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INVESTIGATION ON OCCURRENCE OF INFLUENZA A VIRUS INFECTION IN CAMELS SLAUGHTERED AT AKAKI MUNICIPAL ABATTOIR, ETHIOPIA

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BY:

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September,2020

Bishoftu, Ethiopia

INVESTIGATION ON OCCURRENCE OF INFLUENZA A VIRUS INFECTION IN CAMELS SLAUGHTERED AT AKAKI MUNICIPAL ABATTOIR, ETHIOPIA

MVSc. Thesis

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

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September,2020

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STATEMENT OF AUTHOR

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

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LIST OF ABBREVIATIONS

dNTP	di nucleoside triphosphate
EPHI	Ethiopian Public Health Institute
HA	hemagglutinin
IAV	Influenza A virus
IBV	Influenza B virus
ICV	Influenza C virus
IDV	Influenza D virus
IVs	Influenza viruses
MOH	Ministry of Health
RT- PCR	Reverse Transcriptase – Polymerase Chain Reaction
NA	neuraminidase
NIRL	National Influenza Reference Laboratory
NP	nucleocapsid proteins
RdRp	RNA-dependent RNA polymerase
RIDT	Rapid influenza diagnostic tests
VTM	Viral transport medium
vRNP	viral Ribonucleoprotein

ABSTRACT

Influenza is a contagious respiratory disease of birds and mammals including humans caused by influenza viruses A, B, C and D. Influenza A virus (IAV) subtypes possesses the greatest zoonotic potential. Continuous circulation of Influenza A virus in different animal species may cause influenza virus to acquire adaptive genetic features for efficient human to human transmission through mutations. An abattoir based cross-sectional study was conducted from November, 2019 to August, 2020 to evaluate the prevalence of Influenza A virus infection in camels at Akaki municipal abattoir and assess the public health importance of the virus. The study animals were camels slaughtered at Akaki municipal abattoir, central Ethiopia in which the camels were brought for slaughter from camel rearing pastoral areas of Ethiopia. A total of 392 nasal swab samples were tested for the presence of viral RNA using Reverse transcriptase – polymerase chain reaction (RT-PCR), at the National Influenza Reference Laboratory at EPHI and their result showed no occurrence of Influenza A virus RNA in sampled camels. The public health significance was evaluated using questionnaire survey. A total of 19 participants who had direct contact with the camels at Akaki municipal abattoir (12) and traders (7) participated in the study. In conclusion, the present study showed that there were no camels with current infection. Yet serologic tests should be done to see the presence of antibodies. The zoonotic importance of the disease is not something that can be neglected due to the potential risk factors at Akaki municipal abattoir such as the high frequency of contact with the camels as well as consumption of raw camel meat and milk and lack of appropriate personal protective equipments. Hence, this warrants further study in camel population of pastoralist area of Ethiopia in order to confirm the occurrence of Infulenza A virus in camel and design a control options to reduce its zoonotic chance to the high-risk pastoralist population.

Keywords: *Influenza A virus, prevalence, public health importance,* RT-PCR, *Akaki municipal abattoir, Ethiopia*

1. INTRODUCTION

Influenza (the flu) is a contagious respiratory illness of birds and mammals including humans caused by influenza viruses A, B, C and D. In the last 100 years, influenza viruses in human are generally manifested as seasonal, zoonotic and pandemic with clinico-pathological manifestation that vary from mild to severe illness, and at times can lead to death. However, the most threatening influenza infections are those caused by zoonotic and/or pandemic strains particularly influenza A subtypes H1N1, H3N2 and H2N2, following their introduction usually from animal reservoir into human population (Reperant *et al*., 2016). Although the infection of humans with zoonotic influenza viruses is less frequent than infections with seasonal influenza viruses, there is a global concern that these zoonotic viruses may acquire mutations in animals or humans that favor the efficient animal-to-human or sustained human-to-human transmission (Kandeil *et al*., 2017a).

Of the four known influenza virus genera, Influenza A viruses (IAVs) possess the greatest zoonotic potential as they are able to infect different avian and mammalian animal hosts, from which they can be transmitted to humans. This is based on the ability of IAV to gradually change their genome by mutation or even reassemble their genome segments during co-infection of the host cell with different IAV strains, resulting in a high genetic diversity (Mostafa *et al*., 2018).

Influenza A virus infections have attracted more attention especially since the identification of avian and swine influenza viruses that can (with or without biological or molecular adaptations) be transmitted directly, and cause severe disease in humans and other mammals. This was notable with the occurrence of A/Goose/Guangdong/96 lineage of H5N1, which had infected 860 people and killed 454 (52% case-fatality rate) up till December 2017 (WHO, 2017).

Continuous circulation of Influenza A viruses in different animal species and zoonotic transmission to human may cause influenza virus to acquire adaptive genetic features for efficient human to human transmission through mutations (insertions/deletions), reassortment or emergence of immune or antiviral resistant strains. Those may likely

be precursors of emerging influenza A virus with pandemic potential. Global surveillance for influenza diversity and monitoring reassortment events between potential animal species and human viruses therefore greatly improves the ability for early detection, to identify and anticipate which strains are more likely to evolve (Reperant *et al*., 2016).

One such species that has the potential to harbor these viruses is the *Camelus dromedarius* (Yamnikova *et al*., 2002). More than 80% of the camel population inhabits Africa with 60% being in the Eastern African countries (Sudan, Somalia, Ethiopia and Kenya) which are important exporters of dromedary camels to the Arabian Peninsula and Egypt (Faye, 2015). Ethiopia has an average of 1.42 million camels according to CSA, 2018. In Ethiopia camels are commonly reared by pastoralists who travel across many territories where the animals may be exposed to and adopt Influenza A viruses. The difficulty to control the contact between people and dromedary camels especially in pastoral areas poses a risk for the viruses to develop a zoonotic nature (Tefera, 2004).

Despite reports of serologic activity against influenza A virus among camels in several African countries (Caffar and Kheir, 1985; Olaleye *et al*., 1989), the lack of isolated virus from these populations highlights how little is known about the ecology of influenza viruses in camels. Viruses are known for their high mutation and fatality rate and they might mutate into versions for which effective anti-viral drug or vaccines are unavailable (Chen *et al*., 2015). It is therefore important to have continuous studies on circulating zoonotic viruses in different species of animals and associated risk factors in order to initiate early warning and response systems.

A recent report showed that there is evidence indicating that dromedary camels in Ethiopia can harbor influenza A virus of zoonotic importance (H1N1). In this study conducted in 2017, of 294 nasal swab samples collected from nomadic dromedary herds in Amibara, Asayita and Dubti districts of Afar region, for the study of MERS-CoV, one camel from Amibara district was found to be positive for influenza A virus (H1N1) (Chu *et al*., 2019). In addition, out of the 100 camel serum samples collected from Amibara camels 4 serum samples were positive for antibody against H1N1 (A/dromedary/NV1337/2016) Influenza A virus using hemagglutination inhibition

test (Chu *et al*., 2019). However, specific study on occurrence of Infulenza A virus in camels of Ethiopia and its associated risk factors for zoonotic transmission have not been studied.

In regards of these factors, the objectives of this study are described as:

General objective

- To investigate the occurrence of Influenza A virus in camels slaughtered at Akaki municipal abattoir and the public health importance of the virus at abattoir level.

Specific objectives

- To determine the occurrence of Influenza A viruses in camels slaughtered at Akaki municipal abattoir based on molecular detection of the virus in nasal swab,
- To evaluate the potential risk of exposure to abattoir workers and camel traders and their food safety practices.

2. LITERATURE REVIEW

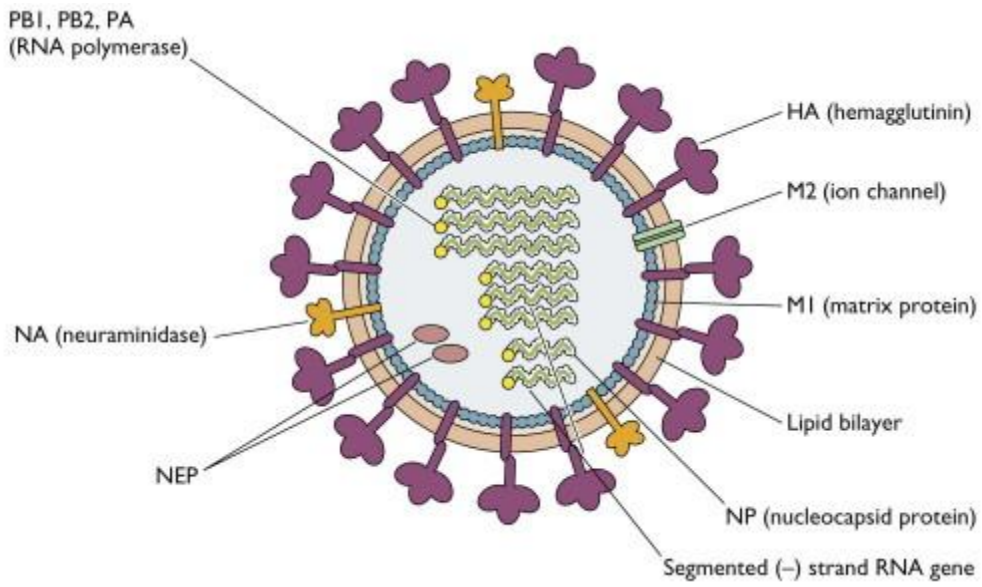
2.1. Influenza A Virus Etiology

Influenza is an infectious respiratory illness caused as a result of influenza viruses (IVs). IVs are categorized antigenically based on the versions of the nucleoprotein (NP) and matrix proteins (unique for every viral type) into 4 genera: influenza A viruses (IAV), influenza B viruses (IBV), influenza C viruses (ICV), and influenza D viruses (IDV) (Hause *et al*., 2014). While both IAV and IBV cause seasonal worldwide epidemics in humans, ICV cases are less recurrent and only cause mild illness with no mentions of epidemics. IDV is typically found in cattle but recent studies show serological evidence in humans (Wright *et al*., 2013; Chiapponi *et al*., 2016; Su *et al*., 2017).

Influenza A viruses are labeled under: realm (*Riboviria*), phylum (*Negarnaviricota*), class (*Insthoviricetes*), order (*Articulavirales*), family (*Orthomyxoviridae*), genus (*Alphainfluenzavirus*). They are negative-sense, single-stranded, segmented RNA viruses. Unlike the other influenza types, IAVs are subtyped consistent with the antigenicity of the surface glycoproteins and are labeled according to an H number for the hemagglutinin type and an N number for the neuraminidase type. There are 18 H and 11 N antigens. So, in theory, there are about 198 distinct combinations of these proteins are probable (Skehel and Wiley, 2000; Shaw and Palese, 2013). Mutation has caused each of the viral subtypes into various strains with particular pathogenic profiles. Some cause diseases in one species but not others, while others are pathogenic to multiple species (Wu *et al*., 2014; Short *et al*., 2015).

The envelope of an IAV (figure 1), that is derived from the host's plasma membrane, carries 3 trans-membrane proteins; 2 surface glycoproteins (HA~80% and NA~17%) and the proton channel matrix protein 2 (M2). The matrix protein 1 (M1) underlies the internal surface of the viral envelope and links with nucleocapsid proteins (NP) and viral ribonucleoprotein complexes (vRNPs). The 8 vRNPs incorporate eight negative-strand RNA segments related to the nucleoprotein and three RNA-dependent RNA

polymerase (RdRp) subunits (PA, PB1, PB2) (Shaw and Palese, 2013; Mostafa *et al*., 2018).



Source: http: //www.virology.ws Vincent Racaniello

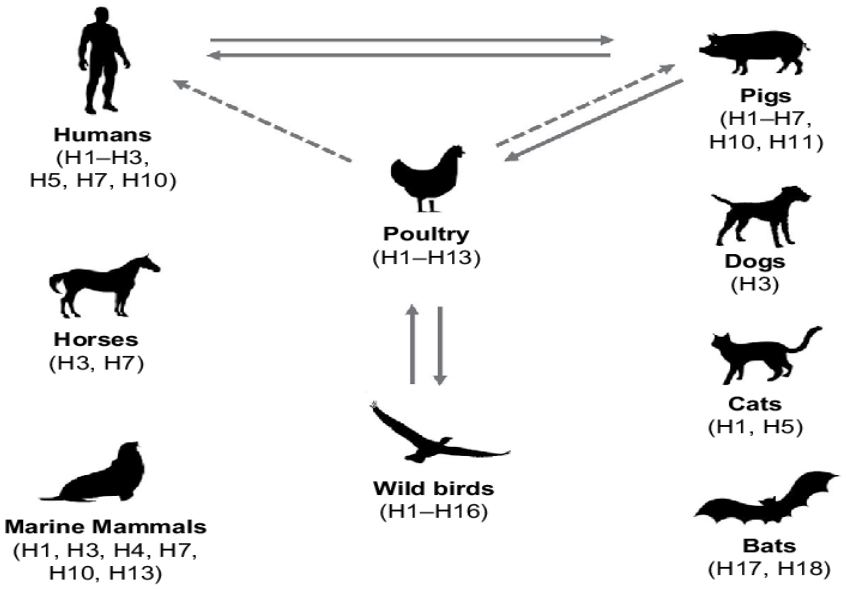
Figure 1: Schematic structure of influenza A virus

The hemagglutinin is significant to the virus's distinguishing and binding capacity to target cells, and additionally to then infect the cell with its RNA by producing agglutination. The neuraminidase, on the other hand, is an enzyme vital for the following launch of the daughter virus fragments created inside the infected cell so that they can spread to adjacent cells by slicing the glycosidic bonds of the monosaccharide sialic acid (Pleschka, 2013; Byrd-Leotis *et al*., 2017). These proteins are frequently the targets for antiviral drugs. Furthermore, they are the antigens to which a host's antibodies bind and initiate an immune response (Wilson and von Itzstein, 2003; Dharmayanti *et al*., 2014).

Variants of IAVs are termed based on a resemblance to a previous isolate. This could be based on the lineage they share (Fujian flu virus-like); based on their typical host (human flu virus); their subtype (H3N2); or their fatality (LP, low pathogenic or HP, high pathogenic) (Chiapponi *et al*., 2016).

2.2. Host Range

Influenza A viruses are in nature capable of causing influenza in birds and mammals which includes human beings, swine, horses, cats, dogs, whales, as well as seal (figure 2). Marine birds are the most significant bunch of faunas in the ecology and epidemiology of IAVs. Almost all certainly circulating subtypes of influenza A virus in birds and mammals (including human) have been traced back to avian ancestors (Alexander, 2000; Lupiani and Reddy, 2009; Fourment and Holmes, 2015) though apparent signs of disease is unusual in them. On occasions, viruses can spread from wild marine birds to native fowls causing an outbreak and giving rise to human influenza pandemics. Except for bat-origin influenza A viruses H17N10 and H18N11, all IAV subtypes were first isolated from avian hosts (Mostafa *et al*., 2018).



Source: https://www.researchgat e.net/figure/The-host-range-of-influenza-A-virus-Notes-The-IAV-HA-subtypes-iso lated-from-each-host.

Figure 2: Host range of Influenza A virus

Over the preceding decades, eruptions of respiratory sickness, assumed to be influenza, amongst camels have been reported in Bactrian camels of Mongolia. Around the 1980s, the virus was characterized by researchers who speculated that it was linked to a reassortant influenza A (H1N1) virus vaccine strain, A/PR-8/34 + A/USSR/77, created in a Soviet laboratory and then given as vaccine to humans in Mongolia who perchance transmitted it to camels in a reactivated form (Yamnikova *et al*., 1993).

Studies additionally demonstrate that the equine influenza A H3N8 has been isolated from camels (Anchlan *et al*., 1996). From 460 nasal swab samples taken from Mongolian camels one sample was found positive for influenza A virus which is phylogenetically associated with equine influenza A(H3N8) virus and can signify the possibility of natural horse-to-camel transmission (Yondon *et al*., 2014).

2.3. Zoonotic Importance of Influenza A Viruses

Influenza A viruses hold an immense zoonotic capacity as seen by their ability to infect diverse avian and mammalian hosts, from which they can be diffuse to human beings. This is primarily based on the capability of IAV to progressively alter their genome by mutation or reassemble their genomic segments in case of co-infection of the host cell with dissimilar IAV strains, ensuing a high genetic multiplicity (Mostafa *et al*., 2018; Mollentze, N., and Streicker, 2020).

The pathogenicity of IAV is a complicated and multigenic attribute, that is laid low by numerous viral and host elements. Nowadays, the existence of sequencing records from a bulky sum of viral strains collectively with the provision of different reverse genetics methods for IAV, permit an improved understanding of a wider host range and virulence factors of IAV (Mostafa *et al*., 2015; Bailey *et al*., 2018).

Rigorous circulation of IAVs in animal reservoirs causes sporadic emergence of genetic traits as a consequence, which are at the minimum adequate to allow the virus to cross the animal-to-human transmission barrier. Furthermore, genetic modifications that enhance the viral aptness for zoonoses possibly will be achieved in the new (human) host (Reperant *et al*., 2012). For instance, different well-studied adaptive genetic variations in human IAV isolates (e.g., Q591K, E627K or D701N) had been detected in avian or other transitional hosts (Cui *et al*., 2017; Xue *et al*., 2018).

2.4.Clinical signs

The appearance of influenza A virus infections in avian is needful of two key features – the species of the affected bird and the pathogenicity of the virus. The causative agents are frequently of the H5N1 (highly pathogenic) and H7N9 (low pathogenic) subtypes. Clinical symptoms appear in chickens and turkeys, ducks and most waterfowl show subclinical signs. Tiredness, loss of need to eat, weight loss, messy feathers and drops in egg production are indicators of infection with avian influenza of low pathogenicity. On the other hand, extremely virulent strains cause lethal systemic illness characterized by edema, internal bleeding and infection of the central nervous system (Aronson, 2017).

In pigs, influenza generally happens in the form of volatile outbursts of acute respiratory disease characterized by high temperature and exhaustion, nasal and eye discharge, coughing, shortness of breath and loss of appetite. The disease persists best for a week, with complete regaining of health. Although mortality is low, swine influenza may result in a monetary loss due to the behind schedule weight gain. Equine influenza is caused by two subtypes of influenza A viruses - H7N7 and H3N8 and clinical symptoms include hyperthermia, nasal discharge and a severe dry cough; seldomly pneumonia in younger foals and donkeys and encephalitis in horses can advance (Meštrović, 2018).

In human beings, symptoms of flu consist of increase in temperature (typically high), headache, extreme weariness, dry cough, aching throat, gooey or stuffy nose, muscle aches, unsettled stomach symptoms, such as nausea, vomiting, and diarrhea - more common in kids than grown-ups. In severe cases troubled breathing or shortness of breath, tenacious pain or heaviness in the chest or abdomen, persistent vertigo, confusion, inability to stimulate, seizures, not urinating, intense muscle ache, severe feebleness or unsteadiness, lapsing cough, and general worsening of chronic health conditions may be detected (Sampson, 2018).

Everyone can get sick with the flu (even healthy people), and serious problems related to flu can happen at any age, but specific groups can be at relatively higher risk of having serious flu-related complications. This includes people who are 65 years and

older, those with long-lasting medical situations (such as asthma, diabetes, or coronary heart disease), pregnant females and children younger than 5 years. Simple cases of influenza typically clear up within 3-7 days for the majority of people, even though cough and malaise can keep on for >2 weeks, specifically in aged people and those with lingering lung disease (CDC, 2019a).

2.5. Routes of Transmission

The majority of influenza A strains reproduce in the lungs and the cells coating the intestinal tract of marine birds with excessive titers of virus expelled with feces into the water leading to an oral-fecal cycle of transmission. Virus will also be released in the animal's saliva and nasal exudation hence lakes and hibernating sites for water birds may aid as viral mixing plates (Wang *et al*., 2015). Birds that travel longitudinally also are crucial in transmission for they were liable for outbreaks in seals, whales, pigs and domestic fowls. The manner of transmission from aquatic to farm birds may be via direct physical interaction of the animals, or with surfaces, water or feed that have been polluted with the virus (Belser *et al*., 2018).

Poultry and pigs are the primary origins of human infections with IAVs. The entry portal for human infections is mostly via the conjunctiva (e.g., rubbing the eyes), nasal and mucosal membranes (e.g., breathing in of dust, droplets) by contact with infected poultry excreta and cadavers, or perhaps swimming in dirty water bodies (Lai *et al*., 2016; Wang *et al*., 2017). The interaction with poultry in live animal markets is an important source of human infections as well as animals with avian influenza viruses like H5N1, H7N9, H9N2 and H10N8 (Yi *et al*., 2015). Plenty of serological investigations have also shown that zoonotic IAV infections are more common in employees of poultry and pig ranches. Likewise, IAV infection of humans (e.g., hunters) through the direct contact with wild birds is an additional risk factor (Khan *et al*., 2015; Fragaszy *et al*., 2016).

Person to person spread is through respiratory droplets (coughing or sneezing) of people with influenza (generally within a distance of a meter). The virus can survive for up to 2 days (Bean *et al*., 1982) on solid surfaces and sometimes people may

become infected by touching such objects with flu viruses and then touching their mouth or nose. Most adults in good physical shape can be able to infect others beginning 1 day before symptoms develop and as much as 5 days after becoming sick (Pfeiffer *et al*., 2013).

2.6.Diagnosis

Influenza is a clinically distinctive illness however; laboratory investigations are indispensable to verify supposed influenza cases and eliminate flu-like illnesses which might be instigated by other viral and bacterial agents. More than a few different procedures are presently existing for diagnosis of IAV infections. To diagnose acute influenza virus infections early on, virus detection by means of rapid procedures for antigen detection and molecular methods have been established. The most commonly used, for screening suspected cases, are rapid influenza diagnostic tests (RIDTs). RIDTs work by detecting antigens or viral proteins that kindle an immune response. These tests can deliver results within roughly 10 to 15 minutes (CDC, 2018).

Traditionally the detection of particular antibodies like IgG and IgM has been broadly used diagnostically. Serological diagnosis is conceivable by means of the complement fixation, hemagglutination inhibition tests, immunofluorescence test and enzyme-linked immunosorbent assay (ELISA). Virus detection is clearly more preferred to antibody determination for diagnosis of recent (up to 1 week) influenza virus infections. However, antibody testing may be useful as a complementary tool to confirm the diagnosis retrospectively since molecular results will be negative by the time immunity kicks in (Allwinn *et al*., 2002).

In addition, there are numerous more accurate and delicate flu tests available that must be performed in dedicated laboratories. These include viral isolation in cell culture, nucleic acid amplification tests, immunochromatography-based rapid diagnostic tests, etc. Newer diagnostic tactics are also being developed to triumph over the restrictions associated with some of the conservative detection systems. All of these tests need swab sample from nose or the back of throat (Vemula *et al*., 2016). The standard for IAV isolation is virus culture in embryonated chicken eggs or mammalian cells, tailed

by a positive result in a hemagglutination assay (HA), and subsequent confirmation of HA-positive specimens using a more explicit test, such as RT-PCR analysis of genomic viral RNA (Eisfeld *et al*., 2014).

2.7. Prevention and Control

Vaccination against IAV

Vaccination still continues to be the number one protection and the most effective way to fight IAV infections (Houser and Subbarao, 2015) and would be required to shield against spread of a zoonotic strain within the human population. Three categories of influenza vaccines are presently certified for human use: inactivated whole- or split-virus; live-attenuated virus; and recombinant vaccines (Ozaki *et al*., 2004; Iuliano *et al*., 2018).

Animal vaccination can lessen the hazard of human infections with IAVs of zoonotic ability. Yet, IAV infections befall even vaccinated birds and pigs and cause disease frequently associated to the vaccines, which were verified by clear antigenic mismatches (Kitikoon *et al*., 2013; Kayali *et al*., 2013; Kandeil *et al*., 2017a), or due to a fresh introduction of a novel strain (Dharmayanti *et al*., 2014). An inherent uncertainty inevitably associated with this is the fact that possible pandemic strains may be antigenically dissimilar from the stockpiled vaccines. To this point, these vaccines stockpiles have to be regularly up to date in response to new discoveries that may become available (Reperant *et al*., 2016; Iuliano *et al*., 2018). Refining vaccination effectiveness in domestic and backyard poultry must also be well thought-out as an imperative measure to avert zoonotic spread of IAV with pandemic risk (Kandeil *et al*., 2017b; Tarigan *et al*., 2018).

Antivirals against IAV

For active control of influenza, good health practices and preventive measures should be followed. To lessen the inappropriate use of antibiotics as well as antiviral drugs primary correct and timely diagnosis should be done. There is no operative remedy in animals, although antimicrobials may be used to reduce secondary bacterial infections.

Sick animals need to be rested and given supportive care. Influenza-specific antiviral drugs are not yet official for use in animals (Reperant *et al*., 2016).

In humans however, the antiviral drugs currently available against influenza viruses are adamantane derivatives (amantadine and rimantadine) and neuraminidase inhibitors like zanamivir (Relenza), oseltamivir (Tamiflu) and peramivir (Rapivab). The neuraminidase inhibitors diminish the capacity of the influenza virus to spread from cell to cell by impeding the infection process at entry, assembly, or release level of viral replication. Whereas, adamantane derivatives hinder virus multiplication by interfering with the transmembrane domain of the matrix protein (M2) of influenza type A viruses and also restricts viral assembly during viral replication (Kumar *et al*., 2015).

In some cases, influenza A symptoms can resolve on their own with sufficient rest and fluid intake (Reperant *et al*., 2016). Despite their effectiveness, these medicines can cause side effects such as nausea and vomiting. Currently, many approaches are being pursued in experimental, pre-clinical work, and at different stages of clinical drug trials (Bouvier, 2018).

Other preventive and hygienic measures include washing hands with antiseptics after contact with suspected animals or people, avoiding large crowds specifically during a flu outbreak, wearing protective clothing like a facemask/respirator and gloves during giving care for patients or when dealing with infected animals, covering mouth and nose when coughing or sneezing, cleaning surfaces frequently, as well as cooking chicken and eggs properly before consumption. Refrigeration and freezing don't work since virus transmission from runny eggs has now been proven (Sampson, 2018).

2.8. Status of Influenza A Virus in humans in Ethiopia

Ethiopia has made it obligatory to file avian-human influenza and pandemic influenza among the 20 priority diseases in the country. The Ministry of Health (MOH) Ethiopia has started sentinel surveillance activities for influenza-like illness (ILI) since September 2008. At present, there are a total of 8 sentinel surveillance sites located in 5 main regions of the nation. In 2011, of 121 influenza specimens from humans 17 of

them tested positive; 8 were influenza B, 7 were seasonal influenza A (H3), and 2 were influenza A(H1N1) pdm09. In Ethiopia, seasonal Influenza A(H3N2), Influenza A(H1N1) pdm2009 and Influenza B viruses have been circulating between 2009–2015 (Woyessa *et al*., 2018).

It can be said that Ethiopia is at the forefront of a future flu pandemic as masses of birds that might be carriers the virus migrate from affected parts of Europe and Asia to East Africa and are thought to pass through lakes and marshland located in the rift valley of Ethiopia (Olsen *et al*., 2006; Nicholas, 2014). This can significantly increase the potential risk of spread into the chicken population. As many families in rural parts of the country practice backyard poultry and commonly live with their poultry in the same residence the chance of contact with infected poultry droppings and corpses is very high (Berhane and Tefera, 2005). Influenza is one of public health problems in Ethiopia and the need to present influenza vaccine and antivirus is vital to thwart upcoming treat of the disease.

Ethiopia is supported through a CDC cooperative agreement in its efforts to enhance laboratory and surveillance dimensions to spot and respond to avian, seasonal, and pandemic influenza. The first supportive contract between the MOH, Ethiopia Health and Nutrition Research Institute and CDC was signed in August 2007. The principal goal of this venture was to sustain and reinforce the influenza investigation, readiness and response to seasonal and pandemic influenza in Ethiopia (Ayele *et al*., 2012).

2.9.Report of Influenza A virus in camels of Ethiopia

In Ethiopia camels are commonly reared by pastoralists who live in arid and semiarid areas of the country and travel across many territories where the animals may be exposed to and adopt different viruses. In a study conducted in 2017, of 294 nasal swab samples collected from nomadic dromedary herds in Amibara, Asayita and Dubti districts of Afar region, for the study of MERS-CoV, one camel from Amibara district was found to be positive for influenza A virus (H1N1) (Chu *et al*., 2019).

There are also serological evidences of Influenza antibody circulation in African countries (Caffar Elamin and Kheir, 1985; Olaleye *et al*., 1989). In a more recent study, Influenza C and D viruses have been isolated in camels of Ethiopia (Murakami *et al*., 2019). In addition, out of the 100 camel serum samples collected from Amibara camels 4 serum samples were positive for antibody against H1N1 (A/dromedary/NV1337/2016) influenza A virus using hemagglutination inhibition test (Chu *et al*., 2019). However, there are no further detail studies showing the occurrence and public health importance of Influenza A virus in camels in Ethiopia which originate from different sites of camel rearing sites of the country. The difficulty to control the contact between people and dromedary camels especially in pastoral areas poses a risk for the pastoralist community to get the virus from camels (Tefera, 2004; Salem *et al*., 2017).

3. MATERIALS AND MEHODS

3.1. Study Setting

The study was conducted at Akaki municipal abattoir found in Akaki town. At the municipal abattoir different species of animals like cattle, sheep, goats and camels are slaughtered daily for public use. The study was conducted on the camels which originated from different camel rearing communities found in the surrounding areas mainly Borena, Metehara and Wenji. The majority of camel population that usually come to the abattoir are from Borena, Minjar, Meiso, Metehara and Shewarobit. However, during the period of this study only camels from those three areas were slaughtered.

Borena is one of the zones of Oromia region of Ethiopia, about 600 kms South of Addis Ababa with semi-arid climate. Camel population in this area has shown double increase in number within the past two decades (Aklilu and Catley, 2011). Metehara is a town in central Ethiopia located in East Shewa Zone of Oromia region found about 250 kms East of Addis Ababa with an elevation of 947 meters above sea level and the prevailing climate in this area is arid. The camels from Wenji originated from Fentale (Metehara) (CSA, 2005).

Pastoralists in Ethiopia frequently take their camels and pass through these areas whereas traders usually buy the camels at live animal markets from the pastoralists (Mirkena *et al*., 2018). Akaki abattoir is the closest abattoir around Addis Ababa that provides camel slaughtering service for consumers in the city. The Somali community and few other Muslim communities who live in the city are the main consumers of the camel meat from this abattoir.

3.2.Study population

The study population was camels slaughtered at Akaki municipal abattoir. Everyday 7-11 camels are slaughtered at the abattoir. About 30 camels are transported form the

pastoral areas once or twice per week and stay in a lairage till they are slaughtered based on customer demands.

3.3.Study Design

Abattoir-based cross-sectional study was used to assess the prevalence of Influenza A virus in camels at Akaki municipal abattoir.

3.3.1. Sample size and Sampling method

Since there were no previous studies on prevalence of the virus in camels that could be used as a reference, simple random sampling method was used to include all possible scenarios. The desired sample size for the study, was calculated using the Thrusfield formula (2005) i.e., single population proportion formula with 95% confidence interval, 5% absolute precision/margin of error and a 50% of expected prevalence was considered.

$$n = \frac{Z^2 P_{exp} (1-P_{exp})}{d^2}$$

Where; n= estimated sample size

Z= 95% CI=1.96

P_{exp} (expected prevalence) = 50%= 0.5

d (margin of error) =0.05

$$n = \frac{(1.96)^2 [0.5(1-0.5)]}{(0.05)^2}$$

$$n = 384$$

The samples were collected whenever new unsampled camels arrive at the abattoir. Risk factors of age, sex, breed and origin of the camels were recorded to measure the association of these factors with the prevalence of IAV. Breed, age and origin were identified by asking the owners (the traders who brought them) and abattoir workers

with years of experience regarding camels. In total, 400 nasal swab samples were collected.

3.3.2. Sample collection and transportation

First viral transport medium (VTM) aliquots of 1.5ul per tube were prepared at the National Influenza Reference Laboratory (NIRL) in Ethiopian Public Health Institute (EPHI). At the abattoir, once the animal was fully restrained, nasal swab samples were taken using sterile break off plastic polyester tipped swabs. The swabs were inserted gently into the camel's nostrils deep enough and rolled over the mucus membrane to scoop enough mucus material. Then the samples were carefully placed inside the phosphate-buffered glycerol-based VTM and kept in an ice box with ice packs during the field trip and stored at -20°C upon arrival at the NIRL until processing.

3.3.3. Questionnaire survey

In order to assess the public health significance of IAV in those who had contact with the camels at Akaki municipal abattoir, a questionnaire survey was conducted on the abattoir workers and camel traders that bring them to be slaughtered in the abattoir. The workers and traders were interviewed face-to-face by means of a structured questionnaire (appendix 2). The questionnaire was categorized into: i) socio-demographic characters and general information, ii) relationship of the participants with the camels, iii) personal hygienic practices iv) health history and v) their knowledge about the disease.

3.4.Laboratory investigation

Following the recommended procedure by CDC (2019b) the nasal swab samples from the camels were tested for the presence of IVA genetic material using Reverse transcriptase – polymerase chain reaction (RT- PCR). Viral RNA was first extracted using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). Extracted RNA was

then tested by a RT-PCR assay targeting a conserved region in influenza A matrix gene. The principles and procedures of RT- PCR test are described below.

There are three different sections of the molecular lab at NIRL. First there is the sample processing room for nucleic acid extraction. Then there is the clean room for master mix preparation. And finally, the room for the viral RNA amplification and detection. Influenza virus is an RNA virus and can be detected by RT-PCR. Purified RNA extracted from respiratory sample is used for detection.

Viral RNA extraction

In order to detect Influenza A virus types and subtypes, the RNA has to be extracted properly from the source sample for PCR analysis as a starting (target) material. Viral RNA was extracted from 392 nasal swab samples (8 of the samples had leaked) using QIAmp viral RNA mini kit (Qiagen, Dusseldorf, Germany). All extraction procedure was carried out in Biosafety Level II (BSL II) biosafety cabinet.

To extract the viral RNA, first a lysis buffer followed by carrier RNA and 140ul of VTM containing nasopharyngeal swab was added to a 1.5ml microcentrifuge. After incubation for 10 minutes at 20 ^{o}C, 95% ethanol (for binding RNA particles) was added to the tube and mixed. Then 630uL of the prepared solution was pipetted into labeled QIAamp spin columns and was centrifuged so that RNA can attach to the membrane of the spin column wall. The spin columns were then placed into a clean 2ml collection tube and the tube containing the filtrate was discarded. Then two wash buffers AW1 and AW2 were added to the spin columns to remove any particles that might have attached to the membrane besides the desired RNA. Finally, the RNA was eluted RNase free water. The detailed procedures are discussed in appendix 1. The tubes containing the sample RNAs were then labeled with sample codes and date and transferred to PCR work station.

RT-PCR

Reverse transcriptase - polymerase chain reaction (RT-PCR) was developed to amplify RNA targets of viruses such as IAV. PCR is based on the enzyme Taq Polymerase but RNA is not an appropriate substrate for this enzyme. This is why its

principle involves first the conversion of the RNA template into a complementary DNA (cDNA) using an enzyme called reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR. Although IAV genomic sequences vary widely across subtypes and species-specific virus groups, the 'M' gene segment is relatively highly conserved. Therefore, the presence of IAV can be evaluated by using RT-PCR with primers that recognize the M gene of viruses of all subtypes (WHO, 2011).

For this study a one-step RT-PCR was performed in which the same reaction tube was used for the reverse transcription as the polymerase chain reaction. First, 20ul of the prepared master mix which contains reverse transcriptase enzyme, primers specific for the target of interest, and nucleotides (dATP, dCTP, dGTP and dTTP) was added to a 96 PCR well plate in master mix room. Then 5ul of the extracted sample was added on it in sample addition room. The primers and probes specific to IAV were used to detect that specific type in a short time period.

Depending on the master mix kit manufacturer's instruction, the RT-PCR was set at specific temperature and time in order to properly do its annealing, denaturation and extension activities. If the target is present, primers anneal to the RNA strand then reverse transcriptase enzyme can synthesize a cDNA strand, extending from the primer. With each repetition of this cycle, the target is doubled and soon, after about 30 cycles, the reaction will yield an excess of 1 million copies of the target RNA fragment (Eisfeld *et al*., 2014).

The specific probes used consistent with the amplified nucleic acids have a reporter and quencher dye. The reporter dyes release fluorescent light as soon as it binds to an amplified DNA section. This released light is captured by the PCR sensor and conveyed as cycle threshold (Ct) value. A sample will be considered positive for influenza A virus if the amplification growth curve passed the threshold within 40 cycles (Ct value < 40) and if the Ct value ≥ 40, it is considered negative (Eisfeld *et al*., 2014) (detailed procedures are mentioned in appendix 1).

3.5.Ethical consideration

The study research protocol was reviewed and approved by the departmental research and ethics review committee for animal subjects of College of Veterinary medicine and Agriculture; Addis Ababa University. Ethical clearance was also obtained from Ethiopian Public Health Institute for human study subjects. The privacy and confidentiality of each individual participant was ensured. Personal name and any identifier of the participants was not revealed at any stage of the research project.

3.6.Data Management and Statistical Analysis

All the collected data was entered into excel spread sheet and analyzed descriptively (frequency and percentage) using IBM SPSS statistics version 20. The results were as summarized and presented using table and graph.

4. RESULTS

In this study, 8 of the collected 400 nasal swab samples were not tested because the collection tubes had leaked by the time of testing. During the sample collection period, the dromedary camels that came to Akaki municipal abattoir originated from three areas; Borena, Metehara and Wenji. From the gathered data by asking the traders and experienced workers at the abattoir, the camels were categorized in two breeds; Afar and Borena. Of the tested 392 nasal swab samples, 44.39% were males and 55.61% were females. The mean age of the study camels was 11.76 years (ranging from 6 – 24 years). The age groups of the study subjects were categorized into two groups; the adults aged between 10 – 24 years and the young ones aged between 6 – 9 years old. The characteristics of the study population are summarized in table 1.

Table 1: *Characteristics of sample population*

CATEGORY		***No. of camels examined n=392***
SEX	Male	174 (44.39%)
	Female	218 (55.61%)
AGE	Young	130 (33.16%)
	Adult	262 (66.84%)
BREED	Borena	224 (57.14%)
	Afar	168 (42.86%)
ORIGIN	Borena	212 (54.08%)
	Metehara	111 (28.32%)
	Wenji	69 (17.6%)

The RT-PCR test result showed that all the samples were negative for IAV.

Out of the 12 abattoir workers that were interviewed, 10 (83.3%) were male and 2 (16.7%) of them were females. Half of them 6(50%) were in the age group 31-40, while the rest were in the group 21-30 and >40 years accounting 25% each. When it comes to level of education, 2 (16.7%) of them were illiterate, 4 (33.3%) of them have attended school up to 8^{th} grade, 3 (25%) of them up to 12^{th} grade and 2 (16.7%) of them have acquired higher education. The 12 abattoir workers were the only ones that had contact of any form with the camels that came to the abattoir. Each one of them was interviewed with 25 questions used for assessment. The results of the questionnaire survey on the workers are indicated in the following tables.

Table 2: *General information on workers at Akaki municipal abattoir*

QUESTIONS	***RESPONSES N=12***						
Gender	Male	Female					
	10 (83.3%)	2 (16.7%)					
Age	15-20	21-30	31-40	>40			
	N=0	3 (25%)	6 (50%)	3 (25%)			
Level of education	Illiterate	Grade 1-8	Grade 10-12	Grade >12			
	2 (16.7%)	4 (33.3%)	3 (25%)	2 (16.7%)			
Type of animals in your vicinity	Camels	Cattle	Equine	Shoat	Chicken	Swine	None
	1 (8.3%)	4 (33.3%)	1 (8.3%)	2 (16.7%)	2 (16.7%)	N=0	2 (16.7%)
Camel contact with other animals	Yes		No		Sometimes		
	N=0		10 (83.3%)		2 (16.7%)		
Camel contact with objects used by animals	Yes		No		Sometimes		
	N=0		12 (100%)		N=0		
Average time in lairage before slaughter	24hrs	3-4 days	>1 week	months			
	5 (41.7%)	2 (16.7%)	5 (41.7%)	N=0			
Animal access to camel lairage	Yes		No		Sometimes		
	N=0		11 (91.7%)		1 (8.3%)		

Table 3: *Worker relationship with camels (frequency)*

QUESTIONS	***RESPONSES N=12***				
	Never	Rarely	Daily	Weekly	Monthly
Contact with camels	N=0	6 (50%)	5 (41.7)	1 (8.3%)	N=0
Cleaning lairage	4 (33.3%)	5 (41.7%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
Contact with camel waste	3 (25%)	4 (33.3%)	3 (25%)	2 (16.7%)	N=0
Camel slaughtering	N=0	6 (50%)	6 (50%)	N=0	N=0
Unloading camels	4 (33.3%)	6 (50%)	2 (16.7%)	N=0	N=0
Feeding camels	5 (41.7%)	3 (25%)	N=0	2 (16.7%)	2 (16.7%)
Contact with sick camels	Yes: 1 (8.3%)	No: 11 (91.7%)			
Consumed Raw milk/meat	Rarely: 1 (8.3%)	Daily: N=0	Weekly: 1 (8.3%)	Never: 10 (83.3%)	
Went to live animal market	Rarely: 2 (16.7%)	Daily: N=0	Weekly: 2 (16.7%)	Never: 8 (66.7%)	

Table 4: *Hygienic practices of workers*

QUESTIONS	***RESPONSES N=12***					
Use of protective gear	Full gear	Gloves	Face mask	Boots	Protective cloths	None
	N=0	N=0	N=0	3 (25%)	8 (66.7%)	1 (8.3%)
Washing hands	At mealtime	Before & after contact	After rest room	Beginning & end of the day	Sometimes	
	N=0	12 (100%)	N=0	N=0	N=0	
Hair	Covered	Not covered				
	10 (83.3%)	2 (16.7%)				
Jewelry	Worn	Not worn				
	1 (8.3%)	11 (91.7%)				

Table 5: *Health history of workers*

QUESTIONS	***RESPONSES N=12***						
Signs of fever or coughing	Yes: N=0	No: 12 (100%)					
Resp. dis. in the last 12 months	Yes: N=0	No: 12 (100%)					
Visited/ stayed at a hospital due to Resp. dis.	Yes: N=0	No: 12 (100%)					
Contact with someone with resp. dis.	Yes: 1 (8.3%)	No: 11 (91.7%)					
Smoking status	Don't smoke	1-2times/day	3-10 times/day	>20times/day	Rarely		
	N=10/12(83.3%)	N=0	N=2/12(16.7%)	N=0	N=0		
Any symptoms of IAV	Fever	Headache	Fatigue	Dry cough	Sore throat	Runny nose	None
	N=0	N=0	N=1/12(8.3%)	N=0	N=1/12(8.3%)	N=0	N=10/12(83.3%)

Table 6: *Knowledge assessment on workers*

QUESTIONS	***RESPONSES N=12***	
Awareness of the disease	Yes: 12 (100%)	No: N=0
Possibility of zoonotic transmission	Yes: N=0	No: 12 (100%)
Received training about food safety	Yes: 10 (83.3%)	No: 2 (16.7%)

According to those in charge at the Akaki abattoir these 7 traders were their longtime customers and they have camel meat shops around 22 Megenagna through which they supply to consumers. They were all given the questionnaire paper and the results are summarized in table 2. Of the 7 traders, 4 (57.1%) of them were males and 3 (42.9%) of them were females. While 3 (42.9%) of them were in the age group of 31-40, the other 2 (28.6%) were in the group 21-30 and the other 2 (28.6%) were above 40 years old. While none of them have acquired a higher education, 3 (42.9%) of them were illiterate, 2 (28.6%) of them have learned up to 8^{th} grade and the other 2 (28.6%) have learned up to 12^{th} grade. The results of the questionnaire survey on the traders are indicated in the following tables.

Table 7: *General information about camel traders*

QUESTIONS	***RESPONSES N=7***						
Gender	Male	Female					
	4 (57.1%)	3 (42.9%)					
Age	15-20	21-30	31-40	>40			
	N=0	2 (28.6%)	3 (42.9%)	2 (28.6%)			
Level of education	Illiterate	Grade 1-8	Grade 10-12	Grade >12			
	3 (42.9%)	2 (28.6%)	2 (28.6%)	N=0			
Type of animals in your vicinity	Camels	Cattle	Equine	Shoat	Chicken	Swine	None
	1 (8.3%)	N=0	1 (8.3%)	2 (28.6%)	N=0	N=0	3 (42.9%)
Camel contact with other animals		Yes		No			Sometimes
		N=0		7 (100%)			N=0
Camel contact with objects used by animals		Yes		No			Sometimes
		N=0		7 (100%)			N=0
Average time camels stay with trader		24hrs	3-4 days	>1 week	months	DK	
		N=0	5 (71.4%)	2 (28.6%)	N=0	N=0	

Table 8: *Trader relationship with camels (frequency)*

QUESTIONS	***RESPONSES N=7***				
	Never	Rarely	Daily	Weekly	Monthly
Contact with camels	1 (14.3%)	4 (57.1%)	N=0	2 (28.6%)	N=0
Cleaning lairage	3 (42.9%)	4 (57.1%)	N=0	N=0	N=0
Camel slaughtering	3 (42.9%)	4 (57.1%)	N=0	N=0	N=0
Loading and unloading camels	2 (28.6%)	5 (71.4%)	N=0	N=0	N=0
Feeding camels	3 (42.9%)	4 (57.1%)	N=0	N=0	N=0
Contact with sick camels	Yes: N=0	No: 7 (100%)			
Consumed Raw milk/meat	Rarely: 1 (14.3%)	Daily: N=0	Weekly: 2 (28.6%)	Monthly: 2 (28.6%)	Never: 2 (28.6%)
Went to live animal market	Rarely: N=0	Daily: N=0	Weekly: 6 (85.7%)	Monthly: N=0	Never: 1 (14.3%)

Table 9: *Hygienic practices of the traders*

QUESTIONS	***RESPONSES N=7***					
Use of protective gear	Full gear	Gloves	Face mask	Boots	Protective cloths	None
	N=0	N=0	N=0	N=0	4 (57.1%)	3 (42.9%)
Washing hands	At mealtime	Before & after contact with animals	After using rest room	Beginning & end of the day	Sometimes	
	N=0	6 (85.7%)	N=0	N=0	1 (14.3%)	
Hair	Covered	Not covered				
	4 (57.1%)	3 (42.9%)				
Jewelry	Worn	Not worn				
	2 (28.6%)	N=5/7(71.4%)				

Table 10: *Health history of the traders*

QUESTIONS	***RESPONSES N=7***						
Contact with someone with resp. dis.	Yes: N=0	No: 7 (100%)					
Signs of fever or coughing	Yes: N=0	No: 7 (100%)					
Resp. dis. in the last 12 months	Yes: N=0	No: 7 (100%)					
Visited/ stayed at a hospital due to Resp. dis.	Yes: N=0	No: 7 (100%)					
Smoking status	Don't smoke	1-2times/day	3-10 times/day	>20times/day	Rarely		
	5 (71.4%)	2 (28.6%)	N=0	N=0	N=0		
Any symptoms of IAV	Fever	Headache	Fatigue	Dry cough	Sore throat	Runny nose	None
	N=0	N=0	N=0	N=0	1 (14.3%)	N=0	6 (85.7%)

Table 11: *Knowledge assessment on camel traders*

QUESTIONS	***RESPONSES N=7***	
Awareness of the disease	Yes: 7 (100%)	No: N=0
Possibility of zoonotic transmission	Yes: 2 (28.6%)	No: 5 (71.4%)
Received training about food safety	Yes: 2 (28.6%)	No: 5 (71.4%)

5. DISCUSSION

In the present study the prevalence of Influenza virus infection in camel was zero. This study has produced a negative result compared to a previous work done in Ethiopia by Chu *et al*., 2019 where only 1 positive result was found of the 102 nasal swab samples collected from Amibara district of Afar region from January – July 2017. The possible reason for not detecting the virus in this study might be related to the difference in site where Chu et al detected the virus in Amibara district of Afar region and in current study no camels where from these sites. Similarly, since serological assessment were not done because of lack of laboratory facility, status of previous exposure of the camels to Influenza A virus cannot be determined at this time. In addition, to the difference in geographic location of the origin of camel, the state of the infection might be another factor as the detection of Influenza A virus in nasal swab can be possible if the infection is recent (less than one week) before the natural immunity develop.

Recent infections (less than a week) could be detected using RT- PCR, infections that have lingered for longer than a week cannot be identified (would be negative by RT-PCR) since by then the camel's natural immunity would have restricted the viral replication (Abdellatif *et al*., 2014; Krammer *et al*., 2018). In this case, serological tests should be done to check for the presence of specific antibodies produced by the camels as a result of previous infection (Malanoski and Lin, 2013; Dziąbowska *et al*., 2018). However, in this study serology studies were not conducted because the desired test kits were not available at NIRL of Ethiopian Public Health institute nor at NAHDIC and National Veterinary Institute. The laboratories were also overcrowded with samples from COVID-19 suspected patients that they were not able to cooperate with other studies.

Influenza A viruses from animal sources are different from seasonal influenza viruses in humans and do not spread readily between humans unless they acquire the sufficient mutation over a period of long frequent exposure (Geoghegan *et al*., 2017). However, zoonotic influenza viruses that do affect humans by direct or indirect contact can cause illness ranging from minor illness to death in humans (WHO,

2020). A questionnaire survey was conducted at Akaki municipal abattoir workers and the traders who bring the camels to assess the public health significance of IAV in relation to the camels.

Generally, the workers have been doing abattoir related work associated with camels for a significant period of time ranging from 1 year to over 15 years. The questionnaire survey result from the abattoir workers showed that there were other animals living near the living areas of all except for the two of them. This shows that most of the workers in addition to their occupation, they are constantly living under a condition that serves as a hot spot (Paige *et al*., 2015) for emerging and re- emerging diseases to jump from one species to another (Neumann and Kawaoka, 2015; Krammer *et al*., 2018; Bailey *et al*., 2018).

At the abattoir, in addition to camels other animals like cattle, sheep and goats are also slaughtered. Not too far from the center there is a large poultry market that can serve as a source of the virus (Thangavel and Bouvier, 2014; Richard, M., and Fouchier, 2016) and camels may be exposed when there is delay during unloading of the camels. As a recent study shows cattle (Sreenivasan *et al*., 2019) can also be carriers of the influenza A viruses. Only 2 of them said there might be a chance other animals in the abattoir may have access to the camels' lairage on occasions when there are too many animals at the abattoir. As mentioned by many researchers (Reperant *et al*., 2012; Mostafa *et al*., 2018; Mollentze, N., and Streicker, 2020; Johnson *et al*., 2020), intensification of multi-species of animals and overcrowding of human populations are one of the major risk factors for emergence of zoonoses.

On average, the camels spend about 4 days in the lairage and during that time the majority of the workers come in close contact with the camels while cleaning the lairage, feeding and watering them, or while unloading the camels. All these show how closely to the camels are the daily lives of the workers where there is an increased frequency of contact which is another critical point in which many diseases may find their way into the human population (Reperant *et al*., 2012; Bueno *et al*., 2015; Mollentze, N., and Streicker, 2020). All of them said the camels and the other animals have no chance of sharing similar materials. However, it was observed that the camels were slaughtered in the same place as the other animals which poses

another critical point where contamination of carcasses can take place (Edmunds *et al*., 2013; Savelli *et al*., 2019).

Regarding consumption of raw camel meat and milk, only 2 of them do so once in a while. It can be said that these 2 consumers are at risk of not only of getting IAV but also for other foodborne diseases (Van Kerkhove *et al*., 2011; European Food Safety Authority (EFSA) *et al*., 2018; Faour-Klingbeil and Todd, 2019). In this case, the chance of exposure to the virus from live animal market is lower as only 4 of them go there as rarely as once in a month (Pusch and Suarez, 2018).

When it comes to the use of protective equipments, the work cloths they have was made of hard fabric yet could only cover them from grossly visible dirt getting on their skin. Although they wash it regularly, it is not enough to protect them from zoonotic diseases that use air and physical contact as mode of transmission. Proper protective cloths like plastic gowns, gloves, face masks and hair masks must be made available not only to protect themselves but also to prevent risk of reverse zoonoses (Nel *et al*., 2004; Kim *et al*., 2014; Abdullahi *et al*., 2016).

Almost all of them were aware of the presence of influenza among humans but none of them knew they could possibly get it from camels. Except the 2 recently hired workers they all said they have received some form of training regarding hygienic practices in abattoir. Despite this fact, they were not able to practice what they learned due to the inconvenient way the abattoir is constructed as it is located in the middle of a crowded town and it lacks a good clean water and waste drainage system.

Only 2 of them said they smoke 3-10 times a day which may have resulted in one of them having a sore throat since the person did not check any of the other boxes mentioned as symptoms of IAV. One of them said he has extreme fatigue which may be related to the hard labor he does at the abattoir.

In a similar manner as the abattoir workers, more than half of them had other animals living close to their living areas. The average time the camels stay with them is 3-4 days which is more than enough time for the virus to transmit to human population. Just like the abattoir workers these traders also have frequent contact with the camels

from the time they buy them from the pastoralists to the stage where they supply the meat to the consumers. This close human – animal interface puts them at risk of many zoonotic diseases (Neumann and Kawaoka, 2015; Krammer *et al*., 2018).

When it comes to consumption of raw camel meat and milk, 5 of them said they do so either on a weekly or a monthly basis which indicates a higher risk of getting the virus through this means (Faour-Klingbeil and Todd, 2019). Almost all of them frequently go to live animal markets where many animal species interact and therefore create a hot spot for cross-species transmission of diseases (Esteban, 2010).

Regarding the use of proper protective cloths, 4 of the participants responded that they use nothing but their bear hands when they handle the camels showing the need for supply and training regarding safety measures. Except one, all of them wash their hands before and after they make any form of physical contact with the camels which should be encouraged.

Of the symptoms of flu infection only one of them said they have a sore throat which may be related to his smocking habit. All of the interviewed traders had knowledge of the disease but only 2 of them knew of the potential of respiratory disease like IAV transmission from animals like camels because they had received a training related to husbandry practices in animal handling. This show how little work is done among the participants regarding awareness creation about zoonoses and food safety measures.

Limitations of the study

One of the major limitations of this study was that serology tests were not done to check for the presence of antibody production by the camels against IAV.

6. CONCLUSION AND RECOMMENDATIONS

In the present study Influenza viral RNA was not detected in none of the nasal swab samples. However, the zoonotic importance of the disease is not something that can be neglected due to the potential risk factors at Akaki municipal abattoir such as the high frequency of contact with the camels as well as consumption of raw camel meat and milk and lack of appropriate personal protective equipments. In addition, RNA viruses are known to have high mutation rates that can enable them to cross-species given a suitable condition. This can then lead to unexpected outbreaks that can jeopardize the health of the workers and those involved as well as have impact on the nation's economy through restriction of export trade of camel.

Based on the above conclusion, the following points are recommended:

- Serology tests should be done to study the occurrence of antibodies in the camels' serum.
- Further surveillance of IAV infection in camels at camel rearing areas should also be conducted in order to understand the potential risk factors for infection and transmission between camels and human populations. Expanded research on camels from previously Influenza A virus detected sites mainly Afar region should be carried out.
- Awareness creation programs should be in place to update the public knowledge about the zoonotic importance of Influenza A virus and preventive measures such as the use of proper protective equipments, food safety and hygienic measures.
- Finally, surveillances systems concerning IAV should be strengthened with long term objectives and should encompass its effects and potential jump to other species besides the already known hosts.

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8. APPENDICES

Appendix1: Laboratory procedures

Principle of RNA extraction:

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

The process involves the sample being lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding to the QIAamp membrane, and the sample loaded onto the QIAamp spin column. The RNA binds to the membrane and the contaminants are washed in two steps using two different wash buffers. High quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage.

Materials and Reagents used:

1. QIAamp viral RNA extraction kit containing;
    a. QIAamp spin columns
    b. Collection tubes
    c. Buffer AVL
    d. Buffer AW1
    e. Buffer AW2
    f. Buffer AVE
    g. Carrier RNA (ploy A)
2. Ethanol (96-100%)
3. Micro-centrifuge tubes (1.5mL)
4. Micropipette 5ul,10ul,20ul,200ul,1000ul capacity
5. Sterile, RNase-free pipette tips with aerosol barrier(filter)

6. Permanent marker pen
7. Powder free Disposable gloves, gown
8. Micro-centrifuge
9. Vortex
10. Masks
11. Timer
12. RNaseZAP,70%alcohol, paper towel(napkin) -for cleaning and disinfection
13. Microcentrifuge racks 2-3

RNA extraction procedure from nasal swab samples:

1. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA by adding 560µl of lysis buffer and 5.6ul carrier RNA into a 1.5ml microcentrifuge tube,
2. Add 140uL of cell culture supernatant or VTM containing nasopharyngeal swab to the Buffer AVL/Carrier RNA in the micro-centrifuge tube. Mix by pulse-vortexing for 15 sec.
3. Incubate at room temperature for 10 minutes and Centrifuge briefly the 1.5mL micro-centrifuge tube to remove drops from the inside of the lid.
4. Add 560uL of ethanol (96-100%) to the tube containing sample and Buffer AVL/Carrier RNA in order to provide optimum binding of the RNA to the QIAamp membrane, and mix by pulse-vortexing for 15sec. Briefly centrifuge the 1.5mL micro-centrifuge tube to remove drops from the inside of the lid.
5. Open the required number of QIAamp spin columns from the paper and plastic wrapper and label with sample identification number. Pipette 630uL of the solution from step 4 above into a labeled QIAamp spin column without wetting the rim. Close the cap of the QIAamp spin column. Repeat this step for all the samples. Then centrifuge at 6,000g (8,000 rpm) for 1 minute.
6. The RNA binds to the membrane, and contaminants are efficiently washed away in two steps using two different wash buffers.
7. Place the QIAamp spin column into a clean 2mL collection tube (provided in kit). The collection tubes containing the flow through will be discarded into jar containing disinfectant and a new collection tube will be replaced.
8. Pipette 500uL of the first wash buffer AW1 to all the QIAamp spin columns. Close the caps and centrifuge at 6,000g (8,000 rpm) for 1 minute. Place the QIAamp spin

column in a clean 2mL collection tube (provided in kit) and discard the tube containing the filtrate into jar containing disinfectant.

9. Pipette 500uL of the second wash buffer AW2 to all the QIAamp spin columns. Close the caps and centrifuge at full speed 20,000g (14,000rpm) for 3 minutes to ensure complete removal of the wash buffer using new collection tubes. The flow through will then be discarded.
10. Place the QIAamp spin column in a clean 1.5mL micro-centrifuge tube (not provided).
11. Carefully open the QIAamp spin column and add 60uL of AVE (RNase-free water) equilibrated to room temperature to elute high-quality RNA. Close the cap and incubate at room temperature for 1minute. Then centrifuge at 6,000g (8,000rpm) for 1 minute.
12. Store extracted viral RNA (elute) at -80oC or proceed to perform RT PCR.

The extracted RNA samples will then be taken to a biosafety level 2 virology laboratory in the institute for the RT-PCR procedure. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors.

Principle of RT-PCR:

Real time reverse transcription-polymerase chain reaction (RT-PCR) is used to quantify RNA. In 1-step RT-PCR, reverse transcription and the PCR is performed in a single buffer system. The reaction proceeds without the addition of reagents between the RT and PCR steps. 1-step RT-PCR offers the convenience of a single-tube preparation for RT and PCR amplification.

Its principles can be summarized as:

Denaturation: When RNA molecule is heated to 95 ^{o}C, the primers can access the single stranded RNA (ssRNA) templates.

Annealing: The reaction mixture is cooled (about 50^{o}C) to allow primers to select and bind (hybridize) to their complementary positions on the ssRNA template molecules.

Elongation: The ssRNA/primer solution is heated to 95^{o}C. In the presence of the heat stable polymerase, PCR buffer, dNTP's and ROX dye, the replication procedure begins. With each repetition of this cycle, the target is doubled and soon, after about 30

cycles, the reaction will yield in excess of 1 million copies of the target RNA fragment. It was then cooled to 58 °C.

Master mix preparation:

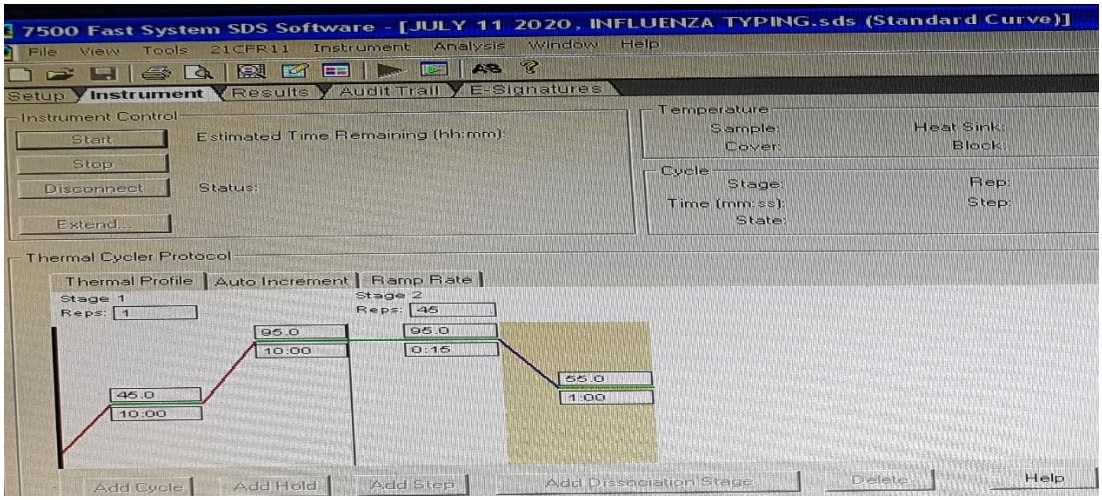
Master mix reagent should be prepared fresh for each batch/run. the reagent volume calculation is performed before the start based on the no of samples analysed per run and the kit type. Master mix was prepared using Ambion AgPath Enzyme Kit reagents to detect flu type A.

Procedure of RT-PCR:

1. Set up a cDNA synthesis (reverse transcriptase) reaction as described below:

Reagents	**Volume of reagent added per reaction (ul)**	**Amount calculated for (N+1) reactions for IAV (N+2=100)**
2x master mix	N x 12.5	1250
Nuclease free water	N x 6.25	625
Forward primer	N x 0.25	25
Reverse primer	N x 0.25	25
Probe	N x 0.5	50
RT-PCR enzyme mix	N x 0.25	25
Total	N x 20	2000ul

2. Vortex to mix, incubate at room temperature for 10 minutes, and then at 42°C for at least 15 minutes.
3. Deactivate the reverse transcriptase by incubating at 95°C for 5 minutes, and then chill the reactions on ice.
4. Set up the RT-PCR reaction in a MicroAmp fast optical 96-well reaction plate as described in the following table. The Primer/Probe Master Mix contains primer/probe sets against highly conserved regions of the IAV M gene.
5. Perform the IAV M-gene specific RT-PCR in a real-time thermocycler as described below:



6. When the reaction is complete, view the amplification plots according to the real-time apparatus manufacturer's instructions. Ensure that the threshold is set just above the background signal and RT-PCR.

The outcomes of real-time RT-PCR analyses are determined by detecting the normalized reporter (Rn) value for every reaction.

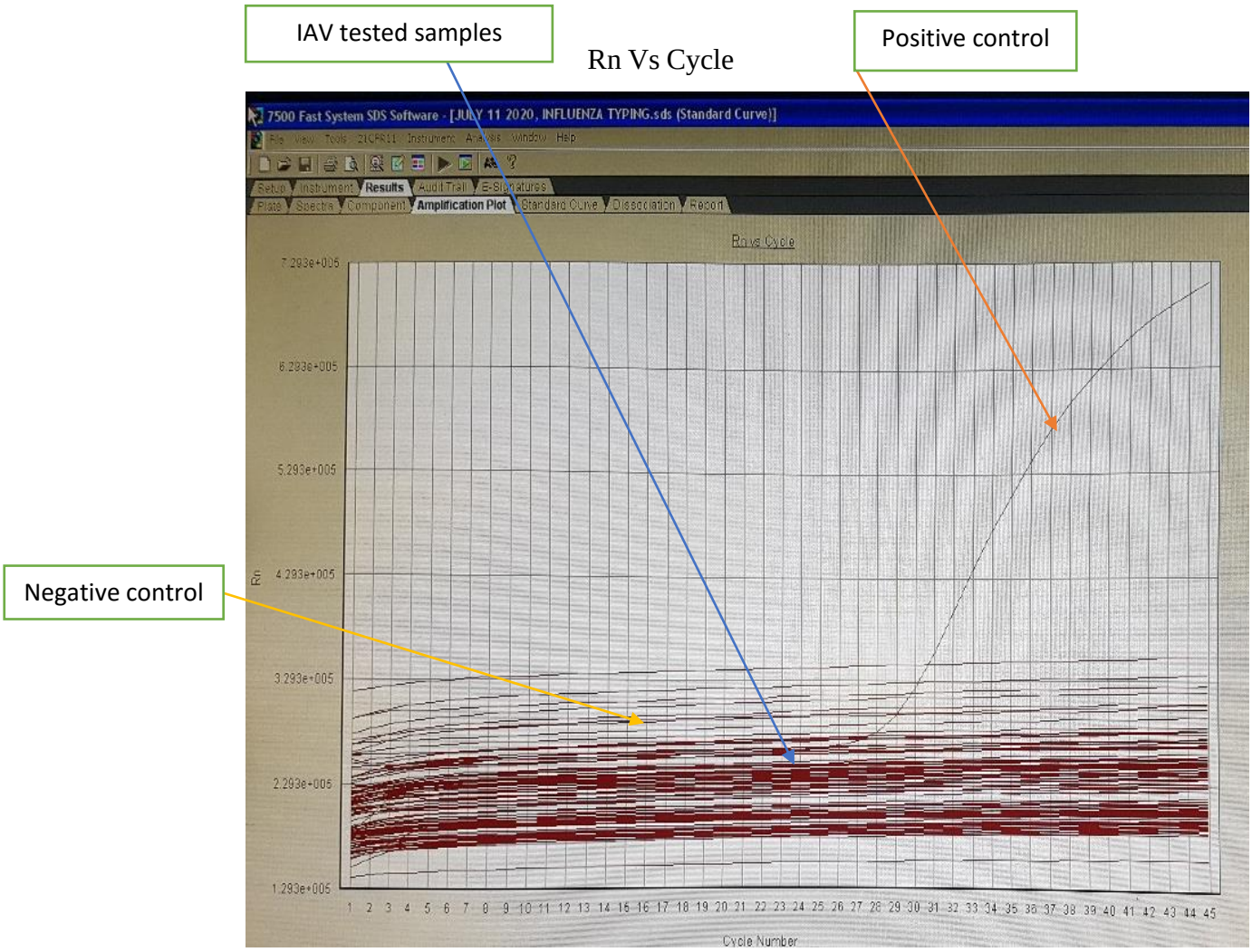
Rn = fluorescence of the FAM™ dye /fluorescence of a passive reference dye (ROX™)

Results are then reported as a Ct (cycle threshold) value, which is the sum of cycles needed for the Rn value to interconnect with the threshold line, and it shows the relative concentration of target RNA in the reaction. A characteristic Ct value for a positive IAV RNA amplification is $\leq$ 35.0, and the Rn value ought to display a sigmoidal curve. Any Ct signal above 35 cycles may be considered suspect and require verification.

Result interpretation:

- CT value < 40 = Positive for specific flu type or subtype
- CT value $\geq$ 40 = Negative for specific flu type or subtype

Appendix 2: RT-PCR result of the camels



Camels IAV negative samples PCR amplification graph

Appendix 3: Questionnaire survey form for human exposure risk assessment of Influenza A virus

1. **General 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: _____

Level of education: _____

1.1. Which of the following animals are raised near your living area? (check all that are there)

□ Camels □ Goats □ Sheep □ Cattle □ Horses □ Donkey □ Chickens □ Pigs □ None

1.2. Do you personally provide care for camels the abattoir? □ YES □ NO

1.3. If "yes", how long have you been doing camel related work?

_____

1.4. Is there a chance the camels might come in contact with other animal at the abattoir or near the area?

□ Yes, there is a chance □ No □ On rare occasions

1.5. Is there a way the camels might come in contact with materials used for other animals in the abattoir?

□ Yes, there is a chance □ No □ On rare occasions

1.6. On average how long do the camels stay in the abattoir's lairage?

□ 24hrs □ 3-4 days □ > a week □ months

1.7. Can other animals access the camel's lairage?

□ Yes, there is a chance □ No □ On rare occasions

2. Worker relationship with the camels (frequency of contact)

2.1. On average, over the last 12 months, how often do you do the following activities? (use "√" sign to indicate your response)

Activity	**Never**	**Rarely**	**Daily**	**Weekly**	**Monthly**
Contact with camels					
Cleaning camels' lairage					
Handel animal waste					
Slaughter camels or assist in slaughtering					
Unloading camels					
Feeding or watering camels					

2.2. In the past 12 months, have you had direct contact with any sick camels?

□ YES □ NO

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

□ YES □ NO

2.4. In the past 12 months, have you drunk raw camel meat or milk?

□ YES □ NO □ Don't Know

2.5. 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

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

□ YES □ NO

2.7. 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

3. Personal Protective Equipment and Hygiene Practices

3.1. What personal protective equipment do you usually wear when handling camels or related works? (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 abattoir (check all that apply)

□ At mealtimes □ Before and after each animal related task

□ At bathroom times □ The beginning and end of the day □ Rarely

3.3. Do you wear head covers when slaughtering camels and while handling meat?

□ YES □ NO

3.4. Do you remove jewelries while working at the abattoir in relation to camels?

□ YES □ NO

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_____

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?

□ 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. In the past few months have you had any respiratory diseases with the following symptoms?

□ fever □ headache □ extreme tiredness □ dry cough □ sore throat □ runny or stuffy nose □ muscle aches □ stomach symptoms (nausea, vomiting)

5.7. Is there any other cause that may have resulted in a respiratory disease?

□ Cigar smoking □ Rhinitis □ Asthma □ other diseases _____

6. Knowledge assessment

6.1. Do you know what IAV infection is and that it can be transmitted from person to person?

□ Yes, I know □ No, I do not know

6.2. Do you know that IAV can be transmitted from camels to humans?

□ Yes, I know □ No, I do not know

6.3. What preventive measures would you use?

_____

6.4. Have you ever received training about food safety and hygienic practices?

□ YES □ NO

II. Questionnaire survey for human exposure risk assessment (Amharic Version)

<u>የተሳታፊው መረጃ</u>

ቀን: ____/____/____

የተሳታፊው ስም፡_____ መለያ ቁጥር፡_____

የመኖሪያ አድራሻ፡ ክልል፡---------- ዞን፡----------ወረዳ፡----------

ጾታ፡---------- እድሜ፡---------- የትምህርት ደረጃ፡----------

1. <u>አጠቃላይ መረጃ</u>

1.1.በሚኖሩበት ቤቶ ወይንም በቤቶት አቅራቢያ ምን አይነት እንስሳት ይገኛሉ?

□ ግመል □ ፍየል □በግ □ከብት □ፈረስ □አህያ □ዶሮ □አሳማ

1.2.በቄራው ውስጥ ለሚመጡ ግመሎች እንክብካቤ ያደርጋሉ?

□ አዎ □ አላደርግም

1.3.መልሶ አዎ ከሆነ ከግመል ጋር በተያያዘ ስራ ላይ ምን ያህል ጊዜ ቆዩ?.....

1.4.ግመሎቹ በቄራ ውስጥ ወይም በአካባቢው ካሉ ሌሎች እንስሳት ጋር የሚግናኙበት አጋጣሚ ይኖራል?

□ አዎ ይኖራል □ ምንም አጋጣሚ የለም □ አልፎ አልፎ

1.5.ግመሎቹ በቄራ ውስጥ ያሉ እንስሳት የተጠቀሙትን ዕቃዎች ጋር ንክኪ ሊኖራቸው ይችላል?

□ አዎ ይችላል □ ምንም አጋጣሚ የለም □ አልፎ አልፎ

1.6.ግመሎቹ እስኪታረዱ በአማካይ ለምን ያህል ጊዜ ይቆያሉ?

□ 24 ሰዐት □ 3-4 ቀናት □ ከሳምንት በላይ □ ወር

1.7.ወደ ግመሎቹ ማቆያ ሌሎች እንስሳት መግባት ይችላሉ?

□ ይችላሉ □አይችሉም

2. ከእንስሳት ጋር ያሎትን ግንኙነት በተመለከተ

2.1. ከዚህ በታች የተዘረዘሩትን ተግባራት ላለፉት 12 ወራት በአማካኝ ምን ያክል ያከናውናሉ? (“√” ምልክት ያድርጉ)

ተግባር	ፈጽሞ አላከናውንም	አልፎ አልፎ	በየቀኑ	በየሳምንቱ	በየወሩ
ግመል መንካት					
የግመል ቤት ማጽዳት					
የግመል እዳሪ ጋር ንክኪ					
ግመል እርድ ማከናወን ወይም መርዳት					
ግመሎች ከመኪና ሲወርዱ እገዛ ማድረግ					
ግመሎች መመገብ/ማጠጣት					

2.2. ባለፉት 12 ወራት ውስጥ ከታመመ ግመል ጋር ንክኪ ነበሮት?

□አዎን □አልነበረኝም

2.3. አዎን ከሆነ መልሰ፤ በዚህ ወቅት ውስጥ ታሞ የሞተ ግመል ያውቃሉ?

□አዎን □አላውቅም

2.4.ባለፉት 12 ወራት ውስጥ ጥሬ የግመል ወተት ጠጥተው ወይም ስጋ በልተው ያውቃሉ?

□አውቃለው □አላውቅም □አላስታውስም

2.5.ጠጥተው/በልተው የሚያውቁ ከሆነ ምን ያህል ጊዜ ያዘወትራሉ?

□1= አልፎ አልፎ (ከወር አንድ ጊዜ ባነሰ)

□2= በየወሩ (ቢያንስ በወር አንድ ጊዜ)

□3= በየሳምንቱ (ቢያንስ በሳምንት አንድ ጊዜ) □4= በየቀኑ

2.6.ባለፉት 12 ወራት ውስጥ የቁም እንስሳት ገበያ ቦታ ሄደው ያውቃሉ?

□አዎን □አላውቅም

2.7. ሄደው የሚያውቁ ከሆነ ምን ያህል ጊዜ?

□1= አልፎ አልፎ (ከወር አንድ ጊዜ ባነሰ)

□2= በየወሩ (ቢያንስ በወር አንድ ጊዜ)

□3= በየሳምንቱ (ቢያንስ በሳምንት አንድ ጊዜ) □4= በየቀኑ

3. <u>የግል መከላከያ ቁስና ንጽህና ተግባራት</u>

3.1.ከግመሎች ጋር የተያያዙ ስራዎችን ሲሰሩ አዘውትረው መከላከያ ቁሶችን ይጠቀማሉ?

□ምንም አልጠቀምም □የእጅ ጓንት □ሙሉ መከላከያ ልብስ □አቧራ መከላከያ ጭምብል

□ቦቲ □የአይን መከላከያ መነጽር □ሌሎች:_____

3.2.ከእንስሳት ጋር ንክኪ ካደረጉ ቡኃላ ምን ያህል አዘውትረው እጆትን ይታጠባሉ?

□ምግብ ልመገብ ስል □ከእንስሳት ጋር የተገናኙ ተግባራትን ከማከናወኔ በፊትና በኃላ

□ሽንት ቤት ስጠቀም □ጠዋትና ማታ □አልፎ አልፎ

3.3.በእርድ ጊዜ እና የግመል ስጋ በሚነኩበት ወቅት የፀጉር መሸፈኛ ይጠቀማሉ?

□አዎን □አለጠቀምም

3.4.በስራ ወቅት ጌጣጌጦችን ያስወግዳሉ?

□አዎን □አላስወግድም

4. <u>ከሰዎች ጋር የሚደረግ ንክኪ</u>

4.1.ባለፉት 6 ወራት መተንፈሻ አካል በሽታ የታመመ ሰው ጎብኝተው ያውቃሉ?

□አውቃለው □አላውቅም

4.2.ጎብኝተው ከሆነ ከታማሚው ጋር ያሎት ግንኙነት ምንድን ነው?

□የቅርብ ቤተሰብ □ዘመድ □ጓደኛ □ሌላ _____

5. <u>የህክምና ታሪክ</u>

5.1.በአሁኑ ወቅት ትኩሳትና ሳል አለቦት?

□ አዎን □ የለብኝም

5.2.ባለፉት 12 ወራት ውስጥ የመተንፈሻ አካል ህመም አጋጥሞት ወደ ሀኪም ቤት ሄደው ነበር?

□ አዎን □ ሄጄ አላውቅም

5.3.በህመሙ ምክንያት ሆስፒታል ውስጥ አድረው ነበር?

□ አዎን □ አድሬ አላውቅም

5.4.በአሁኑ ወቅት ሲጋራ ወይም ትምባሆ ያጨሳሉ?

□ አዎን □ አላጨስም

5.5.የሚያጨሱ ከሆነ በአማካኝ ምን የህል ያጨሳሉ?

□በየቀኑ አላጨስም □በቀን 1-2 ጊዜ □በቀን 3-10 ጊዜ

□በቀን 11-20 ጊዜ □በቀን 20 ጊዜ በላይ

5.6.ባለፉት ወራት ከታች ከተዘረዘሩት ውስጥ የትኛው የጤና ችግር ምልክት አጋጥሞታል?

□ትኩሳት □ራስ ምታት □ከፍተኛ ድካም □ደረቅ ሳል □የጉሮሮ ቁስለት □ቁርጥማት

□የንፍጥ መዝረክረክ □የሆድ ህመም (ማቅለሽለሽ ፣ ማስታወክ)

5.7.ከላይ ከተጠቀሱት የጤና ችግር ምልክቶች ሌላ ካለ

……..

6. <u>ስለበሽታው</u> <u>ዕውቀት</u>

6.1.በሽታው ሰዎችን እንደሚያጠቃ እና ከሰው ወደ ሰው እንደሚተላለፍ ያውቃሉ?

□ አዎ አውቃልሁ □ አላውቅም

6.2.በሽታው ከግመሎች ወደ ሰዎች ሊተላለፍ እንደሚችል ያውቃሉ?

□ አዎ አውቃልሁ □ አላውቅም

6.3.ከዚህ በፊት ንፅህናን እና ትክክለኛ የምግብ አያያዝን በተመለከተ ስልጠና ወስደው ያውቃሉ?

□ አዎ አውቃልሁ □ አላውቅም

Appendix 4: Data Collection Format for cross-sectional abattoir survey in camels

No.	Date of sample collection	Sample No.	Sex	Age	Breed	Origin	Clinical finding	Post mortem Finding