released from the infected cells (Masters, 2006; Susan and Sonia, 2005)

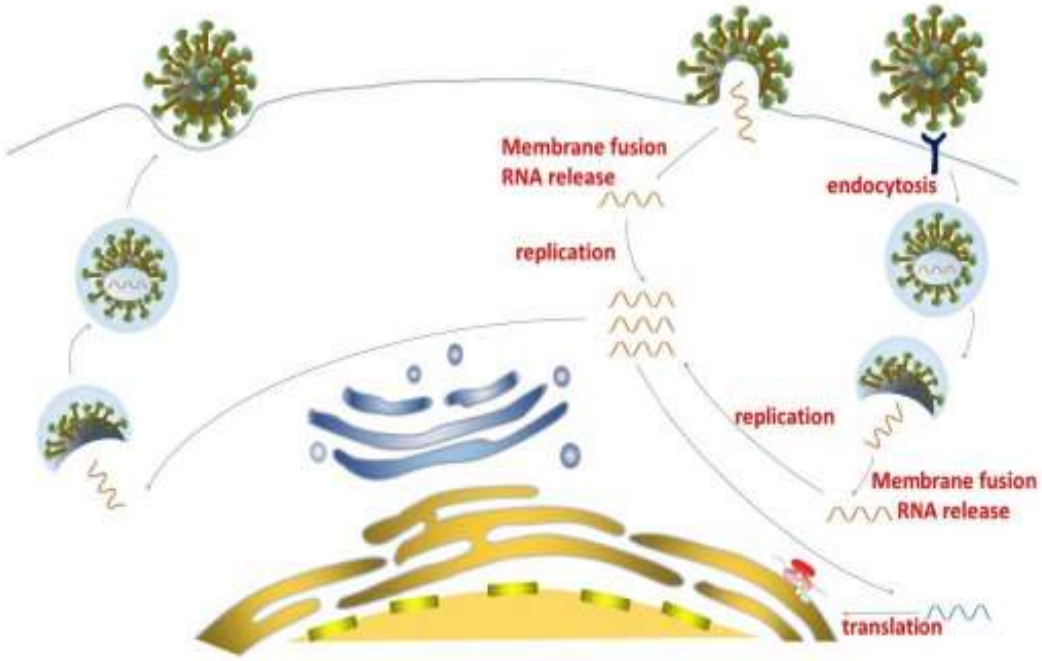

Figure 2: Schematic diagram of (MERS-CoV) infection process. Source: Liang *et al*., (2018)

MERS-CoV enters host cells by plasma membrane fusion (membrane fusion) or endosomal membranefusion (endocytosis), and then releases the viral RNA into the cytoplasm. The RNA genome is replicated and viral proteins are produced. The progeny virus is generated and released from the infected cells.

2.2 Host range of MERS CoV

While coronaviruses are widespread in the animal kingdom, MERS-CoV seems to have a narrow host range (Woo *et al*., 2012a). In the last few years, a large spectrum of domestic species have been negative after MERS-CoV serology tests, including horses, cattle, pig, water buffalo, chickens, goats(Perera *et al*., 2013;Reusken *et al*.,2013a;Hemida *et al* .,2013). Evidence for MERS-CoV infection from domestic animal, has only been found in dromedaries, an exception was published when antibodies were detected in Alpaca (*Vicugna pacos*) in Qatar; this is notably

in a specific region where MERS-CoV is already endemic in dromedary camels (Reusken *et al* .,2016b). Therefore experimental studies indicated that alpacas, similar to dromedary camels, can be infected and potentially serve as a reservoir animal for MERS- CoV (Mohd *et al.,* 2016;Adney *et al* .,2016;Reusken *et al* .,2016b).In general todate, the host rang of MERS -CoV may includes Human, dromedary Camels and alpaca can be concider(Zaki *et al*., 2012; Adney *et al*., 2014;Reusken *et al.,* 2016b;Mohd *et al.,* 2016;Adney *et al*., 2016).

2.3 Reservoire animals of MERS- CoV

2.3.1 Bats as Putative Origin of MERS-CoV:

Bats are known natural reservoirs for several emerging viral infections in humans including rabies, Nipah virus, Hendra virus and Ebola virus (Han *et al*., 2015). Several features enable bats to be efficient sources of emerging human viral infections. MERS-CoV belongs to *Beta corona virus* Lineage c, along with bat corona viruses HKU4 and HKU5 (Woo *et al*., 2007; Corman *et al*., 2014a). It is therefore that initial efforts to identify the origins of MERS-CoV focused on bats (Drexler *et al*., 2014; Omrani *et al*., 2013).

To indicate the relatedness of virus isolate from bat with the novel MERS COV, a number of effort have been made and several findings were reported from different geographical area of the world by sampling bat ,the findings were indicative of bat as an ancestral reservoir of MERS cov, to mention few of them,throat swabs, urine, faces and serum samples were collected from wild bats in Saudi Arabia.The sequenced amplification product was identical to that of the MERS-CoV sequence obtained from the first index human case (Zaki *et al*., 2012; Memish *et al*., 2013a). Another study revealed novel beta corona viruses closely related to MERS-CoV have also been identified from China and mexico (Yang *et al*., 2014; Wacharapluesadee *et al*., 2013) .Recent study in kenya revealed the detection of distinctive HCoV -NL63 -like and HCoV -229E -like sequences in bats by Ying *et al* (2017).There fore the close relatedness of MERS-CoV and various bat viruses allows speculation that MERS-CoV ancestors might exist in Old World bat (Annan *et al.,* 2013).Therefore bats can be concidere as a hypothetical origin of MERS- CoV(Memish *et al*., 2013a).

2.3.2 Dromedary Camels as Reservoirs for MERS-CoV:

Multiple lines of evidence implicate dromedary camels in the emergence and transmission of MERS-CoV. Firstly, MERS-CoV antibodies are highly prevalent in dromedary camels across the Arabian Peninsula, North Africa and Eastern Africa even before the novel human MERS CoV outbreak identified in the middle east in 2012 (Reusken *et al*., 2014a; Hemida *et al*., 2013; Alexanderson *et al*., 2014). Another evidence according to Haagmans *et al* (2014) and Azhar *et al.,* (2014b) MERS-CoV isolate from camels and its sequence analysis were identical with the isolate identified from MERS patients who have a connection with the dromedary camel farms,similarly several supportive research findings reported from Middle east and Africa and had suggested dromedary camels as aconfirmed reservoir animal of MERS CoV and source of infection for human(Azhar *et al.,* 2014b;Haagmans *et al*.,2014,Adney *et al* .,2014).

2.4 Epidemiology of MERS CoV

2.4.1 Global distribution of MERS- CoV in dromedary camels

The first evidence of MERS-CoV in camels described so far is the detection of antibodies to MERS-CoV in camel sera from Somalia and Sudan since 1983 of which 81% tested were positive (Muller *et al*., 2014).Since then , MERS-CoV specific antibodies have been detected in camels across the Middle East and from African continent in sera taken 30 years ago from the emerging of MERS in Middle East, suggesting a geo- graphically widespread and longstanding enzootic circulation in camels,the levels of seropositivity observed in adult dromedaries in Asia and Africa was generally >80% (Reusken *et al* .,2013a;Perera *et al* .,2013;Alexandersen *et al*., 2014;Alagaili *et al* .,2014;Meyer *et al* .,2014;Reusken *et al*., 2013b;Hagmaans *et al* .,2014;Reusken *et al* .,2014a;Corman *et al* .,2014b;Muller *et al*., 2014;Hemida *et al*., 2014b;Chu *et al*., 2014;Wemery *et al* .,2014;Yusof *et al* .,2015). Later studies indicated that MERS-CoV is endemic in dromedaries in many Africa countries and a number of reports showed sero prevalence of camels MERS-CoV reported to be 93% to 99.4% in Ethiopia (Reusken *et al.,* 2014a, Miguel *et al., 2017*) in Kenya 60.8% (Deem *et al*., 2015) and in Tunisia 30% in youngs to 54% in adult (Reusken et al 2014a); Egypt 92.3% (Daniel *et al*., 2014). However; zoonotic

infections have not been reported from Africa (Reusken *et al*., 2016a; Chu *et al*., 2014; Chu *et al*., 2015; Miguel *et al*., 2017). Recent study conducted in few African countries, revealed the presence of genetical and phenotypical differences between circulating virus in northern Africa and prototype strain of Middle east and also similarity between the circulating virus in East Africa and Arabic Peninsula(Chu *et al* .,2018).

According to FAO MERS-CoV update report More than 22 countries from the globe had been confirmed and published camel sero positivity for MERS-CoV (FAO, 2019). These are Bangladesh, Burkina Faso, Chile, Egypt, Ethiopia, Iran, Israel, Jordan, Kenya, Kuwait, Mali, Morocco, Nigeria, Oman, Pakistan, Qatar, Saudi Arabia (KSA), Somalia, Spain (Canary Islands), Sudan, Tunisia, United Arab Emirates (FAO,2019). However, some country like India never reported MERS CoV status in their camels (Saqib *et al*., 2017).

2.4.2 Global distribution of human MERS CoV

Since its identification in the Kingdom of Saudi Arabia (KSA) (Zaki *et al*., 2012) and Jordan in 2012 (Hijawi *et al*., 2013), Middle East Respiratory Syndrome (MERS) has become a global public health threat. Globally it has been affected 2434 people in 5 continents and 27 countries with amortality rate of 36 %(FAO MERS update, May.2019).

In Middle East, Saudi Arabia is considered the epicenter for MERS-CoV infection (ALQAHTANI *et al*., 2017).Middle East have the highest number of cases 88.4%; followed by Asia 10.7%; Europe 0.8% andUSA 0.1%(ALY *et al*.,2017)(Figure 3).There fore**,** The virus has repeatedly spilled over from dromedary camels to humans, principally in countries on the Arabian Peninsula; causing significant morbidity and mortality (World Health Organization, 2017a; Azhar *et al*., 2014a) while the rest affected regions acquired the infection through travel associated source (Nassar *et al*., 2018)(Figure 3).

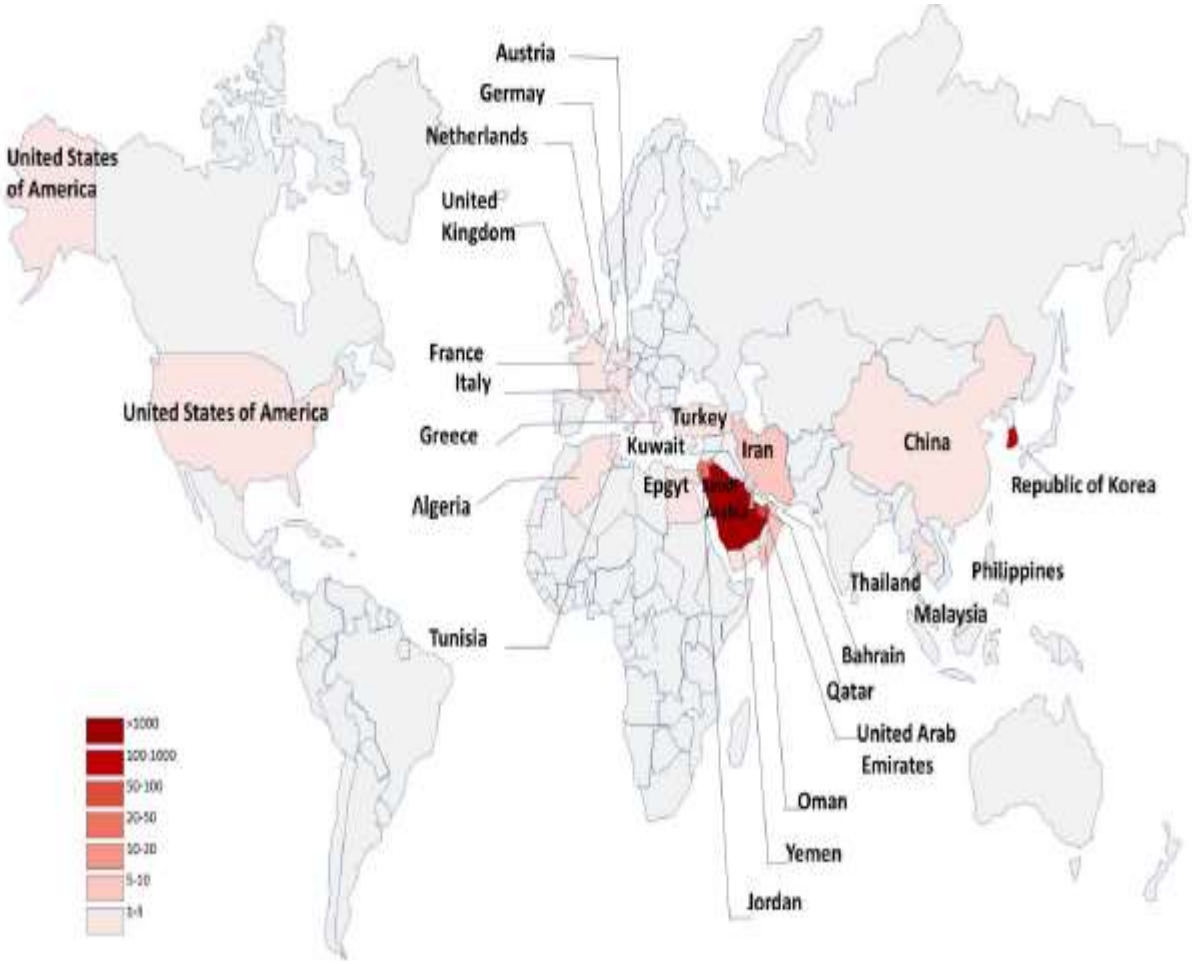

Figure 3: Transmission of human MERS- CoV from Saudi Arabia to different Nations. Source: Liang *et al*., (2018).

2.4.3 Situation of camel MERS CoV in Africa

Camels are widely spread throughout the world, mainly in arid and semi arid areas of the Middle East and in Africa continent. They generate income for their owners' through provision of milk, meat and Draught power and transport (Schwartz, 1992; Faye, 2013).

The unique adaptability of camels to the arid and marginalized areas and the significant contribution to the food security of the nomadic pastoral households are significant (Schwartz, 1992). As the impact of climate change takes hold, it is likely that camels will gain an importance due to their adaptability to arid areas. According to (FAO STAT, 2015) the African continent

harbors the vast majority of dromedary camels. The Food and Agriculture Organization (FAO) of the United Nations estimates the world camel population to be 26,989,193 of which 89% are single-humped dromedary and 11 % are Bactrian (two-humped). Africa has 85% (estimated to be 24 million) of the world's camel population. More than 60% of the world's camel population as described in (Figure 4) is found in the Horn of Africa region (FAOSTAT 2015).

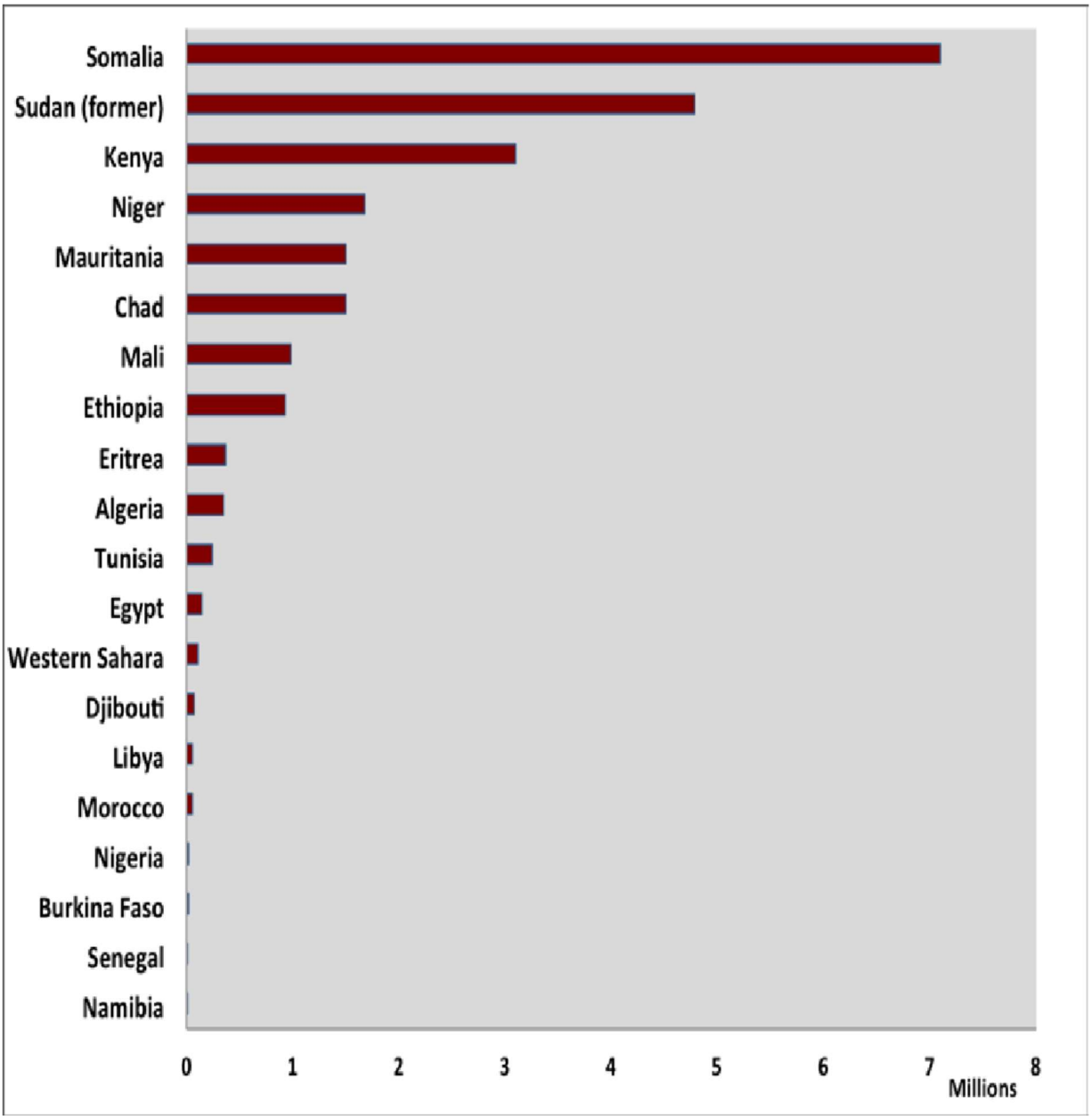

Figure 4: African camel populations Source: FAO STATS, (2015)

Now adays camels are thought to be a natural reservoir of MERS-CoV and can be a source of infection to human (Omrani *et al* .,2015).This was confirmed by different experimental evidences (Adney *et al*., 2014 ;Alagaili *et al*., 2014; Hemida *et al*., 2014a).Acording to omrani *et al*. (2015) review report MERS-CoV specific antibody were detected in seven African countries that was collected from camels several years before 2012 , and the seropositivity ranges from 29 % in Kenya up to 93.7 % in Ethiopia (Omrani et al .,2015).

Currently several serological studies in the continent suggested that MERS-CoV is endemic in dromedaries in many Africa countries but isolation and charactorisation of the virus was very limited in the continent.Based on different studies seroprevalence of camels MERS CoV to be 93-99.4% in Ethiopia (Reusken *et al.,* 2014a,Miguel *et al.,2017*) in Kenya 60.8% (Ommeh *et al*., 2018;Deem *et al*., 2015) and in Tunisia 30-54% (Reusken *et al*.,2014a); Egypt 92.3% (Daniel *et al*.,2014).While MERS-CoV RNA detection had been very limited.Egypt 3.6%(Chu *etal*.,2014),Nigeria 11%,Morocco7.6%,Burkina Faso12.2%,Ethiopia 15.7% (Chu *et al*.,2018) and in Kenya 11 specimens found positive from 1163 swab samples (Ommeh *et al*., 2018).However zoonotic infections had not been reported from Africa (Reusken *et al*., 2016a; Chu *et al*., 2014; Chu *et al*., 2015; Miguel *et al*., 2017);Morocco 48.3-100%;Burkina Faso 73-84%;Nigeria 95%(Chu *et al*., 2018).The reason for the absence of zoonotic disease in Africa is unclear, but recent study conducted by(Chu *et al*., 2018) and(Ommeh *et al*., 2018) revealed some indication for genetic variation of viral strain of African camels MERS-CoV(Nigeria,Burkina Fasso) with the prototype strain of Arabic peninsula, differently the east African camel strain(Ethiopia,and Egypt and Kenya) reported to be similar with Middle east strain (Chu *et al*., 2018,Ommeh *et al* .,2018).

2.4.4 Human MERS CoV in Africa

Some of African camels were found positive for Viral RNA and serum antibody for MERS-CoV, as camels are the source of infection for humans, attention is not well given for public health status especially for those peoples having intimate contact with camels in Africa. In few African countries there are few reports concerning public health status in relation to MERS CoV, the first one is from the east African country Egypt, According to Daniel *et al* (2014), sera samples were

collected in Egypt Cairo from humans working in camel abattoirr and who have intimate contact with MERS-CoV positive camels and tested for MERS-CoV specific antibody, the laboratory result showed all samples were negative.

The other report was from west African country Nigeria, according to So *et al*. (2018),261 camel abattoir workers tested for MERS- CoV antibody, the history of these peoples were they have had repeated occupational exposure to camels at an abattoir in Kano, Nigeria, many of whom also reported: 138 of them drinking fresh camel milk and 94 individual eat raw camel meat and 96 of them using camel urine for medicinal purposes , the laboratory results showed that none of the abattoir workers with exposure to camels had evidence of neutralizing antibody to MERS-CoV (So *et al*., 2018).

In Kenya a total of 486 human plasma samples collected from10 counties in Kenya among those humans 95 % stated that they have had close contact with camels, but all were negative with -neutralization assay for MERS-CoV specific antibody (Ommeh *et al*., 2018).Surprisingly there is no official MERS-CoV related study report from Ethiopia about health status of those peoples having intimate contact and occupational exposure with dromedaries.

2.4.5 Molecular Epidemiology of MERS CoV

Phylogenetic analysis of MERS-CoV has been used extensively to investigate the molecular epidemiology of the virus worldwide. The techniques have assisted in studies of the genetic relationships between different MERS-CoV isolates, geographical distribution of lineages and genotypes.Nowadays MERS-CoV infection is very common in dromedaries across Africa as well as in the Arabian Peninsula, but zoonotic disease appears confined to the Arabian Peninsula.MERS-CoV genomes are phylogenetically classified in to 3 clades, clade A and B and C clusters(Cotton *et al*.,2013a;Chu *et al*., 2018;Ommeh *et al* .,2018).The viral genomes detected in the earliest cases in humans (clade A cluster;EMC/2012 and Jordan-N3/2012) are genetically distinct from the others clade B strains.most of clade B MER-SCoV circulate in Saudi Arabia and Middle east (Figure 5) while Clade C MERS-CoV identified from African continent camels(Cotton *et al*., 2013b;Chu *et al*., 2018,Ommeh *et al*., 2018) .

Clade C Viruses sub divided in to: sub-Cade C1 and sub-Cade C2. Sub-Cade C1 MERS CoV includes camel virus circulate in West African countries; Burkina Faso, Nigeria, and Morroco while Sub-clade C2 circulate in camels of Eastern Africa: Ehtiopia, Egypt and Kenya stated in Figure 5 (Ommeh *et al*., 2018).

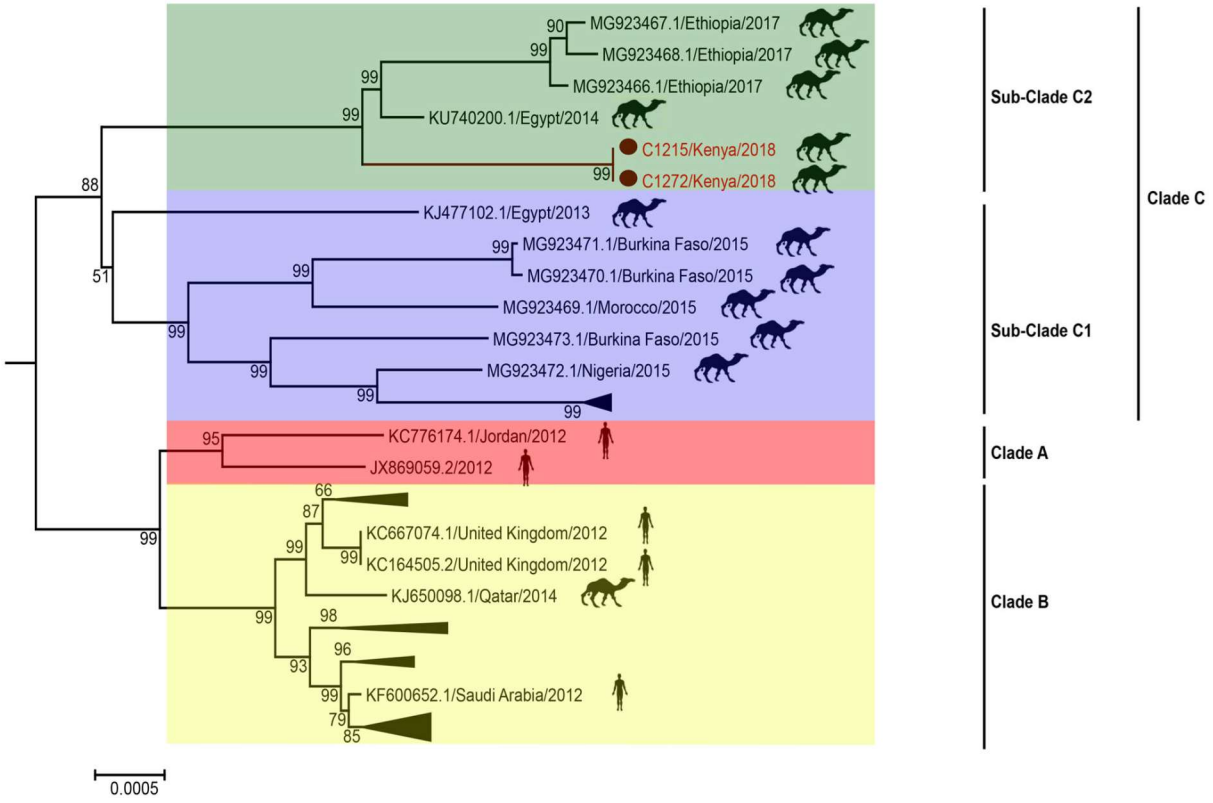

Figure 5: MERS-CoV clades in Middle East and in Africa Source: Ommeh *et al*., (2018)

In recent studies by Chu *et al.* (2018) and Ommeh *et al.* (2018),had characterized MERS-CoV from Burkina Faso, Nigeria, Morocco, Ethiopia and Kenya, they demonstrate that viral genetic and phenotypic differences between West and East African country. MERS CoV isolate of west African indicated basic dis-similarity with isolate of Arabic Peninsula, which may be relevant to differences in zoonotic potential of the viruses between the two region (Chu *et al*., 2018).According to Chu *et al.* (2018) and Ommeh *et al.* (2018) reports camel isolate charactorised from East Africa(Egypt, Ethiopia and Kenya) were phynotypically similar with those of Arabic peninsula prototype isolate. Hence these findings highlighted the need for more

extensive and sustainable surveillance study and monitoring systems,especially in East African camels and public health sector(Chu *et al*., 2018;Ommeh *et al* .,2018).

2.5 Transmission

The disease is contagious because a small dose of the virus is infectious and several routes of excretion and infection have been reported (Adney *et al*., 2014). The primary mode of transmission is via respiratory aerosols since the virus can replicate mainly in the respiratory tract of camels and large amount of the virus particles are excreted from this area, although the virus may occur in all secretions and excretions of infected camels during the acute phase of infection (Adney *et al*., 2014; Alagaili *et al*., 2014; Hemida *et al*., 2014a).

2 .5.1 Infected camels as a source of aerosol and direct contact transmission

Camel's experimentaly infected with MERS-CoV for research purpose confirmed susceptibility, with a large quantity of virus shedding from the upper respiratory tract (Adney *et al*., 2014). Therefore, droplet transmission or direct contact with infected camels can be asource of aerosol transmission. The scientific literature suggests that during the breeding season of camels, the camel farms area potential source of MERS-CoV infections.At that time the main modes of MERS-CoV transmission are by contact, droplet or airborne and its transmission happen among animals, from animals to human, and from human to human. Under certain condition, MERS-CoV can survive on non animate objects for up to 30 hours (vanDoremalen *et al*., 2013). It is therefore possible to predict that in the absence of appropriate precautions, the environment surrounding a MERS-CoV infected camel can become extensively contaminated with viable, potentially infectious viruses(Adney *et al* .,2014,Azhar *et al.,* 2014b)

The viral incubation period is from 2 days to 2 weeks.MERS-CoV directly targets the lower respiratorytract in dromedary camels and continuesto replicate favorably in the airway cells of the upper respiratory tract (Nassar *et al*., 2018). Hence it is the most likely mode of camel to camel

and camel-to-human transmission of MERS CoV (Memish *et al*., 2014; Azhar *et al*., 2014a, Azhar *et al.,* 2014b; Gossner *et al.,* 2016).

2.5.2 Infected Camels milk, meat and urine as source of infection

Dromedary camels are widely present in the Middle East and in Africa and have been part of dry and semi dry or desert cultures for centuries with cultural and economic importance (Gossner *et al*., 2014).Camel milk and meat, in particular row meat from the hump, are commonly consumed while some consider raw organ meet a delicacy (Gossner *et al*., 2014). Hence infected raw camel milk, meat and the habit of drinking camel urine as amedicine can be considered potential source of infection for MERS-CoV (Farag *et al*., 2015). Another source for milk, urine and meat-borne transmission of MERS-CoV is possibly through contamination of the meat, milk during slaughter and milking with respiratory or fecal excreta(Muller *et al* .,2014). Consumption of raw camel milk has been linked to several human MERS cases in KSA and Qatar (Memish *et al*., 2014)

2.5.3 Human to human transmission

In MERS outbreak occurred areast the existence of clusters of infected medical staff and families play great role in the potential of the virus to spread between humans. Aerosol transmission and direct contact is the main route of transmission in hospitals as well as in the environment (Memish *et al*., 2013b; Assiri *et al*., 2013). The Korean out break could be the best example for this mode of transmission. The MERS-CoV was imported by a returning traveler from the Middle East; the infection had spread with in the hospital, and subsequently to other hospitals because of patient movements, resulting in nosocomial spread at 16 hospitals, 186 cases including 38 fatalities with in two month duration (KCDC, 2015).

The exact mechanisms of infection and the minimal infectious dose are not fully studied yet however, MERS-CoV infections acquired in hospital settings lead to clinical signs and have high rate of mortality about 40 % (Cotten *et al*., 2014).The basic reproduction number (R0), which is a measure to determine the contagious nature of the pathogen has been determined to be <1 in

MERS CoV and is relatively low compared to highly infectious diseases (such as Measles, which has an R0 >15) (Cotten *et al.,* 2014). Therefore, MERS-CoV has a very low potential to cause a pandemic but since coronaviruses mutate constantly has the potential to cause a pandemic if a mutation leads to a highly transmissible and virulent virus (Cotten *et al*., 2014).

2.6 Clinical Signs

2.6.1. Clinical sign in human

Corona virus can cause respiratory and enteric infections and how ever in MERS CoV infection patients can present with varying symptoms such as high-grade fever, non-productive cough, and shortness of breath, headache, myalgia, nausea, vomiting and diarrhea and respiratory failure (Sharif *et al*., 2014). Development of Acute renal failures (ARF) is associated with underlying conditions such as diabetes, chronic cardiac disease, chronic renal disease, and chronic lung disease and in immune-compromised persons including pregnant females and infants (WHO, 2015; Mailles *et al*., 2013; Eckerle *et al*., 2013).The number of patients who died was significantly higher among camel source case-patients (2/3, 67%); than among healthcare-associated case-patients (2/27, 7%)most of the patient died due to respiratory and kidney failures (Hunter *et al*., 2016).

2.6.2 Clinical sign of MERS CoV in Dromedary Camels

MERS-CoV infections in dromedary camels are often asymptomatic or associated with short periods of nasal discharge (rhinitis).Experimentally innoculated camels showed minor clinical signs like consisting of rhinorrhea, slight increase in temperature yet no other clinical signs observed (Adney *et al*., 2014;Alagaili *et al*., 2014; Hemida *et al*., 2014a). According to Alagaili *et al* (2014),the fact that significant clinical disease is not recognized in relation to MERS-CoV natural infection in dromedary camels indicated a well-established balance between the camel host and the MERS-CoV(Alagaili *et al*., 2014).

Infected camels can shed the virus through different rout: nasal discharge,oral droplet, in milk and feces and possibly can be detected from bronchial and anal caviyy, as well as found in tissue of lymphnodes, turbinate, olfactory epithelium, larynx, pharynx,and tracheas(Farag *et al*.,2015;Khalafalla *et al*.,2015;Adney *et al* .,2014;Reusken *et al* .,2014b). Ofcourse Sampling of the nasal cavity seems to be the best option for sampling of live camels for viral detection (Farag *et al*., 2015).

2.6.3 *Seasonal variation of MERS-CoV circulation in camel and immune response*

Although in natural infection, MERS-CoV was detected almost year-round in camels, some studies showed presence of relatively higher sero prevalence and viral detection during the cooler winter months (Ali *et al*., 2017a; Khalafalla *et al*., 2015; Ali *et al*., 2017b; Kasam *et al*., 2018).The increase of MERS-CoV circulation in winter and spring can have multiple explanations: the winter is the calving season of camels,this leads to a larger proportion of new borns and young camels that usually have a higher number of MERS-CoV infections and virus excretion in the population, additionally, in winter season, there is a major increase of camel and human movements due to camel racing competitions, camel breeding, trading and movements for grazing grounds, which increases the chance of virus spread (FAO MERS-CoV situation update, 2018). Additionally, cooler temperatures might facilitate MERS-CoV survival in the environment (Vandormalen *et al*., 2013).

In early experimental infection study of camels with MERS-CoV, viral shedding started 1–2 days post-infection, and continued until 7 day post infection while viral RNA remain detectable until 35 days post infection in nasal and lower amounts in oral swab samples,but no infectious virus or viral RNA was detected in fecal or urine samples (Adney *et al*., 2014).

In another study, Viral RNA in nasal and rectal swabs of camels persist after experimental infection for 14 days (Hagmans *et al* .,2016).In field surveys,MERS-CoV RNA has been described in rectal swab samples,though the positivity rate of rectal swabs is lower compared with nasal swab samples (Hemida *et al*., 2014a;Woo *et al*.,2014;Farag *et al* .,2015;Ali *et al* .,2017b; Hagmans *et al*., 2016;Sabir *et al* .,2016;VanDormalen *et al* .,2017;Hemida *et al*., 2017).

Oral swabs are usually negative or show a lower positivity rate even when nasal swabs test positive for MERS-CoV RNA (Hagmans *et al* .,2014; Hemida *et al* .,2014a; Farag *et al* .,2015). Different studies were well documented the presence of MERS-CoV RNA in infected camel milk samples (Ali *et al* .,2017b; Reusken *et al* .,2014b).another Longitudinal studies of camel herds showed, nasal swabs can remain positive after 2 weeks(Ali *et al* .,2017b;Muhairi *et al* .,2016).

Majority of studies indicated that young camels(less than 2 year) with primary infections will most likely play a bigger role in MERS-CoV epidemiology than older animals for which less frequent shedding is observed and who demonstrated higher rates of seroconversion (Hemida *et al* .,2013; Algaili *et al*., 2014; Reusken *et al*., 2014b;Wemery *et al* .,2015; Khalafalla *et al* .,2015), hence higher rate of virus isolation was observed in youngs than subadults (2–4 years of age) implying an increased infectivity of animals less than 2 years (Wemery *et al*., 2015).

According to Farag *et al*. (2015), MERS-CoV infections have also been detected in camels with MERS-CoV antibodies, both in calves with maternal antibodies as well as older camels that had already acquired antibodies from a previous infection. the detection of MERS-CoV RNA in camels with pre-existing antibodies in a herd in KSA and an observed lack of correlation between viral RNA loads and levels of neutralizing antibodies in camels for slaughter in Qatar might indicate limited immune-protection and a potential for re-infection despite previous exposure (Hemida *et al*.,2014a;Farag *et al*.,2015).

Different studied revealed virus replication and virus load is generally lower in infected sero positive camels compared with seronegative one (Hemida *et al* .,2014a; Hagmans *et al*., 2016; VanDormalen *et al*., 2017; Hemida *et al* 2017; Meyer *et al* .,2016; Algaili *et al* .,2014).Hence little is known about the longivity of antibody titres after infection from longitudinal studies (Muhairi *et al*., 2016).Study by Meyers et al.(2016) indicated that neutralizing antibodies remained consistent during a year (Meyer *et al*., 2016).

Mostly MERS-CoV infections are mainly detected in calves and youngcamels (Meyer *et al*., 2016;Algaili *et al*., 2014;).According to Meyers *et al.* (2016) IgG positivity rate increases

gradually in dromedary camels of increasing age while the MERS-CoV RNA detection rate decreases(Meyers *et al*.,2016).

Previous finding by Kamber *et al.* (2001), maternal IgG antibodies in camels will be acquired through the intake of colostrum during the first 24 h post-parturition, after 24h, antibody levels in the dam's milk will decrease gradually.Another similar study also showed that maternal antibodies in calves peak at 7 days post parturition and decline in the following 6 months. After 5–6 months, over half of the calves did not have MERS-CoV specific maternal neutralizing antibodies in their serum any longer (Meyer *et al*., 2016).

In adult camels, a much higher MERS-CoV seroprevalence can be found, which is probably due to a long-lasting immune response against a MERS-CoV infection or multiple re-infections with MERS-CoV.According to Hemida *et al.* (2016) and other researchers, Immunity in camels is not sterilizing, as MERS- CoV infection and shedding have also been shown in adult camels that have MERS-CoV antibodies (VanDormalen *et al*., 2017;Hemida *et al* .,2017;Meyer *et al*., 2016;Algaili *et al*., 2014).

2. 6.4 Pathogenesis of MERS- CoV in camels

Dromedary camels that act as the reservoir species, the virus is suggested to induce only limited pathlogical condition and clinical symptoms. In most cases reported so far, overt clinical disease is absent in dromedary camels positive for MERS-CoV (Chu *et al*., 2014).Hence dromedary camels naturally infected with MERS-CoV may not show disease but still excrete MERS-CoV through nasal fluids, faeces and, potentially, in their milk and urine (Reusken *et al*., 2014b).

Studies have been indicated that the presence of a mild-to-moderate inflammation and necrosis on the upper respiratory tract of experimental infected camels. No viral antigen or lesions were detected in the alveoli (Adney *et al*., 2014).According to gross pathology and histopathology findings the nasal respiratory epithelium is the principal site of MERS-CoV replication in camels (Adney *et al*., 2014; Hagmans *et al*., 2016;Mohran *et al.,* 2016).

2.7 MERS-CoV Diagnosis

There are no pathognomonic clinical, biochemicals, or radiological features that reliably differentiate MERS from other causes of acute infections or hospital acquired pneumonia in humans and no an indicative pathognomonic clinical signs in camels too. Nucleic acid amplification and serology assays are the most widely used method to provide laboratory confirmation of MERS established with in a short period of time early in the epidemic (Corman *et al*., 2012 :CDC, 2014).

The appropriate choice of sample for MERS-CoV specific antibody detection is serum while for molecular studies using real time RT-PCR technology is nasal swab in camels (Mohran *et al*., 2016), but it is possible to collect Milk, urine and feces for viral detection test in camels(Omrani *et al*., 2015).

2.7.1 Enzyme Linked Immune Sorbent Assay

EUROIMMUN anti-MERS CoV camel IgG ELISA kit is the first commercially available kit for specific detection of anti MERS CoV antibody in camels for efficient determination of MERS CoV antibody in serum or plasma based on recombinant S1 protien of MERS CoV which is knowen to well suited for diagnosis as it combines high sensitivity and high specificity and its evaluation is semiquantitative using ratio value (kit protocol EUROIMMUN).

Micro titer strips of each with 8 break-off wells coated with purified S1 antigen of MERS corona virus, in the first ELISA reaction step, diluted serum samples will be incubated in the wells for 30 minutes at 37^0c. In the case of positive samples, specific IgG antibodies will bind to the coated antigens. After washing with a wash buffer, a second incubation will be carried out at the same temperature and time to detect the bound antibodies, using an enzyme-labeled anti-camel IgG (enzyme conjugate). The conjugate catalyzes a color reaction when chromogen or substrate solution is added to it. The amount of color can then be measured with spectro photometer at 450 nm and the color produced is proportional to the amount of antibody found in the sample. The assay included a calibrator which defined the upper limit of the reference range in non-infected

camels and this value was defined as the cut off. The assay was made semi-quantitative. Ratio was calculated by dividing the extinction (OD) of the control or sample by extinction of the calibrator. The results were interpreted according to the manufacturer instruction as follows: Ratio < 0.8: Negative, Ratio ≥ 0.8 to ≤ 1.1: Doubtful (borderline) andRatio ≥ 1.1: Positive, thos doubtful samples were retested for one more time.

2.7.2 Pseudo particle Neutralisation Test

For those pathogens classified to be handled in a bio safety level 3 or 4 (BSL-III or IV) at high level biosafety settings, due to this stringent biosafety requirements, options for clinical diagnosis or epidemiological studies of these diseases by a pseudo viral particle neutralization (ppNT) assay was developed that can conducted in BSL-2 containments. Methods for the pseudo viral particle production and VNT assay were essentially identical (Perera *et al*., 2013).

The principle of the test is Env-defective and luciferase-expressing HIV backbone used for pseudoparticle production is not replication-competent, the resulting pseudo viral particle is capable of achieving only a single-round infection and contains a luciferase reporter gene that can be expressed in infected cells. Unlike virus neutralization test that utilizes the whole genome(live virus) of MERS CoV and require biosafety level III (BSL III) containment,the MERS-spike ppNT assay does not require BSL-III containment and it gave also good correlation with virus neutralization tests result which is the golden standard of all serological tests(Gierer *et al*., 2013;Park *et al*., 2015;Perera *et al*., 2013).

2.7.3 Real-time reverse-transcription polymerase chain reaction (RT-rtPCR)

Reverse Transcription PCR (RT-PCR) is used to amplify DNA from RNA.The Enzyme Reverse transcriptase reversely transcribes RNA into cDNA, which is then amplified by Real Time (rRT-PCR). The technique is highly sensitive with the potential to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis (Corman *et al*., 2012). In MERS CoV diagnosis it is one of the most reliable laboratory technique used as confirmatory

test. The first probe and primer sets for MERS-CoV detection by RT-rtPCR were developed shortly following the first reports of the disease (Corman *et al*., 2012).

Real-time reverse transcription PCR (RT-PCR) targeting upstream of E protein gene of MERS-CoV is used for screening current infections with MERS-CoV from the nasal swabs of camels (WHO, 2015). The open reading frame (ORF) 1a gene was used for confirmation as recommended by the World Health Organization (Corman *et al*., 2012; WHO, 2013).

2.8 Camel MERS CoV in Ethiopia

In Ethiopia, camels represent a subset of major livestock resources with a population estimated at 1.209,321 (CSA, 2016/17). The Ethiopian most dromedaries are found in East, south eastern and north eastern arid and semi arid regions of the country, such as Somali, Afar, and Borena and others (Simenew, 2013). These animals depend on seasonal movement in search of food and water, most of the family members moves with camel herds, traditionally camel owner and most family members intimately reside, sleep, and eat with their camels.drinking raw milk, raw meat is very common culture(Admasu and Kaynata, 2017).

Since the discovery of MERS- CoV in 2012, a cumulative serological and molecular study had been taking place in different part of the country. In Ethiopia an earlier study conducted by Reusken *et al*. (2014a) showed an overall MERS- CoV sero positivity of 93% in adult and 97% in juvenile. Another study revealed 92.3- 93.9% sero prevalence and 7% viral RNA detection in the country (Fekadu *et al*., 2016). Another study by Miguel *et al.* (2017) indicated MERS-CoV RNA detection reported up to 15.7% and sero positivity at high as 99.4%, and another recent reporte showed serological finding of 56% with no RNA detection(Walelign *et al*., 2018).In spite of the overall seropositivity 99% and 15.7% RNA positivity of camels there were no zoonotic report in the country (Reusken *et al*., 2016a; Chu *et al*., 2014; Chu *et al*., 2015; Miguel *et al*., 2017).Recently in 2018, genetic and phenotypic characterization MERS-CoV from dromedaries sampled in four African countries,the study reported high seroprevalence and RNA detection in Ethiopia.The viral RNA strain showed a very close similarity with the east African Egypt camels virus strain which inturn had phenotypic similarity with the Middle east prototype

virus strain, suggested the potential hazard for the country pastoralist people and for those consumers of camel products in the country and indicated the necessity of further study in the area (Chu *et al* .,2018).

2.9 Prevention and control

At present, no effective vaccine or therapeutics are available for the prevention or treatment of MERS-CoV infection; however several MERS-CoV specific vaccines and treatments are in development.In human treatment is supportive and based on the patient's clinical condition. (Falzarano *et al*., 2013; Dyall *et al*., 2018 and Lu *et al*., 2015).

As a general precaution, anyone visiting farms, markets, barns, or other places where dromedary camels and other animals are present should practice general hygiene measures, including regular hand washing before and after touching animals, and should avoid contact with sick camels, the consumption of raw or undercooked camel products, including milk and meat, carries a high risk of infection from of variety of organisms that might cause disease in humans.

Camel meat and camel milk are nutritious products that can continue to be consumed after pasteurization, cooking, or other heat treatments.Until more is understood about MERS-CoV, people with diabetes, renal failure, chronic lung disease, and immune compromised persons are considered to be at high risk of severe disease from MERS-CoV infection. These people should avoid contact with camels, drinking raw camel milk or camel urine, or eating meat that has not been properly cooked. In Africa countries with no human case report, especially in that camel rearing area, awareness creation training should be given for health care professionals working there about the indicator of MERS in patients to watch over clinical cases in their daily base activities.

3. MATERIAL AND METHODS

3.1 Study area

Yabello district is located at the southern part of Ethiopia in Oromia Regional State at about 570 km away from Addis Ababa in southern direction (Lasage *et al.,* 2010). Geographically the district is located at latitude of about 5°23'49″ N and longitude of about 39°31'52″ E and at elevation ranging 1000-1500 meter above sea level (Figure 6). The mean annual minimum and maximum temperatures are 24 and 29°C, respectively. The climate is generally semi-arid with annual average rainfall ranging from 300 mm in the south to >700 mm in the north part of the district **(**Admasu and Kaynata, 2017)**.** The Yabello district comprises about 11 pastoral associations , in which most of pastoralist mainly rear and derives their income from livestock and some of agro-pastoralists dwelling in and around the district practice agro-pastoral activities, they used opportunistic crop farming integrated to their livestock husbandry practices (Coppock, 2010; Habtamu, 2004).

For this study, Yabello district was selected based on stake holder's consultation meetingorganized by FAO Ethiopia MERS -CoV project. The selection of the study area was based on the following criteria: presence of high camel population, presence of mixed and close contact of camels from multiple herds, logistical proximity of the study site. The total camel population of Yabello district is estimated to be 44,042 (Yabello district Agriculture office, April/2015). The study was conducted from Feb, 2018 to April, 2019 in three kebeles of Yabello district: Areri, Dikale and Harwoyu. Dikale, which is located 40 km away from Yabelo town to east direction and with 2590 camel population, another kebelle Harwoyu,which is located 45 km away from Yabello to south direction with 2058 camel population and the third kebele Areri, which is located 20 km away fromYabelo to North direction with 3870 camel population were selected for the study.

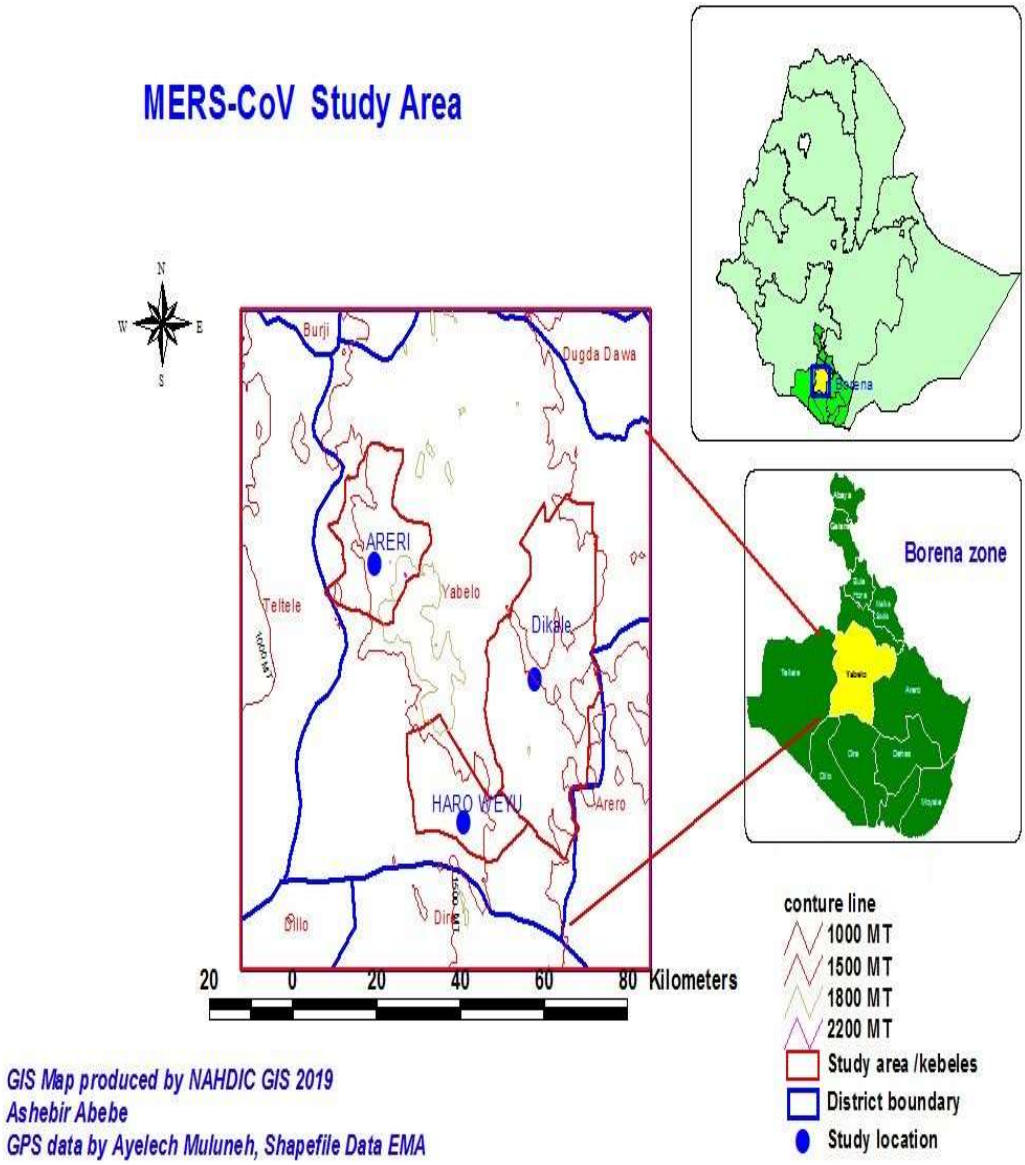

Figure 6: Map indicating study area

3.2 Study population and husbandry

The target populations were dromedary camels found in the three kebeles (Areri, Dikale and Harwoyu) of Yabello district of Borena zone. House holds having dromedary camels keeping under extensive and pastoral production system were included in the study. All age group and both sexs of camels included in the study. The number of juvenile and young camels in the population were small in number while Female camels were larger than males in every herds

,with in 10-40 females camels per herds the number of males were not more than 2-7 including young males, this is because female camels have multipurpose usage for the pastoralists: reproduction and as a source of milk for family feeding as well as for sale,while the male camels used for breeding and for export market to generate income but sometimes they are also used for transportation purpose in changing residence places.

Most of the pastoralist people of the area have no settled home, they depend on the movement of their camels in search of food and water (Yabello district Agriculture office)). The living style may be one of the risk factors for potential transmission of zoontic diseases from camel to human and the habit of drinking raw milk, eating raw meat and same times use of camels urine as medicine may increase exposure to any infectious pathogen.

3.3. Study design and sample size determination

The study design was cross-sectional and the survey was conducted between Feb. 2018 and April, 2019. The required number of camels for the study was determined based on the method described in Thrusfield formula (2005). Expected seroprevalence (Pexp) of 50% was used as there was no previous report based on RNA detection from Yabello district. An absolute precision of 5% and 95% confidence interval considered for the calculation. The minimum number of camels required was computed using the following formula:

$$n = \frac{Z^2\ Pexp\ (1-Pexp)]}{d^2} \qquad n = \frac{(1.96)^2(0.5(1-0.5)}{0.05^2} = 384$$

Where; n = required sample size

P_{exp} = Expected prevalence =0.5

d = margin of error =0.05 Z= 95% CI=1.96

Accordingly, the minimum number of camels required for the molecular study was, 384. However, due to lower probability of pathogen detection, as evidnced from previous recent reports of Waleligh *et al*.,(2018), the sample size was increased to 350 per Kebele totalling to

1050 (Table 1).Similarly the serum sample size was determined based on Waleligh *et al*., (2018) report of 56% MERS CoV seroprevalence ,accordingly the sample size calculated as:

$$n = \frac{Z^2\ Pexp\ (1\text{-}Pexp)]}{d2}$$

$$n = \frac{(1.96)^2(0.56(1\text{-}0.56)}{0.05^2}$$

$$n = 378$$

the minimum required serum samples were 378, to increase the precision it was decided to sample 525 serum sample parallel to the swab samples.Therefore camels participated in the study were 350 per Kebele totalling to 1050 (Table 1) of which serum samples were collected from only 525 camels.The number of camels per sampling round was 50 for swab and 25 for a serum sample per Kebele. As the number of Juvenile and Youngs camels were very small in number in all herds all juvenile and Youngs below three years of age were included for the study and random sampling technique was followed to sample adult camels in each Kebeles. Possiblity of repeat sampling was avoided using marking inks for sampled camels.

Table 1: Study area with respective sample size

Region	**Sample type**	**District**	**Kebeles**			**Total Sample**
Oromia		Yabello	Areri	Dikale	Harwoyu	
	Swab		350	350	350	1050
	Serum		175	175	175	525

3.4 Sample collection

Staffs involved in sampling and handling of samples were trained on biosafety and biosecurity issues relavant to MERS-CoV by appropriate experts. Accordigly, staffs wore appropriate PersonalProtective Equipments's according to standards for handling infectious material such as MERS- CoVspecimens inthe field and in the laboratory, hence used to maintain biosaftey and biosecurity (FAO EPT2 MERS- CoV study Partners manual, 2016).

Nasal swabs and whole blood samples were collected from the study camels.Sample were labeled with area code(name of district and kebele,using only the first two letters), sex (M) for male or (F) for female, sample type (N) for nasal swab and (S) for serum, number of round (1^{st},2^{nd},3^{rd}…) and consecutive number as sample identification using permanent markers. Samples were transported with ice box containing ice pack by maintaining (+4^{o}c) from the sampling site to the field center Yabello regional laboratory.

3.4.1 Swab Collection

Prior to attempt sampling, camels were restrained with traditional method by the owners; nasal swabs were taken using sterile break off plastic polyester cotton tipped swabs. The swabs inserted into the camel's nostril deep enough into (ventral nasal meatus) and rolled over the mucus membrane to scoop nasal material from which RNA of MERS-CoV can be extracted (Figure 7). The sample was then carefully placed inside pre-lebelled sample containers crayovials containing 1.5 ml viral transport medium (VTM) (Chu *et al*., 2014) and kept in an ice box with freeze ice packs during the field trip, and stored in liquid Nitrogen up on arrival the field center, Yabello regional veterinary laboratory, after returning from the field trips to NAHDIC it was transferred and stored at -80°C until testing.Swab samples were collected in dublicate to be tested at NAHDIC,Ethiopia and abroad at Hong Kong Universty(HKU), China.

Figure 7: Nasal swab sampling from camel. Source: Mohran et *al*., (2016).

3.4.2 Blood sample collection

Approximately 10 ml of blood sample was collected from jugular vein of the camels using sterile needle and plain vacutainer tube (without anticoagulants). The blood allowed to clot for 2 hours at room temperature, serum separated from the clot by allowing the collected blood slant standing for up to 1 to 2 hours at room temperature, after clot formation serum samples separated from the clot by centrifugation at 2500 rpm for 5 min and the pure serum decanted to pre -labeled cryo-vial tubes and be stored at -20 ^{0}C. Similar to the swab samples, serum samples were collected in duplicate to be tested at NAHDIC and abroad at HKU in China.

3.*4.3 Disposal of waste material*

All infectious waste materials collected during sample collection from the field were handled according to NAHDIC's SOP for cross contamination and sterilization manuals and incinerated in Yabello Regional Veterinary Laboratory accordingly (NAHDIC-SOP-GEN-015). Field equipments and work surfaces were decontaminated with 5% verikone solution on aroutine basis

always after work. When there was spills, splashes, or other contamination by potentially infectious materials decontamination with 10% bleach were implemented (WHO, Geneva, 2004).

3.4.4 Shipping specimens

Those swab and serum samples which were collected for the study to be tested abroad were sent to referral laboratory (Hong Kong University, China) for confirmation and comparision of tests and for further analysis.The samples were labeled, packed, and transported according to the current edition of International Air Transport Association (IATA) Dangerous Goods Regulations to HKU through Material transfer agreement protocol, following shipping regulations for UN 3373 Biological Substance, Category B infection potential MERS-CoV specimens (CDC, 2017).All specimens were pre-packed to prevent breakage and spillage. Specimen containers were sealed and placed in Ziplocs bags by placing enough absorbent material to absorb the entire contents of the Secondary Container (containing Primary Container) according to (CDC, 2017).

3.4.5 Risk factor data collection

Risk factors like herd size, age, sex, season, origin, and adult female reproduction parameters (lactating, pregnancy, dry) were recorded to assess the association of these factors with the prevalence of MERS-CoV .Sample collection included three seasons: from Winter (February), From Automn (March, April and August) and from summer (June, July and August). Herd size categorization were done according to Yasser *et al.,* (2009) in which camels were categorized into three herd size: small (1-10 camels) medium (11-30camels), large (>31 camels) Yasser *et al* (2009).Additionally according to (Izeldin, 2016), age group of camels divided into three groups juvenile (0-6month), Young (6month -3years) and Adult (above 3 years) (Izeldin, 2016).

3.5 Laboratory investigation

3.5.1 Indirect ELISA

For serology testing of MERS-CoV antibody, indirect ELISA was used to determine sero prevalence of MERS-CoV antibodies in camels according to previously described methodology (CDC, 2014). The indirect S1-ELISA was done at NAHDIC viral-serology laboratory according to the manufacturer instruction.The EUROIMMUN AG made in Germany (Test instructions,2019), which could provides a semi quantitative in vitro assay for antibodies of class IgG against MERS-CoV in serum or plasma of camels were used.
All reagents brought to room temperature +18ºc to 25 ºc approximately 30 min before used. Sample dilution: Samples were diluted 1:100 in sample buffer. Calibrator and controls were prediluted ready for use.

Procedure**:** The test plate contain microtiter strips each with 8 break-off reagent wells coated with purified S1 antigen of MERS coronavirus (MERS CoV S1).In the first reaction steps diluted sampls are incubated in the wells, in case of positive samples specific IgGantibodies (IgA and IgM) will bing to the antigen.To detect the bound antibodies asecond incubation was carried out using an enzyme labelled anti-camel IgG (enzyme conjugate) catalyzing acolor reaction.

Samples were dispensed into dilution plate according to plate layout of the test plate in order to minimize differences of incubation time between samples.To make dilution of the sample into 1:101,first 1:10 dilution was made by dispensing 90µl dilution buffer to the dilution plate then dispensing 10µl of the test sample according to the test plate lay out, from this 1:10 dilution, 10 µl of the mixture transferred to the S1-protien coated plate having pre dispensed 90 µl dilution buffer, the final volume became 100 µl and the ratio of sample to dilution buffer was 1:100, then 100µl of calibrater,positive and negative control were dispensed to the respective wells according to the plate layout of the test plate,sealed with plate sealer and incubated at +37ºc (+/-1ºc) for 30 minute. Wash solution**:**From the (10x) concentrated wash fluid ,the required amount of wash solution(1x) was prepared by diluting in distilled water according to the protocol provided with kit, after 30min incubation the plates were washed 3 times by dispensing 300µl wash solution to

each wells,using multichannel pipette, then tapped in order to remove the remaining fluid from the wells ,100 µl conjugate dispensed to each wells, covered with plate sealer, again incubated at +37^{o}c (+/-1^{o}c) for 30 minute, then washed by dispensing 300 µl wash solution in to each wells three times, after tapping 100 µl substrate solution dispensed to each wells and incubated for 15 min at room temperature(18^{0}c to 25^{0}c),finally the reaction stopped by dispensing 100µl Stop solution to each wells. Photometric measurement of the color intensity made at wavelength 450 nm with BioTech ELISA plate reader that was connected to acomputer loaded with (Gen 5.2) software for automated reading of optical density.Result evaluated semiquantativelly by calculating aratio of the extinction value of of the control or sample over the extinction value of the calibrator.The results were interpreted according to the manufacturer instruction as follows: ratio < 0.8: Negative, ratio ≥ 0.8 to ≤ 1.1: Doubtful and ratio ≥ 1.1: Positive, those doubtful samples were retested for one more time and the result were recorded.

3.5.2 *Pseudo viral particle Neutralization Test (ppNT),*

The MERS Spike ppNT is a sensitive and specific assay used for detecting natural antibodies against MERS-CoV. Pseudo viral particle is capable of achieving only a single-round infection and contains a luciferase reporter gene that can be expressed in infected cells. Anti-GP neutralizing antibodies can inhibit the entry of this pseudo viral particle into cells, thereby inhibiting the expression of luciferase in the assay (Garcia *et al*., 2009; Perera *et al*., 2013*)*.

A total of 75 sera samples that were tested at NAHDIC by ELISA technique were sent to Hong Kong University,China and tested for MERS CoV specific antibody with ppNT according to previously described methodology of Perera *et al* (2013*)* to compare ppNT results with ELISA,

3.5.3 *Molecular characterization*

Real Star MERS-CoV RT-PCR Kit 1.0, made in Germany was used to amplify and detect MERS-CoV RNA in the camel nasal swab samples. Extracted RNA is the starting material for the PCR kit therefore QIAamp Viral RNA mini kit (QIAGEN, Japan) was used, according to the

manual for the extraction of viral RNA from camel nasal swab samples (QIAamp Viral RNA Mini Handbook).

Viral RNA extraction**:** The WHO testing algorithm for MERS-CoV was implemented (WHO, 2014). Viral RNA was extracted from 1050 nasal swab collected in the study area using QIAmp viral RNA mini kit (Qiagen, Dusseldorf, Germany).All extraction procedure was carried out in Biosafty Level II biosaftey cabinate.

Briefly,560 µl lysis buffer containing carrier RNA and 140 µl pre centrifuged supernatant of swab samples were pipeted into 1.5 ml micro centrifuge tube, mixed together by vortexing, then incubated at room temperature for 10 minute, 560 µl of 95% ethanol(absolute alcohol) (for binding RNA particles to the spin columns) pipetted into each tube and mixed by pulse vortexing for 15 sec. then 630µl of the solution transferred to spin column tube with 2ml collection tube to be centrifuged, so that RNA can attach to the membrane of the spin column wall, this step were repeated up to the solution finished, only by changing and using new collection tube . Then AW_1 (wash $buffer_1$)500 µl was used to remove any other particles that might have become attached to the membrane besides the desired RNA and centrifuged at 8000rpm for 1 min. In a similar manner by changing the 2ml collection tube, the second wash buffer, AW2 500µl, was then used for one more washes and centrifuged at 14000rpm, then QIAamp Mini column placed in a new 2 ml collection tube, Centrifuged at full speed for 1 min to eliminate any chance of possible Buffer AW2 carryover. Finally, the RNA eluted by adding 60µl buffer AVE(RNase free water) followed by incubation at room temperature (20^{o}C) for 1 minute,then centrifuged for 1 minute at 8000 rpm. The tubes containing the extracted RNA were labeled with lab codes, date and name of the virus and transferred to PCR work station for immediate use or for safe storage.

MERS-CoV rRT-PCR assay primer/probe sequences:For detection of viral RNA, a one-step real-time reverse transcription PCR (rRT-PCR) targeting the *Up-E* gene was used for screening the samples for MERS-CoV RNA, according to the manufacturer's instructions (Manual for RealStar MERS-CoV RT-PCR kit, 2019).

The following primers were used: UpE forward: GCAACGCGCGATTCAGTT,
UpE Rev. GCCTCTACACGGGACCCATA;
UpEprobe (6-carboxyfluorescein (FAM): CTCTTCACATAATCGCCCCGAGCTCG-6carboxy-NNNN-tetramethylrhodamine (TAMRA) (Corman *et al*., 2012**).**

Real time RT-PCR procedure*:* All 1050 extracted RNA was then tested for the presence of MERS CoV genetic material using the MERS-CoV real time RT-PCR assay as screening test both in NAHDIC and HKU according to Corman *et al* (2012).The rRT-PCR assay was performed using the Invitrogen Super Script III Platinum One-Step quantitative RT-PCR system (Life Technologies). Each 25 µl reaction mixture contains: 12.5 µl of 2 X master mix, 1 µl of Super Script III reverse transcriptase/Platinum*Taq* DNA polymerase, 0.4µl of 50 mM magnesium sulphate solution, 0.5 µl of probe, 1 µl each of the forward and reverse primers, 3.6 µl of nuclease-free water, and 5 µl of RNA extract.Amplification were carried out in 96-well plates on an Applied Biosystems 7500 Fast Dx real-time PCR instrument (Life Technologies).

Therefor 20 µl of the Master Mix dispensed to 96-well reaction plate followed by 5 µl of extracted sample. On the other hand, the Up-E gene standards (positive and negative controls) were prepared by mixing standard templates with RNase free water. Then 25 µl of standard reagents will be dispensed into individual wells according to the plate lay out and sealed with appropriate plate sealer.The real-time PCR machine (Applied Biosystem,USA) was then programmed with the appropriate time and cycles according to the MERS-CoV PCR protocol provided with the kit. Thermo cycling conditions consists of: 20 min at 55°C for reverse transcription, 3 min at 95°C for activation of the Platinum *Taq* DNA polymerase, and 45 cycles of 15 s at 95°C and 30s at 58°C. Finally, the test results were read from the computer with those above the border line considered as positive and those below as negative. Positive viral template control (VTC) and no-template control (NTC) samples were included in all runs to monitor assay performance. A positive test result was defined as a well-defined exponential fluorescence curve that crossed the threshold within 45 cycles.

3.6. Data Analysis

The data obtained from this study were entered and stored in to Microsoft Excel spread sheet.Laboratory results and field information collected during sampling were analysed using STATA version 11 statistical software (College-Statation,Texas-USA).Descriptive statistical analysis was applied to determine association between seroprevalence of MERS CoV and risk factors like age,sex,origine herdsize,season.Multivariable logistic regression was used to analyse the effect of multiplt risk factors on the seroprevalence of MERS CoV.Odds ratio were used to quantify the effect of riskfactors and the significance association among risk factors and the seroprevalence MERS CoV antibody. $P < 0.05$ and 95% CI, (if not include 1 between the intervals), the variation was considered significant in this study.

4. RESULTS

4.1 Description of study animals

From the 525 serology study camels 85% were Females (n=448/525) and 15 %(n=77/525) were male camels.It was not possible to sample an equal proportion of males and females camels during sampling period, mainly because of camel husbandry practices of the pastoralists in Yabello.

The age groups of study camels categorized in to three groups, juveniles which accounts 3% (n=16/524), young camels accounts 30 %(n=159/525) and adult accounts 66.6%(n=350/525). Adult female camels also subdivided according to their reproduction status during sampling: lactating, gravid and dry female

Gravid female camels included in the study were 198 in number while lactating females were 136 and the numbers of dry females participated in the study were only 6 in number.

The samples were collected from dromedary camels which originate from three kebeles of Yabello district. Equal number of camels participated in the study, 33% from Areri, 33% from Dikale and 33% from Harwoyu.

Herd size also categorized in to three groups: Small (containing 1-10 camels), Medium (having 11-30 camels), and large (containing camel population more than 31).All study camels were used for milk and meat and for reproduction but occasionally they used for transport purpose.The sampling period covered three seasones (February from Winter), (March, April and May from Automn) and (June, July andAugust from Summer)

4.2 Seroprevalence of MERS-Cov

Based on indirect ELSIA test result, the overall prevalence of MERS-CoV antibody in camels of the selected kebeles of Yabello district was 74% (390/525) (95% CI 70-78). Association of riskfactors with seroprevalence to MERS CoV showed statistically significant differences among: sex, age, production status of female, herdsize, and season of sampling. Antibodies to MERSCoV were detected from all kebele camels.

The seroprevalence of MERS-CoV antibody was observed to be significantly higher in female camels 77.9 %(n=349/448) than 53 %(n=41/77) in males.The prevalence variation among sexes were stastically significant $\chi 2=20.9$ and p=0.001).

MERS-CoV specific antibody highly prevalent in adult camels 87 %(306/350) compared to youngs 48 %(76/159) and juvenile 50 %(8/16) the difference was statistically significant ($\chi 2=94.98$ and p=0.001).

High prevalence of MERS-CoVantibody recorded in small size herds 80 %(n=92/114) than medium 78.8 %(n=138/175) and large herd size 67.8% (60/236) and the difference was statistically significant ($\chi 2=9.57$and p=0.008).

Seroprevalences in relation to sampling seasons, relatively high seroprevalence recorded in (March, April, May: 83%) than February (69%) and June, July, August (65%) the variation was statistically significant ($\chi 2=16.2$ and P=0.01).

Concerning Sero prevalence in relation to origin of camels, the prevalence of MERS CoV specific antibody in camels originated from Dikale had 76%(134/175) seroprevalence which was slightly higher from Harwoyu 74%(130/175) and Areri 72%(126/175) but the variation was not statistically significant. ($\chi 2=7.47$ and P=0.062)

The seroprevalence of adult females according to their reproduction status were analysed and those gravid females had 91%(180/198) seropostive than lactating 83%(113/136) and dry 66%(4/6),the variation was statistically highly significant($\chi 2 = 7.74$ and $P=0.001$) (Table 2).

Table 2: Association of Risk factors with seropositivity to MERS CoV in camels in Yabello district.

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Variables	Category	No camel tested	No of Positive	Prevalence% (95% CI)	χ2	P value
Sex	Female	448	349	77.9	20.9	0.001
	Male	77	41	53		
Age	Juvenile	16	8	50		
	Young	159	76	48		
	Adult	350	306	87	94.98	0.001
Origin	Areri	175	126	72		
	Dikale	175	134	76.5	0.95	0.062
	Harwoyu	175	130	74		
Hsize	Large	236	160	67.8		
	Medium	175	138	78.8		
	Small	114	92	80	9.57	0.008
Season	Winter	75	52	69		
	Automn	225	187	83	16.2	0.01
	Summer	225	151	67		
Reproductive status of adult female	Lactating	136	113	83		
	Gravid	198	180	91	7.74	0.001
	Dry	6	4	66		

Afemale=Adult female Hsize=Herd Size

According to the multivariable regression analysis as indicated in (Table 3) the possible risk factors: age, herd size and camels origin and female production status indicated a statistically significant association with MERS-CoV seroprevalence in camels of Yabello.Regarding age category, those adult camels were 7.5 times more likely to be seropositive than youngs OR= 7.5; 95% CI= 4.5 - 12.4).In the analysis of seropositivity level based on herd size, those small size herds were 3.4 times more likely to be infected and show detectible antibody than larg size herds (OR=3.44;95% CI=1.76 - 6.75) and medium size herds also 2.01 times more likely to be seropositive than large size herds OR=2.01;95% CI=1.19 - 3.38).Analysis based on Origin of camels those camels found in Dikale kebele were 2.3 times more likely to be positive for MERS-CoV antibody(OR=1.27 - 4.35). Observation on those reproduction status of female camels, gravid camels were 1.5 times more likely to be sero positive for MERS-CoV antibody than lactating and dry femal camels (OR= 1.5; 95% CI= 1.045 - 2.917) (Table 3). Where as there was no statistical significance difference in risk of infection among Sex, P=0.55 and among season of sampling P=0.065, suggesting possible confounding.

Table 3: Multivariable logistic regression analyses of seroprevalence of MERS-CoV antibodies with varies factors.

Variable	Catagory	No of camel tested	No of Positive	Prevalence in % (95% CI)	Crude Odds Ratio (95% CI)	Adjusted odds ratio (95%CI)
Sex	Male	448	349	77.9	1	1
	Female	77	41	53	3.09(1.87-5.1)	1.2(0.64-2.23)
Age	Juvenile	16	8	50	1	1
	Young	159	76	48	0.96(0.2-2.40)	0.90(0.14-1.46)
	Adult	350	306	87	7.5(4.88-11.62)	7.5(4.5-12.4)*
Origin	Areri	175	126	72	1	1
	Dikale	175	134	76.5	1.27(.78-2.05)	2.3(1.27-4.35)*
	Harwoyu	175	130	74	1.12(.70-1.8)	1.65(0.93-2.92)
Hsize	Large	236	160	67.8	1	1
	Medium	175	138	78.8	1.77(1.12-2.78)	2.01(1.196-3.38)*
	Small	114	92	80	1.98(1.15-3.4)	3.44(1.76-6.75)*
Season e	Winter	75	52	69	1	1
	Automn	225	187	83	2.17-(1.19-3.97)	1.95(0.95-3.97)
	Summer	225	151	67	0.90(.51-1.58)	.78(0.395-1.54)
Rep. Afemale	Lactating	142	117	82.4	1	1
	Gravid	198	180	91	2.02(1.04-3.91)	1.5(1.045-2.917)*
	Dry	6	4	66.6	0.4(0.070-2.35	0.2(0.053-1.521)

Rep.Afemale=Reproductive status of adult female, Hsize=herd size, * statistically significant.

4.1.2 Pseudoparticle Neutralization Test (PPNT) Result

A total of 75 serum samples were tested using ppNT at HKU (China) and the result showed that 80 %(60/75; 95% CI 0.70-0.89) of the samples were positive for MERS-CoV antibody.These samples were tested at NAHDIC with indirect S1-ELISA kit .

4.2. Molecular study result

4.2.1 Result of rRT- PCR

Real time PCR test performed at NAHDIC and Hong Kong showed that no MERS-CoV up E genome detected in any of the tested samples (Figure 8).

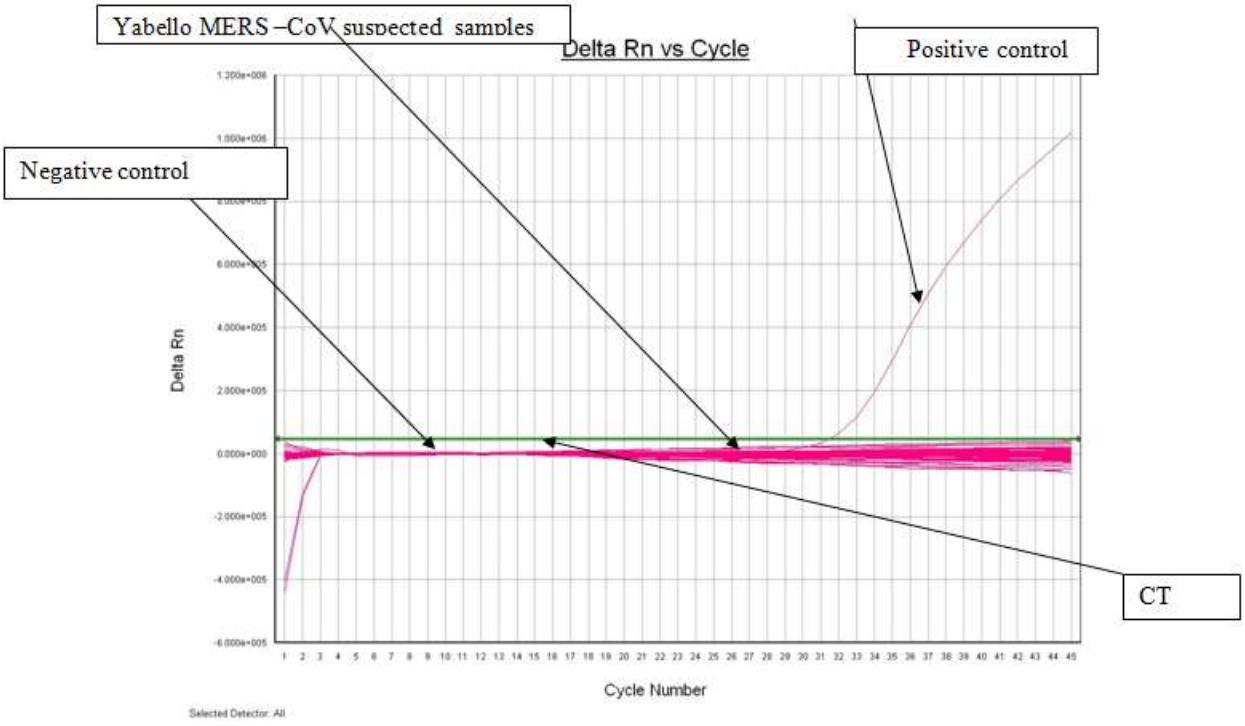

Figure 8: Yabello camels' MERS CoV negative samples PCR amplification graph.

5. DISCUSSION

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection is an emerging zoonotic disease with potential public health significance in camel rearing areas of the world. Therefore active surveillance is key to understand the epidemiology and epizootology of emerging zoonotic viruses like (MERS-CoV).Understanding its epidemiological status and its potential risk factors for infection and transmission in camels has an important role for designing prevention and control options.Hence, crossectional study has been conducted in Borena zone, Yabello district camels aimed at determining the incidence and current status of sero prevalence of MERS-CoV.

The overall seroprevalence of MERS-CoV antibody in this study was 74% (n=390/525, 95% CI 70-78).This seroprevalence to MERS-CoV antibody (IgG) is an indication of prior infection in the camels at some stages of their life (Reusken*et al*., 2014a).Seroprevalence of camels in the current study was inagreement with previous studies conducted in yabello district by Reusken *et al.* (2014a) which was 93-97% ;Fekadu *et al*. (2016),86% ;Miguel *et al* (2017), 85.1% ;Walelign *et al* (2018),56% in the country.Comparing the current study with other places in the country,Yabello district camels MERS CoV sero prevalence was lower than Assayita-Dubti (85.8%) and Melkawerer(99%),Metehara (99.4%),and Akaki abbatoire (99.3%)in Ethiopia (Miguel *et al*., 2017).However; it was relatively higher from some of African countries MERS CoV seroprevalence report : in Tunisia(30- 54%) Reusken *et al*., (2014a) and in Kenya (49.9%) Deem *et al*., (2015); (68%) Ommeh *et al*., (2018).

In this study, seroprevalence was significantly higher in adult camels 87%(n=306/350, 95%CI 4.5-12.4) which is probably due to a long-lasting immune response against previous MERS-CoV infection or multiple re-infections with MERS-CoV, given that older animals knowen for less frequent shedding of virus and demonstrated higher rates of seroconversion(Hemida *et al* .,2013; Algaili *et al*., 2014; Reusken *et al*., 2014b; Hemida *et al* .,2016; Wemery *et al* .,2015; Khalafalla *et al* .,2015).Hence according to Hemida *et al.* (2013) and other researchers, immunity in camels is not sterilizing, as MERS-CoV infection and shedding have also been shown in adult camels that have MERS-CoV antibodies, this might indicate that sterile immunity is only reached at high

levels of antibody titers (Meyer *et al*., 2016). The high seroprevalence of MERS-CoV antibody in adult camels in Yabello was in agreement with different previous research findings in Ethiopia and other camel keeping Africa countries (Fekadu *et al*., 2016; Wemery *et al*., 2015, Ommeh *et al*., 2018,).

The serological finding in the juvenile camels show detectable antibody in 50%(n=8/16) of them, This might be explained by the presence of maternal antibodies via the intake of colostrum during the first 24 hrs post parturition and could play a role in the detection of MERS-CoV-specific antibodies in camels less than 6 months of age and might not reflect actively acquired antibodies (Kamber *et al*., 2001, Meyer *et al*., 2016).

According to Farag *et al*. (2015), MERS-CoV infections have also been detected in camels with MERS-CoV antibodies, both in calves with maternal antibodies as well as older camels that had already acquired antibodies from a previous infection,therefore the detection of MERS-CoV RNA in camels with pre-existing antibodies and an observed lack of correlation between viral RNA loads and levels of neutralizing antibodies might indicate limited immune-protection and a potential for re-infection despite previous exposure or in the presence of maternal antibody (Hemida *et al*.,2014a;Farag *et al*.,2015).Hence antibody detected in juveniles of the study site might be indicative of antibody developed against active infection of MERS CoV.

Another finding during this study was the detection ofMERS- CoV antibodies in 48%(n=76/159) young camels which might indicates the ongoing circulation of MERS-CoV in Yabello area camels with in these three years as the age of the young camels included in the study were below three years.This findings were also inagreement with similar findings in Kenya,Dubai,Ethiopia and in Pakistan (Corman *et al* .,2014b;Wernery *et al* .,2015; Fekadu *et al* .,2016;Saqib *et al* .,2017).

The seroprevalence of MERS- CoV in Yabello camels among different age groups indicated adult camels were more frequently infected and seroconversion was high compared to young camels in the herds,the variation was statistically highly significant(350/525,95% CI 4.5- 12.4).

In the current study area, slightly higher prevalence 76.5 %(n=134/175) was recorded in Dikale area compared to Harwoyu 74 %(n=130/175) and Areri 72%) (n=126/175) and the difference was statistically Significant and camels from Dikale were 2.3 times more likely to be seropositive than camels from Areri area (95% CI 1.27-4.35) Table 3. This finding might be dueto the large camel market located on the neghibouring district,Arero,where camels bought from Dire and Negelle district pass through Dikale kebele to Yabello city which might be a potential source of MERS-CoV infections for those locally resided camels in Dikale kebele .Variations in seroprevalence of MERS-CoV based on origin are an indication of the existence of different predisposing factors such as high camel population, free movment of camels from place to place and poor hygiene at different sites which results in the difference in the exposure of the resideing camels to circulating virus during close contacts with infercted camels (WHO, 2014).

Similarly, higher prevalence was recorded in small herds 80 %(92/114) than medium 78.8 %(=138/175) and large 67.8 %(n=160/236), the variation observed was statistically significant. This finding was not inagreement with similar studies conducted by Fekadu *et al.* (2016)andMiguel *et al.* (2017) in the country who reported statistically significant higher seropositivity in relation to large camel herd size,but thecurrent finding was in agreement with Kenya MERS-CoV study report that showed significantly high prevalence of MERS-CoV antibody recorded in small size herds by Deem *et al*.,(2015),this might be explained by the fact that small number of camels in the herd might have close contact and high interaction for transmission and sustainability of the virus in the herd.

Seroprevalence of MERS-CoV between adult females according to their reproductive status at the time of sampling were investigated and the finding showedthat ahigh prevalence of MERS-CoV antibody was recorded in gravid (pregnant) females (91%)compared to those lactating females(83%) and dry females camels (67%) and the difference was statistically significant p=0.03, this might be explained by the exsistence of physiological stress of pregnancy which might increase susceptibility of female camels to infection,indeed physiological stresses are known to decrease resistance to infection (Susan, 1998).

In comparision of the two serological assays, the ppNT result indicated 80 % of the samples were positive for MERS-CoVantibody while the indirect ELISA resultshowed 69 %(52/75) of the samples were positive for MERS-CoVantibody.The two results indicated 79 %(n=59/75) of them have had result agreement with each other, but inconsistency was seen on 21 %(n=16/ 75) of the samples,of these 13 sera samples which were negative in ELISA became positive by the ppNT, it indicated that thes S1 ELISA assay was a binding assay detecting only IgG alone, rather than a functional neutralizing assay,but the ppNT is capable of neutralizing all functional MERS-CoV specific antibodies,this finding was inagreement with Park *et al*. (2015),in his comparison of serological assays for MERS-CoV who reported MERS-CoV S1 ELISAhad acceptable result but lower correlation with neutralization tests (micro neutralization and ppNT)because of the S1-ELISA assay was a binding assay detecting IgG alone.

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Interestingly, inspite of the high seropositivity in Camels of Yabello,all 1050 nasal swab samples collected for detection of MERS-CoV RNA using qRT-PCR targeting the *Up-E*gene as ascreening test at NAHDIC and inHong Kong University laboratories were negative.The result of the present study was inagreement with those previous studies conducted in Yabello camels by different researchers in different period who reported a 0% viral RNA detection rate even though the seroprevalence of MERS CoV in the camels was ranging from 56% to 86% (Fekadu *et al*., 2016, .Miguel *et al*., 2017; Walelign *et al*., 2018). On the other hand, the finding of the current study was not inagreement with those studies conducted in different region of Ethiopia where 7% RNA detection in Dubti and Fentale area camels and another finding 14.9% RNA detection in Melkaworer area camels(Fekadu *et al*., 2016; Miguel *et al*.,2017). This variation in viral detection at different geographical location might be related to the difference in presence or absence of circulating virus in camels of specific area. The continuous report of lack of detection of viral RNA since the first report in 2014 in Yabello (Reusken *et al*. 2014a) inspite of high seropositivity might indicate previous circulation of the virus in the area, but then the infective camles might have been removed by chance from the area as there has been a higher rate of export (sell) of live camel from Yabello area in recent years.

Moreover, there might be potential factor which also contribute for lack of virus detection in addtion to the above assumption were the anatomical location of the camel posterior turbinate epithelium (predilection site of MERS- CoV) located deep from the nasal opening may inhibit the swab getting in contact with the virus during sampling by using medium length swabbing material (10-15cm).The other possible factor might be , in the camel nostril the gap between adjacent surfaces of the air passage is only 1-2mm in diameter, which is too narrow to allow the cotton tip swab to pass through the projection of the turbinate spongy bone to reach the turbinate epithelium to scoop the epithelium fluid found there.In addition the short viral shedding window 2-5 days per infection with respect to the sample collection days which might not concide by chance with the active viral shedding days of the virus.

6. CONCLUSION AND RECOMMENDATIONS

The occurrence of a significant number of outbreaks of newly emerging zoonotic viruses such as MERS-CoV over the past few years is a matter of concern in global public health. The risk level of the disease in Ethiopia is found to be very high because Ethiopia has high population of camels which is estimated to be more than 1.2 million and these camels are distributed especially in those arid and semi-arid areas of some of the regions and these camels live intimately with the pastoralist and used as asource of meat, milk and transportation for the pastoralist and adjacent communities.

The current study revealed that a high seroprevalence of MERS CoV antibody in Yabello district camels (74%) and the MER-SCoV antibodies were detected in camels from all study sites indicating a wide distribution of the virus. The risk factors such as adult age of camels, small size herds, origin and pregnancy in camels were highly associated with the seroprevalence of MERS CoV in the camel population of Yabello district. .

On the other hand lack of detection of viral RNA using RT-PCR from all 1050 nasal swab samples might indicate the absence of current circulation of the virus in the population at the time of sampling.

Therefore based on the above conclusion, the following points are stated as recommendations:

- Further MERS-CoV studies in camels of Yabello should be continued by considering the calving season of the camels and the small viral shedingwindow (2-5days) of the virus through longutidinal study design all along the whole months of the year.
- Since, MERS-CoV is newly emerging virus, experminetal based studies focusing on its vial replication sites, pathogenesis, incubation time, route of virus sheeding, transmission should be studied inorder to effectively detect and diagnose the diseases at filed level.
- Based on the anatomical compartment of camel nasal cavity,the location of respiratory epithelium(replication site of MERS-CoV in camel) is located far from the external opening of the nose.This challenges the accessibility of the turbinate epithelium with the

common cotton tip swab in live camels, therefore, the study recommends a manufacturing of camel specific swab with long handle and brush type(Flocked)swab material that can pass through the narrow air passage without difficulty to reach the virus site in order to collect the viral particles from turbinate epithelium.

- Continuous epidemiological, virological and molecular surveillance of MERS-CoV infection in camels and human at camel rearing areas with one health approach should be initiated.
- Serological screening and confirmatory methodologies should be adapted and developed in Ethiopia especially in those referral public health centers and veterinary reference laboratories like pseudoparticle Neutralization Test which can be conducted in biosafety level II laboratory facility with relatively high sensitivity and specificity than indirect S1-ELISA.
- To overcome sampling difficulty of accessibility of the predilection site of the virus in live camels, it is advised to conduct parallel study on export abattoir camels sampling after slaughter.

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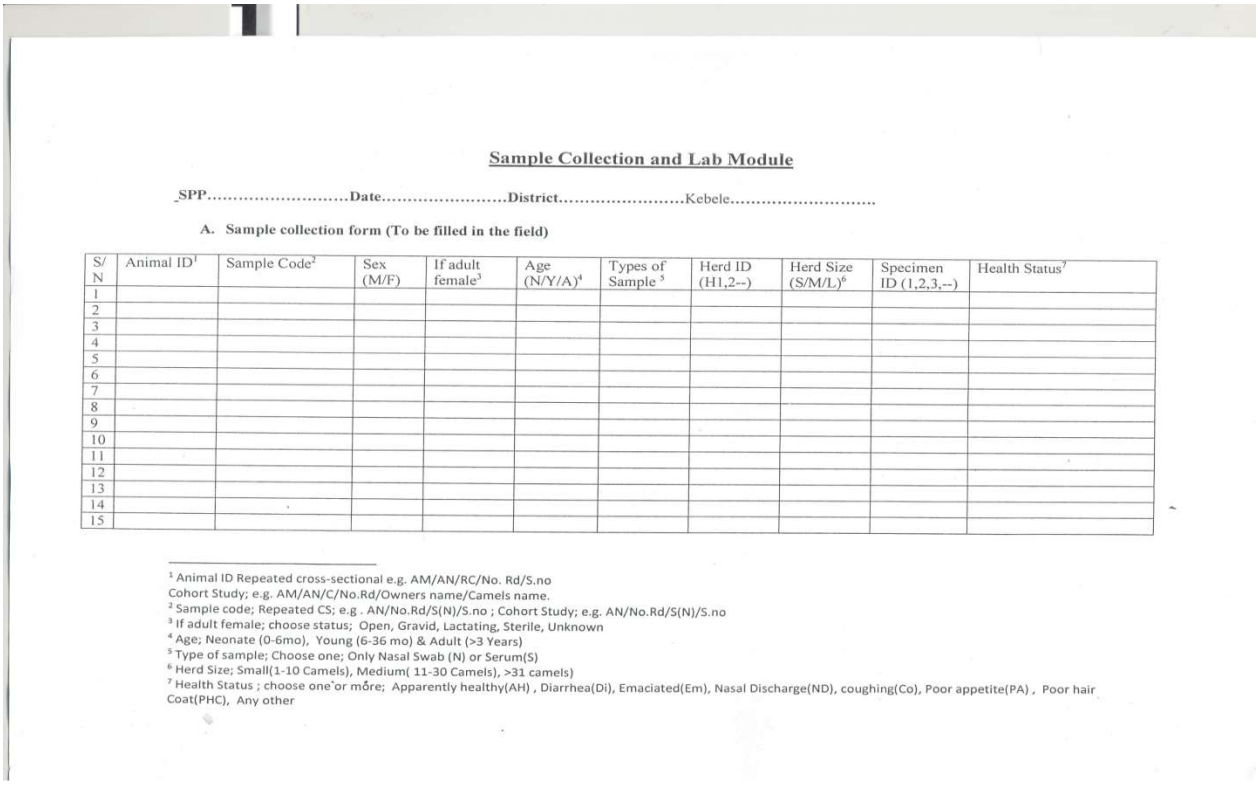
8. LIST OF APPENDIXES

Appendix 1: Study animals age determination table

Species	Age catagory
Dromedary Camels	**Juvenile= 0-6 months**
	Young= 6month up to three years
	Adult =above three years

(Izeldin, 2016**)**

Appendix 2: MERS-CoV Study sample collection data entry format



Sample Collection and Lab Module

SPP.....Date.....District.....Kebele.....

A. Sample collection form (To be filled in the field)

S/N	Animal ID[1]	Sample Code[2]	Sex (M/F)	If adult female[3]	Age (N/Y/A)[4]	Types of Sample [5]	Herd ID (H1,2--)	Herd Size (S/M/L)[6]	Specimen ID (1,2,3,--)	Health Status[7]
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										

[1] Animal ID Repeated cross-sectional e.g. AM/AN/RC/No. Rd/S.no
Cohort Study; e.g. AM/AN/C/No.Rd/Owners name/Camels name.
[2] Sample code; Repeated CS; e.g . AN/No.Rd/S(N)/S.no ; Cohort Study; e.g. AN/No.Rd/S(N)/S.no
[3] If adult female; choose status; Open, Gravid, Lactating, Sterile, Unknown
[4] Age; Neonate (0-6mo), Young (6-36 mo) & Adult (>3 Years)
[5] Type of sample; Choose one; Only Nasal Swab (N) or Serum(S)
[6] Herd Size; Small(1-10 Camels), Medium(11-30 Camels), >31 camels)
[7] Health Status ; choose one or more; Apparently healthy(AH) , Diarrhea(Di), Emaciated(Em), Nasal Discharge(ND), coughing(Co), Poor appetite(PA) , Poor hair Coat(PHC), Any other

Appendix 3: RNA extraction, PCR principle and procedure

QIAamp Viral RNA Mini Kits represent a well-established technology for general-use viral RNA extraction. The kit combines the selective binding properties of a silica-based membrane with the speed of microspin technology and is highly suited for simultaneous processing of multiple samples. The special QIAamp membrane guarantees extremely high recovery of pure, intact RNA in just twenty minutes without the use of phenol/chloroform extraction or alcohol precipitation. All buffers and reagents are also guaranteed to be RNase-free.

RNA extraction procedure for MERS-CoV camel nasal swab samples:

1. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA by adding 560 µl of AVL (lysis buffer) containing carrier RNA to 140 µl of swab sample in a microcentrifuge tube and mix by puls vortexing for 5 second.and incubate at room temperature for 10 min.
2. Then of 560 µl 95% ethanol is added to the mixture to provide optimum binding of the RNA to th e QIAamp membrane, and the sample is loaded onto the QIAamp Mini column 630 µl in a 2ml collection tubeto be centrifuged for 8,000 rpm for 1 min.
3. The RNA binds to the membrane, and contaminants are efficiently washed away in two steps using two different wash buffers.
4. The collection tubes containing the filtrate were discarded and a new collection tube was replaced, then 500 µl of AW_1 wash buffer was added to the spin columns and centrifuged for 1min. at 8000 rpm,
5. Then after replacing the collection tubes, 500 µl of AW2 (second wash buffer) was added to the column and centrifuged at 14,000 rpm for 3min. To ensure complete removal of the wash buffer it is centrifuged at full speed for 1 more minutes using new collection tubes. and discard collection tube with the filtrate.
6. Then plce the QIAamp Mini column in aclean 1.5 ml microcentrifuge tube and add 60 µl of RNase-free water(buffer AVE)equilibrated to room temperature and incubated for 1

minutes at room temperature (20°C) to elute high-quality RNA, followed by centrifugation at 8000 rpm for 1 minute.

The extracted RNA samples were then taken to amplification room for the real time RT-PCR procedure. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors, all extraction procedure were conducted in Biosafty Level II Laboratory.

Master Mix Setup

The master mix used in this study contains:

- 12.5 µl of 2x reaction buffer provided with the superscript III one-step RT-PCR system with platinum Taq polymerase (, 0.4mM of each dNTP's and 3.2 mM of magnesium sulphate)
- 1 µl of reverse transcriptase/Taq mixture from the kit
- 0.4 µl of a 50mM magnesium sulfate solution
- 3.6 µl of Rnase free water/Dnase free water
- 1 µl of UpE Forward primer
- 1 µl of UPE Revers primer
- O.5 µl of UpE Probe and

Principle and procedure of RT-PCR

Real time reverse transcription-polymerase chain reaction (rRT-PCR) is used to quantify RNAand Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR)for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes, there fore one-step RT-PCR offers the convenience of a single-tube preparation for RT and PCR amplification.

All reagents should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use. The reaction reagent is prepared inside PCR work station. After preparing the master mix, then it was taken in to amplification room, 20 µl of the mixed reagent

were dispensed to the Applied Biosystem plate followed by 5 µl of extracted RNA of the swab sample, positive (standard) and negative control (Rnase free water) were included in each PCR test according to the plate lay out of the extracted test sample and controls.

Procedure:20 µl of the reaction reagent was pipetted into each well of an appropriate optical 96-well reaction plate followed by addition of 5 µl eluated RNA.
The positive standard (control) 20 µl of the reaction mixture(master mix) was dispensed into individual well followed by 5 µl of positive standard and 5 µl RNase free water to the positive and negative control respectivelly. The extracted RNA templates were then thoroughly mixed with the Master Mix by pipetting up and down.

The reaction plate was covered by plate sealer and placed in the Applied biosystem real time PCR machine/thermal cycler connected to the computer with it's soft ware. The time and temperature were set according to the protocol mentioned in the amplification Table below.

Table: 1 Amplification Cycle

Temperature ^{o}C	**Time**	**Cycle**	
55 95	20 min 3 min	1	Preheating and reverse transcription
95	15 sec	45	denaturation
58	30 sec		Annealing and extension

Once, the cycles were complete result was displayed on the computer and interpreted as follows:

- Above the base line: positive
- Below the base line: negative

Validity of PCR Test Runs

A.Valid Diagnostic Test Run

For a valid diagnostic test run, the following control conditions must be met:

Control ID	Detection channel
	FAM^{TM} up E
Positive control	+ve
Negative control	-ve

B.Invalid Diagnostic Test Run

A diagnostic test run is invalid, (i) if the run has not been completed or (ii) if any of the control conditions for a valid diagnostic test run are not met. In case of an invalid diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again is recommended.

Appendix 4: Miscilinous photos during study

Yabello district Harwoyu kebele camel owners and MERS CoV sample collection team

Yabello district Dikale kebele camel owners and MERS-CoV sample collection team

Laboratory activity in NAHDIC Molecular laboratory RNA extraction room and MERS -CoV serology laboratory (ELISA)