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Review

Public health risk and transmission route of Middle East respiratory syndrome (MERS): MERS coronavirus in dromedary camel

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Middle East respiratory syndrome corona virus (MERS-CoV) is a novel enzootic beta origin corona virus which was described in September 2012 for the first time. According to the reports of different researchers, the prevalence of MERS-CoV antibodies, during serological examination, is very high in dromedary camels of Eastern Africa and the Arabian Peninsula. However, the infection in camel is mostly asymptomatic. In contrast to the camel case, the clinical signs and symptoms of MERS-CoV infection in humans ranges from an asymptomatic or mild respiratory illness to severe pneumonia and multi-organ failure with an overall mortality rate of about 35%. Therefore, the objective of this paper was to review the public health risk, transmission routes of Middle East respiratory syndrome and to recommend the disease for further research. The identification of MERS-CoV RNA and viable virus from dromedary camels, including samples with respiratory symptoms by different studies have been detected especially in isolation of identical strains of MERS-CoV from epidemiologically linked humans and camels are the suggestive evidence for inter-transmission of the virus, primarily from camels to humans and its public health risks. Though inter-human spread within health care settings is responsible for the majority of reported MERS-CoV human cases, the virus is currently incapable of causing sustained human-to-human transmission (pandemic occurrence). Phylogenetic studies and viral sequencing results strongly suggest that MERS-CoV originated from bat ancestors after evolutionary recombination process, primarily in dromedary camels in Africa, before its exportation to the Arabian Peninsula through the camel trading routes. Currently, there is no specific drug or vaccine available for treatment and prevention of infections due to MERS-CoV in patients. The important measures to control MERS-CoV spread are strict regulation of camel movement, regular herd screening and isolation of infected camels, use of personal protective equipment by camel handlers and awareness creation on the public, especially in African pastoralists where consumption of unpasteurized camel milk is common. Therefore, urgent global epidemiological studies are required, especially in the poor camel rearing African countries to understand the transmission patterns and the human cases of MERS-CoV and also for the proper implementation of the above mentioned control measures.

Key words: Dromedary camel, epidemiology, MERS-CoV, public health, respiratory syndrome.

INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel lineage C beta corona virus derived from bats which can cause acute viral respiratory disease in humans (WHO, 2014a) and in camels (also known

as camel flu) (Richard, 2015). Dromedary camels in the Middle East have high sero prevalence for MERS-CoV and MERS-CoV RNA has been consistently detected in these animals, especially in settings such as camel abattoirs, where camels from multiple origins are assembled, but the exact source of infection in camels has not been identified. More than 60% of the global population of dromedary camels is distributed in African countries and some of these countries are important camel exporters to the Arabian Peninsula (Faye and Bonnet, 2012). MERS-CoV antibodies were also found with high prevalence in dromedary camels in these African countries with positive rates higher than 80% for the animals in Egypt, Ethiopia, Nigeria and Sudan, and 30 to 54% in Tunisia (Reusken et al., 2014).

According to WHO (2015c), there were 1,618 laboratory-confirmed cases of MERS reported with 579 human deaths and camels are believed to be involved in its spread to humans but its way of transmission is unclear (Zumla et al., 2015). This disease in human is zoonotic in origin, although clusters of human-to-human transmission have been reported, especially in health care or family settings (WHO, 2015a). However, this human zoonotic disease has so far been reported only from countries in the Middle-East, the reason for the absence of zoonotic disease report from Africa is unclear (Chu et al., 2014) and it should be of great concern to researchers.

In humans, the virus is graver and opportunistic pathogen associated with the death rate of 40% from infection cases report. It is yet to be established whether infections thought to have been acquired from an animal origin produce a more severe outcome than those spread between humans (WHO, 2013a). Therefore, the objective of this paper was to review the public health risk, transmission routes of Middle East respiratory syndrome and to recommend the disease for further research.

PUBLIC HEALTH RISK AND DESCRIPTIONS OF MERS CORONA VIRUS

History and origin

The first zoonotic introduction of a corona virus into the human population occurred by the severe acute respiratory syndrome corona virus (SARS-CoV) in 2002. According to WHO (2003), SARS-CoV causes a world pandemic, outbreak with 8,400 recorded infection cases and 800 deaths, similarly MERS-CoV marks the second known zoonotic introduction of a highly pathogenic of evidence currently support this theory: the very close phylogenetic similarity with the bat *Beta corona viruses* such as, BtCoV-HKU4 and BtCoV-HKU5 (van-Boheemen et al., 2012); closely related corona virus sequences have been recovered from bats in Africa, Asia, the Americas and Eurasia. Therefore, MERS-CoV uses the evolutionary conserved dipeptidyl peptidase-4 (DPP4) protein in *Pipistrellus pipistrellus* bats for cell entry (Raj et al., 2013).

Middle East respiratory syndrome in human was first identified in 2012, in Saudi Arabia and more than 1000 infection cases of the disease have been reported in May, 2015 and about 40% of those who were infected died due to the disease (Zaki et al., 2012), accordingly, most cases have occurred in the Arabian Peninsula (Zumla et al., 2015). HCoV-EMC/2012 is a strain of MERS-CoV that is detected in the first infected person in London in 2012, which was found to have a 100% identical viral sequencing to the strain identified in Egypt from tomb bats (Zaki et al*.,* 2012); this result is suggestive that bats are the primary origin of MERS-CoV.

Etiology

Middle East respiratory syndrome is caused by the newly identified MERS corona virus (MERS-CoV), with single-stranded RNA belonging to the genus beta corona virus which is distinct from SARS corona virus and the common-cold corona virus (Saey, 2013). According to Eckerle et al*.* (2014), MERS-CoV uses the DPP4 (CD26) receptor to gain entry and effectively replicate in camel cell lines and neutralizing antibodies for MERS-CoV have been detected in dromedary camels from Africa and Middle East (Milne- Price et al*.*, 2014).

Different studies suggested that the mean incubation period of the virus is 5 to 6 days, it will take 13 to 14 days to show clinical illness in infected person and to subsequently spread to another person. In progressively diseased patients, death can occur within 11 to 13 days, sometimes it will range from 5 to 27 days (Chan et al., 2014; Ki, 2015).

Pathogenesis and clinical sign

The disease in human initially showed simple respiratory problem with mild fever, chills, muscle ache and respiratory stress (shortness of breath). Then it leads to severe pneumonia within 2 to 3 days after infection progressively after some period of time it will develop into severe acute respiratory syndrome (SARS) or acute corona virus, probably originating from bats. Three lines

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respiratory distress syndrome (ARDS). In later cases, MERS-CoV causes clinical symptoms of upper and lower respiratory tract infections specific signs and symptoms including high-grade fever, non-productive cough, dyspnea, headache, myalgia, nausea, vomiting, and diarrhea that may precede the respiratory symptoms (Guery et al., 2013). Finally, death may occur in almost half of the cases, especially in unmanaged patients, more death rate will occur (Abdullah et al., 2013).

Laboratory result on admission of patients indicates leukopenia, lymphopenia, thrombocytopenia, and elevated lactate dehydrogenase levels (Assiri et al., 2013). MERS-CoV can also cause severe pneumonia with acute respiratory distress syndrome (ARDS), requiring mechanical ventilation and intensive care admission (Drosten et al., 2013). Incontrast to the human cases, camel showed minor clinical signs of the disease, including of rhinorrhea and a mild increase in body temperature but no other clinical signs were observed (Ahsa, 2013) and the nasal discharge drained from both nostrils varied in character from serous to purulent (Daniella et al., 2014)

Diagnosis

The definitive diagnosis of cases of coronavirus infection basically rely on advanced diagnostic perceptions like the detection of unique sequences of viral RNA by real-time reverse-transcriptase polymerase chain reaction (RTPCR) and immunofluorescence because the disease has no pathognomonic clinical manifestation both in human and in camel (WHO, 2013b). However, antibodies against beta coronaviruses are identified to cross-react within the other species of the genus due to their characteristic antigenic similarity. Therefore, immunofluorescence antibody tests effectively limit their use to confirmatory diagnosis of the disease (Memish et al., 2014).

Throat swabs, urine, faeces and serum samples were collected from wild bats in Saudi Arabia including the area where the first MERS-CoV patient had reported and worked, this study result indicate that, 227of the samples was found positive for nucleotide fragment of the RNA-dependent RNA polymerase region of MERS-CoV genome from the examined different 1003 samples; this result suggest that bats are the evolutionary origin of the virus for camel and human infections (Memish et al., 2013).

EPIDEMIOLOGY OF THE DISEASE

Distribution

According to WHO (2014b) official report, 707 infected patients were identified in 21 countries from three continents of the world. From those detected human cases, 252 patients have died due to MERS-CoV, indicating the case fatality rate of the disease to be about 35% (Drosten et al., 2013). The infection so far has been acquired either directly through a probable zoonotic source, or as a result of human to human transmission through close contacts (Hijawi et al., 2012). An unexplained observation has shown the seasonal variation in the number of reported cases, with a peak occurrence from April to June of each year. The number of cases reported during April, 2014 alone was alarming, because it was greater than the cumulative number of cases reported since the outbreak began (http://coronamap.com, 2014). The possible justification for the seasonal variation of camel case reports may be that it coincides with camel breeding season, and younger camels seem to be more often infected than older camels in the herds (Holmes et al., 2014). Human cases have also been reported in Kuwait, Yemen, Oman, Iran, Lebanon, Tunisia, Algeria, Bangladesh, Malaysia, even in non-camel rearing countries like France, Italy, Germany, the Netherlands, United Kingdom, Greece, Italy and United States (WHO, 2014c), which is suggestive of the presence of other probable intermediate hosts in addition to camel. Furthermore, sero positive camels for MERS-CoV antibodies were identified in Egypt, Kenya, Nigeria, Tunisia and Ethiopia (Figure 1), suggesting that there may be MERS-CoV human cases that are undetected in Africa (Reusken et al., 2014).

Species affected, reservoirs and route of transmission

The disease due to MERS-Cov is thought to be zoonotic because of its close genetic similarity to bat corona viruses (Karesh et al., 2012). Identical strains with human MERS-Cov have been isolated from camels in Egypt, Qatar and Saudi Arabia and MERS-CoV antibodies have also been detected in camels of Africa and the Middle East (Memish et al., 2014). According to Reusken et al. (2013), camels are thought to be a source of human infection, this is because genetic sequence similarities have been determined between human and camel corona viruses (Memish et al., 2014). Transmission from dromedary camels to humans has been shown with RT-PCR and viral genome sequence analysis, molecular detection and phylogenetic analysis have been also performed to investigate transmission (Azhar et al., 2014). Other animals, such as goats, cows, sheep, buffalo, pigs and wild birds have been tested for MERS-CoV antibodies, but no positive findings have been reported, these investigation results provide evidence that camels are a primary probable source of the MERS-CoV zoonotic infection for humans (Reusken et al., 2013). Bats are known natural reservoirs for several

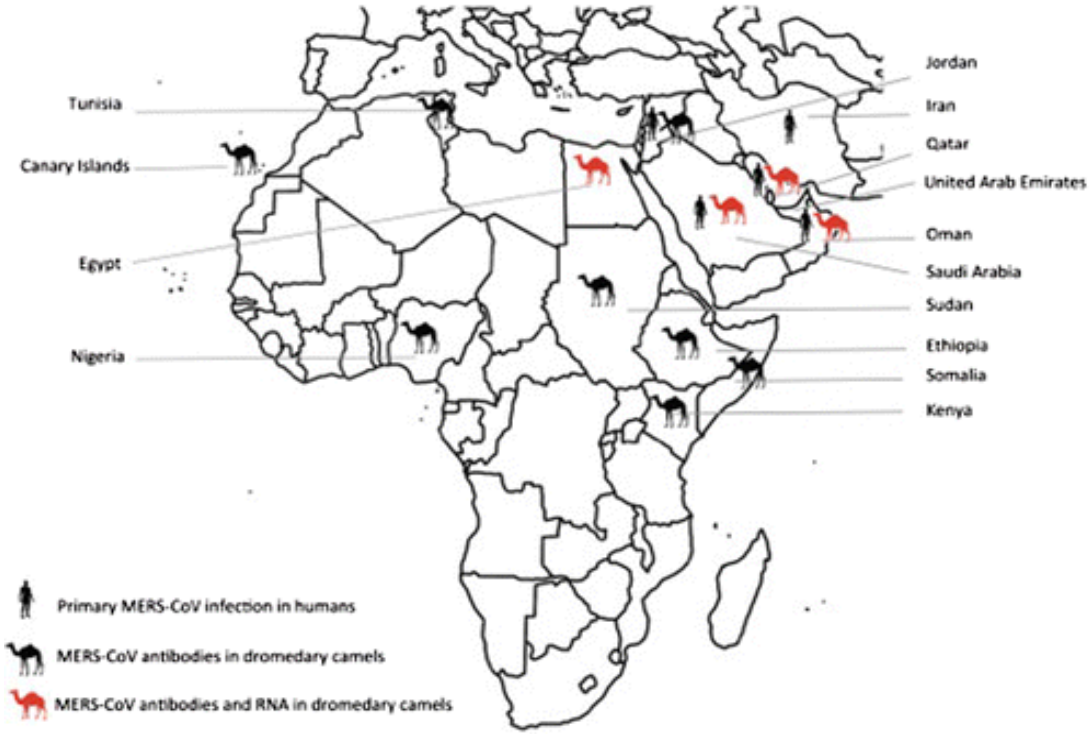

Figure 1. Distribution of human MERS-CoV cases and prevalence of MERS-CoV based on serological and RNA detection in dromedary camels (Source: Taylor and Francis, 2016).

emerging viral infections in humans including rabies, Nipah virus, Hendra virus and Ebola virus (Han et al., 2015). Due to their extremely diverse species with a long evolutionary history, bats have co-evolved with a variety of viruses and due to their lack of B-cell-mediated immune responses which allows them to carry viruses without showing overt clinical signs of viral infections (Dobson, 2005; Karesh et al., 2012). As a result of their low metabolic rate and suppressed immune response during bats' hibernation time, there is delayed viral clearance, which make them the potential reservoirs for different viruses (George et al., 2011). Generally, species affected by Middle East respiratory syndrome and used as source of infection are *Homo sapiens* (human), camels and bats (Augustina et al., 2013).

MERS-CoV is a zoonotic virus that is transmitted from animals to humans (WHO, 2015b). The route of transmission from animal to human is not fully understood(Zumla and Memish, 2014), but researchers hypothesize that camels are a likely reservoir host for MERS-CoV (Figure 2) and most likely, camels are the animal which is source of infection for humans (Saad and Said, 2011).

In some areas of the Arabian Peninsula, the consumption of unpasteurized milk is common and, in some cultural practices of the region, camel milk and urine are consumed for their believed medicinal effects (Saad and Said, 2011). Researchers have proved that MERS-CoV in milk can survive for prolonged periods (van-Doremalen et al., 2014). Contact with a MERS-CoV-infected animal, such as a dromedary, or its bodily fluids may be responsible for zoonotic transmission to humans, but investigations continue to better clarify this question (Aburizaiza et al., 2014).

Transmission of MERS-CoV from human-to-human occurs similarly to other corona viruses. It is believed that it is transmitted by contact with the respiratory secretions of an infected individual that are aerosolized by coughing or sneezing (WHO, 2015a). Many of those infected with MERS-CoV were associated with healthcare settings such as hospitals, but the virus does not have a chance to pass easily from human to human if there is not direct close contact with the infected individual (Oboho et al., 2014).

Group at risk

Studies on MERS-CoV genetic sequences from humans and camels in Egypt, Oman, Qatar and Saudi Arabia point out a close similarity between the virus identified in camels and that detected in human in the same geographic area.

These and other studies also have found MERS-CoV antibodies in camels in Africa and the Middle East and also, preliminary results from an ongoing investigation in Qatar show that people working closely with camels (e.g. farm workers, slaughterhouse workers and veterinarians)

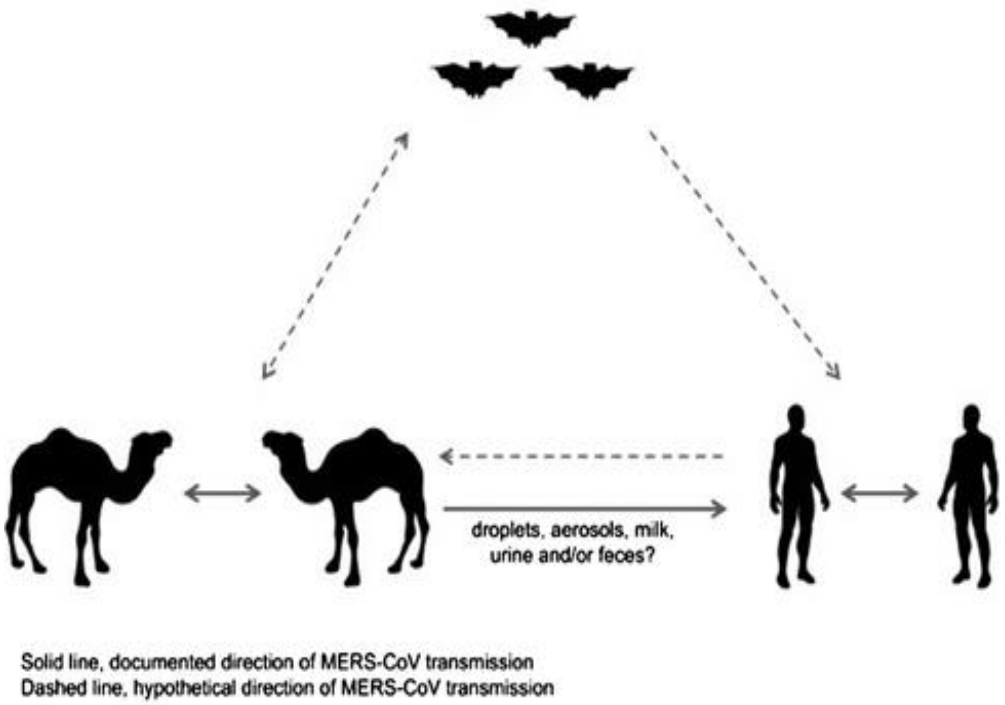

Figure 2. Reported Infection sources and transmission routes of MERS-CoV between bats, camels and human (Source: Taylor and Francis, 2016).

may be at higher risk of MERS-CoV infection than people who do not have regular close contacts with camels and, also health care workers (WHO, 2014d).

MERS coronavirus in dromedary camel

According to Informer East Africa (2016), many outbreak cases of MERS in camels of Kenya is reported and more than 500 camels died due to the disease. An investigation, using real time polymerase chain reaction (RT-PCR) identified MERS-CoV prevalence rate of 3.6% in apparently healthy camels in a slaughterhouse of Egypt. Other research report also indicate that 92% of examined sera collected from camels were reactive to MERS-CoV antibodies and the camels that tested positive were all imported from either Sudan or Ethiopia (Chu et al., 2014).

Studies conducted from 2010 to 2013, in which incidence of MERS was examined in 310 dromedary camels, indicate high titers of neutralizing antibodies to MERS-CoV in the collected serum sample of these animals (Hemida et al*.,* 2013).

A further investigation was conducted on sequenced MERS-CoV on nasal swabs of dromedary camels in Saudi Arabia, result indicate that, MERS-CoV in camels had sequenced exact similarity with previously sequenced human isolates (Briese et al., 2014). The prevalence of MERS-CoV antibodies is significantly higher in older camels when compared with those aged two years or less (Alagaili et al., 2014), which indicates MERS-CoV have been found circulating in dromedary camels for more than 20 years in camel rearing in middle east and African countries (Corman et al., 2014). In addition to the high total prevalence of MERS CoV serum antibody in dromedary camels from Kenya and other African countries, 14.28% of MERS-CoV sero prevalence in dromedary camels from the Canary Islands were also reported by Hemida et al*.* (2014), this result also included three positive camels that were imported from Morocco to the Canary Islands even though there is no current report from Morocco on the disease.

In contrast to the Middle East and African camel infection prevalence report, currently, there is no published report on MERS-CoV antibodies detection in dromedary camels from Australia, Canada, the United States of America, Germany, the Netherlands or Japan, which will be a research question for researchers indicating that further global epidemiological study on the disease is mandatory.

The investigated high sero prevalence of MERS-CoV in dromedary camel of Africa and the Middle East (Table 1) suggests that animal movement has facilitated the transmission and circulation of MERS-CoV (Briese et al*.,* 2014).

Which is also suggestive of the risk of emerging new outbreak in those countries currently free from MERS-CoV.

Table 1. Sero prevalence of MERS-CoV antibodies in dromedary camel in different countries reporting the disease.

Country	Number of camel tested	Prevalence (%)	Year
Saudi Arabia	9	100	2013
UAE	8	100	2015
Oman	50	100	2015
Jordan	11	100	2013
Qatar	105	97	2014
Egypt	110	98.2	2013
Tunisia	158	54	2009
Ethiopia	31	93	2010-2011
Nigeria	358	94	2010-2014
Somalia	86	83.7	1983-1984
Sudan	60	86.7	1984
Kenya	774	29.5	1992-2013
Canary Island	105	14.3	2013

Source: (Taylor and Francis, 2016).

Prevention and control

Understanding the route of transmission of MERS-CoV and its pattern of transmission is important for proper implementation control and prevention of the disease. Currently, as mentioned above, the pattern of the disease transmission will dictate the methods for prevention (Figure 2). The WHO advises people at risk of MERS-CoV infection to avoid contact with camels, to practice good hand hygiene, and to avoid drinking raw milk or eating contaminated food unless it is properly washed, peeled or cooked (WHO, 2014a). Since most of the cases occur in the health care setting, it is thoughtful that all health care workers practice appropriate infection control measures when taking care of patients with suspected or confirmed MERS-CoV (WHO, 2014b).

Currently, there is no specific drug or vaccine available for treatment of infection caused by MERS-CoV. Therefore, medical care is focused on supportive treatment. Even though, a number of antiviral medicines are currently under study (Zumla et al*.,* 2015), there is no licensed vaccine to prevent MERS-CoV infection. However, one company has developed an experimental candidate MERS-CoV vaccine (Novavax, 2013). Almazán et al. (2013) also developed other candidate vaccines which are being studied as full-length infectious cDNA clone of the MERS-CoV genome in a bacterial artificial chromosome.

CONCLUSION

MERS-CoV is a zoonotic emerging disease with bats and dromedary camels as the important animals mainly involved as source for its emergence, outbreak and epidemiological pattern. Transmission of MERS-CoV from camel to human is well documented and studied by different researchers but is generally not very efficient because transmission route of the virus back from human to camel is still hypothetical. The exact mechanism of transmission is not clear, including whether other intermediate hosts are involved, which will be a risk for new incidence of the disease, especially for those countries in which infection cases were not reported. Currently, the most applicable and commonly used control measures of MERS-CoV spread are strict regulation of camel movement, regular herd examination and isolation of positive camels. Thus, case-control studies of humans with MERS-CoV infection are needed to identify important risk factors and possible chains of transmission from camels and other animals and animal products which are sources of human infections. Similarly, urgent epidemiological studies and molecular detection like sequencing of viral RNA by real-time reverse-transcriptase polymerase chain reaction of MERS-CoV are mandatory to better understand the transmission patterns of MERS-CoV both in human and camel samples, in camel rearing African countries like Ethiopia where camel dependent poor pastoral communities are found, even though current human cases are not reported.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Epidemiological investigation of Middle East respiratory syndrome corona virus (mers-cov) among dromedary camels in selected areas of Afar and Oromia region, Ethiopia

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A cross-sectional study was conducted between October 2014 and May 2015, to determine the sero-prevalence, assess the presence of active cases through isolation and identify possible risk factors for the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) in camels, in selected areas of Oromia and Afar regional states of Ethiopia. A total of 569 dromedary camel sera were collected and screened with two serological tests: Pseudo particle neutralization for screening and Micro-neutralization test for confirmation. The overall prevalence of MERS-CoV in the study area was 93% (n=529). Higher prevalence (93.9%) was recorded in female dromedary camels compared to males (91.3%) but the difference was not significant (Chi-Square=1.323 and P=0.250). Age wise prevalence was higher in adult camels (94.6%) than young ones (90.8%), however the difference was statistically insignificant (Chi-Square=3.068 and P=0.080). Similarly, higher prevalence was recorded in larger herds (93.4%) than small herds but the difference observed was not statistically significant (Chi-Square=0.220 and p=0.639). Also, no significant (Chi-Square 0.525 and P=0.469) difference was observed in prevalence between the two regions (Oromia=93.3% and Afar=93.3%). However significant difference was observed in the lower administrations of the regions (Zones, District and Kebeles) in which the highest prevalence recorded in East Shoa, Fentale district of Adis Ketema kebele (99.3%). From total of 100 swabs collected, MERS-CoV was detected in seven (1 Fentale, 4 Amibara and 2 Dubti) districts by Real-time reverse transcription polymerase chain reaction (RT-PCR). Generally, this study showed the existence of high seroprevalence of MERS-CoV among Ethiopian dromedary camels, which was also confirmed by RT-PCR. Therefore further study is required to determine its significance from both animal and public health perspectives and further research should focus on identifying similarity between MERS-CoV viral isolates in neighboring countries and clinical isolates from the Middle East and elsewhere.

Key words: Dromedary camel, MERS-CoV, serum, swab, Real-time reverse transcription polymerase chain reaction (RT-PCR), Ethiopia.

INTRODUCTION

More than 60% of the world population of one-humped dromedary camels (Camelus dromedarius) lives in the Greater Horn of Africa. Their role as one of the most important livestock species for nutrition in arid and semi-arid areas of Eastern Africa is likely to increase since the predicted climate change is in favour of this drought resilient species. Camel milk, for domestic consumption or sale, is often the most important product for female and male pastoralists alike (Marshall et al., 2014). In 2012, a novel coronavirus called Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged on the Arabian Peninsula. Human cases have been reported from 25 countries, with the most recent outbreak in the Republic of Korea (which itself originated in the Middle East). MERS-CoV has caused at least 1,200 laboratory confirmed cases of severe respiratory infection, including more than 400 deaths. Several studies have demonstrated that dromedary camels can act as a source of human MERS-CoV infection (Reusken et al., 2015).

In June 2012, a novel beta corona virus, associated with severe respiratory disease in humans, emerged in the Middle East (Zaki et al., 2012), which is closely related to beta corona viruses, circulating in bats (Anthony et al., 2013). The first isolate of Middle East respiratory corona virus (MERS-CoV), HCoV-EMC/2012, was obtained from a patient with a fatal pneumonia and acute renal failure. To date, 107 additional human cases have been identified, of which 49 were fatal (WHO, 2013). Aside from cases in Saudi Arabia, Qatar, Jordan, and the United Arab Emirates, imported cases have been identified in the United Kingdom, Germany, France, Tunisia, and Italy.

Although no information is available on the source or route of primary transmission of MERS-CoV, human-to-human transmission has been recorded (Assiri et al., 2013). The severity of disease distinguishes MERS-CoV from other corona viruses, circulating in the human population HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV HKU1, which are generally associated with upper respiratory tract infections. Instead, MERS-CoV appears to be more similar to the severe respiratory disease, caused by severe acute respiratory syndrome (SARS)-CoV. *In vitro* studies have shown that MERS-CoV replicates efficiently in non-ciliated cells in the primary human airway epithelium (Kindler et al., 2013), and in *ex vivo* human lung cultures, MERS-CoV replicated in bronchial, bronchiolar, and alveolar epithelial cells (Chan et al., 2013). The MERS-CoV virus is thought to be an animal virus that has sporadically resulted in human infections, with subsequent limited transmission between humans. The evidence for the animal origin of the virus is circumstantial.

Nevertheless, the alternative explanation to explain sporadic appearance of severe human cases with long periods of time between them, and the wide geographical area over which the virus was apparently distributed, is unrecognized ongoing transmission in people. Surveillance efforts, since the discovery of the virus and retrospective testing of stored respiratory specimens suggest this is not the case (Gautret et al., 2013). In August, 2013, the possible source of MERS-COV virus was traced to an Egyptian tomb bat found in a building, in close proximity to an index patient home. The virus genome fragment isolated from the bat was a 100% genetic match to the virus, isolated from the index patient (Jobs, 2013). There had been speculation that, bats might be the source of the virus (Abedine and Saad, 2013). On 9th August, 2013 a report in the journal “The Lancet Infectious Disease” showed that 50 out of 50 (100%) blood serum from Omani camels and 15 out of 105 (14%) from Spanish camels had protein specific antibodies against MERS- COV spike protein and also in Egypt (between 94 and 98% of a total of 110 camels tested positive). A further study on dromedary camels from Saudi Arabia published in December 2013, revealed the presence of MERS–COV in 90% of the evaluated dromedary camels (310), suggesting that dromedary camels not only could be the main reservoir of MERS–COV, but also the animal source of MERS–COV (Hemida et al., 2013).

The Arabian Peninsula remains the site of primary human infection from the zoonotic source of human cases as reported so far and dromedary camels in other parts of North, West and East Africa appear to have serological evidence of MERS infection. The full geographic range of MERS CoV circulation has not been established so far in the above sites including Ethiopia. Sero-prevalence of MERS-CoV in Ethiopia was 97% in adult camels, 93% in young camels and overall prevalence of 96% (Reusken et al., 2013). Therefore the objectives of this study were to determine the seroprevalence of MERS-CoV, to assess the presence of active cases through isolation of MERS-CoV and to identify possible risk factors for theMERS-CoV in

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the study areas.

MATERIALS AND METHODS

Study area and animals

This study was conducted between November 2014 and May 2015 in pastoral areas of Afar (Dubti, Ayssaita, Chifra, Ewa and Amibara districts) and Oromia (Yabello and Fentale districts) regions where camels are populated. Camels of all age and both sexes were randomly selected and included in the study for determination of antibody prevalence against MERS-COV. For the purpose of virus isolation and characterization, nasal swab was taken from 100 camels (Yabelo, Amibara, Fentale and Dubti), 25 from each district. Geographically, the Afar Regional state is located in the northeastern part of Ethiopia. The total geographical area of the region is about 270,000 km^2 (CSA, 2008). It is geographically located between 39°34' and 42°28' East Longitude and 8°49'and 14° 30'North Latitude. The region shares common international boundaries with the State of Eritrea in the north-east and Djibouti in the east, as well as regional boundaries with the Regional States of Tigray in the north-west, Amhara in the south-west, Oromia in the south and Somali in the south-east. Afar is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bi-modal throughout the region with a mean annual rainfall below 500 mm in the semi-arid western escarpments, decreasing to 150 mm in the arid zones to the east. Afar is increasingly in drought prone and the region receives three rainy seasons. The main rain karma accounts for 60% of annual rainfall and occurs from mid-June to mid-September. This is followed by rainy showers in mid-December called dadaa and a minor rainy season during March to April called sugum. Disruptions on the performance of any rainy season will impact on the availability of pasture and water as well as the overall food security situation of the pastoral and agro-pastoral communities (ANRSP, 2010).

Study design and sampling technique

A cross sectional study was conducted for serological investigation of MERS-CoV among dromedary camels found in the study area. Multi stage cluster sampling technique was used to select specific cluster (kebele) for all sites found in the study areas. Finally samples were taken randomly from each selected cluster. Dromedary camels found in Afar and Oromia region of both age groups (young 1 to 3 years and adults > 3 years of age), both sex groups (male and female) and two categories of herd size (large herd size=herd having above 50 camels and small herd size = herd having less than or equal to 50 camels) were included in the study.

Sample size

Based on Thrusfield (2007) formula, the sample size of study animals was calculated.

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

$$n = \frac{1.96^2 (0.96) (1 - 0.96)}{0.0025}$$

n = 59 and the sample size multiplied by the cluster number to get the final sample size 59 x 8 sites = 472 (Total sample size required).

But additional 97 samples were taken to increase the precision of the study and the final sample size became 569. Where n = required sample size, Pexp = expected prevalence (96%) and d = desired absolute precision (95%)

Sample collection, transportation and storage

The samples were taken from randomly selected dromedary camels from each cluster (kebeles) of randomly selected Zones and woredas of Afar and Oromia. Then 10 mm of blood sample was collected from jugular veins of the animals using needle and plain test tube (without anticoagulants). The blood was allowed to clot for2 h at room temperature, stored overnight at 4°C and then, serum was separated from the clot standing for 20 min at room temperature, to allow for clot formation.

Serum was separated from the clot by centrifugation at 3000rpm for 10 min and transferred to crayovial tubes. Then the separated serum was labeled and kept under refrigeration (–20°C) until transported to National Veterinary Institute (NVI) for storage, before taken to the University of Hong Kong for analysis.

The collected 569 sera samples were transported in the icebox to National Veterinary Institute serology laboratory and kept under refrigeration (–20°C) until transported to University of Hong Kong for serological analysis. Hundred swabs were collected by using cotton swab from randomly selected sites (Borena and Metehara) from Oromia region (Dubti and Melka werer). Twenty five swabs were taken from each site mentioned above. The swabs were preserved in buffer solution until transported to NVI virology section for deep freezing at -80°C. Finally the swabs transported to Hong Kong University for analysis.

Laboratory analysis

The blood samples collected were analyzed by two serological tests MERS-CoV spike, pseudoparticle neutralization assay (screening test) and Micro neutralization tests (confirmatory test).

The swabs collected undergo extraction of DNA and Polymerase Chain Reaction (PCR) to identify the presence of MERS-CoV in the sample.

Serology

MERS-CoV spike pseudo particle neutralization assay

A codon-optimised spike gene was designed according to published MERS-CoV genome sequence (GenBank accession number: JX869059.1), synthesised by GeneCust (Luxembourg) and subcloned into pcDNA3.1+ vector to generate pcDNA-S. To produce HIV/MERS spike pseudoparticles, 10 µg pNL Luc E- R- and 10 µg pcDNA-SS were co-transfected into 4x106 293T cells. Supernatants of transfected cells were harvested 48 h later and quantified for HIV p24 viral protein using a p24 ELISA Kit (Cell Biolabs, San Diego, United States).

For ppNT assay, HIV/MERS pseudoparticles containing 5 ng p24 were used to infect Vero E6 cells (ATCC CRL-1586) in a single well (96-well plate format; 1x104 cells/well). Infected cells were lysed in 20 µl lysis buffer and 100 µl of luciferase substrate at 2days postinfection (Promega Corporation, Madison, United States).

Luciferase activity was measured in a Microbeta luminometer (PerkinElmer, Waltham, United States). For ppNT, HIV/MERS pseudoparticles (5 ng of p24) were pre-incubated with serially diluted sera for 30 min at 4°C and then added to cells in triplicate. Residual virus replication was assayed at 2days post infection, as described above. The highest serum dilution giving a 90% reduction of luciferase activity was regarded as the ppNT antibody titre.

Viruses and virus titration

MERS-CoV (strain EMC) virus was obtained from Dr R Fouchier (Erasmus University Medical Center, Rotterdam, and the Netherlands). SARS-CoV (strain HKU-39849) was taken from the virus repository at Hong Kong University. Virus stock for MERS-CoV was prepared in Vero cell culture (ATCC CCL-81) in minimal essential medium containing 2% fetal bovine serum, 100 units/mL penicillin and 100 µg/mL Streptomycin. Virus aliquots were stored at -80°C. Virus was titrated in serial half-log10 dilutions (from 0.5 log to 7 log) to obtain 50% tissue culture infectious dose (TCID50) on 96 well tissue culture plates of Vero cells. The plates were observed in a phase contrast microscope for cytopathic effect (CPE) daily, for three days. The endpoint of viral dilution leading to CPE in 50% of inoculated wells was estimated, by using the Reed Muench method and designated as one TCID50. SARS-CoV was grown and titrated in the same manner with the exception that Vero E6 cells (ATCC CRL-1586) were used.

Micro-neutralization tests

Serial two fold dilutions of heat inactivated sera (56°C for 30 min) were made, starting with a dilution of 1:10. The serum dilutionswere mixed with equal volumes of 200 TCID50 of MERS-CoV or SARS-CoV as indicated. After 1 h of incubation at 37°C, 35 µL of the virus serum mixture was added in quadruplicate to Vero or Vero-E6 cell monolayers for MERS-CoV and SARS-CoV, respectively, in 96 well microtiter plates. After 1 h of adsorption, an additional 150 µL of culture medium were added to each well and the plates was incubated for three more days at 37°C in 5% CO_2 in a humidified incubator. A virus back-titration was performed without immune serum to assess input virus dose. CPE was read at three days post infection. The highest serum dilution that completely protects the cells from CPE in half of the wells was taken as a neutralizing antibody titre and was estimated using the Reed-Muench method. Positive and negative control sera were included to validate the assay.

Extraction of RNA and Polymerase Chain Reaction (PCR)

Real-time reverse transcription PCR (RT-PCR) targeting upstream of E gene of MERS-CoV was used for screening. The open reading frame (ORF) 1a gene was used for confirmation as recommended by the World Health Organization. We also used a previously described pan-CoV nested PCR, targeting the viral RNA-dependent RNA polymerase (RdRp) region (*13*), and PCR products were analyzed by sequencing (online Technical Appendix, wwwnc.cdc.gov).
EID/article/20/6/14-0299-Techapp1.pdf). We detected MERS-CoV RNA in (n=7/100) (7%) of 100 nasal swab specimens from dromedary camels with the upstream of E gene assay (cycle threshold [Ct] 23.2–36.8), confirmed by the ORF1a assay (Ct 23.2–39.1), fulfilling the World Health Organization criteria for diagnosis of MERS-CoV infection. PCR was repeated from a fresh RNA extract to confirm positive results.

Data analysis

Data obtained from the investigations was coded and stored in Excel spread sheets. The independent variables age, sex, herd size and origin (kebele) of the dromedary camels have been compared to evaluate their effect on the magnitude of the disease. The collected data was analyzed using SPSS version 20 software. Descriptive statistics was used to determine the frequency of proportion (prevalence) and chi square, and logistic regression was used to check the association between the sero-prevalence and the independent variables. The precision level used was 5% with the level of significance differences set at $p < 0.05$, chi square(x^2) > 3.841 for association and logistic regression with odds ratio >1 for the magnitude of the risk(effect) of exposure variables.

RESULTS

Seroprevalence of MERS-CoV

From the total of 569 camel sera tested, 93% (n=529/569) were positive for MERS-COV specific antibody with MERS-CoV spike pseudoparticle neutralization assay and Microneutralisation tests. Out of 238 sera tested from young Dromedary, camels (n=216/238) (90.8%) were positive and from 331 adult, camels (n=313/331) (94.6%) were also positive and the difference seen was not significant (Pearson Chi-Square=3.068 and P=0.080) (Table 1).

From 569 dromedaries camel sampled 361 were Female and 208 were Male. (n=339/361) (93.9%) and (n=190/208) (91.3%) of Female and Male were positive respectively. The difference seen between the two sex groups was insignificant (Pearson Chi-Square=1.323 and P=0. .250) (Table 2).Out of 222 Camels tested from small herd sized group (n=205/222) (92.3%) were positive and from 347 large herd sized camels (n=324/347) (93.4%) were positive but the difference observed was not statistically significant (Pearson Chi-Square=0.220and P=0.639 (Table 3).Out of 230 camels tested from Oromia region (n=216/230) (93.9%) were positive and 339 samples from Afar (n=313/339) (92.3%) were positive but the difference was not statistically significant (Pearson Chi-Square =0.525 and P=0.469) (Table 4).

Among Zones included in the study highest prevalence recorded in East Shoa (n=136/137) (99.3%) and the lowest at Borena (n=80/93) (86.0%).The prevalence difference among Zones was highly statistically significant (Pearson Chi-Square=24.285 and P=0.000) (Table 5). Out of 7 districts included in the study the highest prevalence was observed in Fentale (n=136/137) (99.3%) and the lowest in Yabello (n=80/93) (86.0%) with

Table 1. Seroprevalence of MERS-CoV in the two age groups (Young and adult dromedary camels).

Variable	Negative	Positive	Total
Young	22(9.2%)	216(90.8%)	238
Adult	18(5.4%)	313(94.6%)	331
Total	40(7%)	529(93%)	569
	Pearson Chi-Square=3.068	df=1	P=0.080

Table 2. Sero prevalence of MERS-CoV in the two sex groups of dromedary camels.

Variable	Negative	Positive	Total
Female	22(6.1%)	339(93.9%)	361
Male	18(8.7%)	190(91.3%)	208
Total	40(7%)	529(93%)	569
	Pearson Chi-Square=1.323	df=1	P=0. .250

Table 3. Sero prevalence of MERS-CoV in the herd size categories of dromedary camels.

Herd Size	Negative	Positive	Total
Small	17(7.7%)	205(92.3%)	222(100%)
Large	23(6.6%)	324(93.4%)	347(100%)
Total	40(7%)	529(93%)	569(100%)
	Chi-Square=0.220	df=1	P=0.639

Table 4. Sero prevalence of MERS-CoV in the two regions (Afar and Oromia) Ethiopia.

Variable	Negative	Positive	Total
Oromia	14(6.1%)	216(93.9%)	230(100%)
Afar	26(7.7%)	313(92.3%)	339(100%)
Total	40(7%)	529(93%)	569(100%)
	Pearson Chi-Square =0.525	df=1	P=0.469

Table 5. Sero prevalence of MERS-CoV in different zones of the study area among dromedary camels.

Variable	Negative	Positive	Total
Borena	13(14.0%)	80(86.0%)	93(100%)
East Shoa	1(0.7%)	136(99.3%)	137(100%)
Zone 1(Afar)	20(10.4%)	172(89.6%)	192(100%)
Zone 3(Afar)	1(1.1%)	94(98.9%)	95(100%)
Zone 4(Afar)	5(9.6%)	47(90.4%)	52(100%)
Total	40(7%)	529(93%)	569(100%)
	Pearson Chi-Square=24.285	df=4	P=0.000

Table 1. Sero prevalence of MERS-CoV among Dromedary camels in different districts of Afar and Oromia.

Variable	Negative	Positive	Total
Ayssaita	4(6.2%)	61(93.8%)	65(100%)
Chifra	8(13.6%)	51(86.4%)	59(100%)
Dubti	8(11.8%)	60(88.2%)	68(100%)
Ewa	5(9.6%)	47(90.4%)	52(100%)
Amibara	1(1.1%)	94(98.9%)	95(100%)
Fentale	1(0.7%)	136(99.3%)	137(100%)
Yabelo	13(14.0%)	80(86.0%)	93(100%)
Total	40(7%)	529(93%)	569(100%)
	Pearson Chi-Square=27.173[a]	df=6	P=0.000

Table 7. Seroprevalence of MERS-CoV among Dromedary camels in different kebeles of Afar and Oromia.

Variable	Negative	Positive	Total
Surupha	13(14%)	80(86%)	93(100%)
Adis Ketema	1(0.7%)	136(99.3%)	137(100%)
Badule	5(9.6%)	47(90.4%)	52(100%)
Galifage	1(4%)	24(96%)	25(100%)
Geriro	4(17.4%)	19(82.6%)	23(100%)
Gurmudale	8(11.8%)	60(88.2%)	68(100%)
Heladegi	1(1.1%)	94(98.9%)	95(100%)
Jarakontola	4(11.1%)	32(88.9%)	36(100%)
Urmaytu	3(7.5%)	37(92.5%)	40(100%)
Total	40(7%)	529(93%)	569(100%)
	Pearson Chi-Square=28.308	df=8	P=0.000

Table 8. Isolation and Identification MERS-CoV among dromedary camels from selected sites (Yabelo, Fentale, Dubti and Amibara) of the study areas.

Variable	Negative	Positive	Total
Yabelo	25(100%)	0(0.00%)	25(100%)
Fentale	24(96%)	1(4%)	25(100%)
Dubti	23(92%)	2(8%)	25(100%)
Amibara	21(84%)	4(16%)	25(100%)
Total	93(93%)	7(7%)	100(100%)

Pearson Chi-Square=27.173 and P=0.000 value) (Table 6). Among the kebeles (n=136/137) (99.3%) prevalence was the highest prevalence from Adis Ketema of Metehara and the lowest Geriro (n=19/23) (82.6%) of Afar with Pearson Chi-Square=28.308 and P=0.000 values (Table 7).

Molecular identification of MERS-CoV

From the 100 swab randomly sampled (n=7/100) (7%) of them evident circulation of MERS-CoV in the study area 2 from Dubti, 1 from Fentale and 4 from Amibara (Table 8).

DISCUSSION

The overall high prevalence (n=529/569) (93%) evident in this study completely agree with study done in Ethiopia by (Reusken et al., 2013). The prevalence recorded in Young Dromedary camel (n=216/238) (90.8%) and Adult (n=313/331) (94.6%) was also similar with work of the same author (93% in young Dromedary and 97% in Adult). The higher prevalence observed in the Adult Dromedary camel, may be due to their access to MERS-CoV infected animal at common watering place found in the study area. The overall prevalence of MERS-CoV in this study also agree with study done in Egypt by (Chu et al., 2014), (n=48/52) (92.3%) using the same pseudoparticle neutralization assay as the current study. The slight lower prevalence recorded in Female dromedary camels of this study (n=339/361) (93.9%) compared to the Males (n=190/280) (91.3%) may be due frequent interaction of female camels with humans and their calves.

The two herd size categories in the current study have little variation in their prevalence. Camels in the larger herd size have slightly higher prevalence (n=324/347) (93.4%) than the small herd sized (n=205/222) (92.3%), but the difference between the herds categories was not statistically significant. This may be related with the possibility of increment of an infected animal in the herd which further facilitate transmission of the virus. The prevalence observed in Oromia region was higher (n=216/230) (93.9%) as compared to Afar (n=313/339) (92.3%), this may be because of the high contact rate of the camels in each other during watering in Oromia as compared to the Afar camels. Among the five Zones included in the study, the highest prevalence was evident in East Shoa (n=136/137) (99.3%) and the lowest in Borena zone (n=80/93) (86.0%).The difference observed among the zones was highly significant (p=0.000).

Fentale was the district where the highest prevalence of MERS-CoV recorded (n=136/137) (99.3%) the lowest in Yabelo district (n=80/93) (86.0%) both from Oromia regional state of Ethiopia. Nine kebeles were included in the study with highest prevalence from Adis Ketema (136/137) (99.3%) and the lowest from Geriro (n=19/23) (82.6%).The difference observed was statistically significant (P=0.000). Hundred nasal swabs were used for identification of MERS-CoV from Yabelo, Fentale, Dubti and Amibara district by RT-PCR. Twenty five swabs from each district except Yabelo MERS-CoV was recovered from all the districts sampled. From the overall 7 identified MERS-CoV cases, Amibara district account the highest (n=4/100) (4%) this may relate with the highest seroprevalence recorded in the area (n=94/95) (98.9%).

Chu et al. (2014) reported identification of MERS-CoV (n=4/110 swabs) (3.6%) from dromedary camels of Egypt using RT-PCR, the same molecular technique used in the current study except MERS-CoV strain (variant) was not identified.

Conclusion

There was high seroprevalence of MERS-CoV evident in the study area (Afar=92.3% and Oromia =93.9%), regional states of Ethiopia. The seroprevalence evidence was supported by identification of the virus by RT-PCR, which indicates existence of active infection of MRS-CoV in the study area. Among 569 Dromedary camel sampled, highest prevalence was observed in East Shoa, Fentale district and the lowest in Borena, Yabelo district both from Oromia regional state of Ethiopia.

The study also implies the probability of circulation of MERS-CoV in human being in the study areas, due to the zoonotic nature of the virus and is transmissibility between Dromedary camel and human. Therefore, further study should be conducted to identify which specific factors favor the high prevalence observed, identify the strain (variant) of MERS-CoV in the area and serological and molecular characterization to check the status of the disease in human beings in Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Microbiological quality of frozen raw and undercooked Nile tilapia (*Oreochromis niloticus*) fillets and food safety practices of fish handlers in Arba Minch town, SNNPR, Ethiopia

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The study was designed to assess the microbiological quality of Nile tilapia (Oreochromis niloticus) fillets and food safety practices of fish handlers in Arba Minch town, SNNPR, Ethiopia. A questionnaire survey was conducted to assess the food safety practices of fish handlers. The microbiological quality of frozen raw and undercooked (Asa leb-leb) tilapia fillets was assessed by determining the total plate count and isolating hygiene indicator bacteria that is, Escherichia coli and other major foodborne pathogens that is, Salmonella spp. and Staphylococcus aureus. Among interviewed fish handlers, 32.6% and 55.8% were not found to use overcoat and hair cover, respectively. Moreover, 30.2% of the fish handlers didn't take training regarding sanitary handling of fish food. The highest mean bacterial count was observed in frozen raw tilapia fillet sample (4.63x106 cfu/g), which was significantly (p=0.037) different from undercooked tilapia fillet (4.92x103 cfu/g). From 40 frozen raw samples of tilapia fillet examined, 42.5% had E. coli, 7.5% had Salmonella spp. and 65% had S. aureus. In contrast, from 40 undercooked samples of tilapia fillet examined, 7.5% had E. coli and 17.5% had S. aureus whilst Salmonella spp. was not detected. A significant (p=0.000) difference in frequency of bacterial isolates were also observed between frozen raw and undercooked tilapia fillet. Generally, the study revealed that there was a gap on food safety practices and high microbial profile were observed. Hence, it could be wise to advice the government to improve the food safety practices and quality standards of fish sold in the town.

Keywords: Fish, food safety, microbiological quality, Nile tilapia, pathogens, undercooked.

INTRODUCTION

Fish products are essential to food security, providing over 1 billion people with their main source of protein and more than 4.3 billion people with about 15% of their average per capita animal protein intake (FAO, 2012). In

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2010, wild capture fisheries and aquaculture supplied the world with about 148 million tons of fish, worth US $ 217.5 billion. Approximately, 128 million tons or 86% were used for human consumption. Preliminary data for 2011 indicates that production has increased to 154 million tons, of which about 131 million tons, or 85%, were consumed as food (FAO, 2012; World Bank, 2010).

Ethiopia has only inland freshwater capture fisheries. Total annual fish production potential in the country is estimated to be 51,481 tons (FAO, 2012). In 2010/11 the total landing was about 18,058 tons, which is about 35% of the calculated potential (MoA, 2012). But the country overall per capita fish production is less than 240 g per person per year (FAO, 2006). However, in areas and communities where there is regular and sufficient supply, annual fish consumption can exceed 10 kg/person (FAO, 2012). This implies that fish consumption in the country is more highly influenced by supply factors (FAO, 2012). This supply factor is indirectly related to lack of preservation and processing techniques. In the country most (about 73%) of the total fish landed is marketed fresh in nearby markets. The rest reaches distant consumers chilled or frozen (26%), or as dried, smoked and canned (1%) forms (FAO, 2012).

Fish is one of the best protein sources available to the human body in quality and quantity. It is also a valuable source of vitamin A and B, iodine and oils containing polyunsaturated fatty acids (Adebayo et al., 2012). However, the increasing demand for fish and fish products all over the world is greatly challenged by microbial infection of fish and contamination of fish products (Pyatkin and Krivoshein, 1986).

Studies indicate that there are several bacteria that have been isolated from three main parts of fresh fish: slime layer, gills and the gastrointestinal tract. According to Cahill (1990) the microbiological diversity of the fresh fish muscle depends on the fishing grounds and environmental factors around it. Fish from warm waters mostly harbor mesophilic gram-negative bacteria while cold waters harbor mostly psychrophilic, gram-positive bacteria. These bacterial isolates are classified into two groups: indigenous and post-harvest bacteria. Previous studies have also demonstrated the presence of indicator microorganisms of fecal pollution, opportunistic and pathogenic bacteria to humans in fish (Tsai et al., 2002; Ferreira and Pinho, 2006; Tzikas et al., 2007).

The origin of most microbes in fish and fish products may not be precisely known, some workers have related microbial infections and/or contamination of fish and fish products to a number of factors including unfavorable conditions in the fish culture system (Okpokwasili and Ogbulie, 1993), pollution and seasonal changes (Obiajuru et al., 2010), fish handling and processing including personnel and processing equipment (Pelczer and Chan, 1986). Fish contains protein and nutrients favorable for microbial attack even after processing. This often leads to fish spoilage and nutritional deterioration. Recent studies in Owerri, Imo State of Nigeria, showed 20% prevalence of Salmonella in frozen fish (Ohalete et al., 2011). In developing countries like Ethiopia where there is a poor infrastructure for the sector, fish spoilage from microbial contamination is believed to be a critical problem of fisheries and public health (FAO, 1993). Hence, this study was designed to elucidate the microbiological quality of fish meat retailed at Arba Minch town in Southern Ethiopia.

Lake Abaya and Chamo, the two large rift valley lakes, are located about 10 km North and Southeast of Arba Minch town, respectively. The total annual average commercial catch from Abaya and Chamo Lake based on surface area was estimated at 10,100 and 5,500 tons, respectively (FAO, 1993). These two lakes supply fish for local and national markets, and Nile Tilapia (*Oreochromis niloticus*) is the common fish species harvested from them.

According to the data obtained from Arba Minch Zurya Woreda Marketing and Cooperative Office, authorized fishermen associations are the main supplier of fish to hotels and restaurants in Arba Minch town. Fish is transported to the town at the back of pick-up track. However, illegal fishermen have to walk daily to the town carrying fish in sacks on their heads in the sunny and hot weather conditions. Factors that could degrade the safety and quality of fish, such as lack of proper transportation facilities, poor handling and processing are common.

In Arba Minch, fish is processed and presented to the consumer in different types of dish. Among that, Asa leb-leb is the most famous and highly consumed fish dish in the town. The dish Asa leb-leb is lightly fried (undercooked) fish fillet, thus it may contain some pathogenic bacteria of great concern to public health (example, *Escherichia coli*) and their toxic metabolites (example, *Staphylococcus* toxins) which tolerate the temperature and time combination at which this dish is prepared. Moreover, several studies assured that cooking of sea food products would not destroy their toxic metabolites like thermostable enterotoxin of *Staphylococcus aureus* and thermostable enterotoxin of *E. coli* (Vieira et al., 2001; Ayulo et al., 1994). Even if this dish is prepared at the correct temperature and time combination which eliminate the above risks, there may be also a risk of recontamination from improper post-process handling. Therefore, consumers could be at risk of acquiring zoonotic pathogens and/or their toxic metabolites from consumption of Asa leb-leb dish.

Therefore, the general objective of this study was to assess the microbiological quality of Nile tilapia fillets and food safety practices of fish handlers in Arba Minch town, SNNPR, Ethiopia. The specific objectives of the study were:

1. To determine the microbial load of frozen raw and undercooked (Asa leb-leb) Nile tilapia fillets.
2. To isolate hygiene indicator bacteria that is, *E. coli* and

other pathogenic bacteria of great concern to public health that is, *Salmonella* spp. and *S. aureus* from frozen raw and undercooked (Asa leb-leb) Nile tilapia fillets.
3. To assess food safety practices of fish handlers working in the kitchens of different hotels.

MATERIALS AND METHODS

Description of the study area

The study was conducted from June to December, 2013 in Arba Minch town. Arba Minch is the capital town of Gamo Gofa Zone of Southern Nations, Nationalities and People's Regional State (SNNPR). It is located 505 km far from the capital city of Ethiopia, Addis Ababa, in the coordination of 5°57' N latitude and 37°32' E. The area has an average annual temperature of 29.7°C and rain fall of 700 mm (CSA, 2007). Arba Minch is presently experiencing rapid population growth. As a result, the number of commercial food establishments in the town has visibly increased. According to the data obtained from Trade and Industry Office of the town, about 20 registered hotels are currently present in the town. The sources of fish for all these commercial food establishments are fish harvested from Abaya and Chamo Lakes.

Study design

The study involved a questionnaire survey and a laboratory assessment of the microbiological status of frozen raw and undercooked (Asa leb-leb) Nile tilapia fillets collected from the different hotels in Arba Minch town. A questionnaire survey was used to assess the current status of food safety practiced by fish handlers working in the kitchens of different hotels in the town.

Sample and sampling method

In the present study, frozen raw and undercooked (Asa leb-leb) Nile tilapia fillets were collected from selected registered hotels in Arba Minch town. The subjects for this study were fish handlers in the kitchens of hotels, registered under the Trade and Industry Office of the town. Due to limited resources, only representative hotels were selected by purposive sampling strategy.

Sample collection, handling and transportation

A total of 80 (40 frozen raw and 40 undercooked (Asa leb-leb)) Nile tilapia fillets were collected in 10 trips from 10 (50% of total hotels) selected registered hotels of Arba Minch town. In each trip, two hotels were visited to take a total of eight samples of Nile tilapia fillets (two frozen raw and two undercooked from each hotels) to assess their microbiological quality. To make sure that samples were taken without being contaminated, inverted plastic bags were used for collection. The inner surface of the bag was used to touch nothing else but the sample. All samples were labeled with the type of the sample, the place, date of sampling and given an identification code and transported to Wolaita Sodo Regional Veterinary Laboratory in icebox containing ice packs for microbiological analysis. Upon arrival, the samples were immediately processed or stored at -20°C in a refrigerator until use and processed within 24 h of collection.

Microbiological analysis

Sample preparation

The frozen samples were thawed at room temperature for 5 to 6 h before processing (APHA, 2005). T10 g of each Nile tilapia fillet sample was weighed and placed into sterile stomacher bags, diluted with 90 ml of 0.1% sterile peptone water (HiMedia, India) and homogenized in a stomacher for 2 min per procedure describe by Fawole and Oso (2001).

Total plate count (TPC)

From the 10-fold dilutions of the homogenates, 0.1 ml of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions of the homogenates were pour-plated in duplicates on fresh standard plate count agar (HiMedia, India). The plates were then incubated at 37°C for 24 to 48 h. At the end of the incubation period, plates exhibiting 30 to 300 colonies were counted using illuminated colony counter. The counts for each plate were expressed as colony forming unit of the suspension (cfu/g) (Fawole and Oso, 2001).

Isolation and identification of bacteria

Isolation and identification of Salmonella

Salmonella was isolated from 10 g fish samples homogenized in 90 ml of 0.1% sterile peptone water. Aliquots of 1 ml were added to 10 ml of selenite broth (Difco, France). These were incubated at 37°C for 24 h. After gentle mixing, a loopful of culture from the enrichment broth was streaked on to xylose lysine desocholate (XLD) agar (HiMedia, India) and incubated at 37°C for 24 to 48 h. Typical *Salmonella* colonies which were pink with or without black centers were isolated. The colonies were purified on fresh nutrient agar (HiMedia, India), then streaked and stabbed into the butt of triple sugar iron (TSI) (HiMedia, India) slants. These were incubated at 37°C for 24 h. The test tubes that had alkaline (red) slants and acidic (yellow) butts, with or without the production of H_2S (blackening) were presumed to be *Salmonella* isolates. Moreover, two or more colonies from pure isolate were inoculated on urea broth (SRL, India) and incubated at 37°C for 24 h. All test tubes that were urease negative were treated as suspects of *Salmonella* (FDA, 1992). In addition, isolates that were Gram-negative rod, oxidase negative, citrate positive, methyl red positive, indole negative, voges-proskauer negative and lactose and sucrose non-fermenter were accepted putatively as *Salmonella* (Fawole and Oso, 2001).

Isolation and identification of E. coli

For the isolation of *E. coli*, a loopful of homogenized sample was streaked on to eosin methylene blue (EMB) agar (HiMedia, India). The plates were incubated at 37°C for 24 to 48 h. Typical *E. coli* colonies which are blue-black with a green metallic sheen were isolated. The colonies were purified on fresh nutrient agar (HiMedia, India) and then streaked and stabbed into the butt of triple sugar iron (TSI) (HiMedia, India) slants. These were incubated at 37°C for 24 h. All test tubes that had acidic (yellow) slants and acidic (yellow) butts, without the production of H_2S (blackening) were treated as suspects of *E. coli* (FDA, 1992). In addition, isolates that were gram-negative rod, oxidase negative, citrate negative, indole positive, urease negative, methyl red positive, voges-proskauer negative and lactose and sucrose fermenter were accepted putatively as *E. coli* (Fawole and Oso, 2001).

Isolation and identification of S. aureus

For the isolation of *S. aureus*, a loopful of homogenized sample was streaked on to mannitol salt agar (HiMedia, India). The plates were incubated at 37°C for 24 to 48 h. Typical *S. aureus* colonies

which are yellow with a yellow halo around the colony were isolated. The colonies were purified on fresh nutrient agar (HiMedia, India) and then transferred into small tubes with 0.5 mL of rabbit plasma (NVI, Ethiopia). The tubes were rotated gently to mix the content and incubated at 37°C for 2 to 24 h, after which tubes that had clotted plasma were treated as suspects of *S. aureus* (APHA, 2005). Moreover, the smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's Method of staining. The stained smears were observed under microscope. Smear that revealed gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes were considered for *Staphylococci* presumptive. The isolates were further characterized by catalase, voges-proskauer and sugar (mannitol and maltose) fermentation test (Fawole and Oso, 2001).

Questionnaire survey and observation

Hygiene and sanitation conditions were determined by the use of semi-structured questionnaire. Moreover, direct observation was employed to assess the hygienic status and practices by the fish handlers working in the kitchens of different hotels found in Arba Minch town. All fish handlers observed in all 10 purposively selected hotels for this study were interviewed by the use of detailed and semi-structured questionnaire in order to obtain relevant information on food safety practices and fish food handling. Personal information, like educational status and training of fish handlers on sanitary handling of fish food and practices were collected through questionnaire and observation. Informed consent was obtained from owners/managers of the hotels and fish handlers after a brief explanation about the benefits of the study. Confidentiality of the respondent was maintained by assuring that his/her answers would not be released to anyone and would remain anonymous and his/her name would not be written on the questionnaire or be kept in any other records.

Data quality control

The quality of the data was kept by preparing and using standard operational procedures for laboratory investigation and media preparation. Semi-structured questionnaire was tested using pretest before conducting the study. Sample collection and processing were carried out using aseptic techniques. The samples were labeled properly. Culture and bacterial colony count were carried out under supervision of experienced laboratory personnel. All media were prepared according to the manufacturer's specification and sterilized at 121°C 15 psi pressure unit for 15 min. The sterility test of prepared media was checked by incubating at 37°C (APHA, 2005).

Statistical analysis

Basic data entry and handling was done by Microsoft Excel database system. The data were then analyzed using statistical package for social sciences (SPSS) (2012). Descriptive statistics such as, means and frequencies were used to present the findings of both questionnaire survey and laboratory assessment. Also, a *t*-test was used to check whether the mean of two different groups under comparison were significantly different in the normally distributed population from which the samples were drawn.

RESULTS AND DISCUSSION

Practices of fish handlers

Since food handlers may be the source of food contamination either as carriers of pathogen or through poor hygienic practices. Thus all food handlers have basic responsibility to maintain a high degree of personal cleanliness and implement hygienic and safe food handling practices. Among the precautions that a food handler must maintain the major ones are; keeping hands clean and fingernails short, wearing clean working garment and hair cover (hair net and cap) (Kaferstein, 2003). However, the result of this study showed that 32.6% of the fish handlers did not wear appropriate overcoat (Table 1). This finding is higher than the result of similar study done on food handlers in Hawassa (14%) (Teklemariam et al., 2000) but lower than the finding of Fisseha et al. (1999) in Addis Ababa (45.8%). Moreover, 44.2% of fish handlers were also found with covered hair, which is consistent with that of Kumie et al. (2000) done in Zeway (40.1%) but better than the result of Teklemariam et al. (2000) assessed in Hawassa (11.8%).

In addition, nail of 46.5% of the fish handlers in this study were not shortly trimmed and clean (Table 1). This finding is higher than the result of Teklemariam et al. (2000) who found that 21.2% of food handlers in Hawassa city hotels did not trim their nail shortly. It was also observed that 72.1% of fish handlers in this study wore rings on their finger during food preparations, which was markedly higher than the report of Kumie et al. (2000) done in Zeway (28.7%). As food handlers can be the probable sources of contamination for micro-organisms, it is important to take all possible measures so that such contaminations would be reduced or eliminated (Muinde and Kuria, 2005).

Training of fish handlers on sanitary handling of food

According to Adams and Moss (1997), training of food handlers regarding the basic concepts and requirements of personal hygiene and sanitary handling of food play an integral part in ensuring a safe product to the consumer. But, the result of this study showed that 30.2% of the fish handlers have taken no training concerning sanitary handling of food. This result is lower than the previous result of Mekonnen et al. (2012) who obtained 61.5% of meat handlers not to take training on sanitary and food hygiene.

Total plate count (TPC)

Bacterial growth is the main cause of fish spoilage and public health concern therefore total bacterial count is used as a general index of fish quality. In this study the mean bacterial count (cfu/g) was found to be 4.63×10^6 in frozen raw Nile tilapia fillet samples (Table 2). This value was found to be higher than the result of Dhanapal et al. (2012) who found 4.9×10^4 in frozen raw Nile tilapia fillet samples. This high load is mainly the result of poor handling during the transportation, and/or poor personal

Table 1. Practices of fish handlers in relation to food safety, (n=43).

Practice at a time of visit		Frequency	Percentage (%)
Wear of appropriate overcoat	Yes	29	67.4
	No	14	32.6
Wear of appropriate hair cover	Yes	19	44.2
	No	24	55.8
Short trimmed and cleaned nail	Yes	23	53.5
	No	20	46.5
Cleanness of overcoat and visible body part	Kept	20	46.5
	Not Kept	23	53.5
Wear of jewelry or ring	Observed	31	72.1
	Not observed	12	27.9
Nail paint during visit	Observed	24	55.8
	Not observed	19	44.2
Washing of hand before starting work	Yes	43	100
	No	0	0
Discharge from nose, eye, ear and coughing	Observed	5	11.6
	Not observed	38	88.4
Any visible skin rash, boil, cut and wound	Observed	1	97.7
	Not observed	42	2.3

Table 2. Mean bacterial count of frozen raw and undercooked sampled Nile tilapia fillet.

Samples type	Mean bacterial count	Minimum bacterial count	Maximum bacterial count
Frozen raw fillet	$4.63x10^6$	$1.5x10^3$	$6.5x10^7$
Undercooked fillet	$4.92x10^3$	TFTC	$8.5x10^4$

TFTC = Too few to count.

hygiene during filleting.

In the current study, the mean bacterial count (cfu/g) was found to be $4.92x10^3$ in undercooked Nile tilapia fillet samples. This result indicated that low mean value of bacterial load was found in the undercooked Nile tilapia fillet samples, which was significantly different from frozen raw Nile tilapia fillet samples (p=0.037). This is due to the exposure to heat during processing for the undercooked Nile tilapia fillet samples. Almost similar result has been` reported by Salaudeen et al. (2010) and Dhanapal et al. (2012). For ready to eat seafood, the microbiological limit for human consumption proposed by the International Commission on Microbiological Specifications for Foods (ICMSF) (1986) is $5x10^5$ to 10^7 cfu/g in aerobic plate count analysis, which is also in line with this study result.

Bacterial isolation

Table 3 summarizes the frequency of bacterial isolate from frozen raw and undercooked Nile tilapia fillet samples. As it is shown, the lowest frequency of bacterial isolates were observed in undercooked Nile tilapia fillet samples which was significantly different from frozen raw Nile tilapia fillet samples (p=0.000). This could be due to the negative effect of heat on the growth of bacterial contaminants. Regarding the frequency of bacterial

Table 3. Frequency of bacterial isolate from frozen raw and undercooked sampled Nile tilapia fillet.

Samples type	Number of samples processed	Bacterial Isolates (%)		
		***E. coli* (%)**	***Salmonella* spp. (%)**	***S. aureus* (%)**
Frozen raw fillet	40	17 (42.5)	3 (7.5)	26 (65)
Undercooked fillet	40	3 (7.5)	0 (0)	7 (17.5)
Total	80	20 (25)	3 (3.75)	33 (41.25)

isolate from heat treated fish and other ready-to-eat food stuffs similar observations were reported by other researchers (Mezgebu and Mogessie, 1998; Thailambal, 2006; Omenwa et al., 2012).

Finding of this study indicated that 42.5% of frozen raw Nile tilapia fillet samples carried *E. coli*. This result is higher than the result of Vieira et al. (2001) who reported that 12.5% of all samples from Brazilian markets had *E. coli*. The detectable presence of *E. coli* in the present study is a sign of faecal contamination of fish. This is in accordance with results obtained by Monzur-Hassan et al. (1994) and Yousuf et al. (2008) those associated with the detectable presence of *E. coli* in fish to the faecal contamination of fish sourced from the warm blooded animal.

Moreover, this study indicated that 7.5% of undercooked Nile tilapia fillet samples had *E. coli*. This result complies with previous report by El-Gohany (1994) that foods of animal origin either cooked or uncooked were predominantly contaminated with *E. coli*. Besides, Ohalete et al. (2013), reported 58.3% of *E. coli* contamination in fried fish samples from different bars and eating houses in Owerri, Nigeria. This is also in accordance to the assertion of Okonko et al. (2008, 2009a,b) that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affects the health of the consumers.

The result of this study further depicted that 65% of frozen raw and 17.5% of undercooked Nile tilapia fillet samples to harbor *S. aureus.* The occurrence of *S. aureus* in frozen raw Nile tilapia samples in this study was higher than the result of Mhango et al. (2010) who isolated *S. aureus* in 41% of the frozen gutted Nile tilapia samples studied and Ayulo et al. (1994) who isolated *S. aureus* in 20% of fresh fish and fish fillets (*Cynoscion leiarchus*). However, the presence of *S. aureus* in undercooked (Asa leb-leb) Nile tilapia samples in this study indicated that this type of heat treatment was not sufficient to eliminate the pathogen and/or the risk of recontamination from improper post-process handling. Generally, the high prevalence of *Staphylococcus* in the fish samples indicated the unhygienic handling of the fish since this species is found on human skin.

The frequency of isolation of *Salmonella* spp. from frozen raw Nile tilapia fillet samples in this study was 7.5%. This result is lower than the result of Ohalete et al. (2011) who isolated 20% of *Salmonella* spp. from frozen fish samples. Moreover, while in this study *Salmonella* spp. was detected, Rokibul and his team didn't isolate *Salmonella* spp. from frozen fish samples (Rokibul et al., 2012).

In contrast, no *Salmonella* spp. was isolated from undercooked Nile tilapia fillet samples in this study. The absence of *Salmonella* spp. in undercooked Nile tilapia samples in this study agrees with the result of Odu and Ameweiye (2013) that isolated no *Salmonella* spp. from ready-to-eat bole fishes.

But the presence of *E. coli* in undercooked Nile tilapia samples in this study indicated that *Salmonella* could also survive the heat treatment if it was there.

CONCLUSION AND RECOMMENDATIONS

The findings of this study revealed the presence of gap on food safety practices by fish handlers who work in different hotels of the Arba Minch town. The microbiological examination of frozen raw and undercooked (Asa leb-leb) Nile tilapia fillet samples revealed that the frozen raw Nile tilapia fillets are more contaminated than the undercooked Nile tilapia fillets. However, the detection of some zoonotic pathogens in undercooked (Asa leb-leb) Nile tilapia samples in this study indicated that this type of heat treatment was not sufficient to eliminate all zoonotic pathogens and/or the risk of recontamination from improper post-process handling.

In the light of these major findings, the following recommendations are made to safeguard the public against the risks of foodborne infections and intoxication:

1. Fish handlers should be trained on the adverse effect of lack of proper personal and environmental hygiene and sanitation.
2. Proper attention should be paid to the safety of both frozen raw and undercooked (Asa leb-leb) fish through proper handling and use of adequate processing procedures such as proper heating method.
3. Public health authorities in the state should ensure adequate supervision and monitoring of fish handling and sales especially ready-to-eat products like Asa leb-leb dish.
4. Further investigations should be conducted to assess the issues that are not addressed through this study.

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CONFLICTS OF INTEREST

The authors have none to declare.

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