

A Cross Sectional Study of Middle East Respiratory Syndrome: Coronavirus (MERS-CoV)

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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 between January 2020 to August 2020, with the objectives of detection and characterization of MERS-CoV and determining the sero-prevalence and its association with risk factors. The study animals originated from different camel rearing area of Ethiopia: warder, Babille, Ginir, Metehara and Moyale districts. A total of 473 sera sample and 8 nasal swab samples were used for the study. The nasal swab were tested for the presence of specific viral RNA using qRT-PCR and the sera samples were tested for the presence IgG antibody using indirect ELISA at NAHDIC in Ethiopia. Based on ELISA result, the overall seroprevalence of MERS-CoV in Oromia region (Babille, Metehara, Ginir and Moyale was 49.7% (235/473) (95% CI 45-54) while in Somali region(warder)was 100% (8/8). MERS-CoV specific antibody highly prevalent in adult camels 73.2% (63/86) compared to young 44.4% (172/387) the difference was statistically significant ($\chi^2=23.36$ and $P=0.001$). The sero-prevalence of MERS-CoV specific antibody in camels originated from Metehara have 55.2% (84/152) which was slightly higher from Moyale 51.3% (77/150) and Ginir 39.4% (60/152) and the variation was statistically significant ($\chi^2=16.60$ and $P=0.002$). Result showed higher sero-prevalence of MERS-CoV antibody in camels of all study area districts which reflects the evidence of prior MERS-CoV infection while all 8 nasal swab samples originated from Warder districts showed negative result for MERS-CoV RNA and it might suggests absence of active circulation of MERS-CoV at the time of sampling. Therefore further study is required to determine its significance from animal and public health perspectives and further research should focus on characterization of the virus and identifying similarity between MERS-CoV viral isolates in neighboring countries and from the Middle East.

Keywords: MERS-CoV; Camels; Sero-prevalence; Oromiya; Somali; Babille; Ginir; Metehara; Moyale; Warder; Ethiopia

Abbreviations: CDC: Center for Disease Control and prevention; CSA: Central Statistics Agency ELISA: Enzyme Linked Immuno Sorbent Assay; FAO: Food and Agriculture Organisation; IgG: Immunoglobulin G; KSA: Kingdom of Saudi Arabia; MERS-CoV: Middle East Respiratory Syndrom- Corona Virus; PCR: Polymerase Chain Reaction; RNA: Ribose Nucleic Acid; RT-qPCR: Reverse Transcriptase real time Polymerase Chain Reaction; WHO: World Health Organisation

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Introduction

Middle East respiratory syndrome (MERS) is a pneumonic illness of human caused by a novel lineage C, beta-corona virus (CoV). Primary infections have originated from countries within the Arabian Peninsula, although travel-associated cases and some secondary transmission have been reported in other countries [1]. Since its identification in the Kingdom of Saudi Arabia and Jordan, 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 [2-4].

Serological evidence suggests that MERS-CoV or related virus has been circulating in African dromedaries for more than 35 years different studies suggested MERS infections in humans in Africa are sparse, mostly probably due to limited epidemiological surveillance and/or genetic divergence of African MERS-CoV lineages compared to those in the Arabian Peninsula [5-7].

In Ethiopia, dromedary camels represent a subset of major livestock resources with a population estimated at 1, 209, 321 (CSA, 2016/17). According to Hailemariam and his colleagues, the pastoralist and agro-pastoralist areas of Ethiopia such as Borena, Bale, Afar and Somali are considered the traditional source of livestock, supplying 95 percent of camels for export markets [8]. Several studies that focus on viral and serological detection of MERS-CoV in camels were done in some part of the country but not representative of all camels' population. Recent research finding on MERS-CoV in Ethiopian camels revealed that MERS-CoV in East Africa including Ethiopia is genetically distinct from those in the Arabian Peninsula that strengthens the absence of local acquired zoonotic cases in human [9]. Therefore Study should be continued serologically and confirmation of the etiological agent by molecular technique for the preparedness of the continent as RNA virus not known when it became fatal virus to human in Africa.

There for the objectives of the study was:

1. To detect antibody and characterize MERS-CoV RNA from camels of Somali region in Ethiopia
2. To determine the presence of MERS-CoV antibody in serum samples of camels originated from Babile, Ginnir,

Metehara and Moyale districts of Oromia region in Ethiopia.

Materials and Methods

Study area and animals

The study was conducted between January 2020 to August 2020, on dromedary camels originated from different camel rearing area of Somali region (warder, Goladi) and Oromia region (Babile-Harerge ;Ginnir- Bale;Metehara-East showa and Moyale from Borena zone) (Table 1).

For the study, 473 camels' sera were used for antibody detection while 8 swab samples collected from Somali region camels with reported illness were used for genomic detection of MERS-CoV (Figure 1).

Sample collection

Serum and swab samples were collected from the study area and submitted to NAHDIC. 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 °C. Nasal swabs were taken using sterile break off plastic polyester cotton tipped swabs. The swabs inserted into the camel's nostril deep enough and rolled over the mucus membrane to scoop nasal material from which RNA of MERS-CoV can be extracted. The sample was then carefully placed inside pre-labelled sample containers crayovials containing 1.5 ml viral transport medium (VTM) and kept in an ice box during the field trip and kept in -80°C freezer up on arrival to NAHDIC up to conducting the test.

Laboratory tests

Indirect ELISA (Enzyme-Linked ImmunoSorbent Assay): For serological study, indirect Enzyme-Linked Immunosorbent Assay (ELISA) was used for the determination of MERS-CoV antibody according to previously described methodology (CDC, 2014). Enzyme-linked immuno sorbent assay (ELISA) is a semi quantitative test used to detect the presence and concentration of specific antibodies that bind to a viral protein coated on the test plates. The concentration of primary antibody present in

Table 1 Study area with respective sample type and sample size.

Region	Zone	District	Sample type	No of camels
Oromia	East showa	Metehara	serum	152
	Borena	Moyale	serum	150
	Bale	Ginnir	serum	152
	East Hararge	Babile	serum	11
Somali	Warder	Goladi	serum	8
		Goladi	swab	8
Total no. of samples				473

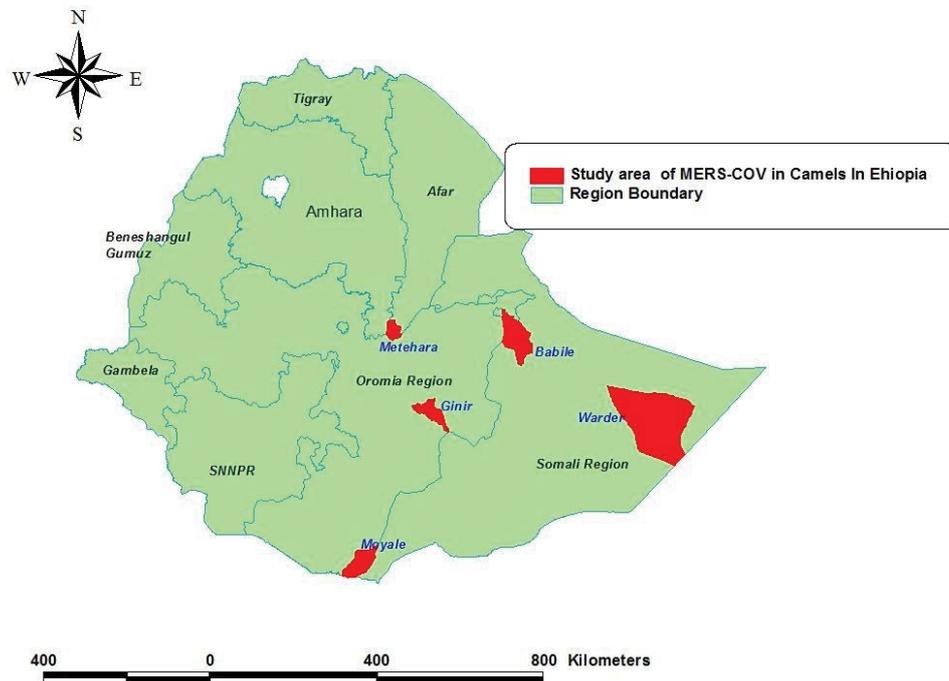


Figure 1 Map showing the study area of MERS-CoV in Camels of Ethiopia.

Table 2 Association of risk factors with sero-positivity to MERS CoV in camels of Oromia and Somali region.

	Category	No camel tested	No of Positive	Prevalence% (95% CI)	χ^2	P-value
Age	Young	387	172	44.4	23.36	0.001
	Adult	86	63	73.2		
Origin	Babile	11	6	54.5	16.60	0.002
	Bale Ginnir	152	60	39.4		
	Metehara	152	84	55.2		
	Borena-Moyale	150	77	51.3		
	Somali	8	8	100		
						Small sample size

the serum directly correlates with the intensity of the color. The indirect ELISA test kit, used for the serological study, has micro titer strips each with 8 break-off reagent wells coated with purified S1 antigen of MERS-CoV provides a useful measurement of antibody concentration in the serum samples. Hence ELISA test conducted for 473 serum samples according to the kit protocol EUROIMMUN AG Germany (Test instructions, 2020).

Real-time Reverse Transcriptase Polymerase chain reaction (rRT-PCR): Routine confirmation of cases of MERS-CoV infection is based on detection of unique sequences of viral RNA by real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. Therefore, according to WHO recommendation rRT-PCR targeting the genomic regions upstream of the *E* protein gene (*Up-E*) was used to screen for current infections with MERS-CoV from the nasal swabs of the camels [10]. Real Star MERS-CoV RT-PCR Kit

1.0, Germany was used to detect MERS-CoV RNA in the nasal swab samples. Extracted RNA is the starting material for the PCR kit therefore, QIAamp Viral RNA mini kit (QIAGEN, Japan) was used for the extraction of viral RNA from the nasal swab samples [11].

Statistical Analysis

All data analyses were performed using STATA version 12 statistical analysis software. The recorded risk factors or variables along with the test results were entered into excel spread sheet. The data was then analyzed and summarized in tables. Pearson chi square statistical analysis methods were utilized to determine the presence and level of association of the prevalence with the risk factors. Statistical significance was considered at 95% confidence level and $P < 0.05$.

Results

Description of study animals

All the study camels are male. The age groups categorized in to two groups, young camels accounts 81% (n=387/473) and adult accounts 18% (n=86/473). The samples were collected from dromedary camels which originate from two administrative regions Somali and Oromia. 1.7% from Somali, 98.3% from Oromia region.

Seroprevalence of MERS-CoV

Based on indirect ELISA test result, the overall prevalence of MERS-CoV antibody in camels of Somali region is 100% (8/8) while in Oromia region the overall prevalence is 49.7% (235/473) (95% CI 45-54). Association of risk factors with seroprevalence to MERS CoV showed statistically significant differences among age groups. Antibodies to MERS-CoV were detected from both regions and all district camels.

MERS-CoV specific antibody highly prevalent in adult camels 73.2% (63/86) compared to young 44.4% (172/387) the difference was statistically significant ($\chi^2=23.36$ and $P=0.001$) (Table 2).

Concerning Sero prevalence in relation to origin of camels, Due to few numbers of camels participated in the study the seroprevalence of MERS-CoV in Somali region is 100%(n=8/8) and in Babile 54% (n=6/11)while the sero-prevalence of MERS-CoV specific antibody in camels originated from Metehara have 55.2% (84/152) which was slightly higher from Moyale 51.3% (77/150) and Ginnir 39.4% (60/152) and the variation was statistically significant ($\chi^2=16.60$ and $P=0.002$) (Table 2).

Result of rRT-PCR

Real time PCR test performed at NAHDIC showed that no MERS-CoV up E genome detected in any of the tested swab samples.

Discussion

Middle East Respiratory Syndrome Corona virus (MERS-CoV) infection is an emerging zoonotic disease with potential public health significance in camel rearing areas of the world. Therefore active surveillance is the 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, a cross-sectional study has been conducted in Borena, Bale, East showa, East Harerge and Warder zone of Oromia and Somali regione camels aimed at determining the sero prevalence and current infection of MERS-CoV.

The overall seroprevalence of MERS-CoV antibody in this study was 49.7% (235/473)(95% CI 45-54). This sero-prevalence to MERS-CoV antibody (IgG) is an indication of prior infection in the camels at some stages of their life [12]. Seroprevalence of MERS-CoV in camels at the current study area was in agreement with previous studies conducted in Ethiopia which was reported

seroprevalence of 93-97%; 86%; 85.1% and 56% [13-15]. Comparing MERS-CoV seroprevalence in camels of the current study area Babille, Ginnir, Metehara and Moyale with other area, these are lower than Assayita-Dubti seroprevalence (85.8%), Melkawerer (99%), and Akaki abbatoire (99.3%) reported in Ethiopia [14]. However; it was relatively comparative with some of African countries camels MERS-CoV seroprevalence report: in Tunisia (30-54%) and in Kenya (49.9%); (68%) [16,17].

MERS-CoV specific antibody highly prevalent in adult camels 73.2% (63/86) compared to young 44.4% (172/387) the difference was statistically significant ($\chi^2=23.36$ and $P=0.001$) 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 known for less frequent shedding of virus and demonstrated higher rates of sero-conversion [18-21]. The high seroprevalence of MERS-CoV antibody in adult camels in Babille, Metehara, Ginnir, Moyale and Warder was in agreement with different previous research findings in Ethiopia and other camel keeping Africa countries.

Another finding during this study was the detection of MERS-CoV antibodies in 44.4%(n=172/387) of young camels which might indicates the ongoing circulation of MERS-CoV in the study area camels within these three years as the age of the young camels included in the study were below three years. These findings were also in agreement with similar findings in Kenya, Dubai, Ethiopia and in Pakistan [22-24].

Interestingly, inspite of the high seropositivity in Camels of warder all 8 nasal swab samples collected for detection of MERS-CoV RNA using qRT-PCR targeting the *Up-E* gene as a screening test were negative. The result of the present study was in agreement with those previous studies conducted in Borena 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% [13-15]. On the other hand, the finding of the current study was not in agreement 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 [13,14]. 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 at the time of sampling [25-28].

Conclusion and Recommendations

In conclusion, the very high prevalence of MERS-CoV neutralizing antibodies in tested camels in different regions with a larger geographical range indicates the widespread nature of the virus in camels of Ethiopia. A systematic active surveillance and longitudinal studies for MERS-CoV are needed to understand the epidemiology of the disease and dynamics of infection. High seroprevalence detected in camels warrants the initiation of an active surveillance study for MERS-CoV in humans, particularly those that are at higher risks of exposure to MERS-CoV infections such as camel keeper, camel traders and abattoir workers.

Ethical Approval and Consent to Participate

No need of ethical clearance

Consent for Publication

No need of permission

Availability of Data and Materials

The data and materials are available

Competing Interests

The authors of this paper have no financial or personal relationship

with other people or organization that could inappropriately influence or bias the content of this paper by any means.

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