SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

PREVALENCE AND FACTORS ASSOCIATED WITH MIDDLE EAST RESPIRATORY SYNDROME-CoV IN HUMAN POPULATION OF CHIFRA WOREDA: CAMEL REARING AREA OF AFAR REGION, ETHIOPIA

A THESIS SUBMITTED TO THE DEPARTMENT OF PUBLIC HEALTHIN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF PUBLIC HEALTH IN FIELD EPIDEMIOLOGY

By DESALEGN BELAY (BSc)

FEBRUARY, 2018
ADDIS ABABA, ETHIOPIA

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GETACHEW TILAHUN (MPH)

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SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE
DEPARTMENT OF PUBLIC HEALTH

DECLARATION FORM

LETTER FOR DECLARATION (body of work with thesis)

I, the undersigned, declared that this is my bona fide original work, has never been presented in this or any other university, and that all the resources and materials used for the body of work with thesis have been fully acknowledged.

Name of the student: _____

Signature_____

Date _____

Pace _____

Date of Submission _____

Approval of Advisors

This body of work with thesis has been submitted for examination with our approval as **candidate's advisors**.

_____	_____	_____
Name of Primary Advisor	Signature	Date
_____	_____	_____
Name of co-advisor	Signature	Date
_____	_____	_____
Name of FELTP coordinator	Signature	Date

DEPARTMENT OF PUBLIC HEALTH GRADUATE STUDIES

FINAL BODY OF WORK WITH THESIS APPROVAL FORM

As member of the Board of Examiners of the final body of work with thesis open defense, we certify that we have read and evaluated the body of work with thesis prepared by **DESALEGN BELAY TAKELE** under the title **"PREVALENCE AND FACTORS ASSOCIATED WITH MIDDLE EAST RESPIRATORY SYNDROME-CoV IN HUMAN POPULATION OF CHIFRA WOREDA: CAMEL REARING AREA OF AFAR REGION, ETHIOPIA "** and recommencement for the ***degree of Master of Public Health in Field Epidemiology***.

_____	_____	_____
Name of Chairperson	Signature	Date
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Name of Primary Advisor	Signature	Date
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Name of External Examiner	Signature	Date

Final approval and acceptance of the body of work with thesis is contingent up on the submission of the final both hard and soft copies of the body of work with thesis to the department of public health.

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Acronyms/Abbreviations

CDC	Center for Disease Control and Prevention
ELISA	Enzyme Linked Immunosorbent Assay
EPHI	Ethiopian Public Health Institute
IgG	Immunoglobulin G
MERS-CoV	Middle East Respiratory Syndrome Corona Virus
Ml	milliliter
RNA	Ribonucleic Acid
S1	Spike 1
SPHMMC	Saint Paul Millennium Medical College
WHO	World Health Organization

Summary

Background: Middle East Respiratory Syndrome (MERS) is an emerging viral zoonosis and considered as one of the major global public health threat. The infected individuals developed severe acute respiratory illness with symptoms of fever, cough, and shortness of breath.So far, 27 countries have reported human laboratory confirmed cases since 2012. Camels are considered as the probable source for zoonotic transmission of the virus. In Ethiopia, high sero and viro-prevalence of MERS-CoV has been reported in camel population; however, human case has been not yet reported. This study was done to determine the seroprevalence and associated factors of Middle East respiratory syndrome coronavirus in Chifra woreda of Afar region, Ethiopia

Methods: Random sampling technique was used at all steps and all persons above 18 years old were enrolled in selected households. Sera were tested for the presence of MERS-CoV antibodies using anti- MERS-CoV ELISA IgG kit. Result was measured at wavelength of 450 nanometers (reference between 620 and 650) and determined using ratio of extinction values (Optical density) of samples to calibrator. Samples with the extinction ratio 1.1 and above were considered positive, 0.8 and above and below 1.1 were borderline, and below 0.8 were negative

Result: Totally, 341 participants enrolled with median age of participants was 25 years (range=18-77 years).Seven (2.05%) specimens were positive, 326 (95.6%) were negative and eight (2.35%) of thespecimens were found on borderline. None of the participants have used personal protective equipments while handling camels and their wastes, and none had travel history to gulf countries. From the total of 341 study participants, 329 (96.5%) had no history of chronic medical problems. In a bivariate analysis, none of our assumed risk factors were significantly associated with the presence of IgG in the participants' specimens.

Conclusion: Our study reveals that the virus has been circulating. For an emerging viral human infection, a single positivity may be considered as public health threat which makes us to say our finding has immense public health importance. This finding should be confirmed by large scale studies involving virus isolation and identification of the sequence of the virus.

Keywords: Ethiopia, Human, MERS-CoV

1. Introduction

1.1. Background

Middle East respiratory syndrome (MERS), which is caused by MERS-corona virus (MERS-CoV), is a believed to be zoonotic disease. The infected individuals developed severe acute respiratory illness with symptoms of fever, cough, and shortness of breath. The original source(s), route(s) of transmission to humans, and the mode(s) of human-to-human transmission have not been determined; however there is clear evidence of person-to-person transmission. The efficiency of person-to-person transmission of MERS-CoV is not well characterized but appears to be low, given the small number of confirmed cases since the discovery of the virus. As of July 2014, no evidence of sustained community transmission beyond small clusters has been reported in any country. Transmission has occurred between patients and healthcare personnel in a hospital setting. Although the exact timing and nature of exposures that result in infection is usually unknown, for those cases for which exposure is known or strongly suspected, the incubation period for laboratory confirmed cases of MERS-CoV is generally less than one week. However, in at least one case, the known exposure occurred 9 to 12 days prior to onset of illness. Further evidence in cases exposed over a range of time suggests that, at least in a minority of cases, the incubation period may exceed one week but is less than two weeks. The period of communicability for MERS-CoV is unknown at this time. Until further guidance is available, follow isolation recommendations used for SARS; persons with MERS should be isolated (for example, by not going to work or to school) until 10 days after fever has resolved, provided respiratory symptoms are absent or improving [1,2,3,4].

Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) is an emerging pathogen of increasing importance. The MERS-CoV was first recognized in 2012 in Saudi Arabia as novel Coronavirus that cause severe acute respiratory disease[5]. The infected individuals developed severe acute respiratory illness with symptoms of fever, cough, and shortness of breath. A small number of the reported cases however also had a mild respiratory illness[6].

As of May 2017, WHO has been notified of more than 1,950 laboratory-confirmed cases of infection and at least 693 deaths related to MERS-CoV. Since September 2012, 27 countries have reported cases of MERS-CoV In total, cases have been reported from 27 countries in the Middle East, North Africa, Europe, the United States of America, and Asia. The high case

fatality rate, large distribution of the reservoir, lack of medical countermeasures, as well as the knowledge gaps in veterinary and human epidemiology, immunity and pathogenesis have placed MERS-CoV as one of the pathogens prioritized in the WHO[7,8].

1.2. Statement of the Problem

Middle East respiratory syndrome coronavirus (MERS-CoV) has been identified from camels in Egypt, Oman, Qatar and Saudi Arabia[8]. Serum samples from camels in a number of countries throughout the Middle East and African countries have been found to have antibodies to MERS-CoV[9].Serological evidence of the early circulation of MERS-CoV in camels in African countries and established camel trade between the Middle East and Africa suggests that African countries are considered as a possible source for the establishment of MERS-CoV in Middle East.As a result, the researchers have now focused on exploring the Epidemiology of MERS-CoV in countries where camels are bred and traded, especially in eastern Africa including Ethiopia[10,11,12]. Camels are thought to play a central role in MERS epidemiology because widespread evidence of MERS-CoV–specific antibodies and virus shedding in camels was found and highly similar viruses have been detected in humans and dromedaries at the same location. The findings of these serological and molecular studies suggested that exposure to camels is one of the risk factor for infection in human [13,14,15].

A total of 2,040 laboratory-confirmed cases of Middle East respiratory syndrome-coronavirus (MERS-CoV) infection were reported to WHOsince its emergence in 2012 up to 21 July 2017, 82% of whom were reported by the Kingdom of Saudi Arabia. Males above the age of 60 with underlying conditions, such as diabetes, hypertension and renal failure, are at a higher risk of severe disease and including death. To date, at least 710 individuals have died (crude CFR 34.8%) [8].

Sero-epidemiological studies have been carried out in domestic livestock (cattle, sheep, goats, horses and poultry) in the Arabian Peninsula and the Middle East, but dromedary camels were the only species from which antibodies specific to MERS-CoV have been detected. Serological evidence of MERS-CoV infection has also been demonstrated in dromedaries from Africa, including Egypt, Ethiopia, Nigeria, Tunisia, Sudan and the Canary Islands, with similarly high seroprevalence except Tunisia and Canary Islands, where adult seroprevalence was 54% and 14%, respectively[10,11,16].

The population based seroprevalence of Saudi Arabia suggests that tens of thousands of MERS-CoV infections have gone unrecognized. Of the total of 28.5 million dromedary camels worldwide, 77% are in Africa, the largest camel populations being found in Somalia (6.2 million), Sudan (4.8 million), Kenya (3 million) and Ethiopia (2.3 million)[15]. Evidences suggests that a dromedary camel was the source of MERS-CoV that infected a patient who had had close contact with the camel's nasal secretions[17].The existence of unrecognized human infections in African or Arabian countries in the past cannot be ruled out. Resource-limited African countries that have been exposed to civil unrest, such as Somalia and Sudan, are not likely to diagnose and report diagnostically challenging infections resembling other diseases[14].

The absence of human cases in countries with high seroprevalence of MERS-CoV in camels is suggested to be associated with poor surveillance system and lack of research focus on humans to address this information gaps in the country.Therefore, this study is aimed at determining the prevalence and factors predicting the disease.

1.3. Significance of the Study

There is a growing speculation that sporadic human cases may have gone undetected since MERS-CoV is not included in list of reportable diseases in Ethiopia[19]. Since the prevalence of MERS CoV in camels is high in Ethiopia, in which Afar region is one[18], knowing the serological status of human population is crucial. However, epidemiological studies are needed to ascertain that whether MERS-CoV has been introduced into the human population of Ethiopia, particularly humans who have close contact with camels. Since the disease is now one of the global public health concerns, and might have been affecting people before, we need to know its seroprevalence in Chifra woreda of Afar region, Ethiopia. Thus, this study will help detect and understand the epidemiology of MERS in high risk human population and investigation of the role of other species including bat in the transmission pattern of the virus. It also will provide evidence to design appropriate control and prevention strategies in pastoral areas of Ethiopia.

2. Literature Review

In this chapter, a critical review of literatures focused specifically on prevalence of MERS CoV and associated factors will be addressed briefly.

In a study conducted in Qatar, 2% of camel farm workers, 14% of camel barn workers at an international racing track and 2 of 5 camel slaughterhouse workers were seropositive while none of 204 people with no camel contact were seropositive[15]. According to a study initiated to identify the virus in camels and to compare it genetically with human-derived MERS-CoV, camel-derived MERS-CoV sequences are clustering independently from each other, but together with the human-derived MERS-CoV sequences from the same geographical areas of Oman[19]. This shows a probable transmission of MERS-CoV from camels to humans.

A study conducted to know the risk of MERS-CoV among people who have contact with camels in Qatar detected MERS-CoV neutralizing antibodies in healthy persons who had daily occupational contact with camels but not in persons without such contact. Only limited evidence is available regarding the presence of MERS-CoV antibodies in the general human population or in specific population cohorts in the study. However, an overall seroprevalence of 0.15% was found in a cross-sectional study in Saudi Arabia, and among slaughterhouse workers, neutralizing antibodies were detected in 3.57% of the participants[9].

Community outbreaks of MERS-CoV have been recorded having transmission in same family in Saudi Arabia. In an initial report of a family cluster, four among 28 extended family members tested positive for MERS-CoV, including the index case[12].

A study was conducted to determine the epidemiology of Middle East respiratory syndrome coronavirus in hospital patients in Kingdom of Saudi Arabia. On multivariate analysis of this study, age >80 years, cardiac disease, and cancer were independently associated with mortality. In this study, compared to primary cases, mortality due to MERS-CoV was lower in household cases and healthcare workers[20]. Additionally, pilgrims attending the 2013 Hajj were also sampled with nasal swab and screened although no MERS-CoV RNA was detected in any of the 5,235 attendants[21].

A retrospective specimen was collected from patients to determine viral shedding and antibody response in 37 patients with Middle East Respiratory Syndrome Coronavirus Infection. In the analysis, all surviving patients, but only slightly more than half of all fatal cases, produced IgG and neutralizing antibodies[22]. In a cross sectional survey in Saudi Arabia, 10,009 serum

samples were collected from healthy individuals older than 15 years who attended primary health-care centers or participated in a national burden-of-disease study in all 13 provinces, shepherds and abattoir workers with occupational exposure to camels. They screened samples by recombinant ELISA and MERS-CoV seropositivity was confirmed by recombinant immunofluorescence and plaque reduction neutralization tests. Finally, Anti-MERS-CoV antibodies were confirmed in 15 (0·15%; 95% CI 0·09–0·24) of 10,009 people in six of the 13 provinces[23].

A similar cross sectional study was also conducted in Kenya to determine the seroprevalence, serum samples were collected as part of a household survey conducted during 2013–2014 in 2 eastern counties of Kenya; Garissa and Tana River. Here, 4 (0.72) in Garissa and 12 (2.13%) from Tana River were found positive by recombinant ELISA. After a confirmatory test by plaque reduction neutralization test, 2 (0.18%) of the sampled were positive[24].

So far, there is no study found that assessed the status of MERS in high risk human population, pastoralists in Ethiopia, despite the high prevalence of MERS in their camel population. Therefore, in general terms, these literatures tell us that anti-MERS-CoV antibody can be detected in the community who has close contact with camels, diseased persons with MERS-CoV, from persons who have underlying chronic illnesses.

2.1. Conceptual framework

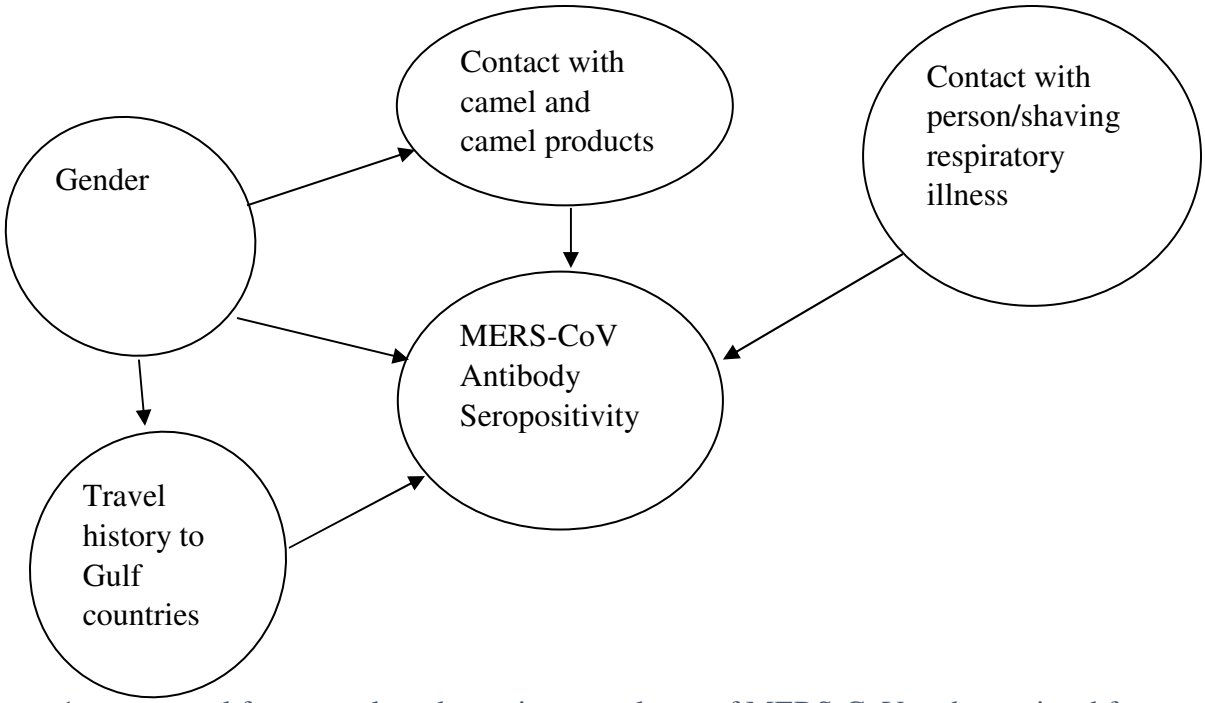

Figure 1: conceptual frame work to determine prevalence of MERS-CoV and associated factors Chifraworeda, Afar, Ethiopia, 2017

3. Objective

3.1. General Objective

- To determine the seroprevalence and associated factors of Middle East respiratory syndrome corona virus in Chifra woreda of Afar region

3.2. Specific Objectives

- To determine seroprevalence of MERS-CoVin pastoral community of Chifraworeda of Afar region
- To identify associated factors for MERS-CoVseropositivity in Chifra woreda of Afar region

4. Methods and Materials

4.1. Study Area and Period

The study was conducted in Chifra woreda of Aar region. Chifra Woreda is one the woredas in Afar region of Ethiopia and is bordered with EwaWoreda in the North, AderWoreda in South, Mile and part of Dubti Woreda in the East and Habru of North Wollo and part of Bati Woreda (Werebabo) in the West[27].It is located 665 Kilometers north of Addis Ababa along a highway to Mekelle. It has a total population of 91,078.The study was conducted fromDecember 01 to 30, 2017.

4.2. Study Design

A community based cross-sectional studywas carried out in Chifra woreda of Afar region.

4.3. Population

4.3.1. Source Population

All residents of Chifra woreda, Afar region, Ethiopia

4.3.2. Study Population

The study population were selected/sampled individuals whose age is18 years and above living in Chifra woreda.

Inclusion and Exclusion criteria

Inclusion criteria: All individuals in selected household who possess camels, andwhose age is 18 years and above was participated.

Exclusion criteria: Individuals who cannot answer questions verbally and cannot provide blood sample due to other health problems were not eligible to participate.

4.4. Sample Size Determination

The sample size was calculated using for single population proportion formula. To estimate the required sample size, a prevalence of 50% was assumed, with a required precision of 5% and an α-error of 5%. Accordingly, a total sample size of 384 is calculated for the study. The formula for single population proportion is:

$$n = \frac{1.96^2 * P(1-P)}{d^2}$$

$$n = \frac{1.96^2 * 0.5(0.5)}{(0.05)^2}$$

$$n = 384$$

Adding 10% non-response rate, the final population was supposed to be 423.

where *n* is the sample size without correction, *Z*α (1.96) is the percentile point relating to the required α error under the *z* distribution, d is the desired absolute precision, Pis the expected prevalence. But due to resource limitations, we tested a total of 341 samples.

4.5. Sampling Technique and Procedure

All Kebeles in Chifra woreda were listed based on their camel population before sample collection. A simple random sampling technique was used to select each level. Then, all persons 18 years old and above in a household were enrolled.

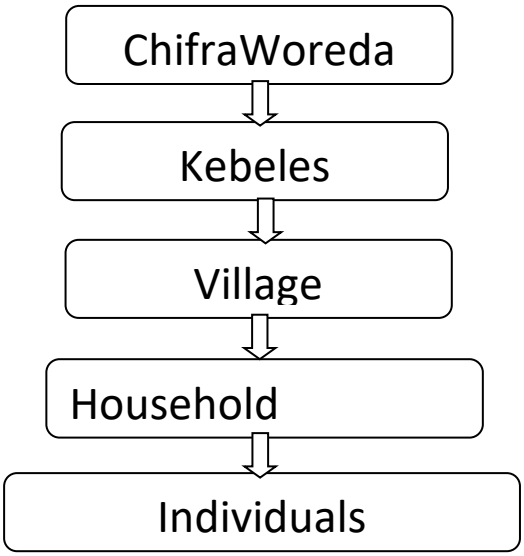

Figure 2: Schematic representation of sampling procedure, Chifra woreda, December 01-30, 2017

4.6. Study Variables

4.6.1. Dependent Variable

Presence of anti-MERS CoV IgG antibody in serum specimens

4.6.2. Independent Variables

Age, sex, occupation, travel history to gulf countries, contact with a person suffering from respiratory illness, handling of camels and their products, consumption of animal products, demographic characteristics, and presence of underlying chronic illnesses (diabetes mellitus, hypertension, asthma, etc.)

4.7. Operational Definitions

Sero-Positive: Individuals whose serum sample is found positive for MERS COV antibodies using both screening and Confirmatory serological tests

Contact with ill person: visiting any person sick with respiratory (cough, breathing problems) in the past 6 months

Contact with camel: caring for camels, cleaning their waste, caring sick camel with nasal discharge, assist in birth, milking and drinking milk, slaughtering and eating meat and others.

Risk Factors: Presence of own camels at home and having a close contact with camels, consumption raw camel milk or a person who has a direct exposure to camel waste material, contact with a person having respiratory illness in the past six months and presence of chronic medical illnesses.

4.8. Laboratory Analysis

4.8.1. Type of Specimen and collection Procedure

Venous blood (2-3 ml) was drawn from the median cubital vein after applying a tourniquet 3–4 inches above the injection site and disinfected with 70% alcohol. Sterilized pre-coded serum vacutainer serum separator tubes were used. After sampling, punctured sites were bandaged using adhesive tapes. Date of collection, location, and name of person collecting the specimen were recorded.

4.8.2. Specimen Handling and Transportation

Sera were separated from clotted blood by centrifugation at 3,000 × g for 15 minutes at nearby health centers' laboratory and stored at -20°C mobile freezer until transported to the laboratory (EPHI).Serum was separated on the date of collection by trained laboratory technologist

4.8.3. Specimen Analysis Procedures and interpretation

Sera were tested for the presence of MERS-CoV antibodies using anti- MERS-CoV ELISA IgG kit produced by EUROIMMUN (Germany Company). Laboratory investigation was done at EPHI (Annex 3). Result was determined using ratio of extinction values (Optical density) of samples to calibrator. Samples with the extinction ratio 1.1 and above were considered positive, 0.8 and above and below 1.1 were borderline, and below 0.8 were negative.

Test Principle of anti- MERS-CoV ELISA IgG kit:

The test kit contains micro titer strips each with 8 break-off reagent wells coated with purified S1 antigen of MERS coronavirus (MERS-CoV S1). In the first reaction step, diluted patient samples were incubated in the wells. In the case of positive samples, specific IgG would bind to the coated antigens. To detect the bound antibodies, a second incubation was carried out using an enzyme-labeled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

4.9. Data Collection Tools & Procedures

To collect epidemiological data structured and pre tested questionnaire was used (Annex 2). Each questionnaire was administered by the investigator to assess the potential risk factors for Middle East Respiratory Syndrome infection in human.

4.10. Data Management and Analysis

Data collected was checked for completeness on the date of collection by the principal investigator. All the data collected from field were coded and entered in to SPSS version 23 by the principal investigator. Quality assurance was maintained by undertaking double data entry and all the hard copy documents are kept in locked file cabinet. SPSS version 23 was used for descriptive and analytical analyses. Descriptive analysis was calculated as counts and proportions, and presented using tables and graphs. Associations between risk factors and past infection were determined by binary logistic regression and expressed as odds ratios with 95% confidence intervals and p value <0.05 was considered as significant. Fisher exact test was also used to determine the significance for variables with a cell count of less than 5. Results were presented mainly in the form of narration, tabular summaries and a figure.

4.11. Ethical Considerations

Ethical approval for the study was obtained from the scientific and ethics review office of the Ethiopian Public health Institute and from the St Paul's Millennium Medical College. Permission for sample collection was obtained from a regional health Bureau, Chifra woreda health office and local administrative bodies. After a thorough explanation about the purpose of the study and its benefits and potential risks to the participants in the Afar language, verbal consent was obtained before enrolment (Annex 1). All questions that arose were addressed.

Participants were clearly informed that their participation in the study is strictly voluntary, and that they can decline not to participate at any time and give no reason for withdrawal. All participants were also informed that withdrawing from this study will not affect the quality of services they receive from any health facility anywhere.

In addition, they were clearly informed that if they consent to participate in the study, 2-3 ml of blood from their arm will be collected.The subjects had been notified that samples could be shipped to external laboratories for specialized screening.

5. RESULT

5.1. Sociodemographic Characteristics

A total of 341 individuals participated in the study making a response rate of 100%. The median age of participants was 25 years (IQR=16). Among the participants, 126(36.9%) were females and 215(63.1%) were males. From a total of 313 pastoral participants, 107(34.2%) were female and 206(65.8%) were males while among 28 student participants, 19(67.9%) were females and 9(32.1%) were male students. Eighty three (24.3%) of the participants had their own camel and the rest 258(75.7%) do not own camels but live with their family who own camels.

Table 1: Demographic characteristics of the study participants, Chifra woreda, December 2017

Characteristic	Category	Frequency (%)
Gender	Female	126(36.9)
	Male	215(63.1)
Age Group	<20	99(29.0)
	20-29	113(33.1)
	30-39	60(17.6)
	40-49	38(11.1)
	50-59	19(5.6)
	>=60	12(3.5)
Locality (Kebele)	Amulli	20(5.9)
	Anderkalo	46(13.5)
	Askoma	76(22.3)
	Chifra 01	45(13.2)
	Geriro	15(4.4)
	Semsem	29(8.5)
	Teaboy	19(5.5)
	Wanaba	28(8.2)
	Woama	63(18.4)
Occupation	Pastoralist	313(91.8)
	Student	28(8.2)

5.2. Seroprevalence of MERS-COV

In the study area, seven [2.05% (95 % CI; 1.00-4.18)] specimens were positive, 326 [95.6% (95% CI; 92.87-97.32)] were negative and eight [2.35% (95% CI; 1.19-4.56)] of the samples were found on borderline by ELISA as indicated on figure 1 below.

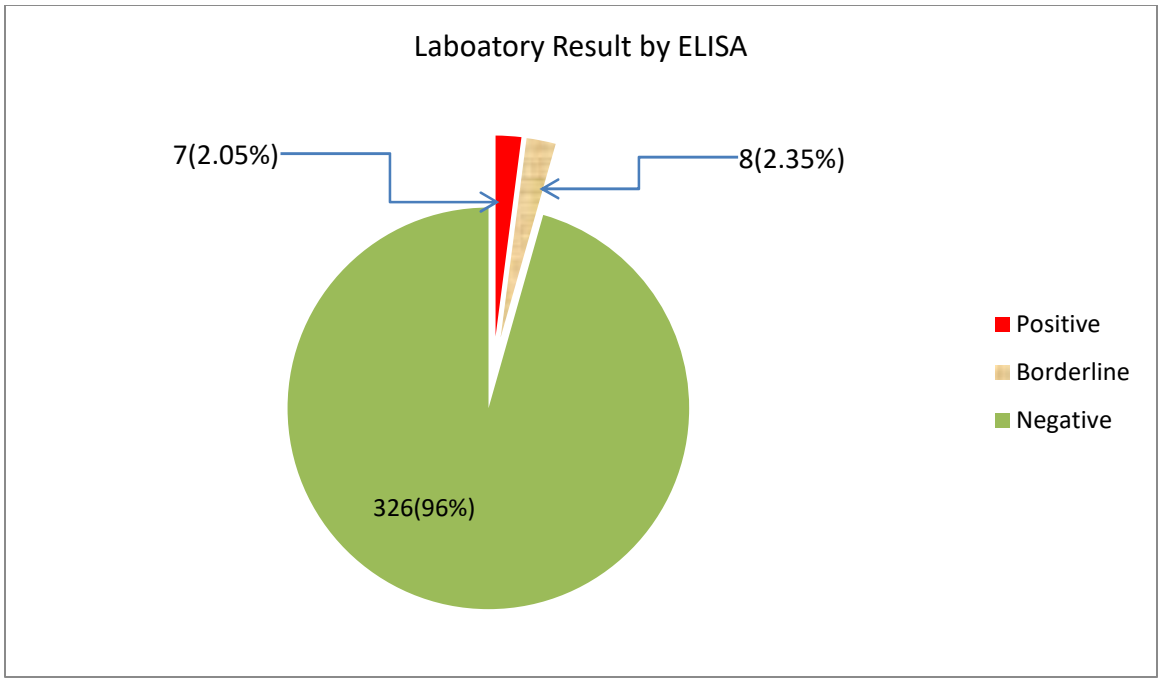

Figure 3: Distribution of results by ELISA, Chifra woreda, Afar, December 01-30, 2017

5.3. Description of Seropositive Participants

Figure 4: Characteristics of the seropositive participants, Chifra woreda, Afar, December 01-30, 2017

Serial Number	Sex	Age	Occupation	Kebele	Bats around home	Chronic medical problems
1	F	44	Pastralist	Askoma	Yes	No/Don't Know
2	F	50	Pastralist	Amulli	Yes	No/Don't Know
3	M	24	Pastralist	Chifra 01	Yes	No/Don't Know
4	M	20	Pastralist	Anderkalo	Yes	No/Don't Know
5	M	29	Pastralist	Wanaba	Yes	No/Don't Know
6	M	18	Pastralist	Askoma	Yes	No/Don't Know
7	M	18	Pastralist	Anderkalo	Yes	No/Don't Know

In addition to the information illustrated in the above table (table 4), the study participants who were seropositive had the following characteristics.

5.3.1. Participant 1

This study participant didn't own camels and camels do not live around her house. She had no contact with camel but drinks raw camel milk. She had no respiratory illness and didn't know if she had any chronic illness.

5.3.2. Participant 2

This study participant owned camel and camels lived near to her house. She touched camels, had contact with sick camel and saw a camel died of illness. She used to drink raw camel milk and has been sick with respiratory illness but didn't visit a doctor.

5.3.3. Participant 3

This participant owned camels and those camels live near to his house. He used to touch camels daily, slaughter camels rarely (more than a month), assisted three camels during birth in the past one year before the interview, administers vaccine rarely, milk camels daily, saw camel died of illness, drink raw camel milk but never been sick with respiratory illness.

5.3.4. Participant 4

This study participant did not own camels but camels live around his house. He rarely touches camels, rarely slaughters camels, assisted one birth of camels in the past one year before interview, rarely administers vaccine, milks camels at most in weekly, had contact with camel, visited camel market rarely and used to drink raw camel milk.

5.3.5. Participant 5

This study participant did not own camels but camels live around his house. He rarely touched camels, rarely had contact with camels' waste, rarely slaughtered camels, has assisted three camel births in the past one year before the interview, rarely administered vaccine, milked camels up to weekly, had contact with sick camel, rarely visited camel market, rarely drank raw milk and was sick with respiratory illness.

5.3.6. Participant 6

This participant owned camels and those camels live near to his house. He rarely touched camels, rarely had contact with camels' waste, assisted in birth of three camels in the past one year before the interview, rarely administered vaccine, rarely milked camels, had contact with sick camel, had seen camel died of illness, rarely visited camel market and used to drink raw camel milk.

5.3.7. Participant 7

This participant owned camels and those camels live near to his house. He used to touched camels at least weekly, had contact with camel waste, assisted in birth of three camels in the past one year before the interview, rarely administered vaccine, milked camels at least weekly, had contact with sick camel, rarely visited camel market and used to drink raw camel milk.

5.4. Associated Factor Analysis

Among the participants, 160(46.9%) of them have never assisted the birth of camels while 35(10.3%) have assisted ones, 36(10.6%) have assisted twice, 13(3.8%) have assisted three times, 24(7.0%) have assisted four times and 73(24.4%) have assisted more than four times. for the past 1 year. One hundred eighty (52.8%) of the study participants have never administered vaccine for camels, 153(44.9%) have administered rarely(less than once in a month), 7(2.1%) have administered at least once a month and the rest one participant (0.3%) administered daily. None of the participants have used personal protective equipments while handling camels and their wastes, and none had no travel history to gulf countries. Thirty eight participants had visited a doctor due to respiratory illness in the past 12 months. None of our participants used to amoke cigarettes. From the total of 341 study participants, 329 (96.5%) had history of chronic medical problems while three (0.9%) had Asthma, four (1.2%) had chronic lung disease, one(0.3%) had a combination of diabetes mellitus and hypertension, three(0.9%) kidney disease and one(0.3%) both kidney disease and hypertension. In a bivariate analysis, we found no statistically significant association between any of the risk factors and the seropositivity (table2).

Table 2: Table describing the relationship between risk factors and sero-positivity, Chifra district, Afar, December 2017

Variables	Laboratory Result by ELISA		COR(95% C.I)	P Value
	Non-Positive (%)	Positive (%)		
Age Group				
<30	205(98.09)	4(1.91)	1	
30-49	97(98.98)	1(1.02)	0.53(0.06-4.79)	0.57
>=50	32(94.12)	2(5.88)	3.20(0.56-18.21)	0.19
Total	334(97.95)	7(2.05)		
Gender				
Female	124(98.41)	2(1.59)	1	
Male	210(97.67)	5(2.33)	1.48(0.28-7.72)	0.64
Touching Camel				
No	96(98.97)	1(1.03)	1	
Yes	238(97.54)	6(2.46)	2.42(0.29-20.37)	0.68
Slaughter Camel				
No	203(98.07)	4(1.93)	1	

Yes	131(97.76)	3(2.24)	1.16(0.26-5.28)	1.00
Assist in Birth of Camels for the past 1 year				
No	158(98.75)	2(1.25)	1	
Yes	176(97.24)	5(2.76)	2.24(0.43-11.73)	0.54
Milking Camels				
No	130(98.48)	2(1.52)	1	
Yes	204(97.64)	5(2.39)	1.59(0.30-8.33)	0.87
Visit Camel Market				
No	179(98.35)	3(1.65)	1	
Yes	155(97.48)	4(2.52)	1.54(0.34-6.98)	0.86
Sickness with Respiratory Illness				
No	210(97.67)	5(2.33)	1	
Yes	124(97.41)	2(1.59)	0.68(0.13-3.54)	0.94

6. Discussion

Overall, this study has determined the seroprevalence of MERS-CoV and assessed expected factors leading to infection by the virus in Chifra woreda, Afar region, Ethiopia. As to the investigators' span of search, this is the first population based survey ever held on humans in Ethiopia with all its short-comings.

This study has used a similar ELISA kit with that done in Kenya and Saudi Arabia. In our study, the prevalence of seropositivity against anti MERS-CoV IgG was higher in men than in woman which fits with a similar study in Saudi Arabia. This might have happened due to the fact that men are more likely to have contact than women. The positivity prevalence (2.05%) is comparable to the two similar studies (0.15% and 1.43% for Saudi Arabia and Kenya respectively) which can also share similarities between the horn of Africa and the gulf countries due to camel trade exchange[25,26,29].

Our study is consistent with a serological investigation conducted among slaughter house workers which showed seropositivity of 0%-5.8% although used different assay. Other seropositivity was revealed among hospitalized patients with the diagnosis of MERS-CoV infection[24,30]. So, this notes that seropositivity is resulted from the infection and meanwhile our study participants who are tested positive have been once infected with MERS-CoV. Some individuals might have been severely ill or died some time, or the infection remained asymptomatic. Due to the rapid genetic changing nature of viruses, we may face a new highly virulent type of MERS-CoV.

In a bivariate analysis, none of our assumed factors were significantly associated with the presence of IgG in the participants' specimens, which is to mean positivity of participants to Anti-MRS-CoV IgG antibody; revealing past infection by the virus. Some of these factors, to the contrary, for example having camels around home or farm, were found associated with evidence of infection in Saudi Arabia. Consumption of raw meat and raw milk were not associated with the positivity like ours [22,28].

7. Strength and Limitation of the Study

We used a validated test kit to determine the seroprevalence as mentioned above. But the study had several limitations. First, it was limited to strictly following WHO's two-step laboratory recommendation [28]. Second, due to the communal dwelling nature of the locality, our participants might have responded similar to the exceeding respondent. Third, due to small number of positives, we couldn't observe an association between the independent variables and seropositivity.

8. Conclusion

Our study reveals that the virus has been circulating in the study area and few of the population remained immunized following the infection. Although there is no cut-off point to say high or low, for an emerging viral human infection, a single positivity may be considered as public health threat which makes us to say our finding has immense public health importance. Based on our data, we couldn't find any statistically significant association between the exposures and seropositivity.

9. Recommendation

Our study should be confirmed by large scale studies involving virus isolation and identification of the sequence of the virus to help for vaccine recommendation. This can be done by the ministry of health through establishing sentinel surveillance involving virological surveillance. Regional and woreda health institutions should be vigilant in identifying the causes of respiratory illnesses.

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ANNEXES

Annex 1: Participant Information Sheet

Introduction

I am _____ from St Paul's Hospital Millennium Medical College working on my Masters of Public Health in Field Epidemiology. We are doing a study to investigate whether or not the newly discovered virus, MERS-CoV, has been introduced in human population in areas where camels are reared in the country. I am now going to give you more information and invite you to be part of this study. Please ask me to stop as we go through the information if there is anything that you do not understand and I will take time to explain. You will also have time to answer questions at the end.

Purpose of the study

The novel coronavirus (MERS-CoV) which caused more than 600 deaths has been reported globally, since it was first discovered in September 2012. It is believed that the virus comes from an animal and camels are implicated to be the source of infection to human but it is not known how the virus gets from animals to humans. In Ethiopia, the presence of MERS-CoV antibody has been reported in camel population. However, there is no report on the status MERS-CoV in human population and circulating virus strain in the country. Understanding the current epidemiology of the virus at animal and human interface is critical to preventing and controlling the disease. Therefore, our study is primarily focused on people living in camel rearing areas to determine the status of MERS-CoV infection in human, to identify potential risk factors for zoonotic transmission of the virus in the country.

People in each of the study areas will be tested for the presence of antibodies against the virus, which will indicated that they have likely been infected with the virus at some time in the past. Learning about the kinds of exposures experienced by people who have had the infection may give us valuable clues about the source of the virus and the activities that result in infection. This information is extremely valuable as it will allow the responsible authorities to provide effective guidance to the public about how to reduce their risk of infection.

Type of Study

This study will involve your participation in a face to face interview that will take about 30 minutes. In addition, a small blood sample will be collected from your arm to test for signs of previous infection with the MERS-CoV. The results of blood test will only indicate previous infection and so will not be urgently shared with you. Blood specimens may be sent out of the country for testing at expert laboratories but will be coded so that only the study team can determine what specimen is yours. Blood specimens may be stored for additional testing in the future.

Voluntary Participation

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you choose to participate, you may change your mind at any time to stop participating in this study.

Procedures

If you agree to participate, you will be asked to answer some questions about your exposures to animals and activities in an interview with myself. If you do not wish to answer any of the questions during the interview, you may say so and I will move on to the next question. No one else but me will be present unless you would like someone else to be there. The information you share is confidential, and no one else except the principal investigator and the research sponsors will access to the information documented during your interview. A blood sample will be taken from you for testing, if you do not want to give a blood sample, however, we will not proceed with the interview.

Risks

The blood collection is very low risk as only a small amount will be taken. You may experience some minor discomfort or bruising at the site of collection.

Benefits

Your participation will help us learn more about the MERS-CoV in order to plan effective measures to prevent others from being infected.

Reimbursements

You will not be provided any payment to take part in the study. But, you will be reimbursed for your working time that you spent to participate in the study.

Confidentiality

The information that we collect from this study will be kept private. Any information about you will be identified by a number on it instead of your name. It will not be shared with or given to anyone except the principal investigator and the research sponsors**.**

Part II: Certificate of Consent

I have understood all information contained in the information sheet and this informed consent form. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Print Name of Participant_____

Signature of Participant _____

Date (DD/MM/YYYY) _____

Print Name of Informed consent provider_____

Signature _____

Date (DD/MM/YYYY) _____

Annex 2: Questionnaire for community based Cross-sectional seroprevalence study of MERS-CoV infection in human population (English Version)

Contact Information of Subject

Date of interview (dd/mm/yyyy): ____/____/____ Name of interviewer:_____

Participant Name: _____ ID Number: (I/HH/V/D):_____

Residence Region: _____Zone:_____District_____Village:_____

Gender (circle one): □ Male □ Female Age: ____ Occupation: _____

1. **General**

1.1. How long have you lived in this area? (in years) ___ ___

1.2. Do you own animals? □ YES □ NO

1.3. What animals do you raise on your farm (check all that are there)

□ Camels □ Goats □ Sheep □ Cattle □ Horses □ Donkey □ Chickens

1.4. Is your animals lived in/near to your house? □ YES □ NO

1.5. If not what is the address of your animals kept: _____

2. **Exposure**

2.1. Do you personally provide care for your camels? □ YES □ NO

2.2. On average, over the last 12 months, how often do you do the following activities?

With 1 = Never 2 = rarely (not even once a month) 3 = Monthly (at least once a month) 4 = Weekly (at least once a week) 5 = Daily

Activity	**Circle the number that is closest to how frequently you perform this activity per day**				
Touch animal	1	2	3	4	5
Clean Animal housing	1	2	3	4	5
Handel animal waste	1	2	3	4	5
Clean farm equipment	1	2	3	4	5
Slaughter animals	1	2	3	4	5
Assist in the birth of animals	1	2	3	4	5
Administer vaccines or medicines to animals	1	2	3	4	5

Milk animals	1	2	3	4	5
Milk Proce9ssing	1	2	3	4	5
Other	1	2	3	4	5

2.3. In the past 12 months, have you had direct contact with camels waste?

□ YES □ NO

2.4. If yes, how often are you in contact with it?

□ 1 = Never □ 2 = Rarely (not even once a month)

□ 3 = Monthly (at least once a month) □ 4 = Weekly (at least once a week) □ 5 = Daily

2.5. In the last 12 months are you aware of being in contact with any sick camels?

□ YES □ NO

2.6. If yes, do you know if any of the camels died of illness?

□ YES □ NO

2.7. In the last 12 months have you visited a live animal market?

□ YES □ NO

2.8. If yes, on average over the last 12 months, how frequently do you go to live animal markets?

□ Rarely (not even once a month) □ Monthly (at least once a month)

□ Weekly (at least once a week) □ Daily

2.9. Is there any cave around you area?

□ YES □ NO

2.10. If yes, How far it is from your home?---------KM

2.11. Did you visit the cave ?

□ YES □ NO

2.12. If yes, How often?

□ Rarely (not even once a month) □ Monthly (at least once a month)

□ Weekly (at least once a week) □ Daily

2.13. For what purpose do you visit the cave?_____

2.14. Have you seen Bats around your home?

□ YES □ NO

2.15. Did you have contact with bats?

□ YES □ NO

2.16. If yes, what type of contact? _____

3. Personal Protective Equipment and Hygiene Practices

3.1. What personal protective equipment do you usually wear when handling animals or their waste? (Check all that apply)

□ No protective equipment used □ Gloves □ Coveralls □ Dust masks

□ Boots or boot covers □ Respirators □ Eye protection (goggles, safety glasses)

□ Others:_____

3.2. How often do you usually wash your hands while staying at the farm (check all that apply)

□ At mealtimes □ Before and after each animal related task □ At bathroom times

□ The beginning and end of the day □ Rarely

4. Human Exposure

4.1. Have you visited any person sick with respiratory (cough, breathing problems) in the last 6 months?

□ YES □ NO

4.2. If yes what was your relationship to the person?

□ Close family □ Extended family □ Friend □ Other_____

4.3. In the past 12 months, have you drunk raw camel milk?

□ YES □ NO □ Don't Know

4.4. If yes, how frequently do you consume raw camel milk products?

□ Rarely(not even once a month) □ Monthly (at least once a month)

□ Weekly (at least once a week) □ Daily

4.5. In the past 12 months, have you eaten uncooked camel meat?

□ YES □ NO □ Don't Know

4.6. If yes, how frequently have you eaten uncooked camel meat?

□ Rarely (not even once a month) □ Monthly (at least once a month)

□ Weekly (at least once a week) □ Daily

5. Medical History

5.1. Are you sick today with Fever and cough? (If yes offer to take respiratory specimens)

□ YES □ NO

5.2. During the last 12 months have you seen a doctor for a respiratory illness (any of the following: cough, fever, runny nose, shortness of breath, rapid or shallow breathing, sore throat, vomiting after cough, or wheeze)?

□ YES □ NO

5.3. During the last 12 months have you had to stay overnight in a hospital for a respiratory illness (any of the following: cough, fever, runny nose, shortness of breath, rapid or shallow breathing, sore throat, vomiting after cough, or wheeze)?

□ YES □ NO

5.4. Are you currently a cigarettes smoker?

□ YES □ NO

5.5. If yes, on average how much do you smoke?

□ Not every day □ 1-2 times/day □ 3-10 times per day

□ 11-20 times per day □ more than 20 times per day

5.6. Do you have any of the following medical problems?

Medical Problem	**YES**	**NO**	**Don't Know**
Diabetes			
Asthma			
Chronic Lung Disease			
Kidney Failure			
Chronic Liver Disease			
High Blood Pressure			
Cancer			
Immune Deficiency			

6. Laboratory Result

6.1. ELISA □ Positive □ Negative

Annex 3: anti- MERS-CoV ELISA IgG test procedure

Preparation

Dilute sample 1:101 with sample buffer

Dilute wash buffer 1:10 with distilled water

Incubation

A, Sample Incubation

1. Transfer 100 microliters of the calibrator, positive control, negative control and diluted serum sample in to individual antigen coated micro plates. Incubate for 30 minutes at room temperature (18-25 ^{o}C)
2. Wash 3 times with diluted washing solution (450 microliters for each well in automated washer)

B, Conjugate Incubation

3. Pipette 100 microliters enzyme conjugate (peroxidase-labeled anti-human IgG) in to each micro plate wells. Incubate for 30 minutes at room temperature (18-25 ^{o}C)
4. Wash 3 times with diluted washing solution (450 microliters for each well in automated washer)

C, Substrate Incubation

5. Pipette 100 microliters chromogen/substrate solution in to each micro plate wells. Incubate for 15 minutes at room temperature (18-25 ^{o}C). protect from direct sun light

D, Stopping

6. Pipette 100 microliters of stop solution in to each micro plate wells in the same order and at the same speed as chromogen/substrate solution was introduced.

E, Measurement

Read at wavelength of 450 nanometers and a reference wavelength between 620 and 650 nanometers within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the micro plate to insure homogeneous distribution of the solution.