ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

BURDEN OF *CAMPYLOBACTER* SPECIES ON LIVESTOCK OWNING HOUSEHOLDS IN PERI-URBAN ADDIS ABABA, ETHIOPIA: A ONE HEALTH APPROACH

BY:

GEMECHU CHALA, DVM

JUNE, 2020

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ABBREVIATIONS

16S rRNA	the 16S ribosomal ribose nucleic acid
C. coli	Campylobacter coli
C. fetus	Campylobacter fetus
C. jejuni	Campylobacter jejuni
C. lari	Campylobacter lari
CBA	Columbia Blood Agar
CDC	Centers for Disease Control and Prevention
CSA	Central Statistical Agency
DNA	Deoxyribose Nucleic acid
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
GDP	Gross Domestic Product
MAL-ED	Malnutrition and Enteric Disease
mCCDA	modified Charcoal Cefoperazine Deoxycholate Agar
OIE	Office of International des Epizooties
PCR	Polymerase Chain Reaction
TBE	Tris/Borate/EDTA buffer
USA	United states of America
UV	Ultraviolet
WHO	World Health Organization

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ABSTRACT

Background: *Campylobacter* is the most common infectious causes of diarrhea and acute gastroenteritis globally, and has been recognized as a significant zoonotic agent. Numerous animals serve as reservoirs and a potential source *Campylobacter* species for food, water and environmental contamination. The rising of antimicrobial resistance amongst*Campylobacter* isolates, is a significant global issue.

Objective (s): The study was aimed to highlight the importance of livestock, poultry and water in peri-urban Addis Ababa, as sources of zoonotic *Campylobacter* species and antimicrobial susceptibility of isolated strains.

Methods: A cross-sectional study was conducted from December 2018 till April 2020 to collect and process 519 samples including feces from livestock, poultry, human and water samples form livestock keeping households of the peri-urban Addis Ababa. Data on houeholds' sociodemographics, knowledge and practices regarding livestock production was collected using a structured questionnaire.

Results: Of the 519 tested samples, 67 (13%) were positive for *Campylobacter* species, in which 10.1%, 18.5%, 13%, 13.3%, 7.1% and 10.5% were from human, cattle, poultry, sheep, goat and water samples, respectively. Regardless of isolation source, *C. jejuni* and *C. fetus* were the most observed species with the prevalence rate of 17 (25.4%) each. *C. coli* was isolated only from 6 (9%) of samples, whereas, none of the tested samples were positive for *C. lari*. The use of stored water and practices of indoor and outdoor manure collecting, taking any specific protection while cleaning animal pen and washing hands before and after cooking were significantly associated with the human *Campylobacter* positivity.All (100%) of *Campylobacter* isolates from human, poultry, sheep, goat and water and 96% of isolates from cattle were resistant to at least one or more of the tested antimicrobials. More importantly, 95.5% the isolates were resistant to three or more classes of antimicrobials.

Conclusion: The study found that multidrug resistant *Campylobacter jejuni*, coli, *fetus* and other *Campylobacter* with unidentified species were prevalent in the study area.Hence, considering the very significant effect of antimicrobial resistance, it is important for the country to have a national plan to advance the rational use of antimicrobials in the view of "One Health" approach.

Keywords:*Addis Ababa; Antimicrobial resistance;Campylobacter; Livestock; One Health;Peri-urban.*

1. INTRODUCTION

Diarrheal diseases are the leading causes of childhood illness and death in developing countries and caused by enteric pathogens such *Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter* spp. and others (WHO, 2015; Whiley *et al*., 2013).*Campylobacter* infection was first implicated in causing human enteritis in the late 1970s (Skirrow, 1977) and has since become recognized as the commonest known cause of bacterial gastroenteritis worldwide. *Campylobacter* species are Gram negative rods, with characteristically curved, spiral, or S-shaped cells and exhibits a rapid and darting motility in corkscrew fashion(Kaakoush *et al*., 2015). *Campylobacter* is one of the most common zoonotic bacteria causes of diarrheal illnesses and acute gastroenteritis globally (Bolton, 2015; WHO, 2013). Studies have shown that *Campylobacter* is responsible for 400–500 million cases of diarrhea each year even surpassing those caused by salmonellosis and shigellosis (Kaakoush *et al*., 2015; WHO, 2013; FrancËois *et al*., 2018).

Epidemiologically, human *Campylobacter*iosis appears to differ between high and low-income countries. In high-income countries symptomatic infection occurs in all age groups whereas in low-income countries, clinical disease mostly affects children under two to five years of age where adults rarely suffer from the disease, but may have asymptomatic excretion (Tafa*et al*., 2014; Osbje*et al*., 2016; FrancËois*et al*., 2018). Predominantly, most *Campylobacter*species have been isolated from both domestic and wild, healthy and diseased animals (OIE, 2017; Hald*et al*., 2016; Whiley *et al*., 2013). Attribution studies had shown that ownership of domestic animals, mainly poultry, is risk factor for *Campylobacter* infections, especially among infants and small children (Brena*et al*., 2016; WHO, 2013; FrancËois*et al*., 2018; Osbje*et al*., 2016;Selesh*et al*., 2015). Thus far, most animals serve as reservoirs and a potential source for the contamination of food, water, and the environment (Szczepanska*et al*.,2017; Osbje*et al*., 2016). Meta-analysis of extensive epidemiological research has shown that contact with farm and pet animals, and poor food hygiene practices are among significant risk factors for contracting *Campylobacter*iosis (Dominguez*et al*., 2012).

Environmental surface water is commonly contaminated with *Campylobacter*due to the sewage discharge, as well as domestic and wild animal fecal input (Chukwu*et al*., 2019; Nilsson*et al*., 2018; Szczepanska*et al*., 2017). These events present an opportunity for transmission of

environment-adapted genotypes to livestock and humans. Among the *Campylobacter* species, thermophilic *Campylobacter*, especially *C. jejuni* and *C. coli*, are the primary causes of human bacterial gastroenteritis worldwide and are major zoonotic agents (Whiley *et al*., 2013; WHO, 2013; OIE, 2017). Most cases of *Campylobacter*iosisare self-limiting rarely fatal infection with enteritis, abdominal cramps, fever, nausea and vomiting as the main manifestations (Kaakoush*et al*., 2015; Whiley *et al*., 2013). However, some cases have been linked to more serious complications like Guillain–Barré syndrome,Reiter's syndrome, reactive arthritis, bacteremia, abortion and other severe complications (Terefe*et al*., 2020; Bolton, 2015; Kaakoush*et al*., 2015).

Macrolides and fluoroquinolones (particularly erythromycin and ciprofloxacin) and intravenous aminoglycosides are drugs of first choice in cases of severe or long-lasting *Campylobacter*iosis(WHO, 2013; Tafa*et al*., 2014; Kovac *et al*., 2017). Contrastingly, over the years, several studies had shown the occurrence and rising of antimicrobial resistance among *Campylobacter* isolates of various sources, especially, to fluoroquinolones, tetracycline, and erythromycin (Ewnetu and Mihret, 2010; Tafa*et al*., 2014; Girum*et al*., 2015; Sproston*et al*., 2018; Abubakar *et al*., 2019). The over use of antimicrobials in veterinary medicine mainly as prophylactic agents and growth promotersenhances selection and spread of antimicrobial resistant pathogens including *Campylobacter*species.This situation is more common in developing countries, where there is widespread and largely uncontrolled use of antimicrobials (Signorini*et al*., 2018; Brena*et al*., 2016; Dominguez*et al*., 2012).

In Ethiopia, a number of *Campylobacter* prevalence and antimicrobial susceptibility test studies have been conducted in humans and/ or animals alone (Gedlu and Assefa, 1996; Asrat*et al*., 1999; Asrat, 2008; Beyene and Haile-Amlak, 2004; Ayalew*et al*., 2013; Mulatu*et al*., 2014; Mitike*et al*., 2009; Tafa*et al*., 2014) and animals (Dadi and Asrat, 2008; Kassa*et al*., 2007; Woldemariam*et al*., 2009; Yeshimebet*et al*., 2013; Selesshe*et al*., 2015; Brena*et al*., 2016). Most of the above studies, however, were conducted either in humans or in animals alone and only few of them focused on both human subjects (hospitalized) and surrounding animals. With the exception of a recent study by Terefe*et al*. (2020) that employed different molecular techniques, all of the aforementioned studies were based solely on culture method.

In the country, although studies have shown the occurrence of *Campylobacter* species in humans, animals and foods of animal origin, those that report on precipitating risk factors on the occurrence of the agent in humans, animals and environment are absent or limited.

Moreover, the role of different environments like water as sources and transmitting agents of *Campylobacter* infection in humans and animals is still far less known in the country. There is alsono published report on assessing the knowledge, attitude and practices of livestock keepers regarding issues like hygiene, biosecurity, husbandry practices and risk of acquiring *Campylobacter*species. Hence, to reduce the risk of human exposure to *Campylobacter* and especially to the strains resistant to various antimicrobials, it is essential to identify and characterize the various *Campylobacter* species circulating in humans, domestic animals and water sources.

This study therefore, aimed to assess the importance of livestock farmed in peri-urban parts of Addis Ababa, Ethiopia as sources of zoonotic *Campylobacter*species and the risk of antimicrobial resistance in theseisolates. The study also assessed the knowledge and understanding practices related to animal husbandry and its impact on *Campylobacter* infection.

2. LITERATURE REVIEW

2.1.Peri-urban Livestock production

In Ethiopia, livestock production as a whole contributes about 45 percent to agricultural GDP. Broadly speaking, peri-urban livestock production in Addis Ababa involves predominantly, Keeping of dairy cows, sheep and goats and poultries. However, in the peri-urban areas, mixed farming is practiced with crop and livestock production complementing one another (Tegegne *et al.*, 2002). The peri-urban livestock production sector in Addis Ababa comprises individual farmers as well as farmers organized in micro-enterprises and cooperatives (Gebremichael *et al*., 2014).

2.2.Importance of livestock in drug resistance and pathogen transmission

Despite the significance of urban/peri-urban livestock to the livestock keepers and to the rest of the community, poor livestock husbandry can present significant health risks to humans (Guendel, 2002). There is growing evidence that proximity of domestic animals and their feces to humans and water serve as sources of zoonotic fecal pathogens (Igwaran and Okoh, 2019; Yirgalem, 2008). In addition to fecal contamination, the presence of livestock in the household environment can increase zoonotic infectious disease risk by leading to more frequent human–animal interactions and possible contamination of food for human consumption (Lowenstein *et al*., 2016). Furthermore, Consumption of animal source foods produced under poor quality control measures (Guendel, 2002). Research suggests that the use of antimicrobials with food animals, which is often used to speed animal growth and decrease the incidence of disease, contributes to the development and transmission of antimicrobial resistant pathogens (Hoelzer *et al*., 2017; Landers *et al*., 2012).

2.3.Campylobacter

2.3.1. Historical Overview

*Campylobacter*infection was described for the first time by Theodor Escherich in 1886, when he found spiral-shaped bacteria in the colons of infants who had died of what he called '*cholera infantum*' (cited in Butzler *et al*. 2004). Twenty-three years later, British veterinarians reported an unknown bacterium frequently isolated from aborted lambs (McFadyean, 1909). The genus *Campylobacter*was created in 1963, when two *Vibrio* species were transferred into *Campylobacter species* based on their low DNA base composition, microaerophilic growth and non-fermentative metabolism (Butzler *et al*. 2004). According to same source it was not until the 1970s, however, that *Campylobacter*was successfully isolated from human feces and recognized as an important human pathogen.

2.3.2. Taxonomy and Special Features

*Campylobacter*species belong to the class Epsilon proteobacteria order *Campylobacterales* family *Campylobacteraceae* and further the genus *Campylobacter* (Vandamme *et al*. 2010). Members of the genus *Campylobacter*are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria (0.2-0.8 μm wide and 0.5-5 μm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility (OIE, 2017). These bacteria require microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), but some strains also grow aerobically or anaerobically (Lastovica *et al.*, 2014; McFadyean, 1909). They neither ferment nor oxidize carbohydrates; nevertheless, they derive energy from amino acids, keto acids and citric acid cycle (also known as tricarboxylic acid or Krebs cycle) metabolic intermediates (Bolton, 2015).

Some speciessuch as enteric campylobacters, *C. jejuni*, *C. coli* and *C. lari*, are thermophilic, growing optimally at 42°C. They can colonize mucosal surfaces, usually the intestinal tract, of most mammalian and avian species tested (Szczepanska *et al*.,2017; Pires *et al*., 2019). Some *Campylobacter*species are ubiquitous in the environment and have been isolated from soil and mud (Lastovica *et al.*, 2014), in untreated drinking water (Dominguez*et al.*, 2012). At present, as it is indicated elsewhere 34 *Campylobacter*species and 11 subspecies have been described, 16 of which have been associated with human disease (OIE, 2017; WHO, 2015; Lastovica *et al.*, 2014). In fact, the continual progress and developments in diagnostic technique the criterion of taxonomy may refine the number of *Campylobacter*species. However, the two most remarkable

species that are responsible for over 95% of all human *Campylobacter* infections worldwide are *C. jejuni* and *C. coli* (Kaakoush *et al.*, 2015; WHO, 2013).

2.3.3. Virulence and Infectivity

*Campylobacter*species have four main virulence properties: motility, adherence, invasion and toxin production. Both flagella and its spiral shape helps *Campylobacter* species to move rapidly thereby enabling it to penetrate through the intestinal lining unlike conventional bacteria (Hansson *et al*., 2018). *Campylobacter*organisms produce two types of toxins: enterotoxin (heat labile) and cytotoxins. It has been suggested that enterotoxin produced by *Campylobacter*species results in watery diarrhoea, as opposed to bloody diarrhoea due to cytotoxin production (Lastovica *et al.*, 2014).

Equally important, the surface of C. jejuni cells are surrounded by a polysaccharide capsule that facilitates survival, adherence and evasion of the host immune system (Chukwu *et al*., 2019). Bolton (2015)detailed that the rates of infection increased with the ingested dose, and ingestion of inocula in suspension buffered appeared to increase rate of illness. In human, it has been estimated that consumption of a small number of organisms (500 or less) may be associated with illness. Therefore, the fact that the organism does not multiply very effectively in most foods does not prevent it from causing foodborne illness (Hansson *et al*., 2018).

2.3.4. Pathogenesis

The pathogenesis of disease in humans is still not fully understood though significant advances have been made in describing various features and mechanisms involved. The pathogenesis involves host and pathogen specific factors, the health and age of the host and pathogen specific humoral immunity from previous exposure which influence clinical outcome after infection (Hansson *et al*., 2018). Motility of *C. jejuni* has been shown as an important factor in the colonization of the intestines providing the ability to traverse the mucous barrier and preferentially target the deep intestinal crypts (Bolton, 2015).

Moreover, experimental infection of humans confirmed the significant role of flagella in which only motile variants were isolated from stools despite volunteers being fed mixtures of motile and non-motile phase variants (Black *et al*., 1988). The diarrheal disease may be due to the production

of a heat-liable toxin. Several putative virulence factors have been identified in *Campylobacter*which contribute to the motility, intestinal adhesion, colonization, toxin production and invasion. Adhesion of the pathogen to the intestinal epithelium is important for colonization and to increase the secretion of bacterial toxins (Bolton, 2015). Furthermore, host factors may have important roles in the pathogenesis of *Campylobacter*iosis in humans (Hansson *et al*., 2018). More importantly, the pathogenic mechanisms of *Campylobacter*species are still an active area of research.

2.3.5. Sources of Campylobacter species

Most *Campylobacter*organisms have been associated with various diseases in human and animals and show a considerable ecological diversity. The main reservoirs of *C. jejuni* are poultry, cattle, sheep and wild birds while *C. coli* is mostly associated with sheep and pigs (Karikari *et al*., 2017; OIE, 2017) whereas *C. upsaliensis* and *C. helveticus* are predominantly detected in dogs and cats respectively (Chaban *et al*., 2010). Apart from these reservoirs, *Campylobacter*species have been also isolated from many environmental sources such as freshwater, seawater, sewage, soil and feedlots (Whiley *et al*., Carron *et al*., 2018; Karikari *et al*., 2017) and feedlots. Moreover, the occurrence of *Campylobacter*species in these environments is considered to arise from contamination with fecal matter of different source animals through direct and indirect routes (Brena *et al*., 2016).

Likewise, it was also shown that processes and practices in production and slaughter of animals colonized by these bacteria leads to the contamination of food of animal origin with *Campylobacter*species (Lastovica, *et al*., 2014). Furthermore, food contaminated with *Campylobacter*species may also serve as a source for cross-contamination of other foodstuffs, surfaces and utensils through various mechanisms (WHO, 2015; Sileshi *et al*., 2015). Contamination with *Campylobacter*species can also occur through vectors such as flies (Hald *et al*., 2004). Therefore, the source of infection, for animals and humans, with *Campylobacter*species may occur through a wide variety of vehicles and transmission routes.

a. Campylobacter in Livestock

In animals, the clinical importance of *Campylobacter*infection is mainly attributed to the species *Campylobacter fetus*, which was most likely the first *Campylobacter*isolated (McFadyean, 1909). The species comprises two subspecies, *C. fetus* subspecies *fetus* and *C. fetus* subspecies *veneralis. Campylobacter fetus* subspecies *fetus* has been isolated from a range of species, including fowl, reptiles and humans, but is mainly associated with abortion in sheep and cattle (OIE, 2017). The pathogen has also been detected in immunodeficient human patients and in neonatal sepsis and septic abortions. *Campylobacter fetus* subspecies *veneralis* causes bovine genital *Campylobacteriosis* and infectious venereal disease that may lead to infertility, abortion and embryo death (Pires *et al.*, 2019).

A high carriage rate of *Campylobacter*as an asymptomatic colonizer is found in a wide range of birds and mammals and is considered a public health threat when occurring in livestock and pets (Kaakoush *et al.*, 2015). Many species of *Campylobacter*are commonly found as colonizers in the intestinal tract in poultry, pigs, sheep and cattle, mainly the species *C. jejuni* and *C. coli*, but also *C. upsaliensis, C. concisus*, *C. lari and C. lanienae*. Once *Campylobacter*is introduced into a flock, it can spread rapidly. In poultry it generally results in life-long colonization (Kaakoush *et al.*, 2015).

b. Role of Livestock in Transmitting *Campylobacter*to Humans

A study using Multi Locus Sequence Testing technique has been compared wild and farmed animal *Campylobacter*genotypes with human isolates and attributed animals as a source of human *Campylobacteriosis* (Wimalarathna *et al*., 2013). Several reports incriminating poultry as major source of *Campylobacter*species to human have been reported (Carron *et al*., 2018). The infection is mainly transmitted from animals through eating undercooked meat from diseased livestock, raw milk from infected lactating animals, contact with infected animals (WHO, 2015; OIE, 2017) and handling of contaminated manure with bare hands and failure to clean hands properly afterwards (reviewed in Osbjer *et al*., 2016). As reviewed in Signorini *et al*. (2018) studies in many countries have shown that natural environment, like soil and water, is also essential in transmission of *Campylobacter*, either directly to humans or indirectly through farm animals or pets.

c. Campylobacter in Humans

In humans, *Campylobacter*are known mainly for causing gastroenteritis and are considered the most common pathogen causing bacterial gastroenteritis worldwide (WHO, 2015; WHO, 2013). Clinical significance of *Campylobacter*species is based on their ability to cause acute, gastrointestinal inflammatory enteritis, known as *Campylobacteriosis*, in humans, which is one of the leading bacterial food-borne zoonosis globally (Igwaran and Okoh, 2019). The most common cause of human infection is *Campylobacter jejuni*, followed by *Campylobacter coli*, but *Campylobacter lari*, *Campylobacter fetus* and *Campylobacter upsaliensis* have also been reported to cause human infections (Kaakoush *et al.*, 2015; Whiley *et al*., 2013; Lastovica *et al.*, 2014). It has been shown that only 500 or less *Campylobacter* cells can cause human illness (Black *et al*., 1988; WHO, 2013; Szczepanska *et al*., 2017).

The incubation period varies from 2 to 5 days (range 1-10 days). Acute, diarrheic symptoms usually last 3 to 5 days, but abdominal pain and stomach rumbling may last for weeks. After the clinical recovery, patient can still excrete the bacteriain feces for several weeks (WHO, 2013). As described elsewhere in this document symptoms of *Campylobacteriosis* vary from mild to more severe and include diarrhea, which in some cases may contain blood, abdominal cramps, fever, nausea, muscle pain, headache and sometimes vomiting. Severe symptoms observed in very young, elderly or immunocompromised or patients with a chronic disease, such as diabetes (Igwaran and Okoh, 2019; Tafa *et al*., 2014).

However, usually *Campylobacteriosis* is self-limiting and antimicrobial treatment is not needed. In case the symptoms are severe or patient is at higher risk, fluoroquinolones like ciprofloxacin or macrolides like erythromycin are used (WHO, 2013; Kaakoush *et al*., 2015). Sometimes, severe post-infectious symptoms may occur as late-onset complications, including rarely occurring Guillain Barré syndrome (GBS), Miller Fisher syndrome (MFS) and reactive arthritis (Igwaran and Okoh, 2019). *Campylobacteriosis* is also associated with post-infectious irritable bowel syndrome (Butzler, 2004) whereas certain strains of *C. jejuni* have been occasionally reported to cause bacteremia among patients (Kaakoush *et al.*, 2015; Szczepanska *et al*., 2017).
Human infection occurs year-round in tropical countries, whereas in temperate countries infection has summer and autumn peaks (Osbjer *et al*., 2016). People of all ages and socio-economic backgrounds may acquire this disease. More important, however, is the distribution of infection in

different age groups. In high-income countries, infection is often symptomatic and all age groups are at risk, although infection is suggested to be more prevalent in toddlers (1-4 years) and young adults (FrancËois *et al*., 2018; Szczepanska *et al*., 2017). In low-income countries, symptomatic infection is usually limited to children below 5 years, with illness/infection ratio decreasing with age (Kaakoush *et al.*, 2015). The clinical picture also differs from that seen in high-income countries, with watery diarrhea being the most common presentation in low-income countries (Erick, 2017; Mitike *et al*., 2009).

Several studies also indicated that a significant burden of disease associated with *Campylobacter*infection in children less than five years of age demonstrating that this bacterium is a risk factor for poor linear growth (Szczepanska *et al*., 2017; Asrat *et al*., 1999; WHO, 2013; Kaakoush *et al.*, 2015; FrancËois *et al*., 2018).

d. Zoonotic Potential of Campylobacter

Most animals serve as reservoirs of *Campylobacter* species and only a small number is afflicted by *Campylobacteriosis* (Divsalar*et al*., 2019; reviewed in Signorini *et al*., 2018) In the majority of *Campylobacter*infections reported to date, transmission to humans occurred through direct contact with livestock or through consumption of contaminated meat, milk or water (EFSA, 2015; Dominguez*et al.*, 2012). According to recent data from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), in terms of zoonoses and food borne outbreaks, human *Campylobacteriosis* is the most commonly detected zoonosis in the EU exceeding salmonellosis cases (EFSA and ECDC, 2015).

Poultry is recognized as the primary source and 50-80% of all human *Campylobacter*infections are suggested to be sourced from poultry (EFSA, 2015). However, the number of human *Campylobacter*infection cases attributed to poultry is higher than the number estimated to be acquired through consumption of poultry meat. Thus, infection may result from indirect transmission from poultrys, and not only through the usual eating or handling routes (WHO, 2015; Signorini *et al*., 2018). Interestingly, in high-income countries international travel, in particular to Southeast Asia, is suggested to be the most important risk factor for Campylobacteriosis (Dominguez*et al.*, 2012).

2.4.Detection and Identification Methods

The conventional identification scheme for *Campylobacter*and related microorganisms is based on classical phenotypic characteristics, such as morphological appearance, biochemical reactions, and growth temperature and tolerance tests (WHO, 2013). However, most laboratories in developing countries do not routinely perform tests to detect *Campylobacter* infection because of the difficulty of the detection methods, especially the growth requirements thatneed selective and expensive culture media and incubation conditions (Jribi *et al*., 2017; Tafa *et al*., 2014; WHO, 2015).

For species identification of isolates, phenotypic methods may be employed but have been shown to be limited. For instance, the use of antibiotic sensitivity testing for speciation is becoming more problematic due to the increasing frequency of resistance. Identification using biochemical tests such as positive hippurate hydrolysis has been a main feature for *C. jejuni* but hippurate-negative strains (up to 10%) have been reported (Kassa *et al*., 2007; Szczepanska *et al*., 2017; Carron *et al*., 2018). As a result, molecular assays using either species-specific or multiplex reactions based on ribosomal 16S gene sequences and other species-specific gene sequences, and microarray-based identification tests have been developed as alternative identification methods (Pintar *et al*., 2017; WHO, 2013).

2.5.Antimicrobial Resistance

Most of the human Campylobacteriosis are usually self-limiting and of short duration, so antimicrobial treatment is only necessary for systemic, severe, and/or prolonged *Campylobacter* infections. In these situations, erythromycin or fluoroquinolones are often used (WHO, 2013). In developing countries, food-borne diseases are under diagnosed and the treatment of bacterial diarrhea is often done without a microbiological diagnosis. In this context, antimicrobial treatment is irremediably done without sufficient information (Carron *et al*., 2018; Brena *et al*., 2016, WHO, 2015). Studies in different countries have hypothesized that the introduction of certain antimicrobials (e.g., quinolones) in livestock (as prophylactic or therapeutic agents) and as

growth promoter in animal farming operations has been associated with the increase in resistance to human isolates (Brena *et al*., 2016; Kassa *et al*., 2007).

Among the different species of the genus *Campylobacter*,*C. jejuni* is able to develop biofilms on various abiotic surfaces and this has been suggested as a factor implicated in the development of bacterial resistance (Divsalar*et al*., 2019). An extensive worldwide systematic review done on antimicrobial resistance of thermotolerant *Campylobacter* species showed that *Campylobacter* isolates from poultry and pigs have shown the highest prevalence of resistance to most of the antimicrobials included in this study (Signorini *et al*., 2018). On the contrary, *Campylobacter* isolates obtained from bovine and ovine showed the lowest levels of resistance prevalence for most of the antimicrobials whereas isolates from humans showed resistance to antimicrobials at intermediate levels, lower than poultry and pigs, but significantly higher than those observed in bovine and ovine isolates.

2.6.Epidemiology of *Campylobacter*iosis

Although the real number of cases of Campylobacterinfections is not understood (WHO, 2013) nowadays, *Campylobacter*is the most common cause of bacterial gastroenteritis in both developed and developing countries. It is responsible for 400–500 million cases of diarrhea each year (Whiley *et al*., 2013; FrancËois *et al*., 2018). As the result of an epidemiological study of human health burden of food-borne infections in Japan, the estimated burden of *Campylobacter* infections was the highest among the other pathogens. The estimated incidence per 100,000 per year in this region was 237 cases for *Campylobacter*(FrancËois*et al*., 2018).

*Campylobacter*continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the European union since 2005. The Food-borne Diseases Active Surveillance Network (Food Net) of the Centers for Disease Control and Prevention (CDC) in the USA estimated that, in 2009, the number of reported infections and incidence per 100,000 populations by *Campylobacter*was 6,033 and 13.02, respectively. However, many more cases remain undiagnosed or unreported (Kaakoush *et al*., 2015; EFSA and ECDC, 2015).

The epidemiology of *Campylobacter*in humans is primarily dependent on the socioeconomic status that distinguishes two patterns in global incidence of the disease. *Campylobacteriosis* in developing countries is endemic and marked by common asymptomatic infection and seasonality (Carron *et al*., 2018; Kaakoush *et al*., 2015). In these regions, *Campylobacter*is associated with symptomatic infection only in the early childhood life and rarely in adults (FrancËois *et al*., 2018; Signorini *et al*., 2018). In the setting of developing countries, the pathogen is ubiquitous in the environment; hence, risk factors are frequently associated with environmental routes of transmission, especially drinking water (Lengerh *et al*., 2013). On the other hand, in the developed world *Campylobacteriosis* is sporadic, except for common source outbreaks, asymptomatic excretion is low and all age groups can be affected by clinical disease (Amour *et al*., 2016). Nevertheless, while differences in reporting, climatic conditions, and livestock production systems may explain differences in incidence, the epidemiology of the *Campylobacter* remains poorly understood and other factors may be involved (Carron *et al*., 2018; WHO, 2015).

In low and middle-income countries, surveillance for *Campylobacter*seldom exists in people and animals, and data regarding the organism's presence, risk factors and impacts are scarce. In a multisite birth cohort study from 2009 to 2012 (MAL-ED study) in Asian, Latin American and African countries, *Campylobacter*species were the most frequently detected pathogens, occurring in 84.9% of 1892 children, and contributed the highest burden of diarrhea in the first year of life. *Campylobacter*infection in children was associated with growth deficits across sites (Carron *et al*., 2018; FrancËois *et al*., 2018). Reports from limited number of countries in Africa, (especially sub-Saharan African countries) indicated a considerable burden of the disease (Signorini *et al*. and Carron *et al*., 2018). According to available data from these countries *Campylobacter*infection is most prevalent in the pediatric population (Kaakoush *et al*., 2015; WHO, 2013).

In Ethiopia likewise, the occurrence and susceptibility testing of *Campylobacter* strains to antimicrobials was reported on human (Terefe *et al*., 2020; Asrat, 2008; Asrat, *et al*., 1999; Gedlu and Assefa, 1996; Tafa*et al*., 2014; Mulatu *et al*., 2014), animals and foods of animal origin (Brena *et al*., 2016; Girum, 2015; Seleshe *et al*., 2015; Yeshimebet *et al*., 2013; Woldemariam, *et al*., 2009; Dadi and Asrat, 2008;Kassa, *et al*., 2007).

2.7.Zoonosis and One Health

One health is defined by the One Health Commission as “the collaborative effort of multiple disciplines to obtain optimal health for people, animals, and our environment.” This approach plays a significant role in the prevention and control of zoonoses (Rousham *et al.,* 2018). It has been noted that more than 60% of human infection diseases are defined as zoonotic with important and increasing impact on human health. Zoonoses, agriculture, and food safety are all interconnected topics in that they all directly impact the health and livelihoods of humans. However, they are the poor livestock keeping households of most developing countries that suffer the most from the threat of zoonosis (FrancËois *et al*., 2018; Rousham *et al.,* 2018).

Ethiopia, a country which is mostly dependent on agriculture in which most of the households have direct contact with animals, remained vulnerable to the effect of zoonotic diseases (Pieracci *et al*., 2016). Moreover, One Health interventions have the potential to overcome some of the existing social, political and economic challenges that render healthcare delivery in most of low-income countries of the world. One Health interventions advocate close inter-sectoral cooperation, interdisciplinary expertise and the involvement and empowerment of multiple stakeholders to ensure the health of persons, animals and environment (Clevelan*et al*.,2017). One health has gained momentum and now encompasses zoonotic infections, food safety, and even health delivery systems and in fact its future is a one world approach with the continued effort towards integration of the contributing parts that form the whole which is health.

3. OBJECTIVE (S) OF THE STUDY

3.1. General Objective

- ✓ It was to investigate whether livestock and chickens contribute to zoonotic *Campylobacter* species and environmental load of *Campylobacter* species by examining feacal samples from livestock, humans and drinking and environmental water of the households in the peri-urban Addis Ababa.

3.2. Specific Objectives

- ✓ Was to determine the occurrence of *Campylobacter* species in humans, domestic livestock, poultry and water samples in the per-urban areas of Addis Ababa.
- ✓ Was to determine antimicrobial susceptibility patterns of *Campylobacter* species.
- ✓ Was to examine risk factors associated with human *Campylobacter* species positivity.
- ✓ Was to describe households' knowledge, and practices related to livestock keeping and occurrence of zoonotic *Campylobacter* species in peri-urban Addis Ababa

4. MATERIALS AND METHODS

4.1.Study Area

The study was conducted in the peri-urban areas of Addis Ababa, the capital city of the Federal Democratic Republic of Ethiopia. Administratively, the city is a chartered city having three layers of government: city government, sub-city administrations, and district (Woreda) administrations. The city has ten (10) sub-cities of which five of them are considered as peri-urban areas. Among the peri-urban areas of Addis Ababa, AkakiKality which is considered as the major livestock raising area, was selected in order to provide a representative picture of Addis Ababa's livestock production system and major types of urban landscape found in the city (CSA, 2013).

AkakiKality, one of the very thriving sub-cities in Addis Ababa selected to investigate the burden of *Campylobacter* in livestock keeping households in the context of transitioning urban landscape and livestock production systems. This community was selected for the study because it is characterized by high level of small-scale livestock production, and research has documented a high prevalence of zoonotic enteric infections in children(Agajie*et al*., 2020; Hassen *et al*., 2018; Gebremichael *et al*., 2014).The sub-city is experiencing both crop and animal production systems, characterized by a rural-like landscape with pockets of residential areas and moderate population density (Yohannes and Elias, 2017). The study was purposively conducted on two woredas (Woreda 02 and 03) representing different wealth levels and production systems. These Woredas were selected on the basis of operational convenience, but also represented a range of social and physical differences across the peri-urban areas of Addis Ababa.

4.2.Study Designand Determination of Study Subjects

A cross-sectional study was conducted from December 2018 until April 2020 in order to assess the burden of *Campylobacter* species in households of the peri-urban Addis Ababa. Study participants were selected from available lists of livestock keeping households in both woredas using random numbering. Randomization of selected household lists and households was done using random numbers generated in a spreadsheet program (Microsoft Excel 2019, Microsoft

Cooperation, USA). Households were included in to the study as long as they had at least 1 free-roaming poultry in the compound or owning at least one ruminant animal. When the consent is refused, or the household head or representative is unreached, the next household was approached. Each Woreda was visited for several days and 5-7 households were interviewedeach day and samples were collected from same households on same day of interview.

Additionally, questionnaire data (information related to the socio-demographic characteristics of the households; their knowledge, attitude and practices regarding animal husbandry and its impact on human and environmental health and/or risk factors that predispose to the contamination and spread of pathogenic *Campylobacter*and development of antimicrobial resistance) were gathered using a structured questionnaire (see Annex I-IV).

4.2.1. Sample Size Determination

Sample sizes were calculated for two independent populations based on an expected *Campylobacter* species prevalence of 50%, 5% confidence limit and 95% confidence interval using the total number of households owning livestock in Woreda 2 and 3, which was 227. Accordingly, the minimum calculated sample size was 143 humans and 372 animals, respectively; however, in order to adjust for possible confounding and sampling error and interaction in the statistical models thereby increasing the precision of the estimates, 150 humans and 400 livestock samples were recruited in the current study. Sample size for water samples was conveniently determined based on the available type of water sources that was in use for both household daily activities and drinking water for humans and animals in the study area as described elsewhere (Szczepanska*et al*., 2017).

Humans and animals that were on antimicrobial treatment or who were treated during the previous two weeks at the time of contact were excluded from the study (see Annex-I).

4.3.Sample Collection, Handling and Transport

A total of 347fecal samples were collected from humans (n=99), cattle (n=135), sheep (n=30), goat (n=14) and poultry (n=69). Water samples (n=172) were collected from 99 households and their sources; Surface water (n=9), Municipal pipe water (n=84), Ground water (n=16) and Stored

water (n=63) (**Figure1**) (see Annex V). When it was difficult to obtain stool samples from all of the household members at time of data collection, pooled stool samples were collected only from individuals who were most closely associated with the management of the livestock and houses.All animal and human samples were collected as pooled samples using a sterile cotton swab (see Annex V).

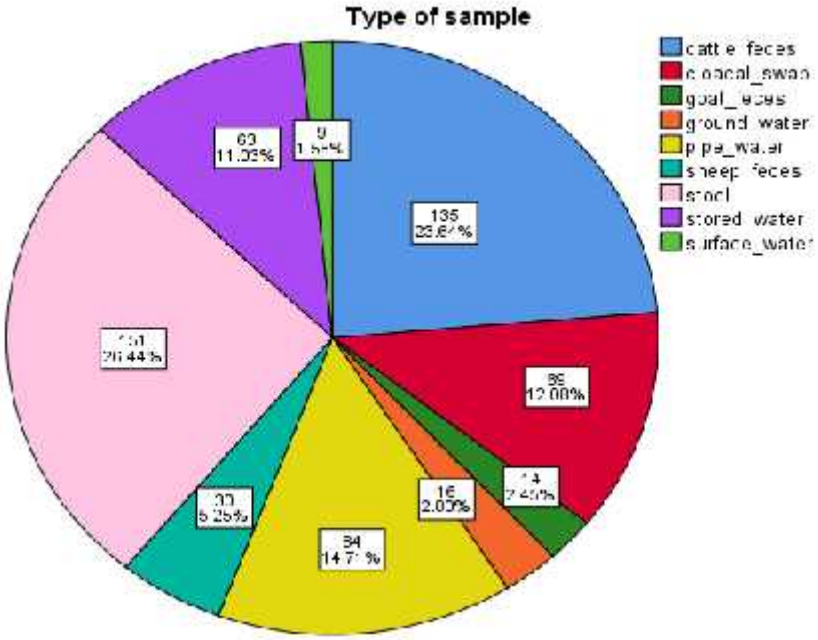

Figure 1. Pie chart showing type and number (%) of collected samples.

Fecal samples from animals were collected either directly from rectum or floor of animal's pen immediately while the animal was voiding the fecal matter and all samples were collected as pooled sample for each animal species using cotton swab into clean 15ml sterile screw-capped falcon tubes containing Cary-Blair Transport Medium (Oxoid). All collected samples were kept in a cool box containing ice bags and transported to Microbiology Laboratory, of Akililu Lemma Institute of Pathobiology, Addis Ababa University where all laboratory work was conducted. All samples wereprocessed within 4-6 hours of collection.

Householdsociodemographic data, knowledge about *Campylobacter*iosis, livestock management, personal and environmental sanitary practices and use of antimicrobials were gathered using a structured questionnaire (see Annex III). Both closed and open-ended questions were prepared with English language and translated to local languages (AfaanOromoo and Amharic) to interview selected households. The questionnaire was pretested to assess clarity and time

requirement by selecting 20 households from Woreda 09 of Yeka sub-city which was not part of the study area; however, the area is similar in many parts with the current study sub-city regarding the livelihood of target households. The questionnaire was then adjusted in line with the feedback from the pre-test before the study began. Filled questionnaire were reverse translated to English language from the local languages before database was created (ANNEX-V).

4.4.Isolation and Identification of *Campylobacter*Species from Various Sources

For isolation of*Campylobacter* speciesfrom all samplesincluding water selective enrichment broth was used to favor detection of stressed or low numbers of thermophilic *Campylobacter*s and followed by subculture onto selective culture media as described elsewhere (Jokinen *et al*., 2012; Divsalar*et al*., 2019). Water samples were filtered through a 0.45 µm sterile nitrocellulose filter (Pall Corporation, Ann Arbor, MI). For the enrichment of *Campylobacter* species membrane filtered water, homogenized human and animal fecal samples were inoculated in to a 30ml of selective Bolton broth (HiMedia Laboratories; Mumbai, India), with 5% (v/v) defibrinated cattle blood and incubated microaerophilic conditions at 37°C for the first 4 hour, then increasing the temperature to 42°C during 48 hours of incubation period for resuscitationas described by Szczepanska *et al*.(2017) and Chukwu *et al*.(2019). After 48 hours of incubation, a loopful of aliquot of culture was spread onto a modified charcoal cefoperazone deoxycholate agar (mCCDA, CM739 Oxoid, Hampshire, England) plate with *Campylobacter*selective supplements (see Annex VI).

The plates were incubated under microaerobic conditions (CO_2, 10%; O_2, 5%; N_2, 85%) created by Campy Gene gas generating kit (Thermo Scientific, Waltham, MA, USA) for 48 to 72 hours at 42°C. Following incubation period, mCCDA plates were examined for growth and all *Campylobacter*-like colonieswere selected and checked for motility, oxidase, catalase and gram character as described elsewhere (Siddiqui *et al*., 2015; Chukwu *et al*., 2019). All suspected colonies were sub-cultured to Columbia blood agar (Difco, USA) with 5% defibrinated sheep blood for separate colony isolation following the aforementioned incubation environment, temperature and time. All isolates with typical colony characteristics of *Campylobacter*were screened using biochemical tests (see Annex-VI). Gram negative isolatespositive for both

oxidase and catalase tests and those with *Campylobacter* characteristic motility were further investigated using multiplex polymerase chain reaction (PCR) for confirmation and speciation of *Campylobacter* isolates.

4.5.Identification and Speciation of Campylobacter by conventionalPCR

All presumptive colonies from the fresh Columbia blood agar culture plates were first consistently confirmed using simple conventional PCR assay as being *Campylobacter* by targeting the 16S rRNA gene sequences for the *Campylobacter* genus identification as described previously (Khan *et al*., 2009; Yamazaki-Matsune*et al*., 2007). Next, Species specific detection of *Campylobacter*species isolates in various sources was further confirmed using the multiplex PCR assay. Multiplex PCR was conducted targeting the region of 16S rRNA for detection of *Campylobacter* species as well as amplification of species-specific regions of genes *cj0414*, *glyA*, *cstA* and *ask* genes to categorize positive isolates as being either *C. jejuni*, *C. lari*, *C. fetus* and *C. coli*, respectively, as previously described (Yamazaki-Matsune*et al*., 2007). Sequences of the forward and reverse primers used in this study are shown in Table 1.

Table 1. Primer pairs used for detection and speciation of Campylobacter species using multiplex PCR

Species	Expected amplicon Size (bp)	Target gene	Primer	Sequence (5' to 3')
Genus *Campylobacter*	816	16S rRNA	C412F	5'-GGATGACACTTTTCGGAGC-3'
			C1228R	5'-CATTGTAGCACGTGTGTC-3'
C. coli	502	*Ask*[¥]	CC18F	5'-GGTATGATTTCTACAAAGCGAG-3'
			CC519R	5'-ATAAAAGACTATCGTCGCGTG-3'
C. jejuni	161	*cj0414*[f]	C-1	5'-CAAATAAAGTTAGAGGTAGAATGT-3'
			C-3	5'-CCATAAGCACTAGCTAGCTGAT-3'
C. lari	251	*glyA*[β]	CLF	5'-TAGAGAGATAGCAAAAGAGA-3'
			CLR	5'-TACACATAATAATCCCACCC-3'
C. fetus	359	*cstA*	MG3F	5'-GGTAGCCGCAGCTGCTAAGAT-3'
			MG3F	5'-AGCCAGTAACGCATATTATAGTAG-3'

All PCR reactions and cycling parameters were the same as those outlined in Yamazaki-Matsune*et al*.(2007) with only slight modification (see Annex-VII). Each reaction mixture was

analyzed by gel electrophoresis through 1.8% (w/v) agarose in 1X TBE buffer for 1:30 hour, and visualized by UV transillumination after staining with ethidium bromide (0.5 µg/ml). The DNA bands were photographed using an ultraviolet transilluminator (BTS-20), and a1 Kb DNA ladder was used as a molecular size marker (Figure 2).Isolates that were positive for the genus-specific PCR but negative for the *C. lari*, *C. fetus*, *C. coli* and *C. jejuni*-specific PCR fragments were designated as unidentified thermophilic *Campylobacter* species.In this study a household was considered positive for *Campylobacter* species if at least one isolate was obtained and confirmed by PCR from the pooled samples of any of the animal samples or human samples obtained from that household.

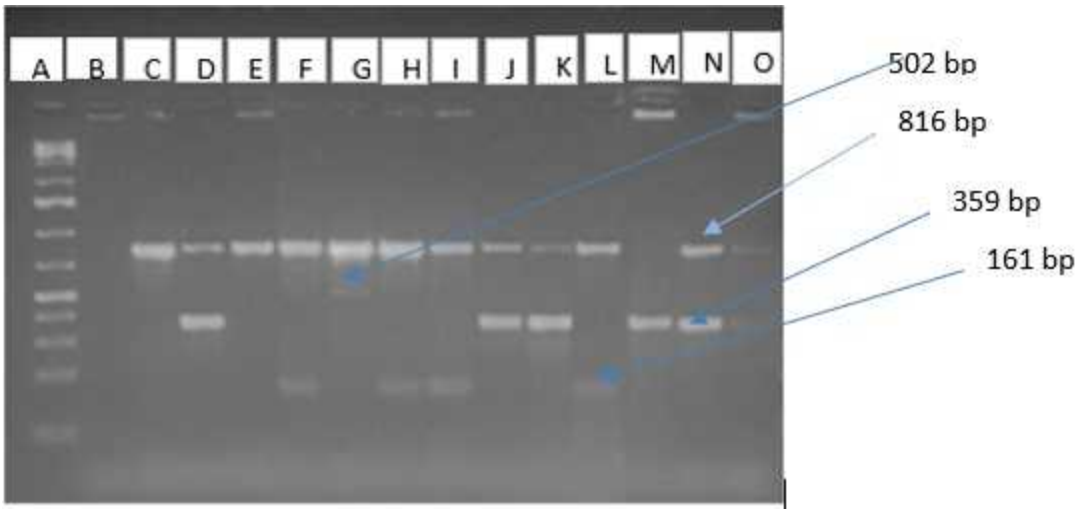

Figure 2. Representative gel image of multiplex PCR showing the identification of C. jejuni, C. coli, C. lari, C. fetus and genus level Campylobacter species

Lane A= 100 bp DNA ladder; Lane B= Distilled water as negative control; Lanes C, E shows genus level identified *Campylobacter* species; Lanes D, J, K, M, N and O shows *C. fetus*; Lanes F, H, I and L shows *C. jejuni* and Lane G shows *C. coli.*

4.6.Antimicrobial Susceptibility Test

All PCR confirmed isolates were subjected to phenotypic antimicrobial susceptibility analysis using the standard disk-diffusion method (Kirby-Bauer) on Mueller-Hinton agar (HiMedia Laboratories; Mumbai, India) (see Annex-VIII). The phenotypic antimicrobial susceptibility test was performed for the following antimicrobials obtained from Oxoid which are of importance

both in human and veterinary medicine: Ciprofloxacin (CIP) (5µg), Nalidixic acid (NA) (30µg), Erythromycin (E) (15µg), Azithromycin (AZM) (15µg), Tetracycline (Te) (30µg), Gentamicin (GM) (10µg), Ampicillin (AM) (10µg), Amoxicillin and clavulanic acid (AMC) (20µg), Chloramphenicol (C) (30µg)and Trimethoprim-sulfamethoxazole (SXT) (25µg), and Amikacin (AN) (30µg).The test was performed in accordance to the guidelines and recommendations of the Clinical Laboratory Standards Institute in conjunction with the European Committee on Antimicrobial Susceptibility Testing guidelines and breakpoint interpretive criteria (CLSI, 2016; EUCAST, 2020). In this study an isolate was defined as multidrug-resistant (MDR) when it was found resistant to three or more classes of antimicrobials (Meistere*et al*., 2019; Schiaffino*et al*., 2019).

4.7.Data handling and Analysis

A database was entered to Microsoft Excel 2019and imported to SPSS software version 25 for further statistical analysis. Descriptive summary statistics (frequencies and cross-tabulation) were computed for prevalence, antimicrobial resistance and general household characteristics data. The Chi-square (χ^2) and/ or Fisher's exact two-tailed tests (when applicable) were used to compare differences in the antimicrobial resistance rate between *Campylobacter* species isolates and between strains isolated from different animals and humans, humans and water isolates, water isolates and animals. Differences were considered significant at values of $p<0.05$.

Risk factor data associated with the occurrence of pathogenic *Campylobacter*in human samples were analyzed in two steps using logistic regression analysis: (i) univariate logistic regression was used to screen all potential risk factors for statistical significance at a P-value of <0.2 and (ii) statistically significant variables in univariate logistic regression were included in a multivariable logistic regression analysis based on a forward variable selection approach utilizing the likelihood ratio statistic and a P-value <0.05. Goodness of fit of the final model was assessed using the Hosmer and Lemeshow test and Omnibus tests of model Coefficients. The odds ratios for each of the significant risk factors were obtained from the model.

4.8.Quality control

A type strain Campylobacter jejuni and Campylobacter coli obtained from the Ethiopian Public Health Institute, department of microbiology laboratory were used as a positive control in all the isolation, identification, speciation and antimicrobial susceptibility test procedures.Before inoculation of the specimens, the mCCDA, CBA, Mueller Hinton agar plates and all the enrichment broths were checked for their sterility by incubating at both 37 and 42 ^{o}C for 24 to 48 hours.During PCR analysis a standard-grade laboratory water was used as a template negative control.Maximum care was taken to keep all the laboratory and field activities as sterile.

The questionnaire was pre-tested to assess its validity and questions were prepared in local languages to ensure the reliability of the information obtained from the household's. At the end of interview reverse translation was done by two independent individuals to keep the consistency of obtained information.

4.9.Ethical Consideration

Prior to data collection, ethical approval was obtained from College of health sciences of Addis Ababa University, Department Research Ethics Review Committee (DRERC)of the Department of Microbiology, Immunology and Parasitology (DMIP). Permission to interview people was obtained from the Addis Ababa Bureau of agriculture and livestock and Addis Abba Bureau of Health. The corresponding sub-letters were also obtained from the respective sub-cities and Woredas. The study objectives and participants' rights were explained in local languages (Amharic and Afaan Oromo) to each household and Woreda facilitators upon arrival at the site. Verbal and written consent to participate in the study were considered before initiating data collection. Written informed consent was obtained prior to enrolment from the head of each household and an informal assent was obtained from parents or guardians of children when available to collect stool samples.

5. RESULTS

5.1.Description and Socio-Demographic Characteristics of Households

For this study a household was defined as a group of people living together by making a common arrangement for sharing of shelter, food and house commodity. Of the 121 contacted livestock keeping households in the study area, 99 of them were consented and participated on to the study with a response rate of 82%. In woreda 3, eight households were without livestock when contacted due to the fact that they had recently lost or sold their livestock and 9 households from the same woreda refused to participate. Five households from woreda 2 were willing to be interviewed, however, they refused to provide their stool and livestock samples. Questionnaire was administered to these 99 livestock keeping household from which human, animal and environmental samples were also collected.

The median household size of the studied household was 5.0(ranging from 2-15 persons), with a mean of 5.72 ±2.5 standard deviation (SD).Over half (56.6%) of the households were female headed. Overall, 37.4% of respondents had attained primary school, while 34.3% of the respondents completed high school or above, whereas 22.2% of the respondents had no formal education.Of the 99 studied households, 87.9% raised cattle, 37.4% raised poultry, 27.3% raised sheep and 12.1% raised goats were raised by 87.9% of them whilst37.4%, 27.3% and 12.1% of the households raised poultry, sheep and goat, respectively, whereas 58.6% of the studied householdsraised two or more livestock species.

5.2.Households' Knowledge, Understanding and Practices about *Campylobacter*iosis

The majority (97%) of study households had no information of either animal or human *Campylobacter*iosis. Although 85.9% of households recognized that they can get any disease from animals, only 2% of them were aware of the modes of a transmission *Campylobacter*iosis from animal to human (unprotected contact with diseased animals, eating of undercooked meat and or/ drinking of raw milk, etc.). About 74.7% of the studied household responded that they would like to eat raw meat, however, only 2% of the household had known that consumption of raw meat/poultry could expose them to the disease.

Majority (81.5%) of the households mentioned ways of preventing disease transmission from animals to humans, (avoiding unprotected contact with animals, drinking well boiled milk and eating well cooked meat, using hand protections while cleaning animal pen, etc.). Almost all (98%) the studied households were aware that they could get any disease from not washing hands and only a quarter (25.3%) of the households could treat their drinking water. Furthermore, 93.9% of the studied households responded that they need further information regarding the disease *Campylobacter*iosis and other diseases that could be transmitted from animals to humans.

5.3.Livestock Management

The result from questionnaire revealed that in the 99 households studied, cattle were by far most common species raised (87.9%), followed by poultry (37.4%), and, less often goats(12.1%). The responsibility for taking care of ruminants and poultry was shared between women, children and other daily working personnel though wife took more (59.6% and 29.3%, respectively) responsibilities in both cases.

5.4.Prevalence of *Campylobacter* species in various sources

In total, 519 samples from human, cattle, sheep, goat, poultry and different water sources were analyzed from 99 livestock keeping households as shown in Figure 1.Of thesesamples, 93/519 (18%)were found to be positive for *Campylobacter*specieson basis of colony morphology. But it was difficult to get pure *Campylobacter*species cultures due to contamination with other organisms, despite samples were inoculated on selective media and incubated under microaerophilic environment at 42°C. Presumptive culture isolates were further verified by simplex PCR targeting the 16SrRNA and speciation was done by multiplex PCR. The result showed that 67/519 (13%) samples were confirmed positive for *Campylobacter* species (Table 2).

Table 2. Campylobacter isolation rate and species distribution in various samples.

Sample type	Number tested	Total number (%) positive	Number (%)positive samples for				
			C.jejuni	*C.coli*	*C.fetus*	*C.lari*	Other
Cattle feces	135	25 (18.5%)	6 (24%)	0 (0%)	10 (40%)	0 (0%)	9 (36%)
Sheep feces	30	4 (13.3%)	1 (25%)	0 (0%)	3 (75%)	0 (0%)	0 (0%)
Goat feces	14	1 (7.1%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Cloacal swabs	69	9 (13.0%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	0 (0%)	4 (44.4%)
Human feces	99	10 (10.1%)	5 (50%)	1 (10%)	0 (0%)	0 (0%)	4 (40%)
Water samples							
Municipal Tap water	84	7 (8.3%)	2 (28.6%)	2 (28.6%)	1 (14.3%)	0 (0%)	2 (28.6%)
Stored water	63	7 (11.1%)	1 (14.3%)	1 (14.3%)	0 (0%)	0 (0%)	5 (71.4%)
Ground water	16	2 (12.5%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	1 (50%)
Surface water	9	2 (22.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Total	519	67 (13%)	17 (25.4%)	6 (9%)	17 (25.4%)	0 (0%)	27 (40.3%)

Of the total (99) enrolled households, 42(42.4%) were positive for *Campylobacter* at least for one of the samplestypes tested.The isolation rate of *Campylobacter* species among the 99pooled human samples, (248) pooled animal samples and (172) testedwatersamples was 10(10.1%), 39(14.1%) and 18(10.5%), respectively.Among these samples, the proportion of burden of *Campylobacter jejuni* and *C. fetus* was 25.4% (17/67) each, whereas, 9% (6/67) and 40.3% (27/67) of the isolates were categorized as *C. coli* and unidentified *Campylobacter* species (Table 2).

Regardless of the sample source, none of the tested samples were positive for *Campylobacter lari*. Collectively, the *Campylobacter*species carriage rates varied considerably between the different sources. The highest prevalence was found in cattle (18.5%) followed by sheep (13.3%) and poultry (13.0%) whilst the lowest prevalence was recorded in humans (10.1%) followed by water (10.5%).However, there was no statistically significant difference in the proportion of *Campylobacter* species positivity across all sample types($p \geq 0.05$).

5.5.Distribution of Campylobacter Species in Different Sources

Overall, of the 519 tested samples, multiplex PCR analysis of the positive isolates revealed that *Campylobacter jejuni* was isolated more frequently than *C. coli* across all sample types with an isolation rate of 17 (3.3%) and 6 (1.2%), respectively. On the other hand, 17(3.3%) and 27(5.2%) of the isolates were identified as *C. fetus* and unidentified *Campylobacter* species, respectively. Isolation rate of *Campylobacter* species in cattle samples varied from 0(0.0%) to 10(40%) in which *C. jejuni*, *C. coli*, *C. lari*, *C. fetus* and other unidentified *Campylobacter* species accounted for 6 (24%), 0%, 0%, 10(40%) and 9 (36%), respectively. Similarly, of the 4 sheep *Campylobacter* isolates, 1(25%) was found to be *C. jejuni* and 3(75%) were *C. fetus*, whereas the single *Campylobacter* isolate from goat was found to be *C. fetus* (Table 2).

Of the 9 *Campylobacter* isolates from poultry, 2(22.2%) of them were *C. jejuni* and *C. coli* each, 1(11.1%) *C. fetus* and 4(44.4%) were unidentified *Campylobacter* species. Likewise, from the 10 identified human *Campylobacter* isolates, 5(50%), 1(10%), and 4(40%) were *C. jejuni*,*C. coli*, and unidentified *Campylobacter* species, respectively, while none of the human stool samples were positive for *C. lari* and *C. fetus*. Overall, 10.5%(18/172) of the water samples were positive for *Campylobacter* species of which 8.3% (7/84), 12.5% (2/16), 11.1% (7/63) and 22.2% (2/9) was from municipal tap water, ground water, stored water and surface water samples, respectively. *C. jejuni* and *C. coli* were isolated from 2(2.4%) and 1(1.6%) of municipal tap and stored water samples each, respectively, whereas *C. fetus* was isolated from 1(1.2%) of municipal tap water and 1(6.3%) of ground water samples. Unidentified *Campylobacter* species were isolated from 2(2.4%)fo municipal tap water, 5(8%) stored water, 2(22.2%) surface water and 1(6.3%) ground water samples (Table 2).

5.6.Analysis of Risk Factors Associated with Human *Campylobacter*Positivity

Analysis of the 12 explanatory factors obtained from the questionnaire data was undertaken to investigate the potential risk factors associated with *Campylobacter* species positivity in human samples (Table 3). Accordingly, the univariable logistic regression analysis was run for human *Campylobacter* species positivity (outcome variable) and any of the 12 selected explanatory

factors. The results of the univariable logistic regression analysis showed that eight of the 12 selected explanatory variables were significantly ($p < 0.2$) associated with the *Campylobacter* species positivity as shown in Table 3. However, there was no significant ($p > 0.2$) association observed between the human *Campylobacter* species positivity and the four explanatory variables.

Table 3. Univariate logistic regression analysis of explanatory variables for human Campylobacter species positivity.

Explanatory variables/Risk factors	B	p-value	OR (95% CI)
Municipal tap water as sole household source of water	1.764	**0.101**	5.83(10.71– 48.08)
Stored water as common household source of water	3.345	**0.000**	28.36(5.32– 151.2)
Protect oneself when cleaning animal pen	-3.032	**0.000**	0.05(0.011– 0.22)
Heard disease transmitted from animals to human	3.029	**0.005**	20.67(2.49 – 171.3)
Slaughter domestic animals	0.959	0.377	2.61(0.31– 21.84)
Eat and/or taste raw or undercooked meat	1.499	**0.067**	4.48(0.9 – 22.27)
Collect manure indoor and outdoor daily	3.963	**0.000**	52.62(6.2 – 450.5)
Wash hands with soap before and after cooking	-2.982	**0.000**	0.05(0.01 – 0.26)
Treat drinking water	-0.292	0.729	0.75(0.14 – 3.89)
Owning mixed animal species	1.903	**0.077**	6.71(0.81– 55.22)
Occurrence of gastrointestinal disease symptoms	0.461	0.524	1.59(0.38 – 6.54)
Cull sick animals for consumption	1.635	0.576	5.13(1.03 – 25.53)

OR = Odds ratio; CI = confidence interval

In the multivariable logistic regression model, no associations were found between human *Campylobacter* species positivity and self-reported gastrointestinal disease symptoms (Table 4). Likewise, there were no significant association observed for household practice of raw and or under cooked meat consumption and owning of different animal species with human *Campylobacter* species positivity. The household practice of collecting manure indoors and outdoors was significantly associated with the human *Campylobacter* species positivity and found

to increase the odds of human *Campylobacter* species positivity by 31.49(p= 0.007; OR: 31.49; 95% CI: 2.63–378.46).

On the other hand, the household practices of 'wash hands with soap before and after cooking' and 'take any action to protect oneself while cleaning animal pen' were associated with decreased odds of the human *Campylobacter* species positivity. Accordingly, the odd of human *Campylobacter* species positivity was 0.01 less in households who did practice washing hands with soap before and after cooking than those households who did not (p = 0.006; OR: 0.01; 95%CI: 0.000 – 0.26). Similarly, the odd of human *Campylobacter* species positivity was 0.023 less in households who did take any specific action to protect themselves while cleaning animal pen than who did not take any specific action to protect themselves during cleaning of animal pen (p = 0.009; OR: 0.023; 95%CI: 0.001– 0.38).

Table 4. Multivariate logistic regression analysis showing significantly associated explanatory variables for human Campylobacter species positivity.

Explanatory variables	Category	Number of observations	Number (%) *Campylobacter* positive	B	p-value	OR (95% CI)
Indoor-outdoor manure collecting	Yes	36	9 (25)	3.45	0.007	31.49(2.62-378.46)
	No	63	1 (1.6)			
Take any action to protect oneself while cleaning animal pen	Yes	83	3 (3.6)	-3.78	0.009	0.023(0.001-0.38)
	No	16	7 (43.8)			
Wash hands with soap before and after cooking	Yes	76	2 (2.6)	-4.61	0.006	0.01(0.000-0.26)
	No	23	8 (34.8)			
Stored water as common source of water for household	Yes	19	8 (42.1)	4.16	0.005	64.18(3.58-1149.54)
	No	80	2 (2.5)			

Note. B: Estimate; OR: Odds Ratio; CI: Confidence Interval

More importantly, the odd of human *Campylobacter* species positivity was 64.18 times less in households who did not use stored water either for drinking or for food preparation (p= 0.005; OR: 64.18; 95% CI: 3.58–1149.54) (Table 4).

5.7.Antimicrobial Susceptibility Pattern of CampylobacterIsolates

a. Overall Susceptibility Pattern

Disk diffusion was performed on 67 campylobacter isolates confirmed by PCR. Over 98% of isolates were resistant to one or more antimicrobial agents. With the exception of *Campylobacter* isolates recovered from cattle where 24/25 (96%) showed resistance to one or more antimicrobials used, all isolates (100%) from human, sheep, goat, poultry and water were resistant to all tested antimicrobials (Table 5).

Table 5. Antimicrobial susceptibility pattern of Campylobacter species isolates from various sources

Antimicrobial classes	Antimicrobial	Number (%) resistant						
		Human (n=10)	Cattle (n=25)	Sheep (n=4)	Goat (n=1)	Poultry (n=9)	Water (n=18)	Total (n= 67)
Aminoglycosides	Amikacin	9(90)	18(72)	4(100)	1(100)	7(77.8)	14(77.8)	53 (79.1)
	Gentamycin	3(30)	9(36)	2(50)	0(0)	2(33.3)	4(22.2)	21 (31.3)
Macrolides	Azithromycin	7(70)	14(56)	2(50)	1(100)	5(55.6)	11(61.1)	40 (59.7)
	Erythromycin	8(80)	14(56)	1(25)	1(100)	6(66.7)	11(61.1)	41 (61.2)
Penicillin	Amoxicillin/clavulanic acid	7(70)	15(60)	2(50)	0(0)	8(88.9)	15(83.3)	47 (70.1)
	Ampicillin	6(60)	15(60)	2(50)	0(0)	6(66.7)	14(77.8)	43 (64.2)
Phenicol	Chloramphenicol	2(20)	5(20)	2(50)	1(100)	0(0)	3(16.7)	13 (19.4)
Potentiated sulfonamides	Sulfamethoxazole/trimethoprim	2(20)	13(52)	3(75)	1(100)	2(33.3)	6(33.3)	28 (41.8)
Quinolones	Ciprofloxacin	5(50)	14(56)	4(100)	1(100)	7(77.8)	11(61.1)	42 (62.7)
	Nalidixic acid	6(60)	13(52)	1(25)	1(100)	6(66.7)	16(88.9)	43 (64.2)
Tetracycline	Tetracycline	7(70)	17(68)	4(100)	1(100)	5(55.6)	11(61.1)	45 (67.2)

Regardless of the isolation source, majority (79.1%) of the isolates were resistant to amikacin followed by Amoxicillin-clavulanic acid (70.1%), Tetracycline (67.2%), while 64.2% isolates were resistant to Ampicillin and Nalidixic acid each. On the other hand, a lower resistance rate was observed to Chloramphenicol (19.4%) and Gentamycin (31.3%), whereas a moderate (41.8%) resistance rate was observed for Sulfamethoxazole-trimethoprim. Of the 67 isolates tested, 1(1.5%) isolate was found to be resistant to only Erythromycin and Amikacin and was statistically significant (P = 0.016) while 1(1.5%) was resistant to only Erythromycin, Azithromycin and Amikacin. Overall, the antimicrobial susceptibility test revealed that the majority of isolates were found resistant to amikacin and sensitive to Chloramphenicol with a resistance rate of 79.1% and 19.4%, respectively.

The distribution of antimicrobial resistance according to species showed different patterns disregarding the source with resistance ranging from 11.1% to 100% (Figure3). Collectively, the current study showed that *C. coli* exhibited relatively more resistance rate to most of tested antimicrobials than *C. jejuni* and other identified isolates. The resistance rate of *Campylobacter coli* to Nalidixic acid was significantly higher than *C. jejuni* (P = 0.019). Irrespective of the isolation source, there was a significant difference in the resistance rate of *Campylobacter jejuni* and *Campylobacter fetus* to Nalidixic acid), Amoxicillin-clavulanic acid, Erythromycin and Ampicillin (P < 0.05). Analysis of resistance pattern indicated that there was no statistically significant difference observed to the rest of tested antimicrobials among the different species tested (p> 0.05). Moreover, one unidentified *Campylobacter* species isolate from cattle was found to be pan-susceptible, whereas one *Campylobactercoli*isolate from water was resistant to all antimicrobial tested.

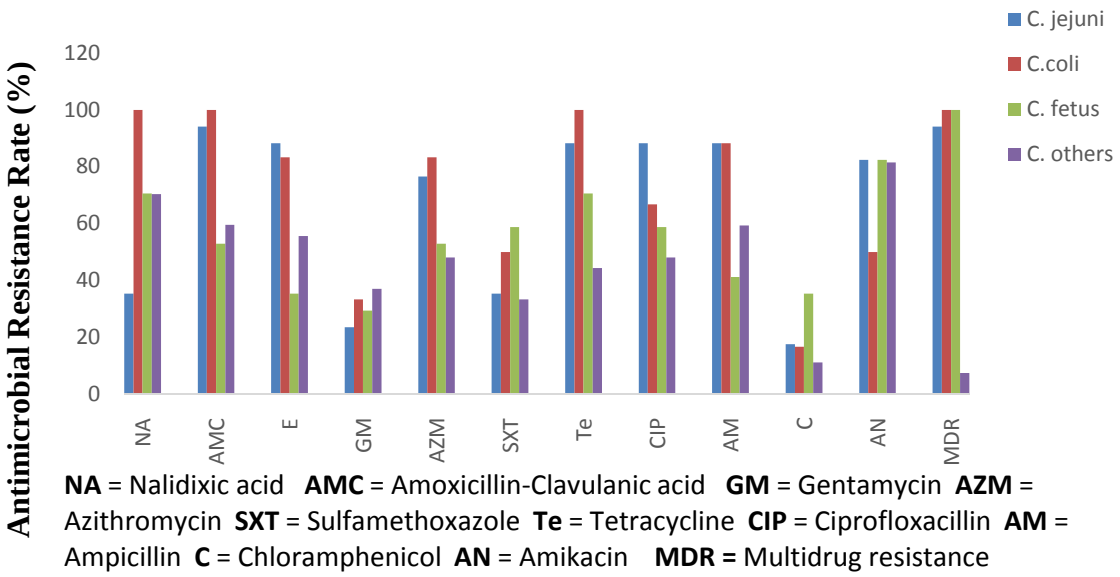

Figure 3. Antimicrobial resistance profile of Campylobacter species

b. Multidrug resistance patterns of Campylobacter isolates

Sixty-four (95.5%) of the 67 isolates were found to be resistant to three or more antimicrobial classes. Of the observed multidrug resistant isolates, 24(37.5%), 18(28.1%), 4(6.3%), 1(1.6%), 8(12.5%) and 9(14.1%) were from cattle, water, sheep, goat, human and poultry isolates, respectively. Furthermore, regardless of the tested antimicrobials, the highest multidrug resistance rate was observed in sheep, goat, poultry and water where all isolates from each of them showed 100% resistance to three or more antimicrobial classes.

Isolates from human, cattle and poultry were resistant to 3-6 antimicrobial classes whereas isolates from water and sheep were found resistant to 3-7 and 4-7 antimicrobial classes, respectively. Furthermore, the single isolate from goat was found resistant to six different antimicrobial classes (**Figure4**). However, no statistically significant difference was observed between the different source types with their multidrug resistivity pattern ($P > 0.05$).

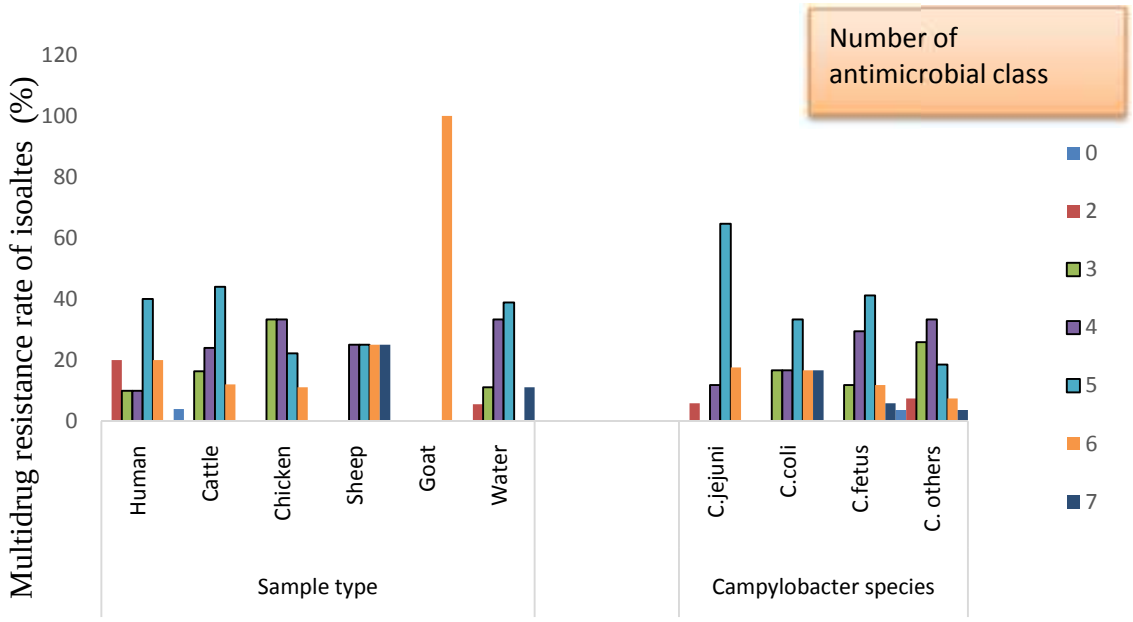

Figure 4. Multidrug resistance rate of isolates considering sample type and Campylobacter species

Species level analysis of multidrug resistance pattern showed that regardless of the source type *C. jejuni* isolates were resistant to 4-6antimicrobial classes whereas, each of the *C. coli*, *C. fetus* and unidentified *Campylobacter* species were found resistant to 3-7 antimicrobial classes (Figure4). All *C. coli* and *C. fetus* isolates showed 100% multidrug resistance pattern, whereas multidrug resistance pattern was observed in 94.1% (16/17) of *C. jejuni* and in 92.6% (25/27) of unidentified *Campylobacter* species (Figure 3). Nonetheless, no statistical significance was observed across the *Campylobacter* species regarding multidrug resistance pattern ($P > 0.05$).

Resistance analysis showed that fifty-four different multidrug resistance pattern ranging from 3 to 7different antimicrobial classes (Table 6). Of these multidrug resistance patterns, NaAmcEAzmTeCipAm resistance pattern was observed in 7.4% (4) isolates followed by AmcEAzmSxtTeCipAmAn and AmcESxtTeCipAmAn each occurring in 5.6% (3) isolates. Yet, Macrolide (Erythromycin and Azithromycin) was the most dominant antimicrobial class appearing in 74.1% (40) (86.2%) of the fifty-four observed multidrug resistance patterns (Table 6).

Table 6. Antimicrobial resistance pattern of Campylobacter species isolated from animals, humans and water in peri-urban Addis Ababa.

Campylobacter species	Source							Antimicrobial resistance pattern and source
	Cattle	Poultry	Sheep	Goat	Human	Water	Total	
C. jejuni	6	2	1	--	5	3	17	EAzmAn (**H**), AmcEGmAzmTeCipAmCan (**H**), AmcEAzmTeCipAmCAn (**H**), NaAmcSxtTeCipAmAn (**H**), NaAmcEAzmTeCipAm (1**H**, 1**W**, 1**P**), AmcEAzmTeAmC (**C**), AmcEGmTeCipAmAn (**C**), AmcEAzmSxtTeCipAm (**C**), AmcEAzmSxtTeCipAmAn (1**C**, 1**P**), NaAmcEGmAzmSxtCipAmAn (**C**), AmcEAzmTeCipAmC (**C**), AmcETeCipAmAn (**W**), NaAmcGmAzmTeCipAmAn (**W**), AmcESxtTeCipAmAn (**S**)
C. coli	--	2	--	--	1	3	6	NaAmcEAzmSxtTeCipAm (**H**), NaAmcTe (**W**), NaAmcEAzmTeAmAn (**W**), NaAmcEGmAzmSxtTeCipAmCAn (**W**)$^{\beta}$, NaAmcEAzmTeCipAm (**P**), NaAmcEGmAzmSxtTeCipAmAn (**P**)
C. fetus	10	1	3	1	--	2	17	NaGmSxtTeCipCAn (**C**), NaAmcETeCipAmCAn (**C**), NaAzmSxtAmAn (**C**), AmcAzmSxtAmCAn (**C**), NaAmcEGmTeCipAm (**C**), AmcESxtAmAn (**C**), NaEAzmSxtTe (**C**), NaSxtTeAn (**C**), NaEAzmTe (**C**), NaAmcAzmTeCipAn (**C**), AzmSxtTeCipAmCAn (**S**), NaGmSxtTeCipAn (**S**), AmcGmAzmTeCipCAn (**S**), NaEAzmSxtTeCipAn (**G**), NaAmcSxtCipAn (**W**), NaAmcGmAzmTeCipAmAn (**W**), AmcCipAn (**P**)
Other species	9	4	--	--	4	10	27	EAn (**H**), NaGmAzmAn (**H**), NaAmcEGmAzmTeAmAn(**H**), NaAmcETeCipAmCAn (**H**), NaEAzmSxtTeAmCAn (**W**), NaAmcEAzmCipAmAn (**W**), NaTeCip (**W**), EGmTeAn (**C**), NaAmcEAzmSxtTeCipAm (**W**), NaAmcAzmCipAmAn (**W**), NaAmcESxtAm (**W**), NaEAzmCipAn (**W**), AmcESxtTeCipAmAn (1**W**, 1**C**), NaAmcCipAmCAn (**W**), NaGmAzmSxtTeAmAn (**C**), NaGmAzmTeCipAn (**C**), AmcGmAzmAn (**C**), NaAmcSxtCipAmAn (**C**), NaEAzmSxtTeCipAn (**C**), AmcGmCipAmAn (**C**), AmcTeCipAm (**P**), NaECipAn (**P**), NaAmcGmSxtAn (**P**), NaAmcEGmAzmAmAn (1**P**, 1**W**), Pan susceptible (1**C**)

Notes. Amc: Amoxixillin-clavulanic acid; Na: Nalidixic acid; E: Erythromycin; Gm: Gentamycin; Azm: Azithromycin; Sxt: Sulfamethoxazole + trimethoprim; Te: Tetracycline; Cip: Ciprofloxacin; Am: Ampicillin; C: Chloramphenicol; An: Amikacin; **C**: Cattle; **S**: Sheep; **H**: Human; **P**: poultry; **W**: water; **G**: Goat; **β**:Resistant to all tested antimicrobials

6. DISCUSSION

In the current study, 42 (42.4%) out of 99 studied households were positive for *Campylobacter* species. Unfortunately, the absence or limited number of similar or related studies both in Ethiopia and other countries make it difficult to compare the results of the current study directly with other studies. This report however, is relatively higher than that reported by Osbjer*et al*. (2016) who reported a prevalence rate of 24.2% (66/269 households) in Cambodia. This difference can be explained by the fact that the current study only considered 99 livestock keeping households compared to the higher number of households (269) examined by Osbjer*et al*. (2016). The other reason may be the difference in geographical and ecological location in which Osbjer*et al*. (2016) studied rural settings where the prevalence is expected to be less as compared to the peri-urban areas like the current study.

The 10.1% prevalence of *Campylobacter* species found in humans in this study is in close agreement with previous prevalence reports in Ethiopia byEwunetu and Mihret (2010) (11.6%),Beyene and Haile-Amlak (2004) (10.5%) and Mitikie*et al*. (2000) (8%). Contrastingly, higher prevalence report was documented byTerefe *et al*. (2020) (50%); Tafa*et al*. (2014) (16.7%); Lengerh *et al*. (2013)(15.4%) and Asrat*et al*. (1999) (13.7%).Similar findings were also reported from Poland (9.6%) (Szczepanska *et al*., 2017)and Tanzania (11.4%)(Komba *et al*., 2015). However,a higher rate was reported by Chuma*et al*. (2016)(19%) and Schiaffino *et al*. (2019) (79.9%) whereas Meistere *et al*. (2019) (5.3%) and Rawat *et al*. (2018) (3.4%) reported a lower isolation rate.

The 18.5% prevalence of *Campylobacter* species in cattle in this study is relatively in line with a previous report of Selesh*et al*. (2015) (21.5%) but, higher than the 12.7% found by Kassa*et al*. (2007) and lower than the 48% found by Abamecha*et al*. (2015).This 18.5% recovery rate is comparable to a report by Kim *et al*. (2015) (17.3%) from Republic of Korea and Meistere*et al*. (2019) (17%) from Latvia but, is higher than reports from Iran (5.3%), Ghana (13.2%)and California (15.9%) (Rahimi *et al*., 2017; Karikari*et al*. 2017; Pires *et al*., 2019). On contrary, the present prevalence rate is lower than studies in Republic of Korea (25.6%) (An *et al*., 2018) and USA (72.2%) (Tang *et al*., 2017).

The 13% overall prevalence of the *Campylobacter* species observed in poultry in the study area is lower than earlier reports from Ethiopia in which prevalence rate ranging from 18.4% to 86.6% was reported (Brena *et al*., 2016; Abamecha*et al*., 2015; Kassa*et al*., 2007). However, the result of the current study is higher than the findings of Pires *et al*. (2019) (11.3%) from California and Rawat *et al*. (2018) (5.9%) from Indiawhereas a higher isolation rate was reported in Kenya(69%) by (Nguyen *et al*., 2016), Cambodia (56%) by (Osbjer*et al*., 2016) and Tanzania (42.5%) by (Chuma*et al*., 2016). On the other hand, the present finding is in line with the result reported by Meistere*et al*. (2019) (12.9%) in Latvia.

The 13.3% *Campylobacter*species prevalence in sheep is higher than the report from Ethiopia (10.6%) by (Chanyalew*et al*., 2013), and Iran (10%) by (Rahimi *et al*., 2017), but lower than the report from Ethiopia by Kassa *et al*. (2007) (38%) and Abamecha*et al*. (2015)(39%)and 18.6% (Karikari*et al*., 2017) reported in Ghana.

Even though different prevalence rates have been reported across different study sites in Ethiopia, the very limited number of available comparable study types makes direct comparison of results difficult. The variation in study result may be explained by the actual differences in study duration and periods, seasonality, animal management system, sanitary practices and agro ecological variations (Chuma*et al*., 2016; Osbjer*et al*., 2016). Moreover, while precise cause of prevalence difference is not clear, however, it can arise as a result of several methodological variables including difference in the number of samples and sampling type, sampling technique, sampling units and laboratory methodologies employed (Meistere*et al*., 2019; Szczepanska*et al*.,2017).

To this end, the current study was a community level-based study employing pool sampling method and sampling from asymptomatic sampling units. On contrary, most of the previous studies conducted in Ethiopia were based on symptomatic human subjects and/ or farm-level animal studies that may result in observing of different prevalence rates across the studies. Additionally, except for Terefe*et al*. (2020) that used molecular techniques for isolation and identification, in all of the previous reports in Ethiopia, culture was the only isolation and identification method used. The current study, however, employed both culture and molecular

(PCR) methods for isolation and identification of *Campylobacter* species, respectively. Therefore, the employed methodological difference in different studies may result in variation of the prevalence rate.

The overall isolation rate of *Campylobacter* species in water in this study was 10.5%. Yet, there were no previous studies conducted in Ethiopia to be compared with the current result. The result of the current study is similar with the study from Turkey (10.3%) (Elamli and Can, 2019) and lower than a report from South Africa (21%) (Chukwu *et al*., 2019), and Poland (16%) (Szczepanska*et al*., 2017). The observed discrepancies across the different studies may be emanated from the source of water, technique of water collection, season, geographical location, method of isolation and in fact the amount and size of included water samples.

The current study area is impacted mainly by both agricultural operations (mainly cattle and poultry production and vegetable production) and urban wastewater treatments which mayinfluence the prevalence of different microorganisms like *Campylobacter*(Yohannes and Elias, 2017; Gebremichael *et al*., 2014). Although the contributions of water to the burden of sporadic cases of *Campylobacter* infections in human might be unknown while not all cases lead to severe illness (Elmali and Can, 2019; Pitkänen, 2013), the result of the current study may provide a baseline information regarding the circulation of *Campylobacter* species between livestock, human and water in the area. Further studies, however, on this part with the use of molecular typing methods need to be conducted to ascertain the transmission of *Campylobacter* within the environment.

With the exception of isolates from cattle and poultry, the current study result showed that *C. jejuni* was the most frequently isolated species than other thermophilic *Campylobacter* species with the observed prevalence rate of 50% (human), 24% (cattle), 25% (sheep), 22.2% (poultry) and 22.2% (water)positives. This finding is in agreement with various previous reports (Terefe*et al*., 2020; Chukwu *et al*., 2019; Pires *et al*., 2019; Kassa *et al*., 2007). It was documented that in most of domestic animals,*C. jejuni* can be more prevalent and also naturally has longer viability in biological milieu thereby increasing its chance of recovery than other thermophilic *Campylobacter* species (Pires *et al*., 2019; Kashoma*et al*., 2016; and Nguyen *et al*., 2016).

The difference in species distribution can also be explained by the differences in the mechanism of pathogenesis and elimination between the different thermophilic *Campylobacter* species within the host cells in which unlike *C. jejuni* other thermophilic *Campylobacter* species, mainly *C. coli* is more readily phagocytosed and killed by peritoneal macrophages (Elmali and Can, 2019; Szczepanska*et al*., 2017). Yet, differences in isolation proportions of microorganisms might also be related to the actual variations in the composition of common *Campylobacter* species in local environments. Nonetheless, *Campylobacter lari* was not recovered in this study that echoes previous reports (Terefe *et al*., 2020; Pires *et al*., 2019; Kashoma *et al*., 2016; Asrat*et al*., 1999).

Many of the risk factors identified in the literature for human *Campylobacter*species positivity were included in the present study (Chukwu *et al*., 2019; Osbjer *et al*., 2016; Komba *et al*., 2015). These factors were found to have a significant association with the outcome of interest in the univariable analysis or were identified as associated risk factors in the multivariable analysis.

In multivariate analysis households using stored water (water with in different storage containers) frequently for drinking, food preparation and cleaning of kitchen utensils were shown to have higher odds of *Campylobacter*species positivity, compared to those who did not (P= 0.005; OR: 64.18; 95% CI: 3.58-1149.54). This result is in line with the reports of previous studies (Chukwu *et al*., 2019; Elfadaly *et al*., 2017). Studies have been shown thatuntreated stored water is a significant source of *Campylobacter* infections and outbreak (Nilsson *et al*., 2018; Pitkänen *et al*., 2013). Factors like source of water and type of storage container have been linked to the poor microbial quality of stored household water (Elfadaly *et al*., 2017). However, the high odds ratios presented in this study should be interpreted with caution as the association is significant with a wide confidence interval.

The current study result showed that indoor and outdoor manure collecting was associated with increased odds of human *Campylobacter* species positivity (P= 0.007; OR: 31.49; 95% CI: 2.62–378.46). This is in agreement with previous reports (An *et al*., 2018; Osbjer *et al*., 2016). Peoples could store manure for various reasons including the later use in irrigation or collect for some days in door and/or outdoor to clean it together. *Campylobacter* can survive at variable rates in this stored manure and even in composted manure (reviewed in Pires *et al*., 2019 and Hald *et al*., 2016). Consequently, human exposure to *Campylobacter* species may occur in cases when

drinking or ingesting of contaminated drinking water, animal and/or vegetable products (An *et al*., 2018).

Multivariable analysis also identified that taking any specific action to protect oneself while cleaning animal pen and washing hands with soap before and after cooking were associated with decreased odds of human *Campylobacter* species positivity. Similar finding was reported in previous studies (Osbjer *et al*., 2016). This is not surprising as poor hygiene and sanitation are associated with increased odds of multiple adverse health outcomes (Mbuya and Humphery, 2016).

Globally, several studies had shown the occurrence and rising of antimicrobial resistance among *Campylobacter* isolates (Abubakar *et al*., 2019; Sproston *et al*., 2018; Ewnetu and Mihret, 2010). The issue has been also recognized as a public health problem by World Health Organization (Ogbor *et al*., 2019; Silva *et al*., 2011). Antimicrobials, mainly macrolides, (fluoro) quinolones and tetracycline, are drugs of choice in the case of severe human *Campylobacter* gastroenteritis. Occurrence of antimicrobial resistance in both humans and animals leads to limitation of treatment choice and treatment failure (reviewed by Hlashwayo*et al*., 2020). In recent years, several studies confirmed the increased number of *Campylobacter* isolates resistant to these classes of antimicrobials (Hlashwayo*et al*., 2020; Rahimi *et al*., 2017; Mitikie*et al*., 2000).

In the present study, higher number of isolates were resistant to amikacin (79.1%) and amoxicillin-clavulanic acid (70.1%), whereas the lowest resistance rate was observed against chloramphenicol (19.4%) and gentamicin (31.3%). Similar study results have been documented from different countries (Divsalar*et al*., 2019; Signorini*et al*., 2018; Girum, 2015). The study also showed a higher level of resistance to fluoroquinolones (Nalidixic acid) (64.2%) and (Ciprofloxacin) (62.7%). This is at a similar level with the report of (Wieczorek*et al*., 2019; Nguyen *et al*., 2016;Abamecha*et al*., 2015). In contrast, various studies reported lower resistance rate than the present study (Chukwu *et al*., 2019; Lengerh*et al*., 2013).

The resistance to macrolides (erythromycin (61.2%) and azithromycin (59.7%)) observed in this study is higher than previous reports from various parts (Schiaffino *et al*., 2019; Szczepanska *et*

al., 2017; Tafa*et al*., 2014; Lengerh*et al*., 2013). The current resistance rate corroboratesthe reports from various studies elsewhere (Divsalar *et al*., 2019; Du *et al*., 2018; Kashoma *et al*., 2016; Abamecha*et al*., 2015).Therate of resistance against tetracycline (67.2%), ampicillin (64.2%) and sulfamethoxazole-trimethoprim (41.8%) observed in this study agrees with previous study reports (Chukwu *et al*., 2019; Schiaffino *et al*., 2019; Tafa*et al*., 2014; Lengerh*et al*., 2013). However, resistance rate less than the current report has been privouslydocumented (Rawat *et al*., 2018; Mulatu *et al*., 2014; Kassa*et al*., 2007).

This study revealed that *C. coli* was highly resistant to Nalidixic acid (100%), Amoxicillin-clavulanic acid (100%), Azithromycin (83.3%) and Ampicillin (88.3%) than other species, whereas *C. jejuni*showed relatively higher resistance to Erythromycin (88.2%), Ciprofloxacin (88.2%) and Amikacin (82.4%) than other species. This result is comparable with reports from previous studies (Igwaran and Okoh, 2020; Chukwu *et al*., 2019; Gharbi*et al*., 2017), however, the current result disagrees with the results of other previous studies (Elhadidy *et al*., 2020; Painset *et al*., 2020; Girum, 2015). On the other hand, *C. fetus* showed higher resistance to Sulfamethoxazole-trimethoprim (58.8%) and Chloramphenicol (35.3%) than the other isolates. It should also be emphasized that in this study all poultry isolates were susceptible to chloramphenicol whereas one isolate (unidentified *Campylobacter* species) from cattle showed pan susceptibility and one isolate (*C. coli*) from water was resistant to all tested antimicrobials.

In the current study regardless of the isolation source a higher (>95%) number of *Campylobacter* isolates exhibited multiple antimicrobial resistance. The study found a 100% multiple antimicrobial resistance rate in both poultry, water, sheep and goat isolates whereas multiple antimicrobial resistance rate was observed in 96% of cattle isolates and 80% of human isolates. This finding is concordant with various previous study reports (Schiaffino *et al*., 2019; Chukwu *et al*., 2019; Abamecha*et al*., 2015; Lengerh*et al*., 2013;). However, lower rate of multiple antimicrobial resistance than the present study has been reported from various studies in different countries (Rahimi *et al*., 2017; Kashoma *et al*., 2016; Dadi and Asrat, 2008; Kassa*et al*., 2007).

In this study all the *Campylobacter* species analyzed exhibited multiple antimicrobial resistance. Irrespective of the isolation source, the highest (100%) multiple antimicrobial resistance was

observed in *C. coli* and *C. fetus* isolates each followed by *C. jejuni* (94.1%). This finding is consistent with multiple reports from previous studies both in Ethiopia and other countries (Raeisi *et al*., 2017; Li *et al*., 2016; Kassa*et al*., 2007). However, the current study findings disagree with the findings from previous reports (Schiaffino *et al*., 2019; Karikari *et al*., 2017; Abamecha*et al*., 2015). The reason why *C. coli*was resistant to several antimicrobials than other *Campylobacter* species may be explained differently. It is widely accepted that the *C. coli* strains could acquire horizontal resistance genes better than other species and/or those target genes could mutate faster in the *C. coli* (Raeisi*et al*., 2017; Jamali *et al*., 2015). The present study results, however, need forethought during interpretation as the number of *C. coli* isolates was relatively low.

The higher antimicrobial resistant rate observed in the current study might be associated to the overuse and misuse of antimicrobial agents in both human and animals. To our knowledge and available evidences, oxytetracycline, tylosin, and penicillin alone or in combination with streptomycin are used widely for therapeutic purposes in livestock and poultry in Ethiopia, which increases the risk of resistant *Campylobacter*species to these drugs (Eguale, 2018; Beyne *et al*., 2015).Moreover,available evidences also indicate that extensive application of antimicrobials in animal industry for therapy, prophylaxis, and growth promotion has been associated with the advance of resistance (Szczepanska*et al*., 2017). This significantly reduces the number of available antimicrobials that can still be used effectively for the treatment of human and animal infections (Elhadidy*et al*., 2020; Chukwu *et al*., 2019).

The differences in isolation rate of the different *Campylobacter*species among the different sources may make it difficult to compare levels of resistance between the sources. Nonetheless, observed resistance discrepancies in this study compared to previous investigations could be due to periodical changes but also an attribute of differences in exposure rates of the bacteria to the different antimicrobials. The inevitable human-animal-environment interaction in the current study area and along with misuse of antimicrobials in both animals and humans might have led to pronounced selection pressure for resistant strains.

There are a number of reasons related to the mechanism of multidrug resistance in *Campylobacter*s. The presence of a major efflux system that is responsiblefor resistance to occur to a broad range of antimicrobials (Whitehouse *et al*., 2018). The destruction or inactivation of

antimicrobials (by enzymes encoded by chromosomal or plasmid genes) and low-level access of antimicrobial agents to their targets are the other reasons for multidrug resistance to exist among *Campylobacter* species (Raeisi*et al*., 2017; reviewed in Nguyen *et al*., 2016).

7. CONCLUSION AND RECOMMENDATIONS

Overall the present study has shown the presence, associated risk factors and antimicrobial resistance profiles of human, animal and water-derived *Campylobacter*isolates to different antimicrobials in peri-urban part of Addis Ababa, Ethiopia. The study found that *Campylobacter jejuni*, *coli*, *fetus* and other *Campylobacter* with unidentified species were prevalent in the study area. Nevertheless, in the study, relatively a high proportion of *C. jejuni* positive samples than otherthermophilic *Campylobacter* species in both livestock and water samples were observed suggesting the potential risk for humans in the current study area where contact with livestock and their environment is inevitable.More importantly, the study alsoindicated that most of the *Campylobacter* isolates were found to be resistant to most of the antimicrobial agents tested, where many of the isolates were showing resistance to three or more of these antimicrobial agents.Moreover, questionnaire data also revealed that shortage of land, livestock disease and poor feed availability are major concerns of urban livestock keepers. Given the above conclusions in to considerations, the following recommendations can be forwarded:

- Taking the significant effect of antimicrobial resistance into consideration, it is important for the country to have a national plan to advance the rational use of antimicrobials in the view of “One Health” approach.
- There is a need for awareness creation of the livestock keeping households on *Campylobacter*iosis and other zoonoses, antimicrobial resistance and environmental sanitation among other issues that may contribute to reduction of livestock infections thereby minimizing zoonotic diseases.
- Taken together, the current study findings can help guide future robust community-based studies with the use of advanced molecular techniques to be exploited for insights into the role of different livestock species and environmental factors in the human acquisition of *Campylobacter* species in the livestock producing areas of the country.

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ANNEXES

ANNEX I: Participant information sheet

Title: 'Burden of *Campylobacter* Species on Livestock owning Households in Peri-urban Addis Ababa, Ethiopia: A One Health Approach'

Investigator: Gemechu Chala (DVM, MSc fellow at Addis Ababa University College of Health Science). Mobile phone: +251-941393996; email: game33chala@gmail.com

Advisor: Professor Daniel Asrat

Co-Advisor (s): Dr. Andrew Stringer; Dr. Fufa Abunna; Dr. Tadesse Eguale

This participants information sheet is for livestock owning households in the Peri-urban areas of Addis Ababa, Ethiopia and who I am inviting to participate in a research entitled " **Burden of *Campylobacter* Species on Livestock owning Households in Peri-urban Addis Ababa, Ethiopia: A One Health Approach".** I am Gemechu Chala, an MSc fellow at Addis Ababa University College of Health Science. I am conducting a research on the zoonotic disease '*Campylobacter*iosis' which is common in this country and in Addis Ababa as part of the requirement for the partial fulfillment of the master's degree in Medical Microbiology at Addis Ababa University. I am going to give you information and invite you to be part of this research. Your participation in this research is entirely voluntary and it is up to you to decide whether or not to take part. This form may contain words that you do not understand, please ask me to stop as we go through the information and I will take time to explain; or if you wish to ask questions later, you may contact me by the above contact information.

This research is proposed to investigate the presence and burden of zoonotic *Campylobacter* species and associated risk factors thereby generate valuable information which would be used to contain the problem. This research will involve your participationand I believe that you can help and contribute your part by allowing me to sample fecal matter from family members and animals and water samples as well as answering some following questions. You are being invited to take part in this research because I feel that your experience as a livestock owner can contribute much to the available understanding and knowledge of local livestock production practices and risk of contracting zoonotic diseases.

The time to complete the survey questionnaire will vary, however, it is anticipated that no more than thirty minutes will be necessary.You are required to answer the questions based on your personal experience during the interview. You may decline to answer any or all questions and you may terminate your involvement at any time if you choose regardless of any precondition. I don't believe there are any risks from participating in this research. Nevertheless, to protect your privacy, all information which is collected about you during the course of the research will be kept strictly confidential. Your name and any other information that can directly identify you will be represented using anonymous**.**Even though there is no direct benefit to you during the research, you will be beneficiary from the information to be generated from this research in such a way that obtained information will be used to design appropriate disease prevention and control measures at public health and animal health sectors.

ANNEX I. Participant's consent (Assent; when applicable) form

Title: 'Burden of *Campylobacter* Species on Livestock owning Households in Peri-urban Addis Ababa, Ethiopia: A One Health Approach'

Investigator: Gemechu Chala (DVM, MSc fellow at Addis Ababa University College of Health Science). Mobile phone: +251-941393996; email: game33chala@gmail.com

Advisor: Professor Daniel Asrat

Co-Advisor (s): Dr. Andrew Stringer; Dr. Fufa Abunna; Dr. Tadesse Eguale

I (_____) have read (has been read to me) the foregoing information. Accordingly, I understood that I am kindly invited to participate in a research entitled '**Burden of *Campylobacter* Species on Livestock owning Households in Peri-urban Addis Ababa, Ethiopia: A One Health Approach'**, which is conducted by Gemechu Chala.

Hence, I have been asked to participate in this research entirely voluntarily and during this participation I will give my stool sample and also allow the researcher to take water and my animal (s) fecal samples. Furthermore, I understood that I will be asked questions and will answer as to my knowledge. Moreover, I have perceived that I can withdraw from participating in the research at any time or point during the research activity and also, I am not going to be enforced to answer questions that I do not want to answer. I clearly understood that there will be no risks to me, my family and my animals from participating in this research and all my personal information that directly identify me and my family will be kept strictly confidential. Additionally, it is also clear to me that I do not get any direct benefit from the research.

Therefore, as a participant of this research I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction and I understand that I will be given a copy of this consent form. Hence, I understood and agree with all the information given to me and I voluntarily consent to be a participant in this research by my signature.

Participants' signature_____ Date_____

Investigators' signature_____ Date_____

Witnesses' signature (if any) _____ Date _____

ለጥናቱተሳታፊዎችየተዘጋጀመረጃ

የጥናቱርዕስ:Burden of *Campylobacter* species on livestock owning Households in peri-urban Addis Ababa, Ethiopia: A One Health Approach

የጥናቱባለቤት: ገመቹጫላ (በአድስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሁለተኛድግሪተማሪ). ሞባይልቁጥር: 0941393996; E-mail: game33chala@gmail.com

ዋናአማካሪ: ፕ/ር ዳንኤልአስራት.

ተባባሪአማካሪዎች: ዶ/ር ፉፋአቡና፤ ዶ/ር ታደሴእንሌእና ዶ/ር አንድሪውእስትሪንገር

ይህመረጃበአድስአበባከተማውስጥለምኖሩእናየተለያዩየቤትእንስሳትንበማርባትለምተዳደሩእናበዝህጥናትላይለ መሳተፍፍቃደኛለሆኑትለግንዛቤተብሎየተዘጋጀነው፡፡. ከላይበርዕሱእነደተገለፀውእኔ (ገመቹጫላ) በአድስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሁለተኛድግሪተማሪየሆንኩኝስሆንይህንጥናትሁለተኛድግሪዬንለማ ጠናቀቅእንደአንዱማሚያነው፡፡ ይህጥናትየምያቶኩረውካምፓይሎባክቴርዎስስየምባልበሽታስሆን፤ ይህበሽታከእንስሳት፤ ከውሃእነድሁምከተለያዩየአከባቢቆሻሻወደሰውልተላለፍየምችልስሆንበይበልጥህፃናት፤ ልጆችንእናበሽታንየመከላከልአቅማቸውየቀነሰሌሎችሰዎችንየምያጠቃነው፡፡

ይህጥናትይሳካዘንድያንቱ/ች ተሳትፎእጅግአስፈላጊነው፡፡ በዝህጥናትላይለመሳተፍፍቃደኛከሆንክ/ሽ ለናመ-ናየምሆንያንቱን/ችንእናየቤተሰቦችክን/ሽንአይነ-ምድር፤ እንድሁምከእንስሳቶችምለናመ-ናየምሆንእበት፤ ከምትጠቀመ-ትውሃ/የውሃሚንጭየምወሰድይሆናል፡፡ በተጨማሪምእንስሳትዕርባታእናእሱንተከትሎልከሰቱበምችሉትበሽታዎችዙርያ ከ20-25 ደቂቃልወስድየምችልጥያቄናመልስይኖራለ፡፡

በእንስሳትዕረባታያለክን/ሽንልምድእናተሞክሮለዝህጥናትስኬታማነትጉልህአስተዋፆዕልያበረክትይችላልበምልአ ንቱን/ችንመርጠንካል/ሻል፡፡ በዝህጥናትላይለመሳተፍወይንምላለመሳተፍመ-ሉ-መብትአለክ/ሽ፡፡ በተጨማሪምበጥናቱላይላለመሳተፍከፈለክ/ሽ በፈለግከው/ሽውግዝማቆምትችላለክ/ሽ፡፡

በዝህጥናትላይስለተሳተፍክ/ሽ ወይንምስላልተሳተፍክ/ሽ ባንቱ/ች ላይወይንምበቤተሰቦችክ/ሽ ላይልደርስየምችልተፅዕኖ/ችግርአይኖርም፡፡ መምለስያልፈለክውንጥያቄእነድትመልስአትገደድም/ጅም፡፡ በዝህጥናትላይየምትሰጠው/ጭውማንኛውምመረጃበምስጥርየምያዝስሆንናያንቱንም/ችንምሆኔየቤተሰቦችክን/ ሽንማንነትየምገልጥመረጃበኮድየምመዘገብይሆናል፡፡ አንቱ/ች ለዝህጥናትብለክ/ሽ የምትሰጥ/ጭ ማንኛውምመረጃለሌላሰውአይታይም፡፡

ከዝህጥናትላይየምገኘውመረጃየተባለውንበሽታለመከላከልእናለመቆጣጠርየምዉልስሆንእዝህጥናትላይስለተሳ ተፍክ/ሽ በቀጥታየምታገኘው/ኝውጥቅምየለም፡፡

ለጥናቱተሳታፊዎችየተዘጋጀየስምምነትቅፅ

የጥናቱረዕስ:Burden of *Campylobacter* species on livestock owning Households in peri-urban Addis Ababa, Ethiopia: A One Health Approach

እኔ (_____)
ከላይየተጠቀሰውንመረጃእናገለፃሙሉበሙሉአንብቤ (በሌላሰውተነቦልኝ) ሙሉበሙሉተረድቻለሁኝ፡
፡

በዝሁመሠረትከላይበሪዕሱየተጠቀሰውእናበገመቹጫላበምጠናውጥናትላይበፍቃደኝነቴተሳታፊእነድ
ሆንበትህትናጠይቆኛል፡፡
ስላዝህበዝህጥናትላይሙሉበሙሉበፍቃደኝነቴእንድሳተፍየተጠየኩስሆንእኔምበጥናቱላይለመሳተፍ
ፍቃደኛነኝ፡፡ በዝሁመሠረትለናሙናየምሆንአይነ-
ምድርየምሰጥስሆንበተጨማሪምከእንስሳቶቹምለናሙናየምሆንዕበት፤
እንድሁምቤተሰቤከምጠቀመውውሃናሙናእንደምወሰድገብቶኛል፡፡
በተጨማሪምጥናቱበምካሄድበትግዜየተለያዩጥያቄዎችንእንደምጠየቅእናጥያቄዎቹንምበምችለውመ
ልኩእንደምመልስተረድቻለሁኝ፡፡

በተጨማሪምጥናቱላይመሳተፍካልፈለኩኝበፈለኩበትሰዓትእናሁኔታጥናቱንማቋረጥእንደምችልእና
መመለስየማልፈልገውንጥያቄምለመመለስእንደማልገደድተረድቻለሁኝ፡፡
እንድሁምበዝህጥናትላይስለተሳተፍኩኝበእኔምሆኔበቤተሰቤእንድሁምበእንስሳቶቼላይየምደርስጉዳት
እንደሌለእናበጥናቱወቅትምስለራሴ/ቤተሰቤየምወሰደውማንኛውምመረጃበጥብቅምስጥርየምያዝመሆ
ኑንተረድቻለሁኝ፡፡
በተጨማሪምበዝህጥናትላይስለተሳተፍኩኝእኔምሆኔቤተሰቤምንምአይነትቀጥተኛጥቅማጥቅምየማና
ገኝመሆኑንለመረዳትችያለሁኝ፡፡

ስለዝህእንደጥናቱጥናቱተሳታፊነቴየፈለኩትንጥያቄየጥናቱንባለቤትጠይቂበቂምላሽለማግኝትችያለሁ
ኝ፡፡ እንድሁምየዝህውልአንድግልባጭእንደምሰጠኝተገንዝበዋለሁኝ፡፡
ስለዝህበዝህጥናትላይየተሰጠኝንመረጃበአግባቡተረድቼእናተስማምቼበሙሉፍቃዴጥናቱላይለመሳተ
ፍእናየምፈለገውንእገዛለማድረግመስማማቴንበፊርማዬአረጋግጣለሁኝ፡፡

የጥናቱተሳታፊፊርማ _____ ቀን _____
የጥናቱባለቤትፊርማ_____ ቀን _____
የእማኝስምናፊርማ (ካለ)_____ቀን _____

ODEEFFANNOO QOODA FUDHATTOOTA QORANNOO

Mata Duree: **'DhiibbaaBaakteeriyaan*'Campylobacter'*maatiihorsiiseebultootanaannoohandaaramagaalaa Finfinnee jiraatanirrattiGeessisu'**.

QorataaQorannichaa: GammachuuCaalaa (kolleejjiiFayyaaYuuniversiitii Addis Ababaattibarataadigirii 2ffaa). Lakk. Moobayilii: 0941393996; E-mail: game33chala@gmail.com

Gorsaaaddaduree: Pirof. Daani'elAsraat

Gorsitootadabalataa: Dr. FufaaAbbuunnaa, Dr. TaaddeseeIguwaalee, Dr. AndiriwIstiriinger

Odeeffannoonkunimaatiibeeyladootaaddaaddaahorsiisaajiraniifinaannoohandaaramagaalaa Finfinnee jiraataniifiqorannoo kana irrattihirmaattotata'uufkaadhimamaniifkanqindaa'edha. Akkumaarmaanoliittiibsametti, ani (GammachuuCaalaa) KoolleejjiiFayyaaYuuniversiitiiFinfinneettibarataadigirii 2ffaayeroonta'u, qorannoonkunileenjiikoo kana xumuruufakkabarbaachisummaatokkooti. Caalmaattiqorannoonkunidhukkuba '**Campylobacteriosis'** jedhamuufibeeyiladaafiwantootanaannoobeeyiladaatti argaman kanneenakkabishaan, biyyoofibalfabeeyiladairraaargamuugaranamaattikandarbuufikeessumaadaa'immman, ijoolleefinamootadandeettiindhukkuba of irraaittisuunisaaniigadibu'aata'anirracaalaankanmiidhuirrattixiyyeeffata.

Fiixaanba'umsaqorannookanaafhirmaannaankeedaraanbarbaachisaadha. Qorannoo kana fiixaanbaasuufqoodafudhataayootaatebobbaatiikeeirraaakkasumasbeeyiladootakeeirraaakkasumas bishaanfayyadamtanirraahangaxiqqooakkafudhadhuufakkasumasgaaffileetokkotokkosigaafadhuu fheeyyamakeebarbaada.

Muuxannoonatihosriisabeeyiladaairrattiqabduusababeeffachuunhubannoofi beekumsa horsiisabeeyilladaa fi carraadhukkubaaddaaddaatiinqabamuuilaalchiseeargamuirrattiga'eekeeakkabaatu hubannoo keessagalchuunqorannoo kana irrattiqoodafudhataataateefilamteetta. Qorannoo kana

irrattihirmaachuufidhiisuunfeedhiikeeguutuuirrattihundaa'a. Yoohirmaachuuffeedhiidhabdeyeroofeeteaddaankutuunidandeessa.

Gaaffileejirandeebisuufyoobaay'atedaqiiqaa 25 fudhachuudanda'a. sababaqorannoo kana irrattihirmaatteefmiidhaansirraga'utokkoyyuuhinjiru. Gaaffiideebisuufhinbarbaanneedhiisuunidandeessa. Odeeffannoonatiqorannoo kana irrattikennitusicciitiinkanqabamuufimaqaankeeakkasumasodeeffannoodhuunfakeesta'ekanmaatiikeekanilaallatukamiyyuukallattiinakkakanibsamumiti; kanaa manna bakkabuuteeaddaaddaattigargaaramuungalmeeffama. Odeeffannooatiqorannoo kana irrattikennitekamiyyuunamootaqorannoo kana irrattihirmaachaajiranmaleeeenyuttuuhinmul'ifamu. Sababaqorannoo kana irrattiqoodafudhataataateeffaayidaakallattiiargachuubaattus, odeffannoondhumairrattiqorannoo kana irraaargamudhukkuba kana akkabiyyaattidursaniiittisuufito'achuufkanooluunfayyaabeeyiladaafinamootaamirkaneessuufkanfaayyaduta'a.

UNKA WALII GALTEE QOODA FUDHATTOOTA QORANNOO

Ani (_____) odeeffannooarmaanoliirratidhiyaatehaalagaariindubbisehubadheera. Haalumawalfakkatuunqo'annoomatadureenisaa:

'DhiibbaaBaakteeriyaan*'Campylobacter'*maatiihorsiiseebultootanaannoohandaaramagaalaa Finfinnee jiraatanirrattiGeessisu' ta'eefiGammachuuCaalaatiingaggeeffamuirrattifedhiinakkanhirmaadhuufafferamuukoohubadheera. Haalumakanaanqorannoo kana irrattihirmaachuufisdhiisuufisfeedhiikooakkata'e natty himamee hubannoo argadheera. Yerooqorannoonkunigaggeeffamuttisbobbaatiikookennuufiakkasumasbobbaatiihoriikooakkafudhatamu, bishaanittimaatiinkoofayyadamuirraasakkafudhatamuhubadheenjira. Kana malees, gaaffileeqorannoo kana waliinwalqabateakkangaaftamuufigaaffileekunnenishanga beekumsa kooqofadeebisuuakkandanda'unaafgaleera. Dabalataanisyeroonfedhettiqorannoo kana irrattihirmaachuudhaabuuakkandanda'uyookiingaaffiindeebisuuhinbarbaannekamiyyuudhiisuufmirgaguutuuakkanqabuhubachuudanda'eera. Sababiinqorannoo kana irrattihirmaadheefanayookiinmaatiikooakkasumasbeeyiladakooirrabalaanyookiindhiibbaanga'uakkahinjirree fi odeeffannoon ani kennumartuuicciitiinakkakatabamuufiqabamu hubannoo argadheera. Kana malees, odeeffannoonkallattiinmaalummakooyookiinmaatiikooibsukamiyyuunamasadaffaagarabiraaf (namootaqorannoo kana irrattihinhirmaannee) kanhinmul'ifamneta'uuisaahaalaanhubadheera. Akkasumassababaqorannoo kana irrattihirmaadheeffaayidaan/kaffaltiin anis ta'emaatiinkookallattiinargannuakkahinjirrebeekeera.

Kanaafuu,

akkaqoodafudhataaqorannookanaattigaaffiingafachuuffedhehundaagaafadheedeebiiquubsaafikallattiiargadheera. Kana malees, waraqaanwaliigalteekunifuullitokko (garagalchitokko) akkanaafkennamuhubadheenjira.

Kanaafodeeffannoonaafdhiyaatehundaahubachuunifiittiwaliigaluun, qorannoo kana irrattifedhiinhirmaachuufiwantaqorannoo kana irrattigochuunnairrajiruhundaagochuufguutummaanwaliigaluukoomallattookootiin nan mirkaneessa

Mallattooqoodafudhataaqorannoo_____ Guyyaa _____

MallattooQorataaQorannoo_____ Guyyaa _____

Mallattoo fi maqaaRagaa (yoo jiraate) _____ Guyyaa _____

ANNEX III. Household KAP assessment survey Questionnaire

This questionnaire will take approximately 20-25 minutes to answer. Please, be assured that any information you provide will be anonymous and no personal information collected will appear in any documents or reports based on this survey.

Interview Date: ___ /___/_____ (DD/MM/YYYY)

Sub-city: _____Woreda: _____ Household Number: _____

Respondent Status:

Female Head of household Male head of household Other adult (>18)

<u>Demographic Questions</u>

1. Marital status:

   Single Married Divorced Widowed
2. Age: _____
3. What is the highest educational level you have attained?

   No formal education Read and write Elementary
   High school College level University level
4. How many people (including children) are in your household? _____
5. How many children < 5 years of age live in your household? _____
6. What is your occupation?

Employed: A) Government B) Private C) NGO Builder/carpenter
Farmer Unemployed/retired/Housewife

7. Does your household have:

Electricity Television Radio
Refrigerator Telephone/Mobile phone

8. What kind of toilet facility do members of your household usually use?

Flush, connected to latrines Pit latrine with cement slab
Pit latrine, without cement slab Canal or open defecation/bush/field
Other (please, specify_____)

9. Is there any separate toilet for children?

Yes No

10. Who primarily care for cattle/sheep/goat?

Wife Husband Children Other (please, specify)

11. Who primarily care for poultrys?

Wife Husband Children Other (please, specify)

12. What is the main water source of the household for the following activities?

	Piped water	Surface water	Ground/bore-hole	Rain water	Canal water	Bottled water
Drinking water for household						
Drinking water for animals						
Water for food preparation						
Water for cleaning kitchen, utensils						
Water for hand washing and laundry						
Water for cleaning of animal pen						

13. Are there any times during the year when water is not readily available?

Yes: (please specify the time_____) No Don't know

14. Do you have any of the following animal species?

Animal species	Number of animals
Sheep	
Goat	
Cattle	
Pig	
Horse/Donkey/Mule	

Poultry	
Others (specify)	

15. What is the source (s) of feed for your animals?

Scavenging	Household leftover	Purchase feed
Graze by road side	Bring in forage	Swill

Questions related to the knowledge, attitude and practices of households

16. To whom do you talk about your family's health issues?

Family doctor	Other health professionals	Neighbors/relatives
Family member/friends	Local health service centers	Don't know

17. Do you think that animals be source of human diseases?

Yes No

If yes;

18. Have you heard of diseases transmitted from domestic animals to people?

Yes No Don't know

If yes, can you mention some of the diseases?

Brucellosis	Tuberculosis	*Campylobacter*iosis	Typhoid fever
Rabies	HIV/AIDS	Q-Fever	Taeniasis
Leptospirosis	Toxoplasmosis	Others (please,specify)	

19. If you answered 'yes' on question 19, do you know how a human get infected from animals?

Direct contact of animals	Eating raw or undercooked meat/milk products
Drinking raw or under boiled milk	Other (please, specify)

20. Have you ever seen any of the following disease symptoms on yourself or your family members during the last six months or year?

Nausea	Vomiting	Abdominal pain	Diarrhoea
Blood in stool	Tenesmus	Fever	

21. Have you heard of the disease *Campylobacter*iosis? A) Yes B) No C) No idea

If yes,

22. Which animals do you think can get infected with it?

Cattle Sheep/goat Horse/donkey/mule Poultry Dogs/cats

23. Do you think that any of your family members are at risk of acquiring *Campylobacter*iosis?

Yes No

If yes, which family member(s) do you think is/are most susceptible to infection?

24. If a person in your household gets *Campylobacter*iosis, what do you do?

Seek medical attention Try to manage at home Do nothing Other

25. What do you think the way human contract *Campylobacter*?

Handling/eating undercooked/raw poultry or meat.	Consumption raw milk or dairy products.
Contaminated and untreated drinking water.	Unprotected contact with animals/poultrys
Person-to-person transmission	Other (please, specify)

26. Do you think that consumption of raw poultry/meat/milk be source of the disease?

Yes No Don't know

27. Do you like to taste or eat raw meat or raw minced meat while preparing food?

Yes No Don't remember

If yes, please specify what kind of meat?

1) _____2)_____3)_____4)_____

28. Do you know if there is any treatment for *campylobacter*iosis in humans?

Yes No

29. Do you know how diseases transmitting from animals to humans prevented/controlled?

Yes No No idea

If yes, could you mention it?

Avoiding contact with animals/infected animals	Vaccination of animals
Eating well cooked meat	Drinking well boiled milk products
Other (please, specify)	

30. Do you think that you need to have more information on *Campylobacter*iosis/diseases transmitted from animals to humans?

Yes No

If yes, how do you like to receive the information?

Group discussion	Via news/media	School training
Telephone communication	Other (please, specify)	

31. If you suspect an animal having a disease, what do you do?

Seek veterinary assistance	Sell the animal	Slaughter the animal
Self treat the animal	Do nothing	Others (please, specify)

32. Do you take any specific action to protect yourself when dealing with animals/diseased animal?

Yes No

If yes, what kind of action (s) do you take?

Use gloves Wash hands with soap Others (please, specify)

33. Do you think you/family members become sick from not washing hands?

Yes No Don't know

34. How do you and your family members wash your hands?

Washes hands in a bowl of water (sharing with other people)	With someone pouring a little clean water from a jug onto one's hands
Under running water	Washes hands with soap or ashes
Other	Don't know

35. What are the key moments when you need to wash your hands to prevent germs from reaching food?

After going to the toilet/latrine	After cleaning the baby's bottom/changing a baby's nappy
Before preparing/handling food	Before feeding a child/eating
After handling raw food	After handling garbage
Other (please, specify)	

36. Do any of the children in your family play with/ help feed any of the animals or clean-up animal stalls/pens?

Yes No

If yes, do they wash their hands with soap afterwards?

Yes No I don't know

37. Do you treat your water in any way to make it safe for drinking?

Yes No

If yes, how do you make the water safe for drinking?

Boil water	Use water filter	Strain it through cloth
Chlorination	Let it stand/settle	Other (please, specify)

38. Have you heard of diseases that you can get from contact with water?

Yes (please, specify No

39. Which of these practices do you employ in this household?

Eat undercooked meat	Cull sick animals for consumption
Eat animals found dead	Allow animals sleeping in food preparation areas
Slaughter domestic animals	Capture and slaughter wild animals for consumption
Wash hands with soap before and after cooking	Wash hands with soap after handling live animals

Bury or burn meat waste products Collect manure indoors and outdoors daily

This is the end of the questions. Thank you for agreeing to take part in this valuable study. Please feel free to mention any additional comments regarding the study or information you provided.

_____

_____

<u>የጥናቱተሳታፊዎችንእዉቀት፤ ግንዛቤእናተግባራቸዉንለመለካትየሚጠየቅጥያቄ</u>

ጥያቄውበግምት ከ20-25 **ደቂቃየሚፈጅይሆናል፡፡**

እዝህላይየሚትሰጡትመልስምሆኔሀሳብበምስጥርየምየዝስሆን፤

ማንኛውምስለአንቱምሆኔስለቤተሰቦችክየሚገልጽማንኛዉምመረጃበኮድየምቀመጥስሆንእነኝዶኩመን

ቶችያላንቱፍቃድከጥናቱአባላትውጪየማይታይ/የማይገለጽይሆናል፡፡

ቀን: ___ /___/_____ (ቀን/ወር/ዓ. ም)

የአጥኝዉስም: _____

ክፍለከተማ: _____ወረዳ: _____ ቀበሌ: _____ ኮድ _____

የተጠያቂውሁኔታ:

ሴትእናየቤቱእማወራ ወንድእናየቤቱአባወራ ሌላ (>18)

<u>የተሳታፊዉየኑሮሁኔታየሚለዉጥያቄዎች</u>

1. **የጋብቻሁኔታ:**

ያላገባ/ች ያገባ/ች የፈታ/ች በሞትየተለያዩ

2. **ዕድሜ (በዓመት):** _____

3. **ያጠናቀከውከፍተኛየትህምርትደረጃ፡**

ያልተማረ ማንበብእናመፃፍ አንደኛደረጃ
ሁለተኛደረጃ ኮሌጅደረጃ ዩኒቨርሲቲ

4. ልጆችንጨምሮቤታቹውስጥስንትሰውይኖራል? _____

5. ከ5 ዓመትበታችስንትልጆችቤታቹውስጥይኖራሉ? _____

6. ስራክ/ሽ ምንድነዉ?

የመንግስትሥራ	ግንበኛ/አናፂ
የግልመስርያቤት	ጡረተኛ/የቤትእመቤት
አርብቶ/አርሶአደር	ስራየሌለዉ

7. ቤትክ/ሽ ውስጥከታችየተዘረዘሩትመገልገያዎችአሉ?

ኤለክትርክ	ተሌቪዥን	ራዲዮ
ፍሪጅ	የገመድ/ሞባይልስልክ	

8. ቤቱሰቦችክ/ሽ በምንአይነትሽንትቤትይጠቀማሉ?

በውሃየምሰራእናከጉድጓድጋርየተገናኘ	የተቆፈሬጉድጓድእናበስምነቶየተደፈነ
የተቆፈሬውናበስምንቶያልተደፈነ	ሽንትቤትየለም
ሌላ (ግለፅ/ጭ)	

9. ለእንስሳቶቹማንእንክብካቤያደርጋል?

10. ለ ሚስት ባል ልጆች ሌላሰው (ግለፅ/ጭ)
ምክተሉትተግባራትየምትጠቀሙትየውሃአይነትየቱነዉ?

	የቧንቧ	የወንዝ/ኩሬ/ሐይቅ	የጉድጓድ	የዝናብ	የሀይላንድ	ሌላሚንጭ
የምጠጣዉሃለሰዉ						
የምጠጣዉሃለእንስሳት						
ለምግብዝግጂት						
ለምግብቤትፅቃፅዳት						
ለእጅእናልብስ						
የእንስሳትንበረትለማፅዳት						

11. በአመትውስጥውሃየምታጡበትወቅትይኖራል?

አዎ፤(ግለፅ/ጭ) _____ የለም አላዉቅም

12. ከታችከተዘረዘሩትእንስሳትዉስጥየትኛዉንይኖርካል/ሻል?

እንስሳትአይነት	ብዛት
በግ	
ፍየል	
ከብት	
አሳማ	
ፈረስ/አህያ/በቅሎ	
ዶሮ	

13.ለእንስሳቶችክ/ሽ የምግብምንጭምንድናው?

ወድያወድህብሎያገኛሉ የቤትትራፊ የምገዛ
መንገድዳርይግጣሉ የታጨዬሳር በጉድጓድውስጥየምዘጋጅ

በጥናቱላይተሳታፊየሆኑትንሰዎችእዉቀት፤

አስተሳሰብእናተግባራቸዉንለመገምገምየምዉልጥያቄዎች

14.ብዙዉንግዜስለቤተሰብክ/ሽ የጤናሁኔታከማንጋርትወያያለክ/ሽ?

ለቤተሰቡሐኪም ለሌሎችየጤናባለሙያዎች ለጎሮቤታችነ/ለዘመዳችን
ለቤተሰብ/ጓደኛ በአከባቢያችንለምገኘዉጤናጣብያ አላዉቀዉም
ከቤተሰብአባል/ዘመድ/ጓደኛ በትህምርት ከልላ (ግለጽ/ጭ)

15.እንስሳትለሰዉልጅየበሽታመንስዐይሆናሉብለክ/ሽ ታስባለክ/ሽ?

አዎ አይደለም

16.ከምከተሉትበሽታዎችዉስጥየትኞቹከእንስሳወደሰዉልጅይተላለፋልብለክ/ሽ ታስባለክ/ሽ?

ብሩሴሎስስ ቲቢ ካምፓይሎባክተርዎስስ ታይፎይድ
የዉሻበሽታ ኤች-አይቪ/ኤድስ ኮሶ
ለፕቶስፓይሮስስ ቶክሶፕላስሞስስ

17.በሽታእንዴትከእንስሳትወደሰውልጅይተላለፋልብለክ/ሽ ታስባለክ/ሽ?

እንስሳቶችንበመነካካት ጥሬ/በደንብያልበሰሌስጋበመመገብ
የልተፈላ/በደንብየልፈላወተትበመጠጣት በሌላመንገድ (ግለጽ/ጭ)

18.ባለፈውዓመት/6ወራት ከምከተሉትየትኛውንምልክትበራስ/በቤተሰብክ/ሽ ላይአየክ/ሽ?

ማጥወልወል ማስመለስ የሆድሕመም ጠቅማጥ
ሰገራውስጥደም ማማጥ ሙቀትመጨመር

19.ካምፓይሎባክተርስለምባለውባክቴርያ/ካምፓይሎባክቴርዎስስስለምባለውበሽታሰመተክ/ሽ ታውቃለክ/ሽ?

አዎ አልሰመውም

መልስህ/ሽ አዎከሆኔእንዴትሰማክ/ሽ?

ከሚድያ/ዜና በትህምርት ከልምድ
በሽታውተከስቶ በስራ ከሌላ (ግለጽ/ጭ_____)

20.ይህበሽታየትኛውንእንስሳያጠቃልብለክ/ሽ ታስባለክ/ሽ?

ከብት በግ/ፍየል የጋማከብት ዶሮ ዉሻእናድመት

21.የሰውልጅበዝህበሽታልታመምይችላል? አዎ አይታመም

22.እንዳንተ/ች አስተሳሰብለዝህበሽታይበልጥታጋላጭየሆኑትየቤቴሰብአባላትአሉ?

አዎ የለም

መልስክ/ሽ አዎከሆኔየትኛውየቤተሰብአባልነው? _____

23.ይህበሽታበምንመንገድወደሰውልተላለፍይችላልብለክ/ሽ ታስባለክ/ሽ?

ጥሬስጋንበመብላት/በመንካት በደንብያልፈላወተት/ወተትተዋፅያበመመገብ
ንፁያለሆኔውሃንበመጠቀም እንስሳትንበባዶእጅበመነካካት
ከሰውወደሰውበመተላለፍ በሌላመንገድ (ግለፅ/ጭ)

24. ጥሬየከብት/የዶሮስጋካምፓይሎባክተርባቴርያንተሸካሚናቸውተብሎየታመናል፤

ይህሁኔታምንያክልያሳስብሃል/ሻል?

በጣምያሳስበኛል ያሳስበኛል
ትንሽያሳስበኛል አየሳስበኝም አላውቅም

25. በምግብዝግጂትግዜጥሬ /የተከተፈስጋንየማሽተት/የመቅመስልማድአለክ/ሽ?

አዎ አይደለም አላስታውስም

መልስክ/ሽ አዎከሆኔ፤ የምንስጋእንደሆኔታስታውሳለክ/ሽ?

1) _____2)_____3)_____4)_____

26. የምከተሉትንየስጋአይነትምንያክልብበስልትወዳለክ/ሽ?

	ጥሬ	ትንሽስበስል	መካከለኛ	በደንብየበሰለ
ዶሮስጋ				
የተከተፈስጋ				
የበሬ/ጥጃስጋ				
የአሳማስጋ				

27. በበሽታውሉተያዘሰው /እንስሳመድሃኒትእንዳለታውቃለክ/ሽ?

አዎ አላውቅም

28. በሽታውንበተመለከተበአገርደረጃተቋቁሞያለውንፕሮግራምታውቃለክ/ሽ?

አዎ አላውቀውም

መልስክ/ሽ አዎከሆኔ፤ መጥቀስትችላለክ/ሽ: _____

29. በሽታውለሰውክትባትአለው? አዎ የለውም

30. ከእንስሳትወደሰውየምተላለፉትንበሽታዎችእነዴትመከላከል/መቆጣጠርየቻላል?

እንስሳትንአላግባብከመንካትመቆጠብ እንስሳትንበማስከተብ
በደንብየበሰለስጋንበመመገብ በደንብየፈላወተት/ወተትተዋፅያበመመገብ
በሌላመንገድ (ግለፅ/ጭ)

31. በዝህበሽታዙሪያተጨማሪግንዛቤየሚያስፈልግክ/ሽ ይመስልካል/ሻል?

አዎ አይመስለኝም

መልስክ/ሽ አዎከሆኔ፤ ግንዛቤውንበምንመልክመውሰድትፈልጋለክ/ሽ?

በጋራምክክር በዜና/በሚድያ በትህምርትስለጠና
በስልክ በሌላ (ግለፅ/ጭ)

32. እንስሳትበበሽታመያዙንብታውቅምንታደርጋልክ/ሽ?

የእንስሳትሐኪምዕርዳታመጠየቅ እንስሳቱንመሸጥ እንስሳቱንማረድ

በራስእንስሳቱንማከም ምንምአላደርግም ሌላ (ግለፅ/ጭ)

33. ሁሉምየቤተሰብክ/ሽ አባልእጃቸውንሁልግዜይታጠበሉ?

አዎ አላስብም

መልስክ/ሽ አዎከሆነ፤ እጃቹሁንእንዴትእነደምትታጠቡመግለፅትችላለክ/ሽ?

በእጅመታጠብያስንከንላይ ሌላሰውበእጃችንላይውሃበመቅዳት
እየወረደባለውሃስር እጃችንበሳሙና/ ዓመድመታጠብ
በሌላ (ግለፅ/ጭ) አላውቅም

34. አላስፈላጊጀርሞችምግብጋርእንዳይደርስስባልእጃቹንበሳሙናመታጠብያለባቹወሳኝሰዓትመቹ መቹነው?

ከሽንትቤትመልስ ለህፃንዳይፐርስቀየር/መቀመጫቸውንስናፀዳ
ምግብከማዘጋጀት/ከመንካትበፊት ከመብላት/ህፃናትንከማብላትበፊት
ያልበሰለምግብከነካቹበኋላ ቆሻሻንከነካቹበኋላ
ሌላግዜ (ግለፅ/ጭ)

35. እንስሳትንከነካቹ/

ከእንስሳትጋርየተያያዘውንስራካከናወናላቹበኋላምንያክልእጃቹንበሳሙናትታጠባላቹ?

ሁልግዜ አንዳንዴ ዕምብዛም በፍፁም ምንምሀሳብየለኝም

መልስክ/ሽ አንዳንዴ፤ ዕምብዛም/በፍፁምከሆነ፣ ለምን?

ብዙምአያስፈልግም ንፁውሃስለሌለ ሳሙናስለሌለ
አላውቅም ሌላምክንያት (ግለፅ/ጭ)

36. በቤተሰባቹውስጥልጆችምንያክልከእንስሳትጋርይጫወጣሉ/የእንስሳትንበረትያፀዳሉ?

ሁልግዜ አልፎአልፎ አንዳንድግዜ በፍፁም

37. ልጆችእንስሳትንወይንምየእንስሳትንበረትከነኩበኋላእጃቸውንበሳሙናይታጠባሉ?

ሁልግዜ አልፎአልፎ አንዳንድግዜ በፍፁም

38. ውሃንለመጠጥምቹእንድሆንለማድረግታክማላቹ?

አዎ አናክምም

መልስክ/ሽ አዎከሆነ፤ በምንሁኔታታክማላቹ?

በማፍላት የውሃማጥልያመጠቀም በልብስማጥለል
ክሎሮንበመጨመር እንድሰክንማድረግ ሌላ (ግለፅ/ጭ)

39. ከምከተሉትተግባራትባንቴ/ች ቤቴሴብውስጥየትኛውይተገበራል?

ጥሬ/በደንብያልበሰለስጋመብላት የታመሙእንስሳትንለምግብነትማረድ
የሞቱእንስሳንማረድእናመብላት እንስሳትየምግብማዘጋጃስፍራእንድተኙመፍቀድ
የቤትእነስሳትንማረድ የዱርእንስሳትንማደንናለመብላትማረድ
ምግብከማዘጋጀትበፊትናቧላእጅመታጠብ እንስሳትንከነኩበኋላበሳሙናእጅንመታጠብ
የስጋተረፈ-ምርትንመቅበር/ማቃጠል የእንስሳትንቆሻሻበየቃኑማፅዳት

ጥያቄውእዝህላይያልቃል፤

እናምበዝህአስፈላጊጥናትላይለመሳተፍፈቃደኛስለሆኑእጅግበጣምአመሰግናሁኝ፡፡ እባክህ/ሽ ሌላማንሳትየምትፈልግ/ጊ ካለለመጠየቅትችላለክ፡፡

_____

Gaaffiilee beekumsa, hubannoo (ilaalcha) fi gochaahorsiisee bultootaa madaaluufqophaa'an

Walumaagalattigaaffiinkuniyoobaay'atedaqiiqaa 20-25 kanfudhatuta'a. Adeemsaqorannoo kana keessattista'egaafileedhiyaatankeessattideebiinyookiinodeeffannoonatikennituakkasumasmaatiike ewaliinkanwalqabatujirumartubifaicciitiinkangalmaa'uufiodeeffaannoonatikeennitumartuunamas adaffaagarabiraattikanhinbeeksifamneefiqorannoo kana keessattisodeeffannoonakkamaqaa fi kanneendhimmadhuunfaailaallatanmartuubifagabajeenyookiinimmoomallattooaddaaddaatiinkanb akkabuufamanta'a.

Guyyaagaaffiifideebiinittita'u: _____/_____/_____ (Guyyaa/Ji'a/Waggaa)

Nama gaaffiileegaafatu: _____

KutaaBulchiinsaMagaalaa _____Aanaa_____Lakk. _____

Maalummaaqoodafudhataa:

Abbaa manaa HaadhaManaa Nama garabiraa (ga'eessaawaggaa 18 olii)

Gaaffilleewaliigalaa

1. Maalummaamaatii:
   Baaqqee Kan fuute/kanheerumte kanhiike/te kanirraadu'e/te
2. Umuriinkeemeeqa?
3. Sadarkaabarnootaaol'aanaaqaqqabde;
   Kan hinbarannee dubbisuufibarreessuukandanda'u sadarkaa 1ffaa

sadarkaa 2ffaa Sadarkaakoolleejjii sadarkaaYuuniversiitii

4. Maatiinkeesssannamameeqaqaba (ijoollotadabalatee)? _____
5. Ijoolleenumurii 5 gadiimeeqatumaatiikeessajira? _____
6. Hojiinkeemaali?

Hojjetaamootummaa Hojjetaadhaabbatadhuunfaa Hojjetaadhaabbata-mitimootummaa

Qotee/horsiiseebulaa Hojiidhuunfaa Kan biro

7. Mana keekeessaatajaajilakanneengadiikamqabdaa?

Humnaelektrikii Televitsiinii/Raadiyoo

Qabbaneessaa Bilbilamanaa/ Moobaayilii

8. Maatiinkeessan mana fincaaniiakkamiittigargaaramaa?

Kan mana keessaa Boollaqabiyyeenisaasimmintoo

Boollaqabiyyeenisaabiyyee Bo'ii/ tajaajilabakkeetti

Garabiraa (addabaasi_____)

9. Maatiikeessankeessaaeenyutubeeyiladootakunuunsa?

Haadha manaa Abbaamana a Ijoollee Kan biraa (addabaasi_____)

10. Maddi bishaantajaajilaaddaaddaaarmaangadiifoolumaali?

	Tuubboo	Bishaan kuusaa	Boolla	Bokkaa	Jallisii	Pilaastika	Kan biraa
Dhugaatiif (namaaf)							
Dhugaatiif (beeyiladootaaf)							
Nyaataqopheessuuf							
Qulqullinabakkanyaataittiqopheessaniif							
Harkaa fi uffaatamiicuuf							
Mana galmaabeeyiladootaaqulqulleessuf							

11. Waggaakeessattihanqinnibishaaniiyeroonittimul'atunijiraa?

Eeyyee: (ibsi_____) Lakki Hinbeeku

12. Beeyiladootaarmaangadiikeesssaakamqabda?

Gosabeeyiladaa	Baay'inabeeyiladaa
Hoolaa	
Re'ee	
Sa'ayknSangaa	
Booyyee	
Farda, Harree, Gaangee	
Lukkuu	
Kan biraa (ibsi	

13. Maddi nyaatabeeyiladakeemaali?

Oliifigadideemuunargatu | Hafteenyaatanamaa | Nyaatabitamu

Daandiicinaadheeduu | Okaafimargahaamame | Nyaatabulbulame

<u>Gaaffilee beekumsa, hubannaa fi gochaaqoodafudhattootaamadaaluufqophaa'e</u>

14. Yeroobaay'eewaa'eefayyaamaatiikeeeenyuunmarii'atta?

Ogeessafayyaamaatii Maatii/hiriyyaa | Ogeessafayyaakanbiroo Buufatatajaajilafayyaanaannoo | Ollaa/fira Hinbeeku

15. Bineeldimaddadhukkubanamaanita'ajetteeniyaaddaa?

Eeyyee Lakki

16. Akkayaadakeettidhukkubootaarmaangadiikeessaakamtubineeldairraanamattidarbaa?
17. Kanneenarmaangadiikeessaaakkayaadakeettinamnikaraakamiinbineeldairraadhukkubaanqabamuudanda'aa?

Bineeldaqaqqabuun | Foonhinbilchaatiinnyaachuu

Aannanhaalaanhindanfiindhuguu | Karaabiraa (ibsi)

18. Waggaadarbekeessattimallattooleedhukkubaaarmaangadiikeessaakamiin of irrattiyookiinmaatiikeeirrattiargiteejirtaa?

Garaahammaachuu Hooqqisa Garaadhukkubbii Garaakaasaa

Bobbaatiidhiigaanmakaa Ittansuu Dabaluuho'inaqaamaa

19. Waa'eebaakteeriyaa '***Kampaayiloobaakteer***' yookiindhukkuba '**Kampaayiloobaakteeriyoosisii**' jedhamuudhageesseebeektaa?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta'e, eesssaadhageesse?

Oduuirraa Barumsaan Muuxannoon

Dhohinsadhukkubichaa Karaahojiiidilee Karaabiraa (ibsi)

20. Gosabeeyiladaaisakamtudhukkubakanaanqabamajetteeyaadda?

Saawwan Hoolaa/Re'ee Kotteeduudaa Lukkuu Saree/Adurree

21. Miseensamaatiikeessaadhukkubakanaafcaalaattisaaxilakanbahannijiruu?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta'e, warren kamfa'i (ibsi) _____

22. Osoonamnitokkomaatiikeekeessaadhukkubakanaanqabameehagamsidhiphisa?

Badaamiti Hagatokkonayaaddessa Baay'eenayaaddeessa

23. Baakteeriyaankunikaraaleekamiingaranamaattidaddarbajetteeyaadda?

Foonloonii/lukkuudheedhiiqaqqabuunyknnyaachuun Aannanyookiinbu'aawwanaan

Karaabishaanfaalameyknkilooriniihinqabneen Horii yknlukkuu of eegganno

Nama irraanamatti Karaabiraa (ibsi)

24. Foonhorii/lukkuudheedhii, aannanhindanfineyeroohedduubaakteeriyaa kana of keessattiqabatajedhameetuamanama; kana akkamittiinhubatta?

Gadifageenyaan Gidduugaleessaan

Xiqqoo Hubannoo hinqabu Hinbeeku

25. Foongosaarmaangadiicaalaattiakkamiinqopheessuufilatta?

	Dheedhii	Darbeedarbee	Gidduugaleessa	Akkagaariittibilcheessa
Foonlukkuu				
Foonkukkutame				
Foonsangaa				
Foonbooyyee				

26. Nama dhukkubakanaanqabameefqorichaakkamiiakkakennamunibeektaa?

Eeyyee Lakki

27. Dhukkuba kana ilaalchiseesagantaaakkabiyyaattihundaa'eejirubeektaa?

Eeyyee Lakki

YoodeebiinkeeEyyeeta’e, ibsuudandeessaa?_____

28. Namnidhukkubakanaanakkahinqabneeftalaalliinkennamuakkajirubeektaa?

Eeyyee Lakki

29. Dhukkubootnibineeldairraagaranamaattidaddarbanakkamittiittifamuyookiinto’atamujettee yaadda?

Bineeldadhukkubsatuqaqqabuuirraa of qusachuu

Bineeldotatalaaluun

Foonsirriittibilchaatesoorachuun

Aannansirriittidanfedhuguun

Karaabiraa (ibsi)

30. Odeeffannoodabalataawaa’eedhukkuba ‘**Kampaayiloobaakteeriyoosisii**’ jedhamuuyookiindhukkubootahoriiirraanamattidarbanbirooargachuunifeetaa?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta’e, karaakamiinodeeffannoonkunyoosiifkennamewayya?

MariiGareen Karaaoduu/miidiyaa Leenjii mana barumsaa

Bilbilaan Karaa biro (ibsi)

31. Horiinkeeakkadhukkubsatteyooshakkitemaalgoota?

Ogeessafayyaabeeyiladaansoqa Nan gurgura Nan qala

Ofiinhorichayaala Homaahingodhu Kan biroo (ibsi)

32. Yeroohoriidhukkubsategargaartuofeeggannooaddata’enitaasiftaa?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta’e, maalgoota?

Golgaaharkaanfayyadama Harkadhiqadha Kan biraa (ibsi)

33. Maatiinkeeyookiinatiharkadhiqachuudhiisuundhukkubanifidajetteeniyaaddaa?

Hinyaadu Naafhingalu Sirriittinyaada

34. Atiyookiinmaatiinkeeyeroomaraaharkanidhiqatuu?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta’e, akkamittiakkaharkadhiqattanmeeibsi.

Bakkanamootnihedduunharkadhiqatanittidhiqachuu

Bishaanyaa'aattidhiqachuu

Kan biraa

35. Akkajarmoonniaddaaddaasooratahinfaalleefyeroomurteessaanatiharkaittiqulqulleeffachuu qabajetteeyaaddukamfa'i?

Deemsa mana fincaaniinbooda

Nyaataqopheessuun/qaqqabuun dura

Erganyaatahinbilchaatiinqaqqabanneebooda

Yeroo biro (ibsi)

Ergauffatadaa'imaajijjiiraniinbooda

Daa'immannyaachisuun dura/nyaatan dura

Kosiiqaqqabuunbooda

36. Ijoolleenkeehagambineeldotaqe'eewaliintaphatuu/hagamhoriifnyaatadhiyeesu/hagammooraaisaaniiqulqulleessu?

Yeroomaraa Darbeedarbee Yerootokkotokko Tasumaa

37. Atiyookiinmaatiinkeeergahoriiqe'eeyookiinbakkajireenyaisaaniiqaqqabataniiboodaharkaisaaniisaamunaannidhiqatuu?

Yeroo maraa Darbeedarbee Yerootokkotokko Tasumaa

38. Dhugaatiifakkatoluttibishaanniqulqulleesitaa?

Eeyyee Lakki

YoodeebiinkeeEeyyeeta'e, akkamiinqulqulleessita?

Bishaandanfisuu Calaltuubishaaniifayyadamuun Uffatakeessancalaluu

Kiloorineessuun Akkajalattiitituugochuun Mala biroo (ibsi)

39. Gochaawwanarmaangadiikeessaakamtumaatiikeebirattiamaleeffatama?

Foondheedhiisoorachuu	Horii dhukkubsatesoorataafqaluu
Foonhoriidu’eeargamesoorachuu	Horiingolanyaatiittiqophaa’uakkaciisaneeyyamuu
Beeyiladaqaluu	Bineensotabosonaaadamsuufisoorataafqaluu
Nyaatabilcheessuunduraa fi boodasaamunaanharkadhiqachuu	Ergahoriiqaqqabaniinboodasaamunaanharkadhiqachuu
Hafteefoonsameeawwaaluu/gubuu	Kosiihoriimooraakeessaafialaaguyyuunsassaabuu

Gaaffiinkeenyaasumairrattixumurama. Waanqorannoobu’aqabeessa kana irrattihirmaatteefhedduugalatoomi. Hadaraayaadadabalataaqorannoo kanas ta’eodeeffannoonaafqooddeirrattiqabdukamiyyuunaafdheeruunidandeessa.

_____

_____

Date:				Interviewer /sample collector name:									
Sub-city:				Enumeration Woreda:									
Starting time:													
HH code	Consent given	Tel #	# persons in HH	Sample type						Sample code	Interview completed		Remark
				stool	Feces				water		Yes	No	
					cattle	sheep	goat	poultry					
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
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ANNEX IV. Field Recording Sheet Format

ANNEX V. Sample Collection Protocols for different sample types

Collecting human and animal fecal samples

For this study, both human and animal samples were considered as pooled samples. To clarify, in all pooled samples, the pooled samples consisted of approximately equal fresh fecal matter from all individual animals in each category or humans and put onto a sterile flat plastic and gently mixed. A sterile cotton swab moistened with nutrient broth was used to transfer about 7- 10g of the pooled samples into a 15ml screw-capped falcon tube containing Cary Blair transporting medium.

While it was difficult to obtain stool samples from each of the household members at time of data collection stool samples were obtained only from representative individuals. Accordingly, pooled stool samples were only collected from individuals who were most closely associated with the hands-on managementof the livestock and houses. More importantly, at least one pooled stool sample was obtained from the 99 included households.

In total, 248 animal fecal samples were collected from cattle, sheep, goat and poultry. Fecal samples from sheep and goats were collected only per rectum using lubricated latex gloves and mixed to get the final pooled samples. However, cattle fecal samples were collected both per-rectum and whenever it was available, fresh feces immediately voided on to the floor of animal's pen also collected. Nevertheless, if no fecal sample could be collected rectally due to inaccessibility of the fecal matter or when there was no freshly voided fecal matter with in the animal's pen at time of collection or when the owners are refused to do so then rectal swabs were collected using sterile cotton swabs pre-soaked in to nutrient broth.

Cloacal swab samples from individual poultrys were obtained with a sterile cotton wool swab moistened in nutrient broth. Whenever we were disallowed to the poultry house, available fresh feces immediately dropped to the ground were collected using sterile glove by the owner or care

taker of the farm. Thence, samples were obtained from these fecal drops with sterile cotton swabs and mixed with other samples from another poultry in order to obtain a pooled sample for final *Campylobacter* isolation.

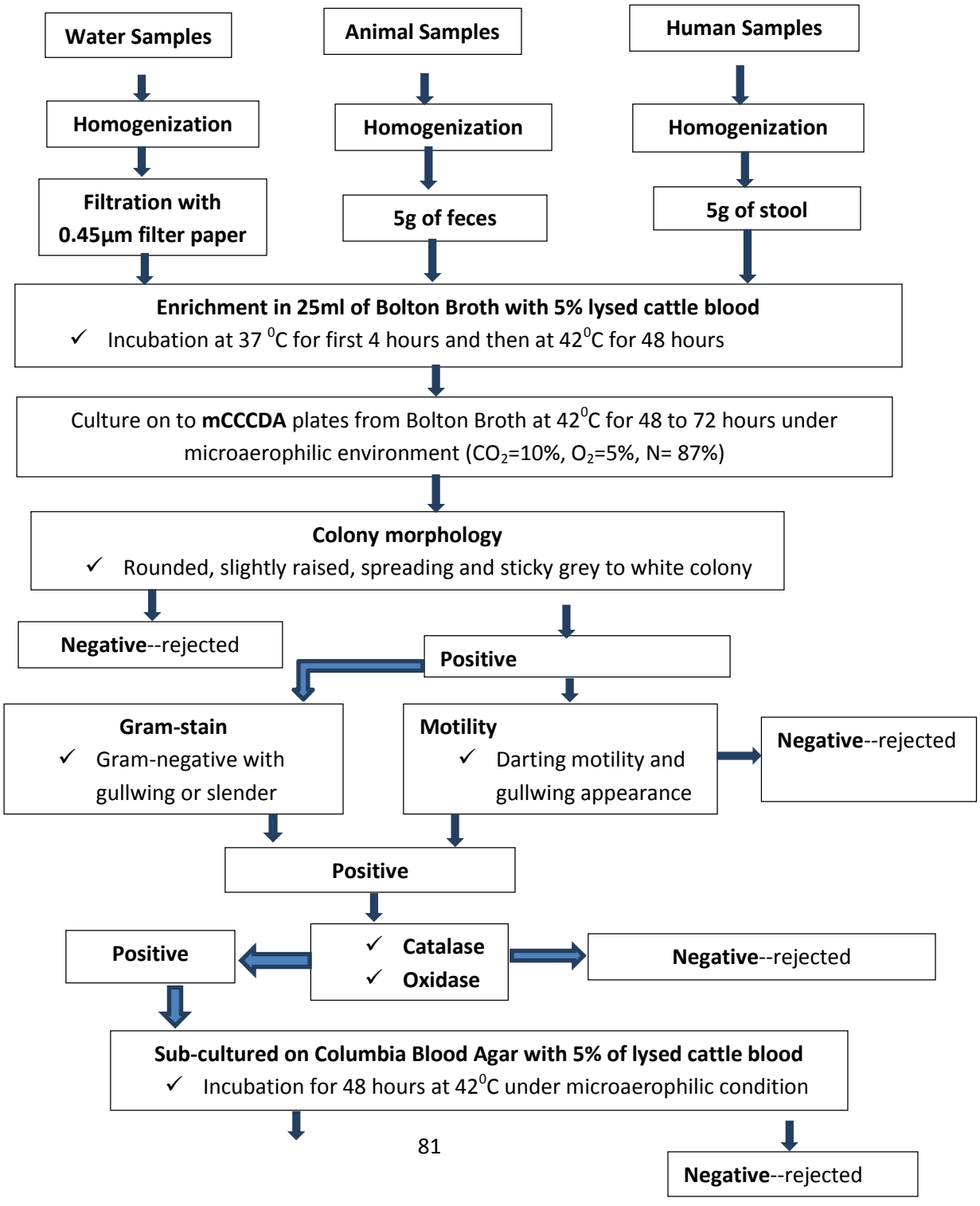
For all households owning two or more than two animals of same species, samples from corresponding animals were considered as pooled in order to make sure that all animals were sampled and in fact in some cases the number of animals of same species was too large to sample each of them individually. For convenience, different animal species were classified as small, medium and large (for this study only) before the commencement of sample collection. Accordingly, ruminants were classified at household level as small when number of animals was categorized as (1-19 and 1-29), medium (20-49 and 30-69) and large (≥50 and ≥70), for cattle and sheep or goat, respectively. Poultry flocks were also classified small when the number of poultrys in the flock was 1- 99, medium (100-199) and large (≥200). Therefore, 1-3, 4-5 and up to six pooled samples were collected from small, medium and large classes, respectively.

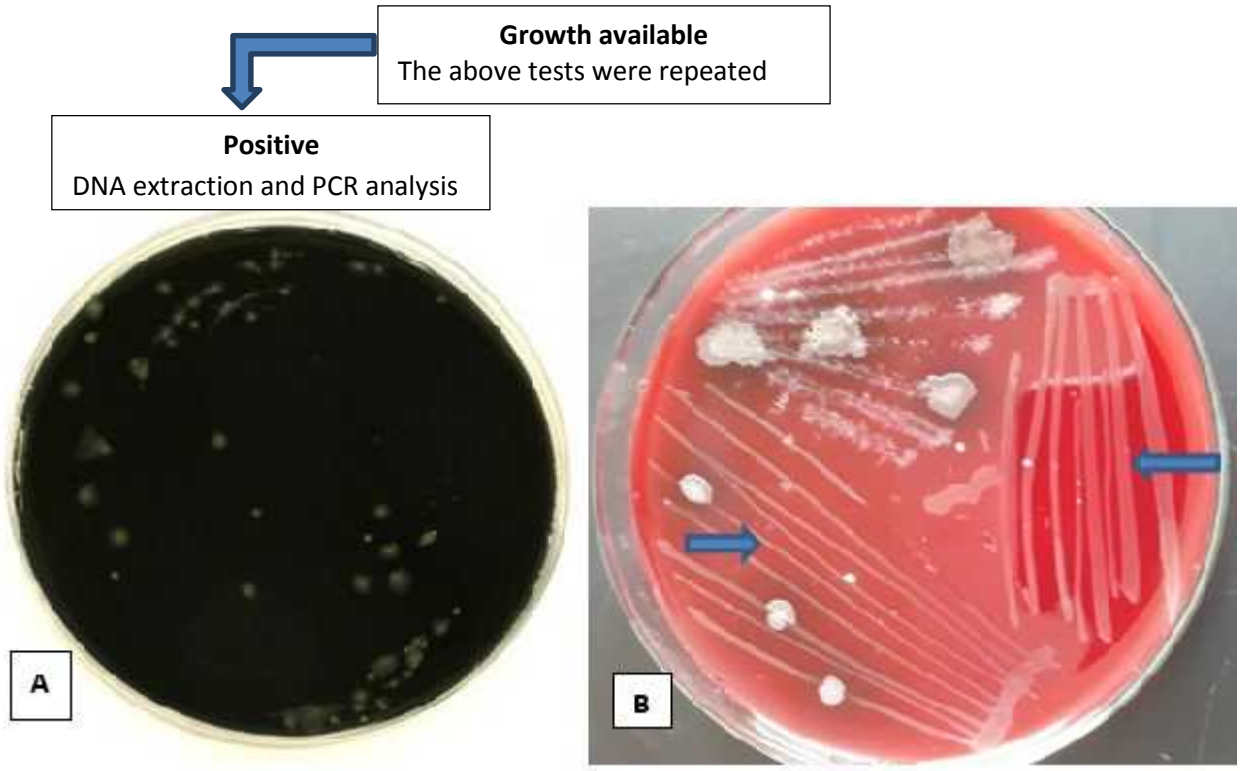
Water sampling

A total of 172water samples from different sources were collected during the studyperiod. Samples were collected using different protocols adopted for the different water samples. Surface and stored water samples were collected by submerging a sterile capped plastic bottles to a depth of 0.5-1m as according to ISO 19458 (ISO, 2007) as described elsewhere (Wilkes *et al*., 2011; Denis *et al*., 2011; Chukwu *et al*., 2019). Bottles were tightly capped and packaged on ice for shipping and processed within 24 hours. A sample of stored water that was intended for drinking or cooking was collected from each household in a 350ml plastic bottle. Collectively, 172 water samples were collected from 99 households and their surroundings of which 84, 63, 16, 9 were directly from a municipal tap water, stored, ground and surface water, respectively. Importantly, when collecting surface water samples, sampling locations were chosen based on surface water flow as opposed to livestock density.

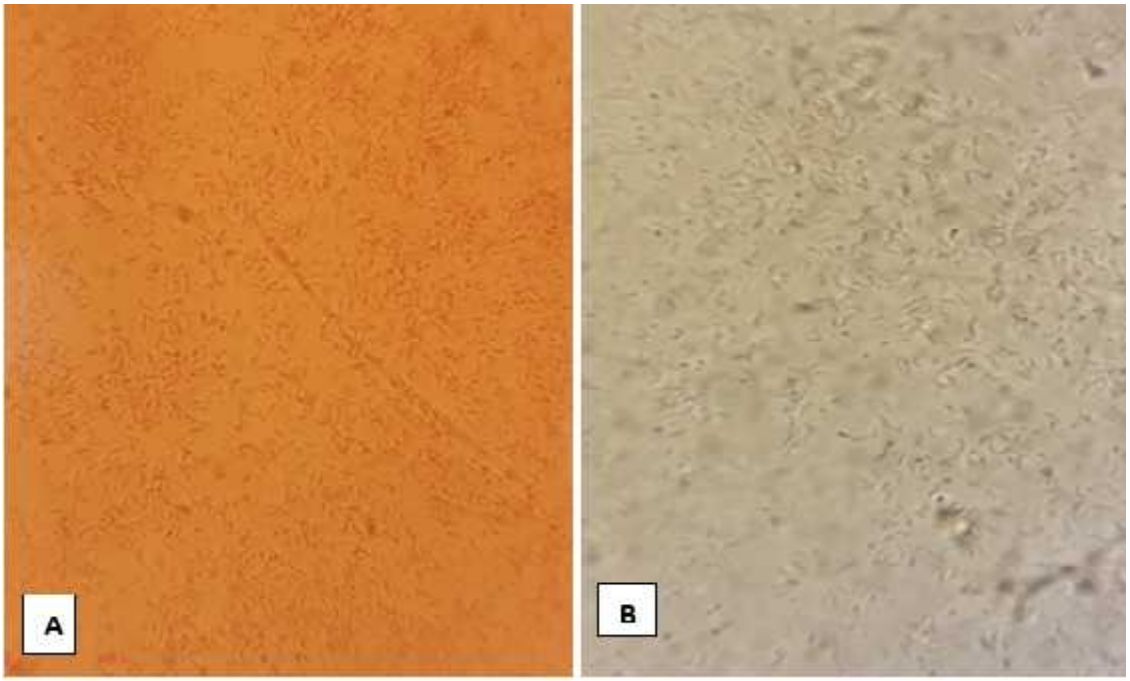
Finally, all collected samples of animals, humans and water kept in a cool box containing ice bag and transported to Akililu Lemma Institute of Pathobiology, medical microbiology laboratory, where all laboratory works undertaken. All samples were processed within 4-6 hours of collection.

ANNEX VI: Flow chart showing sequence of isolation, identification, and characterization of Campylobacter species using selective enrichment plus streak plate





Growth of Campylobacter species on mCCDA (**A**) and Columbia blood agar (**B)**



Gram stain (**A**: slender, curved, seagull appeared) and microscopic result of wet smear of Campylobacter species (**B**)

ANNEX VII. General procedures and protocols followed for PCR analysis

DNA extraction

Genomic DNA from presumptive isolates was extracted by boiling from fresh cultures grown on Columbia blood agar with 5% defibrinated cattle blood for 48 hour as earlier described (Khan*et al*., 2009; Jokinen *et al.,* 2012; Kashoma*et al*., 2016). Briefly, a loopful of suspected*Campylobacter* species isolate from a test culture was suspended within 100µl of sterilized DNAse and RNAse free water. To obtain a DNA lysate, the suspension was vortexed for homogenization of cells and boiled at 95^{o}C for 15 minutes using the thermocycler PCR machine and cooled to 4 ^{o}C until the next step.

DNA amplification

In order to get the final volume (25µl) of PCR product for gel electrophoresis, the following steps were followed.

I) Preparation of the premix (mixture of all primers): 0.6µl of each of the forward and reverse primers of *C. coli*, *C. jejuni*, *C. fetus*, *C. lari* and the genus specific primers was used for a single DNA lysate (1 sample). Accordingly, the mixture of all the primers was prepared based on the number of the samples to be amplified and in fact to get the final 25µl. All the forward and reverse primers are presented in Table 1. above)

II) Adding all the constituents in to the PCR tubes (total volume): the master mix (containing all the bases, cofactors and polymerase), premix, template DNA (1µl) and DNase/RNase-free water all mixed together with in a single tube and the final 25µl volume of the mixture was dispensed to each of the PCR tubes.

The DNA amplification was performed using ccc thermocycler with the cycling conditions as described by Yamazaki-Matsune*et al*. (2007) with only slight modification as follows;

- Initial denaturation cycle at 95^{o}C for 15 minutes

- 30 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 58^{o}C for 90 seconds and extension at 72^{o}C for 1 minute and
- The final extension at 72^{o}C for 7 minutes
- Samples were then held at 4^{o}C prior to analysis.

Gel preparation and Electrophoresis

Each reaction mixture was analyzed by gel electrophoresis through 1.8% (w/v) agarose in 1x TBE buffer for 1:30 hour, and visualized by UV transillumination after staining with ethidium bromide(0.5 µg/ml).The DNA bands were photographed using an ultraviolet transilluminator (BTS-20), and a1 kb DNA ladder was used as a molecular size marker.

1x TBE buffer preparation

- ✓ 10.8gm of Tris-base and 5.5gm of boric acid was dissolved in 805ml distilled water.
- ✓ 4ml of 0.5M EDTA at P^{H} = 8.0 was added to the above mixture
- ✓ Finally, the volume was adjusted to 1L by adding distilled water

Preparation and loading of a 1.8% agarose gel

- 4.5gm of agarose was added to a 500ml Pyrex flask containing 250ml of 1xTBE buffer
- Boiled with in microwave until the agarose was fully dissolved
- 10µl of Ethidium bromide was added to the dissolved agarose at 45^{o}C and dissolved by slowly shaking the flask not to form air bubble
- Then it was poured on to the 25cm gel making plate
- Allowed to solidify to form a gel cast for about 40 minutes
- The gel cast was placed in to the gel doc after removing the combs and then overlaid with 1xTBE buffer until the gel cast is fully covered
- Finally, the gel was loaded with the amplified PCR product along with the loading dye and 1Kb DNA ladder and allowed to run for 1 and half hours.
- At the end, the DNA bands were photographed

ANNEX VIII. Antimicrobial susceptibility test procedures

A 48 hours old *Campylobacter* isolates from Mueller-Hinton broth (HiMedia Laboratories; Mumbai, India) were resuspended in sterile saline to attain a turbidity value equivalent to 0.5 McFarland. Mueller-Hinton agar (HiMedia Laboratories; Mumbai, India) plate was then seeded with these suspensions. Using sterile tweezers, antimicrobial discs were placed aseptically on the surface of Mueller Hinton agar plates. Tweezers were re-flamed after application of each disc. The plates were then incubated in microaerophilic condition created by Campy Gen gas generating kit at 42°C for 24 to 48 hours. Following incubation, the diameter of zone of inhibition was measured and recorded to the nearest millimeters for each disc used using a digital ruler.

Table: Antimicrobials, their classes and concentration used

Classes	Antimicrobials	Concentration (µg)	Zone diameter interpretive standards (mm)		
			Resistant	Intermediate	Sensitive
Quinolones	Nalidixic acid	NA; 30	≤13	14-18	≥19
	Ciprofloxacin	CIP; 5	≤15	16-20	≥21
Potentiated sulfonamides	Trimethoprim-sulfamethoxazole	SXT; 25	≤12	13-14	≥15
Aminoglycosides	Gentamycin	GM; 10	≤12	13-14	≥15
	Amikacin	AN; 30	≤14	15-16	≥17
Macrolides	Erythromycin	E; 15	≤13	14-22	≥23
	Azithromycin	AZM; 15	≤13	14-17	≥18
Tetracycline	Tetracycline	Te; 30	≤14	15-18	≥19
Penicillin	Amoxicillin-clavulanic	AMC; 20	≤13	14-17	≥18

	acid				
	Ampicillin	AM; 10	≤13	14-16	≥17
Phenicol	Chloramphenicol	C; 30	≤12	13-17	≥18

Farm observation (A); Interview with household (s)(B); Laboratory work (C)

DECLARATION

I, the undersigned, declare that this MSc Thesis is my original work and has not been presented for a degree in any of other University and all sources of materials used for the Thesis have been dully acknowledge.

Principal investigator

Gemechu Chala (DVM)

Signature _____Date of Submission_____

Place of submission: Addis Ababa, Ethiopia

Supervisor

Professor Daniel Asrat (MD, M.SC, PhD:)

(AAU, CHS, SOM, Dep of Microbiology, Immunology & Parasitology)

Signature _____ Date _____