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Identification and Risk Factor Assessment of Campylobacter Species in Backyard Chickens in Households with Diarrheic Children In Bahir Dar Zura District, Ethiopia

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SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

MASTERS IN VETERINARY PUBLIC HEALTH

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MSc Thesis

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A Thesis Submitted to the Graduate Program in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Public Health

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THESIS APPROVAL SHEET

This thesis entitled **"Identification and Risk Factor Assessment of *Campylobacter* Species in Backyard Chickens in Households With Diarrheic Children in Bahir Dar Zura District, Ethiopia"**, by Andargachew Misganaw Adugna has been evaluated by the board of examiners and was accepted in partial fulfillment of the requirements of Degree of Masters of Science in Veterinary Public health.

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I

DECLARATION

This is to certify that this thesis entitled "**IDENTIFICATION AND RISK FACTOR ASSESSMENT OF *CAMPYLOBACTER* SPECIES IN BACKYARD CHICKENS IN HOUSEHOLDS WITH DIARRHEIC CHILDREN IN BAHIR DAR ZURA DISTRICT, ETHIOPIA**", submitted in partial fulfillment of the requirements for the award of Degree of **Master of Science In Veterinary Public Health** to the Graduate Program of the College of Agriculture and Environmental Sciences, Bahir Dar University by **Andargachew Misganaw Adugna** (ID. No. BDU1206722) is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

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Co Advisor	Signature	Date

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LIST OF ABBRIVATIONS

AFLP	Amplified Fragment Length Polymorphism
CAT	Cefaperazone Amphotericin Teicoplanin
CBA	Columbia blood agar
CDC	Center for Disease Control and Prevention
CDT	Cytolethal Distending Toxin
GBS	Guillain-Barre Syndrome
IBD	Inflammatory Bowel Disease
mCCDA	Modified Cefoperazone Charcoal Deoxycholate
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electropherosis
PHE	Public Health England
REA	Reactive Arthritis
WHO	World Health Organization

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Isolation and identification of *Campylobacter* species and its associated risk factors in backyard chickens in Bahir Dar zuria district, Ethiopia

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ABSTRACT

Campylobacter is one of the most significant foodborne pathogens in the world and poultry is considered as the main reservoir of the bacteria. Although the significance of commercial poultry in the development of campylobacteriosis is well documented, little is known about the possible contribution of backyard chickens as a direct animal/faecal contact or exposure pathway for consumption. A cross-sectional study was conducted from February 2021 to February 2022 to isolate and identify Campylobacter species and assess its associated risk factors from backyard chicken feces in Bahir Dar zuria district. Using standard cultural and biochemical techniques, a total number of 179 samples were subjected to Campylobacter isolation and identification. During sample collection data on backyard chicken management, biosecurity, and hygiene practices were collected using a structured questionnaire. The risk factors were analyzed using univariable and multivariable logistic regression with the significant levels of P-value less than 0.05. Out of 179 fecal samples processed, the rate of recovery of Campylobacters was 71 (39.7%); of which 51 (71.83%) were found to be C. jejuni, 9(12.67%) were C. coli, 4(5.63%) were C. lari and 7(9.86%) were unidentified isolates. Regarding to risk factors the multivariable logistic regression analysis revealed that Campylobacter positivity was significantly increased by the following factors: chickens kept with other animals (OR = 3.26; 95% CI = 1.42- 7.47), backyard spread of manure (OR: 11.29; 95% CI: 2.36 - 54.02) and chickens from rural areas (OR = 9.06; 95% CI = 3.59-22. 84). However, the presence of working latrine had protective effect (OR = 0.25, 95% CI: 0.12 - 0.60). The present study revealed the presence of high level of Campylobacter species in backyard chickens which necessitate drawing attention to the implementation of biosecurity

measures, good hygiene practices and animal management to reduce this potential zoonotic risk.

***Key Words**: Backyard, Bahir Dar zuria, Campylobacter, Chickens, Risk factors*

Chapter 1. INTRODUCTION

1.1 Background and Justification

Gastroenteritis is a major public health concern, with over 800 000 fatalities in children annually, most occurring in Asia and Africa. Despite a global decline, diarrheal mortality accounts for one in ten child deaths in developing countries (Osbjer *et al*., 2016). According to studies in Ethiopia, diarrhoeal diseases are significant causes of child mortality and morbidity. In Ethiopia, an estimated 39,000,000 episodes of diarrhea occur each year, with 230,000 deaths among children under the age of five (Ayalew Lengerh *et al*., 2013).

Different enteric pathogens cause gastrointestinal infections in humans such as bacteria, viruses, and parasites. Of the bacterial aetiologies, *Campylobacter* is a leading cause of gastrointestinal infections worldwide (Gahamanyi *et al*., 2020; Tizazu Zenebe *et al*., 2020) responsible for 400–500 million cases of diarrhea each year (Gahamanyi *et al*., 2020) and causing more than 37, 000 deaths per year worldwide (WHO, 2015).

Campylobacters are small gram-negative, non-spore-forming, helical bacteria with a particular `darting' motility, and are catalase and oxidase positive (Lemma Dadi and Daniel Asrat, 2008). They commonly have a single polar unsheathed flagellum at one or both ends (Hassan *et al*., 2019). Currently, the genus has 39 species and 16 subspecies (Hlashwayo *et al*., 2020) from this at least a dozen of *Campylobacter* species has been associated with human (Hassan *et al*., 2019).

The gastrointestinal tract of farm and wild animals considered as natural reservoir of *Campylobacters*. Thus, the bacteria are frequently isolated from a range of animal species like` poultry, cattle, pigs, sheep, pets, wild birds, and rodents (Begum *et al.*, 2015). However, it is estimated that about 50%–80% of strains infecting humans originate from the chicken reservoir (Wagenaar *et al*., 2013). Although poultry can act just as a carrier without clinical symptoms, they serve as a possible source of human infection (Yohans Hagos *et al*., 2019).

There are several pathways for *Campylobacter* species colonization of poultry flocks, including the persistence from previous productive cycles, horizontal transfer from other

animals (wild or domestic), insects, fomites, and contaminated feed and water (Behailu Assefa *et al*., 2022). Once *Campylobacter* enters the chicken flock, it spreads rapidly and colonizes the intestinal tracts of most chickens after one week. Other risk factors that raise the risk of *Campylobacter* species colonization includes; flock size, the use of untreated water, and disposal of poultry wastes on the farm (Rukambile *et al*., 2021; Behailu Assefa *et al*., 2022).

The transmission of *Campylobacter* from animal reservoirs to humans can occur through multiple routes, including contaminated food (especially poultry meat) and water, the environment, and contact with infected animals. Children may be exposed to *Campylobacter* species directly or indirectly through exposure to animal feces (Yitagele Terefe *et al*., 2020). *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are the predominant cause of human campylobacteriosis and are responsible for approximately 95% of all infections (Zhang *et al.,* 2020).

There seems to be a difference in the epidemiology of human campylobacteriosis between high and low-income countries. In low-income countries, the majority of symptomatic *Campylobacter* infections are detected in young children, and adults appear to develop a level of protective immunity with repeated exposure. In high-income countries, symptomatic infection occurs in all age groups (Osbjer *et al*., 2016). In humans, campylobacteriosis could be a self-limiting infection characterized by fever, abdominal cramps, and diarrhea with blood and leukocytes (Morsy *et al*., 2017). However, in immunocompromised individuals, the infection can cause life-threatening conditions like Guillain-Barre syndrome (GBS), reactive arthritis (REA), hemolytic uraemic syndrome, meningitis, and abortion (Lone *et al*., 2016).

The bacterial isolation and identification of *Campylobacter* is considered the gold standard for disease identification; however, it is tedious and laborious due to the complex nature of *Campylobacter* (Ghoneim *et al*., 2020). Furthermore, differentiation between species through conventional bacteriology is difficult, as very few biochemical characteristics differ among species (Frasao *et al*., 2017). As a result, molecular-based assays, such as polymerase chain reaction (PCR) and sequencing, can enable easy, rapid, and specific detection and epidemiological applications (Barakat *et al*., 2020). While most cases of *Campylobacter*

enteritis are self-limiting, in situations where antibiotic therapy is indicated either erythromycin or ciprofloxacin are the drugs of choice (Komba *et al*., 2013). However, there is an escalating number of *Campylobacter* isolates resistant to these drugs due to the misuse of antimicrobials in animals and humans (Gahamanyi *et al*., 2020).

The burden of disease due to *Campylobacter* in low-income countries like Ethiopia is under-reported because of the absence of a national surveillance program, limited routine culture availability for the isolation of *Campylobacter* species at clinical and research settings, and the need for selective media and a unique growth atmosphere. As a result, the real number of cases is thought to be up to ten times higher than the documented case numbers (Ayalew Lengerh *et al*., 2013; Belay Tafa *et al*., 2014). Backyard chickens are popular as a cost-effective means of subsistence in Bahir Dar zuria distict; however, there is no information on the status of *Campylobacter* in this type of production. Therefore, this study aimed to isolate and identify *Campylobacter* species and assess its associated risk factors from backyard chickens in Bahir Dar zuria district, Ethiopia.

1.2. Statement of the Problem

Human exposure to animal feces is more common in developing countries where domestic animals and their animal excrement may not be effectively controlled or isolated from home environments. These conditions may lead to fecal-oral transmission of zoonotic pathogens through direct contact with humans and/or fecal contamination of fingers, food, and water sources. Several pathogens of zoonotic origin are associated with acute gastrointestinal symptoms that may arise from contact with animal feces (Penakalapati *et al*., 2017). Among pathogens *Campylobacter* species has become a growing concern worldwide (Vandeplas *et al*., 2008). Indeed, poultry is a major source of *Campylobacter* species to humans, causing an estimated 50 to 80% of campylobacteriosis cases (Wagenaar *et al*., 2013).

Currently, around 96 million Ethiopians reside in rural households, where sharing space with domestic livestock is commonplace. Most rural families' livelihoods depend on chicken production (an estimated two-thirds of Ethiopian peasants keep poultry) (Brena *et al*., 2016). Due to the lack or poor quality of chicken houses, which cannot guarantee the safety and physical security of chickens, most households secure chickens inside their houses overnight.

This system predisposes the environment, the house, and the kitchen utensils to contamination with chicken feces, which can increase the risk of acquiring chicken-related *Campylobacter* infections (Budge *et al*., 2020).

In Ethiopia, a few studies have indicated that campylobacteriosis is common in human and domestic animals. In chickens, studies on the prevalence of *Campylobacter* have revealed a range of 13% (Gemechu Chala *et al*., 2021) to 86.6 % (Abdulhakim Abamecha *et al*., 2015)**.** In addition the isolation rates of these bacteria in children range from 8% (Desalegne Ewnetu and Adane Mihret, 2010) to 50 % (Chen *et al.*, 2021). In the current study area chickens are mostly managed under the small extensive scavenging production system with human houses mostly functioning as both children's playgrounds and chickens' scavenging and housing locations. Thus frequent screening for fecal carriage is an important step in preventing the spread of this pathogen to humans via the fecal-oral route. This study will give information that may help to establish surveillance programs and intervention measures regarding the prevalence and risk factors of *Campylobacter* in backyard chickens in Ethiopia.

1.3. Objectives of the Study

1.3.1. General objective

To investigate the presence of *Campylobacter* species in backyard chickens and assess its associated risk factors in Bahir Dar zuria district.

1.3.2. Specific objectives

- To isolate *Campylobacter* species from backyard chickens in Bahir Dar zuria district
- To determine thermophilic *Campylobacter* species among *Campylobacter* isolates using phenotypic test***s***
- To assess potential determinant factors associated with the occurrence of *Campylobacter*

1.4. Research Questions

- Could *Campylobacter* be isolated from fecal samples of backyard chickens?
- Which one was the predominant species among thermophilic *Campylobacter* species?
- What were potential determinant factors associated with the occurrence of *Campylobacter*?

Chapter 2. LITERATURE REVIEW

2.1. General Properties of *Campylobacter*

The family Campylobacteraceae (proposed in 1991) includes four closely related genera; *Campylobacter, Arcobacter*, *Dehalospirillum*, and *Sulfurospirillum (*Shad and Shad, 2019). The genus *Campylobacter* belongs to the family Campylobacteraceae, the order Campylobacterales, the class Epsilon proteobacteria, and the phylum Proteobacteria. Since its first description, the genus has grown to include several important human and animal pathogens that are primarily characterized through phylogenetic methods (Marroki and Leila, 2019).

Currently, 39 *Campylobacter* species are recognized (Hlashwayo *et al*., 2020), but this number is anticipated to rise over because of advancements in diagnostic methods and genomic analyses (OIE, 2017). Pathogenic *Campylobacter* species known to be implicated in human infections include *C. jejuni*, *C. concisus, C. rectus, C. hyointestinalis, C. insulaenigrae, C. sputorum, C. helveticus, C. lari, C. fetus, C. mucosalis, C. coli, C. upsaliensis, and C. ureolyticus*. Major veterinary pathogens are *C. fetus subspecies venerealis* and *C. fetus subspecies fetus* (Igwaran and Okoh, 2019).

Campylobacters are slender, spirally curved, and non-spore forming Gram-negative rods. The size of bacterial cells is small and ranges from 0.2 to 0.9 μm in width and 0.5 to 5 μm in length (Figure 2.1). Some species such as *C. gracilis* and *C. hominis* form straight rods (Ammar *et al*., 2019). The majority of organisms use a single polar unsheathed flagellum at one or both ends of the cell to move in a corkscrew-like motion. The only exceptions are *C. howae*, which possesses numerous flagella, and *C. gracilis*, which is non-motile (Silva *et al*., 2011).

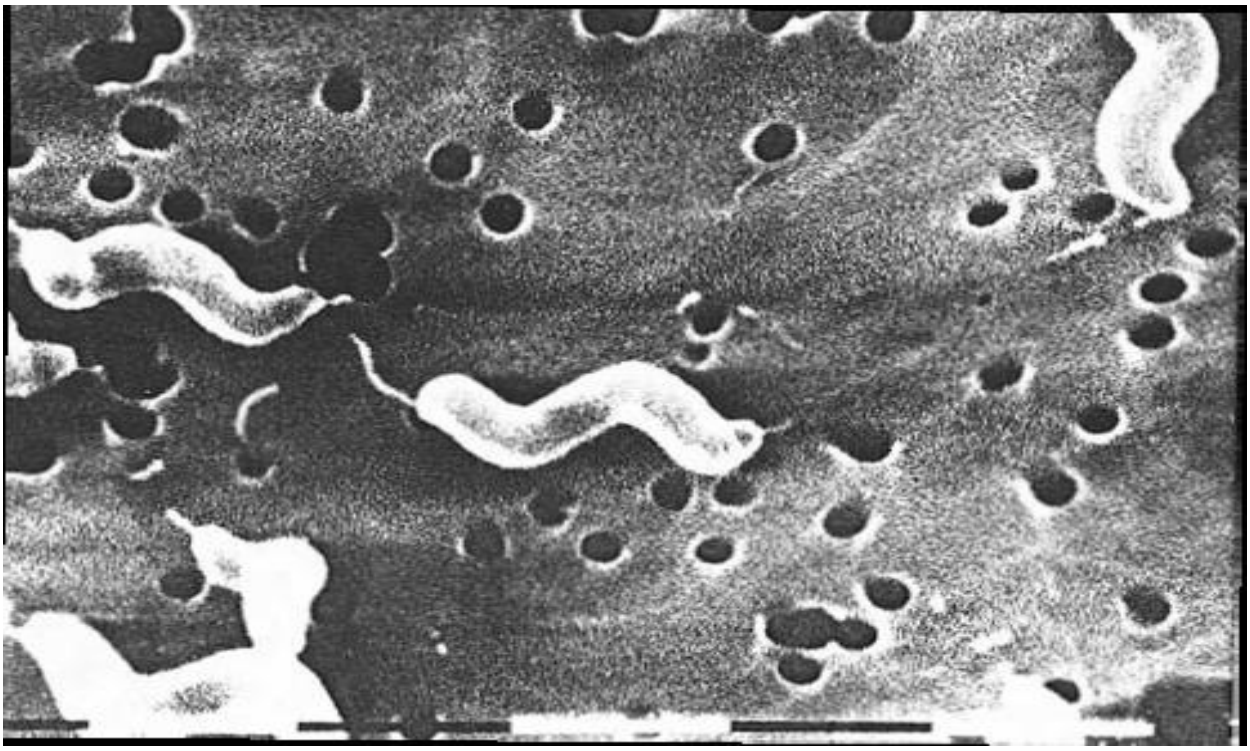

Figure 2. 1. Scanning electron micrograph of *C. jejun* (Altekruse and Tollefson, 2003).

Campylobacters are nutritionally fastidious and grow under strictly anaerobic or microaerobic (containing approximately 5-10% O2 and 5-10% CO2 for recovery) conditions but several *Campylobacter* species including *C. concisus, C. curvus, C. gracilis, C. mucosalis, C. rectus, C. showae,* and some strains of *C. hyointestinalis* require a hydrogen enriched atmosphere (3-7% H2 is required) for growth, a condition not routinely used in the diagnostic laboratories. Their optimum growth temperature is 37 - 42°C (PHE, 2018).

On selective agar, modified charcoal cefoperazone deoxycholate agar (mCCDA), colonies are grey/white or creamy grey in color and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented. On blood agar, translucent colonies are produced. They also appear slightly pink, round, and convex with a regular edge. Agar pitting is dependent on the medium used, but most strains exhibit this trait after a few days of anaerobic growth on blood agar (PHE, 2018).

Numerous variables affect *Campylobacter* species growth and survival. The bacteria have a limited ability to withstand environmental stress and are susceptible to factors like temperature, the availability of water, and oxygen. These traits limit the capacity of the bacteria to grow in food throughout processing and storage as well as outside of an animal host (Park, 2002). Environments with water activity (aw) lower than 0.987 do not support growth, and 0.997 is the critical value for optimal growth. *Campylobacter* species are quickly inactivated by heat treatments with their D-value being less than1 min. Additionally, freezing and thawing also decrease the population of the bacteria (Silva *et al*., 2011).

They die more rapidly on a dry surface at room temperature than under refrigeration conditions. They can survive at refrigeration temperatures (4°C) and in meat stored frozen (at -18°C to -22°C) for several weeks and in general, survive better at cooling temperatures. The growth of *C. jejuni* and *C. coli* thought to be optimum at 0.5% NaCl concentration. However these bacteria are sensitive to NaCl concentrations of 2% and higher. The pH range of 6.5 to 7.5 is ideal for the growth of *Campylobacter* species, but the bacteria cannot survive PH below 4.9 or above 9 (Yohans Hagos *et al*., 2019).

2.2. Virulence and Pathogenesis

Several different potential factors put on specific influences in the pathogenesis of disease like motility and chemotaxis, adhesion, invasion, and toxin production (Shad and Shad, 2019). Colonization and intestinal epithelium adherence are the initial and crucial steps of disease pathogenesis. Thus, the characteristic motility of the bacterium by polar flagella that the cell possesses is critical. The flagella regulate a rotational propulsive movement of the cell body while the helical shape determines a typical movement like a corkscrew. The colonization of the intestinal epithelium follows a chemotaxis process in which the mucins and glycoproteins that make up the intestinal mucus serve as the primary chemoattractant (Facciolà *et al*., 2017).

A number of adhesins present on the surface of the bacteria facilitate the subsequent bacterial adherence to the intestinal epithelial surface. Generation of various cytotoxins is linked to the resulting cell damage. Among cytotoxins cytolethal distending toxin (CDT) is the most studied one. This toxin has deoxyribonuclease activity and determines the cell cycle block in the G2 phase and fragmentation of the nucleus resulting in cell death (Facciolà *et al*., 2017).

2. 3. *Campylobacter* in Humans

Campylobacter organisms are etiologic agents of diarrhea in humans (Barbour *et al*., 2012). The most important *Campylobacter* species in human gastroenteritis is *C. jejuni,* which accounts for 90 to 95% of all campylobacteriosis cases recorded. The majority of the remaining cases are caused by *C. coli,* but the importance of *C. coli* as an enteric pathogen varies between regions, degree of urbanization, and age of the patient (Pitkanen and Hanninen, 2017).

The main route of transmission is generally believed to be food borne, via undercooked meat and meat products, as well as raw or contaminated milk. Contaminated water or ice can potentially be source of infection. A proportion of cases occur following contact with contaminated water while engaging in recreational activities. Although it is uncertain how much each of the above sources contributed to the overall burden of disease, eating infected, undercooked poultry is thought to be a significant factor (WHO, 2015). There has also been speculation about fly-borne transmission (Butzler, 2004). In addition even though it is rare, person-to-person transmission can happen (Marroki and Leila, 2019).

The onset of disease symptoms usually occurs 2 to 5 days after infection with the bacteria but can range from 1 to 10 days. The most common clinical symptoms of *Campylobacter* infections include diarrhea (frequently bloody), abdominal pain, fever, headache, nausea, and/or vomiting. The symptoms last after 3 to 6 days. Death from campylobacteriosis is rare and is usually confined to very young Children or elderly patients, or those already suffering from another serious disease such as Acquired Immune Deficiency Syndrome (AIDS) (WHO, 2015).

Campylobacter species infection can be burdened by some major complications. The most recognized squeals are GBS, REA, and irritable bowel syndrome. The Miller Fisher syndrome, a variant of GBS, can also be associated with previous *Campylobacter* infection. Evidence suggests a possible association with Inflammatory Bowel Disease (IBD), and there is evidence that other functional gastrointestinal disorders may be related to gastroenteritis in general (not specifically caused by *Campylobacter*) (Facciola *et al*., 2017).

2.4. *Campylobacter* in Poultry and other domestic animals

Campylobacter is considered to be a commensal organism in many avian species, including those grown commercially. Poultry is frequently colonized with *C. jejuni* (65–95%), less often with *C. coli*, and rarely with other *Campylobacter* species Colonization rates in broiler chickens are age-related. Most flocks are negative until 2 weeks of age. Once *Campylobacter* colonization occurs in a broiler flock, transmission, via exposure to fecal contamination, is extremely rapid and up to 100% of birds within a flock can become colonized within a few days (OIE, 2017).

There are 2 modes of transmission of *Campylobacter* in poultry: horizontal and vertical. Both have been shown to occur. Horizontal transmission is believed to be mainly through contaminated water, litter, insects, wild birds, rodents, fecal contact, and farm personnel via there. The feed has not been implicated in the spread of the bacteria because it is too dry to favor survival. Chickens can harbor very high levels of *Campylobacter* in the gut, up to 9.0 log10 CFU/g of cecal content, without symptoms, and the microorganism can be transmitted among birds within a flock (Keener *et al*., 2004).

According to most studies, horizontal transmission from the poultry farm environment is the major source of exposure of flocks to *Campylobacter*. Notably, many studies concluded that vertical transmission from breeder flocks via eggs was not a major source in the introduction of the bacteria to broiler houses although some controversy still exists (Sahin *et al*., 2015).

The factors commonly associated with *Campylobacter* colonization in broiler flocks include lack of overall biosecurity on farms, presence of other animals close to poultry houses (including other poultry species, livestock, pets, and wildlife), age and number of houses on a farm, slaughter age, size of flocks, the practice of partial depopulation (thinning), seasonal and climate changes, use of ventilators, fly population (and lack of fly screens), use of old litter, farm equipment, transport vehicles, and farm workers (Sahin *et al*., 2015).

Chickens colonized with *Campylobacter* species generally do not exhibit clinical disease. Many chickens are colonized with the bacteria early in life with no associated clinical signs or pathology. Some studies have reported that challenged chicks may exhibit distention of the

jejunum, disseminated hemorrhagic enteritis, and in some cases, focal hepatic necrosis. However, infected flocks seldom exhibit these lesions, increased mortality rates, or decreased feed conversion (Lee, 2019).

In addition to poultry, *Campylobacters* are frequent colonizers of the intestine of livestock such as cattle, sheep, and pigs. Cattle and sheep are found to be colonized mainly by *C. jejuni*, *C. coli*, *C. hyointestinalis*, and *C. fetus*, whereas pigs are predominantly colonized by *C. coli*. In young animals, the numbers are higher than older animals. In older animals, the organisms can be intermittently detected in feces, probably due to low numbers or due to intermittent shedding (OIE, 2017).

Even though, in most species *Campylobacter* exists as an intestinal commensal without causing clinical diseases, it may induce localized enteritis or systemic infections in some circumstances. Reproductive losses (e.g. abortion and infertility) in ruminants are among the most significant clinical conditions associated with *Campylobacter* infection in animals. *C. jejuni* and *C. fetus subspecies fetus* are the primary *Campylobacter* species associated with outbreaks of sheep abortions worldwide, and they also cause sporadic abortion in cattle and goats (Dai *et al*., 2020).

Campylobacter jejuni and occasionally *C. coli* cause enteritis in dogs, cats, calves, sheep, mink, ferrets, poultry, pigs, and some species of laboratory animals. The clinical signs may be more severe in young animals, such as kittens, puppies, or calves. The feces are usually watery or bile-streaked, with mucus and sometimes blood. The clinical signs generally last 3 to 7 days, but some animals may have intermittent diarrhea for weeks and occasionally for months. Calves typically have thick, mucoid diarrhea with occasional flecks of blood, either with or without a fever (Spickler and Leedom, 2013).

In cattle, *C. fetus* subspecies *venerealis* and *C. fetus* subspecies *fetus* can cause bovine genital campylobacteriosis; this disease is characterized by infertility, early embryonic death, and a prolonged calving season. Abortions are uncommon but are occasionally seen. Infected cows may develop mucopurulent endometritis but do not usually have other systemic signs. Bulls are asymptomatic (Spickler and Leedom, 2013).

2.5. Status of *Campylobacter* in Ethiopia

Campylobacter is a leading cause of diarrhea in both the developing and the developed world that has become increasingly prevalent. In developing countries, *Campylobacter* isolation rates for foodborne illnesses are between 5% to 20%. There is quite an epidemiological difference between low, middle, and high-income countries which likely arise from differences in diagnostic techniques, biocontrol protocols, food practices, nutritional status, environmental hygiene, climatic condition, and the abundance of natural reservoirs (Kula Jilo *et al*., 2021)

In Ethiopia Reports of *Campylobacter*-positive chicken flocks vary widely from 13% (Gemechu Chala *et al*., 2021) to 86.6% (Abdulhakim Abamecha *et al*., 2015). The variations of prevalence are seen between regions, seasons, productions system, and flock age. In Bahir Dar, the prevalence was 72.7 % (Desalegn Ewnetu and Adane Mihrt, 2010), whereas in Gondar (Seleshe Nigatu *et al*., 2015), Mekele (Yohans Hagos *et al*., 2021), and Bishoftu (Behailu Assefa *et al*., 2022) was respectively 28.9, 43.93, and 70% (Table 2.1.).

Table 2. 1. Previous works on isolation of *Campylobacter* species on chickens in Ethiopia

Sample Size	**Sample type**	**Study area**	**Overall prevalence (**%)	***C. jejuni* (%)**	***C. coli* (%)**	***C. lari***	**References**
Chicken (60)	Meat	Addias Abeba and Debre zeit	13(21.7)	11(84.0)	1(8.0)	1(8.0)	Lemma Dadi and Daniel Asrat, 2008
Chicken (220)	Feces	Bahir Dar	160(72.7)	148 (92.5)	12(7.5)	-	Desalegn Ewnetu and Adane Mihrt, 2010
Chicken (97)	Feces	Nuer zone	84(86.6)	73(86.9)	10(11.9)	1(1.2)	Abdulhakim Abamecha *et al*., 2015
Chicken (90)	Feces	Gondar	26(28.9)	26(28.9)	- -	- -	Seleshe Nigatu *et al*., 2015
Chicken(66)	Meat	Mekele	29(43.93)	23(80)	4(13.79)		Yohans Hagos *et al*., 2021
Chicken (20)	Cloacal Swab	Bishoftu and Mojo	14(70)	10(71.4)	-	-	Behailu Assefa *et al*., 2022
Chicken (69)	Cloacal swab	Adddis Abeba	9 (13.0)	2 (22.2)	2 (22.2)	-	Gemechu Chala *et al*., 2021

2.6. Laboratory Diagnosis of *Campylobacter* Species

2.6.1. Sample collection

Campylobacters can be isolated from fresh feces/cecal droppings or cloacal swabs. For reliable detection of the bacteria by culture, freshly voided feces (preferably without traces of urine) should be collected. Such samples must be prevented from drying out before culture (OIE, 2017).

2.6.2. Sample transportation

Campylobacters are sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight, and elevated temperature. Transport to the laboratory and subsequent processing should therefore be as rapid as possible, preferably on the same day, but within at least 3 days. The samples must be protected from light, extreme temperatures, and desiccation. No recommendation on the ideal temperature for transportation can be made, but freezing or high temperatures can reduce viability. High temperatures (>20°C), low temperatures (<0°C), and fluctuations in temperature must be avoided. When the time between sampling and processing is longer than 48 hours, storage at 4°C (±2°C) is advised. When samples are collected on boot swabs or rectal swabs, the use of a commercially available transport medium is recommended. Transport media that can be used for swab sample shipment include Cary-Blair, modified Cary-Blair, modified Stuart medium, Campythioglycolate medium, alkaline peptone water, and semisolid motility test medium. Good recovery results have been reported using Cary-Blair (OIE, 2017).

2.6.3 Isolation and detection of *Campylobacters*

Efficient and reliable techniques for the isolation and identification of *Campylobacter* species in poultry are essential to facilitate clinical and epidemiological studies. The use of the conventional method for detecting and isolating *Campylobacters* has been mostly relied on. The conventional method involves enrichments and/or plating onto selective media and biochemical confirmation (Corry *et al*., 2003). Enrichments broths used for isolating *Campylobacters* include Cefaperazone Amphotericin Teicoplanin (CAT), Hunt and Radle, Bolton, Exeter, Hunt, Preston, Park-Sanders, Doyle and Roman, Rosef, blood-free

enrichment, and *Campylobacter* enrichment broths. While plating has been achieved on mCCDA, Columbia blood (CBA), Campy-Cefex, CAT, blood, Karmali, Abeyta-Hunt, Blaser, and Skirrow agars. Biochemical tests carried out for *Campylobacters* also include oxidase, catalase, and glucose utilization. Incubation is done between 25 to 42°C under a microaerobic (5% oxygen, 10% carbon dioxide, and 85% nitrogen) condition. Thermophilic *Campylobacters* cannot grow below 32°C (Frederick and Huda, 2011), But grow optimally at 42°C which is nearer the body temperature of birds. This perhaps favors the growth of thermophilic *Campylobacters* (Horrocks *et al*., 2009).

Conventional methods for the detection and isolation of *Campylobacter* species are said to be relatively slow, laborious, and less efficient (Keramas *et al*., 2004). As such, various rapid methods categorized broadly into immunological (e.g., latex agglutination test, Enzyme Linked Immunosorbent Assay (ELISA), nucleic acid methods (e.g PCR), and growth-based methods have been applied. With thermophilic *Campylobacters,* flagellin typing (FlaA/FlaB), Pulsed Field Gel Electrophoresis (PFGE), and Amplified Fragment Length Polymorphism (AFLP) are commonly employed to identify and compare distinct genotypes among humans and animals. These methods determine specific thermophilic *Campylobacter* strains based on precise identification of genomic Deoxyribonucleic Acid (DNA). Nevertheless, conventional methods are widely used and have the advantage that they are cheaper, detect only viable bacteria, and also yield isolates that can be studied and further characterized (Frederick and Huda, 2011).

Generally isolation methods for *Campylobacter* vary between laboratories, even in the same country. Most use selective agar plates, developed principally for *C. jejuni-coli*, containing antibiotics to suppress normal fecal flora, incubated at 42 °C in a microaerophilic atmosphere, like the mCCDA, the preferred media for isolating *C. jejuni-coli* But those conditions inhibit the growth of most of the *Campylobacter* species other than *C. jejuni or C. coli* . Due to their specific growth conditions, slow growth rates, and susceptibility to antibiotics present in the *C. jejuni-coli* selective agar plate, like *C. concisus, C. curvus, C. upsaliensis*, and *C. fetus*, are more difficult to isolate in culture. Specific isolation methods, such as the filtration method (FM) on antibiotic-free agar, are thus warranted, with incubation at a temperature lower (37 °C) than for *C. jejuni* and *C. coli* An increased hydrogen (H2) concentration is required to

better isolate several *Campylobacter* species other than *C. jejuni* or *C. coli* like *C. concisus, C. curvus, C. sputorum*, and *C. hyointestinalis* (Tilmanne *et al*., 2019).

2.7. Treatment

In animals many cases of campylobacteriosis are self-limiting and require only supportive therapy. Antibiotics may be useful for some cases of enteritis, especially those that are severe. Macrolides and fluoroquinolones are commonly prescribed for campylobacteriosis; however, resistance to these and other antibiotics also occurs. Treatment of healthy animals is not recommended for several reasons: there is a high likelihood of re-exposure and there is no evidence that treatment is effective (Spickler and Leedom, 2013).

Antibiotic treatment may not completely prevent shedding in colonized animals, though it may prevent exposed sheep from aborting during an outbreak. Bulls with bovine genital campylobacteriosis are sometimes treated; cows usually are not, due to practical considerations (Spickler and Leedom, 2013).

Without using antibiotics, most people recover from a *Campylobacter* infection. As long as the diarrhea persists, patients should increase their fluid intake. Some persons who have severe illnesses or are at high risk for developing infection may require antibiotic therapy. These individuals include those who are 65 years of age or older, pregnant women, and those who have compromised immune systems, such as those who have AIDS, blood disorders, or are undergoing chemotherapy. If individuals have compromised immune systems or exhibit severe symptoms, the CDC has advised initiating treatment regimens for *Campylobacter*. Fluoroquinolones and macrolides are often recommended medications, with macrolides favoured due to their low resistance rate (CDC, 2019). The prognosis for *Campylobacter* enteritis is often very good, and treatment is not necessary when these organisms are isolated from feces. For two to seven weeks following sickness, feces remain positive in the absence of chemotherapy (Butzler, 2004).

2.8. Prevention and Control

Control of *Campylobacter* along the food chain is most effective when the colonization of living animals can be prevented. Reducing the prevalence of *Campylobacter* infection in the primary production phase decreases high numbers of the bacteria in the following steps. This may result in a low concentration or absence of the bacteria in the final product (Wagenaar *et al*., 2006).

Strict biosecurity, decontamination of housing between subsequent flocks, removal of rats and wild birds, and insect eradication are the foundations for the preharvest prevention of *Campylobacter* infection in commercial poultry. Chlorination of drinking water to 2 ppm and operation of farms on a strict "all-in/all-out" basis occasionally reduces the prevalence of infection. Innovative methods of prevention, such as competitive exclusion, bacteriophage therapy, bacteriocins, and the use of vaccines, are under intensive investigation (Lee, 2019).

Commercial poultry processing, improved washing of carcasses, use of counter-flow mechanical advances in scalding, elimination of immersion chillers, and reduction in manual handling by the installation of advanced automated equipment have reduced *C. jejuni* contamination on poultry meat. Chemical disinfectants in the washes, such as chlorine, peracetic acid with hydrogen peroxide; trisodium phosphate, glutaraldehyde, and succinic acid; and organic compounds such as lactic and acetic acids may effectively also reduce *C. jejuni* on poultry carcasses in the processing plant. Concurrent measures in food preparation, hygienic storage, handling, and preparation are necessary to prevent contamination of prepared foods, work surfaces, and utensils by raw poultry and other meats. The risk of foodborne *C. jejuni* infection can be reduced by cooking poultry to achieve a core temperature of 74°C for 1 minute (Lee, 2019).

Chapter 3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Bahir Dar zuria district as indicated in the figure 3.1. Bahir Dar Zuria district is situated around Bahir Dar city, the capital city of the Amhara National Regional State, and is located at a distance of 560 kilometers from the capital city of the country, Addis Ababa (Zelalem Dejazmach *et al*., 2021). The district is bordered on the east by the South Gonder zone, on the west by the Mecha and Achefer districts, and on the north and south by Lake Tana and the Yelimanadensa district respectively. The topographic features of the district indicate that approximately 48% may be defined as rolling, 32% hilly, 13% mountainous, and 7% valleys (Bimrew Asmare, 2018). The topographic features of the district indicate that approximately 48% may be defined as rolling, 32% hilly, 13% mountainous, and 7% valleys (Bimrew Asmare, 2018). The district has a mean annual rainfall of 1035 mm. The minimum and maximum temperatures lie at 10˚C and 32˚C, respectively (Zelalem Dejazmach *et al*., 2021). The altitude ranges from 1,750 to 2,300 m above sea level (m.a.s.l). Its latitudinal and longitudinal extension is 11°25 N- 11°55 N and 37°.04 E- 37.39 E. The district has 32 rural sub-districts (*kebeles*) (CSA, 2015). Teff, corn, sorghum, cotton, and sesame are important cash crops. The majority of the population is engaged in primary occupations and 84% of small farmers perform livestock husbandry. Backyard chickens are popular as a cost-effective means of subsistence in Bahir Dar zuria distict (Mushir Ali and Mulugeta Neka, 2012).

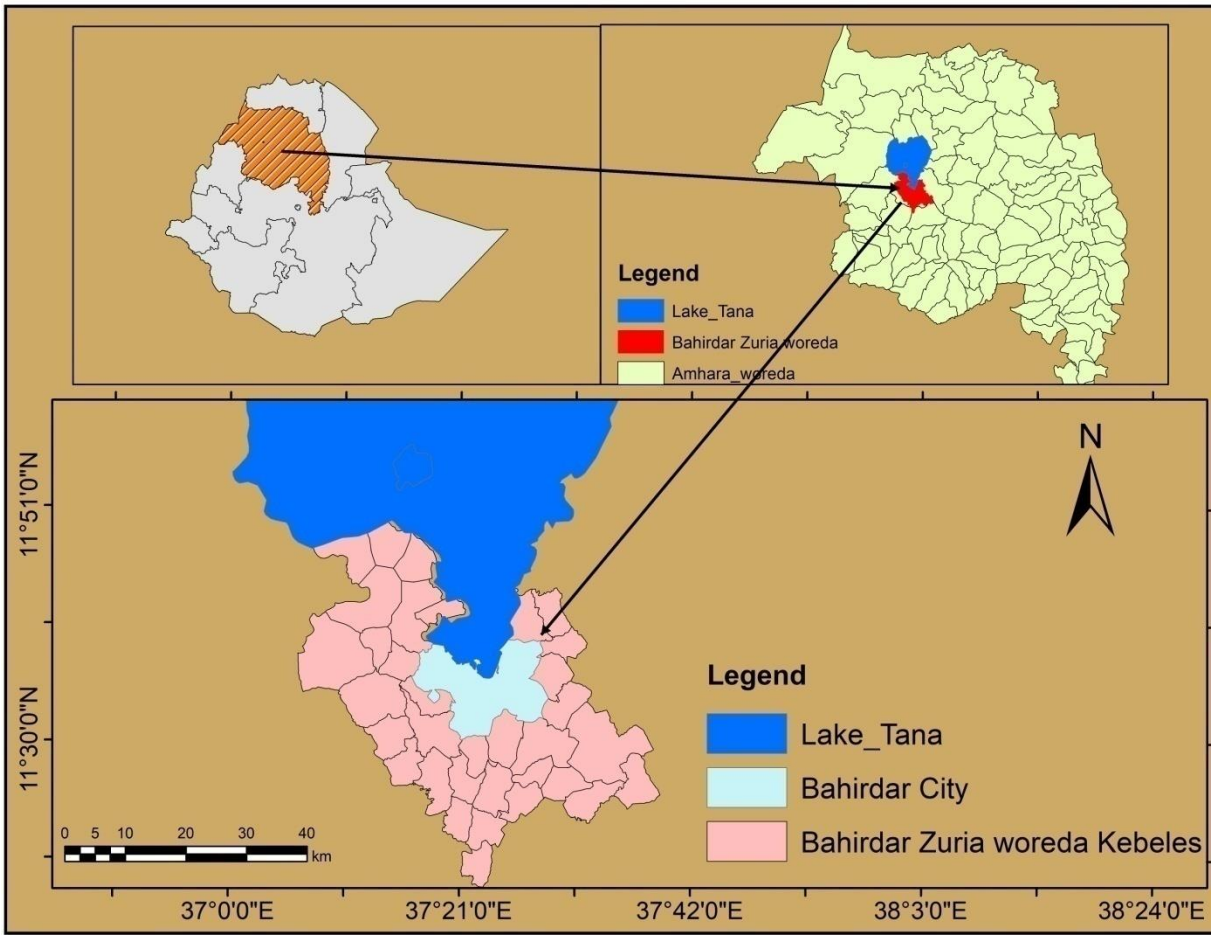

Figure 3. 1. Location of the study area

3.2. Study Design and Population

A cross-sectional study was conducted from February 2021 to February 2022 to isolate and identify *Campylobacter* species and to assess its associated risk factors from feces of chickens in Bahir Dar zuria district. The study populations were chickens which were from households with under-five children diagnosed with cases of diarrhea in the study area.

3.3. Sample Size Determination and Sampling Techniques

A purposive sampling method was used to select households that had backyard chickens and under-five children diagnosed with cases of diarrhea. Chicken fecal samples were selected by a convenient sampling method. The sample size was determined based on the expected prevalence of 86.6% by Abdulhakim Abamecha *et al*. (2015) with absolute desired precision

of 5% at a confidence level of 95% according to formula provided by Thrusfield (1995). The formula was:

$$N = \frac{(1.96)^2 x\ p\ (1-p)}{D^2}$$

Where N=sample size; P=expected prevalence; D= desired absolute precision
Thus, based on the formula the total sample size was179.

3.4. Inclusion and Exclusion Criteria

Chickens were required to be owned by individuals that had under-five children with diarrhea. On the other hand chickens from commercial farms and households without diarrheic children were excluded.

3.5. Sample Collection and Transportation

Freshly voided fecal and cecal excretions were immediately taken, either after observed excretion from individual chickens, or on the stable floor. Fresh feces were determined to be those feces that were soft, shiny, and warm (Schets *et al*., 2017). During the collection of samples aseptic measures were taken. The spoon of stool cup was used to collect fresh feces by picking from the top to the middle part of feces (avoiding urate and floor contamination). After collection, all the samples were labeled, kept in ice boxes held over ice packs, and brought to the Applied Microbiology laboratory of Bahir Dar University within 4 hours.

3.6. Isolation and Identification of *Campylobacter* Species

Isolation of *Campylobacter* species was carried out by filtration method as described by Shiramaru *et al.* (2012) with slight modification. Fecal samples were diluted at 1:10 in saline solution and vortexed for 30 seconds at 2500 revolution per minute (rpm). The 0.60-µm pore size PC filters (Nuclepore™, Whatman, Germany) were placed on mCCDA without the addition of selective antibiotics. Six drops of the fecal suspension were placed on top of the membrane and allowed to filter passively for 30 min at 37 °C in aerobic conditions. After filtration, filters were removed and plates were then placed in a glass jar with a candle. The candle was then lit and the jar was covered and waited until the flame was extinguished. This

method creates a microaerophilic environment suitable for the growth of *campylobacter* species (Davis and Dirita, 2008). The jar was then placed in the incubator at 37°C for 48 hours. After 48 hours, the plate was then examined for the growth of bacteria. Grey, flat, and irregularly spreading colonies were observed. The suspected colonies were picked up and subcultured again onto mCCDA through the same procedure to get a pure colony. Thus, pure isolates obtained were Gram-stained and observed for Gram-negative spiral rods. Differentiation of isolated *Campylobacter* species based on growth characteristics including biochemical tests, such as the catalase test, oxidase test, hippurate hydrolysis test, and sensitivity to Nalidixic acid and Cephalothin were performed according to the standard procedures described earlier (Foster *et al*., 2004) (Annex, 3).

To identify *C. jejuni* hippurate hydrolysis test was done according to Hwang and Ederer (1975) with slight modification, a 1% aqueous solution of sodium hippurate (Eastman) was prepared and dispensed in 0.4-ml amounts in screw-cap tubes (Annex, 3.4). Portions not used on the day of preparation were frozen at -20°C until used. For the test, a large loopful of *Campylobacter* culture grown on mCCDA microaerobically for 48 hours at 37°C was added, agitated with the loop to produce a suspension. The tubes were incubated for 2 hours at 37°C aerobically. After incubation, approximately 0.2 ml of 3.5% ninhydrin solution was added to the tubes, and incubation was continued at 37°C aerobically for 10 min before the results were checked. Deep purple color was developed by C. *jejuni* species (Annex, 6).

3.7. Questionnaire and Observational Survey

During the sample collection, data on hygienic and management measures were recorded by observation of the examined backyards and interviews with 179 backyard chicken owners. Most of these questions were close ended. Verbal consent was obtained, and the objective of the study was also explained to the respondents. Risk factors considered in the current study were sanitary conditions of the house, water safety and source, presence of other animals, location, bedding type, manure disposal system, and the presence of a working latrine (Annex, 1). Due to the collection of fecal dropping, the present study could not consider chicken factors as risk factors for the occurrence of *Campylobacter*.

3.8. Data Analysis

Data from field surveys and laboratory tests were recorded in Microsoft Excel spreadsheets and data were cleaned, and coded. The data were further exported into SPSS version 20.0 software (SPSS INC. Chicago, IL) for statistical analysis. The odds ratio (OR) was calculated through a univariable logistic regression model for estimating the relationship between the selected exposure variables and the presence of *Campylobacter* species, and a p-value of <0.05 was considered statistically significant. All variables with a p-value lower than 0.2 were retained for assessment in the multivariable analyses. These variables were further utilized in the multivariable logistic regression analysis. Descriptive analysis was done to describe the study population concerning risk factors; the outputs were presented in frequencies and proportions.

3.9. Ethical Review

The study protocol was reviewed and approved by the Institutional Review Board of Bahir Dar University. A letter of support was obtained from, the School of Animal Science and Veterinary Medicine, Bahir Dar University. Permission from backyard chicken owners was asked to participate in the study. After thoroughly explaining the objectives and relevance of the study, the procedure, benefit, and their right, informed consent was obtained from the participants.

Chapter 4. RESULTS

4.1. Isolation and Identification of *Campylobacter* Species

Out of 179 fecal samples collected from chickens and screened for the presence of *Campylobacter*, 71(39.7%) samples were found positive using filter technique on mCCDA plate and biochemical tests. The hippurate hydrolysis test revealed that among the 71 *Campylobacter* species isolated from feces, 51 (71.83%) were *C. jejuni*. While 9(12.67%) nalidixic acid-susceptible, hippurate negative isolates were identified as *C. coli*, and 4(5.63%) isolates were nalidixic acid-resistant, hippurate-negative isolates were identified as *C. lari*. In addition, there were 7(9.86%) unidentified isolates (Table 4.1).

Table 4. 1. *Campylobacter* species Identification

Tests/ species	**Gram staining**		**Oxidase**		**Catalase**		**Hippurate test**		**Nalidixic acid**		**Cephalothin**	
	+	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**S**	**R**	**S**	**R**
C. jejuni	0	51	51	0	51	0	51	0	51	0	0	51
C. coli	0	9	9	0	9	0	0	9	9	0	0	9
C. lari	0	4	4	0	4	0	0	4	0	4	0	4
Unidentified	0	7	7	0	7	0	0	7	7	0	7	0

R=Resistance, S=sensitive, + = Positive, - = Negative

4.2. Potential Risk Factors Associated with *Campylobacter* Occurrence

Univariable analysis was performed to identify possible associations between the 10 selected exposure variables and the positivity of *Campylobacter* species Five variables were found to have a significant association ($p < 0.2$) with a positive *Campylobacter* culture from fecal sample: chickens kept with other animal, location, the spread of manure inside the backyard, the presence of working latrine, and the presence of pets (Table 4.2). These variables were further used in multivariable logistic regression analysis.

Table 4. 2. Univariable logistic regression analysis of *Campylobacter* occurrence with risk factors

Explanatory Variables	**Levels (No of observations)**	***Campylobacter* frequency**	**OR**	**95% CI**	**P values**
Chickens kept with other animals	No (58)	12	1		
	Yes (121)	59	3..53	1.71 - 7.31	0.001
Cleanness of the house	Clean (79)	30	1		
	Not clean (100)	41	1.10	0.60 - 2.01	0.761
Spread of manure in the backyard	No (23)	2	1		
	Yes (156)	69	8.14	1.86 - 35.91	0.006
The presence of a working latrine	Absent (128)	51	1		
	Present (51)	20	0.30	0.14 – 0.63	0.001
Location	Urban (57)	7	1		
	Rural (122)	64	7.903	3.323-18.78	0.000
Water treated with chlorine	Not treated (52)	20	0.97	0.53-1.77	0.92
	treated(127)	51	1		
Bedding type	Earth (117)	45	1.21	0.67-2.18	0.524
	Other materials(62)	26	1		
The presence of cattle	Present (73)	23	1.74	0.93-3.24	0.82
	Absent (106)	48	1		
The presence of pets	Present (42)	25	2.78	1.38-5.6	0.004
	Absent (137)	46	1		
The presence of shoats	Present (51)	20	0.92	0.47-1.77	0.79
	Absent (128)	51	1		

1= Reference variable, Clean = litter removed +cleaned, Not clean = the presence of litter on ground according to Carron *et al*. (2018), OR= Odd Ratio, CI = Confidence Interval

Multivariable logistic regression analysis was carried out to observe the independence of each risk factor concerning the occurrence of *Campylobacter species* in backyard chickens. Thus, a high culture-positive rate of *Campylobacter* species had been observed in chickens from rural

areas than in urban areas. Chickens from rural areas were found 9.06 times (OR = 9.06; 95% CI = 3.59-22. 84; p = 0.000) colonized than chickens from urban areas. The odds of *Campylobacter* species presence was higher when chickens were kept with other animals (OR = 3.26; 95% CI = 1.42- 7.47; p = 0.005) compared to when only chickens were kept. Chickens that were from households that had working latrine had a 0.25 times less likely probability of being infected by *Campylobacter* species as compared with those chickens from households without working latrine (OR = 0.25, 95% CI: 0.12 - 0.60; P = 0.002). In addition, the odds of *Campylobacter* species positivity was 11.29 times higher in chickens from households that spread manure in the backyard than outside the backyard (OR: 11.29; 95% CI: 2.36 - 54.02; p = 0.002) (Table 4.3).

Table 4. 3. Multivariable logistic regression analysis of significantly associated explanatory variables for Campylobacter occurrence in chickens

Explanatory variables	**Levels**	**OR**	**95% CI**	**P values**
Chickens kept with other animals	No(58)	1		
	Yes(121)	3.26	1.42- 7.47	0.005
Spread of manure in the backyard	No(23)	1		
	Yes(156)	11.29	2.36 - 54.02	0.002
The presence of a working latrine	Absent (128)	1		
	Present (51)	0.25	0.12 - 0.60	0.002
Location	Urban (57)	1		
	Rural(122)	9.06	3.59-22. 84	0.000
The Presence of Pets	No (137)	1	0.87- 4.7	0.102
	Yes (42)	2.03		

1= Reference variable, OR= Odd Ratio, CI = Confidence Interval

Chapter 5. DISCUSSION

In the present study, the rate of recovery of *Campylobacter species* in backyard chicken feces was 39.7%. This finding was comparable to a previous study done in Mekelle of Ethiopia 43.93% (Yohans Hagos *et al*., 2021). Similarly, this result was proportionally in agreement with other reports in different countries with 41.7% in Ecuador (Ochoa *et al*., 2016) and 35% in Canada (Schweitzer *et al*., 2021). However, in Ethiopia, it was higher than the findings reported from Addis Abeba and Debre Zeit (21.7 %) (Lemma Dadi and Daniel Asrat, 2008), Gondar (28.9%) (Seleshe Nigatu *et al*., 2015), and Addis Abeba (13%) (Gemechu Chala *et al*., 2021). In addition, a lower prevalence than the present finding was reported in South Africa (5.9%) (Bissong and Ateba, 2019) and Sweden (14.72%) (Frosth *et al*., 2020).

On the other hand, this data indicated a low frequency of occurrence relative to reports from Bahir Dar (72.7%) (Desalegn Ewnetu and Adane Mihrt, 2010), Nuer zone (86.6 %) (Abdulhakim Abamecha *et al*., 2015**),** and Bishoftu and Mojo (70%) (Behailu Assefa *et al*., 2022). Furthermore, reports from different regions of the world which were higher than the present study were 96 % from Algeria (Guessoum *et al*., 2016) and 67.96 % from Burkina Faso (Kagambèga *et al*., 2018). The variation in *Campylobacter* isolation frequency observed between different studies would be attributed to several factors such as animal management system (Seleshe Nigatu *et al*., 2015), season, geographic location, differences in bacterial culture conditions and sampling methods, as well as the sample size (Williams and Oyarzabal, 2012; Lone *et al*., 2016).

The present study showed that out of 71 tested isolates the occurrence of *C. jejuni* was 51 (71.83%) which was higher than 9 (12. 67%) *C. coli* and 4 (5.6%) *C. lari*. In Ethiopia these results were in line with the findings of Desalegn Ewnetu and Adane Mihrt, 2010; who reported 92.5% C. *jejuni* and 7.5% *C. coli,* Abdulhakim Abamecha *et al*., 2015; who reported 86.9% *C. jejuni* 11.9%, *C. coli* and 1.2% *C. lari* and Yohans Hagos *et al*., 2021; who reported 80% *C.jejuni* and 13.79% *C. coli*. Higher prevalence of *C. jejuni* than *C. coli* coupled with low or lack of *C. lari* isolation was consistently reported in different countries like India (33.9 % *C. jejuni*, 94.8% *C. coli and* 1.6% *C.lari*) (Lone, *et al*., 2016), Tanzania (87.1% *C. jejuni and* 12.9% *C. coli*)(Chuma *et al*., 2016), and Cambodia (52% *C. jejuni* and 14% *C. coli*)

(Osbjer *et al*., 2016). In contrast, certain studies revealed a higher percentage of *C. coli* than *C. jejuni*. In India Malik *et al*. (2014) reported (2% *C. jejuni and* 98% *C. coli*) and in Canada Schweitzer *et al*. (2021) reported (18% *C. jejuni and* 20% *C. coli*). On the other hand findings of Gemechu Chala *et al*. (2021) indicated that the percentage of *C. jejuni* (22.2%) and *C. coli* (22.2%) was equal. The higher prevalence of *C. jejuni* in the above studies including the present study might be due to its longer viability in the environment compared to other thermophilic *Campylobacter* species, therefore increasing its chance of recovery. The difference could also occur due to variations in the mechanism of pathogenesis and elimination amongst the different thermophilic *Campylobacter* species within the host cells. Differences in the isolation of *Campylobacter* species might also be related to their actual compositional variations in local environments (Chuma *et al*., 2016; Gemechu Chala *et al*., 2021).

In the present study, *Campylobacter* species was isolated from chickens in both urban and rural settings. However, its occurrence differed statistically. The odds of being colonized by *Campylobacter* species were more likely to occur in chickens from rural areas than those in urban areas. This finding agrees with a study conducted by Mdegela *et al*. (2006) in Tanzania which stated that chickens in rural areas were significantly more infected with thermophilic *Campylobacters* than those in urban areas. This might be due to treatment and other disease control measures, which are commonly practiced, in chickens kept in urban areas. In contrast, a crossectional study carried out by Tesfaye Kassa *et al*. (2005) revealed that there was a high prevalence of *Campylobacter* bacteria in chickens in urban settings that have extensive indoor systems than the rural settings where animals are reared under free-range conditions. The reason for more probability to be positive with *Campylobacter* species in chickens from rural areas might be the increased risk of exposure to multiple sources of contamination from environmental reservoirs such as farm or wild animal populations, untreated water sources, or soil.

Findings from the present study showed that the odds of chickens kept with other animals were more likely to be *Campylobacter* infected than those chickens kept separated. Consistent with this finding a study conducted in Senegal reported a similar observation, in which keeping chickens with other animals on the farm significantly increase the risk for flock

infection with *Campylobacter* (Cardinale *et al*., 2004). On the contrary, a study conducted in Iceland indicated that the presence of other domestic livestock on the farm was associated with a decreased risk of *Campylobacter* colonization (Guerin *et al*., 2007). The justification for this according to Guerin *et al*. (2007) was that Icelandic producers that raise domestic livestock in addition to broilers take precautions that prevent contamination of the broiler houses, such as increased efforts at biosecurity and sanitation practices. Since, other domestic livestock species act as reservoirs; they could contaminate the environment thereby providing a continual source of bacteria to the chickens. This might be the possible explanation for this finding in the present study.

The present study confirmed that chickens from households that had working latrines were less likely to be *Campylobacter* positive than those from without working latrines. This might be because people from households that had no working latrine had the practice of open backyard defecation which could contaminate the environment as result scavenging chickens in that areas could get colonized with *Campylobacter*. A study conducted in Gondar reported that high infection rates were seen in under-five children whose families didn't use the latrine regularly and those whose families had no latrine in their home (Ayalew Lengerh *et al*., 2013). However, this finding was inconclusive due to a lack of reference data on chickens.

The present study found that the risk of *Campylobacter* colonization was higher for chickens from the household that had practiced backyard spread of manure compared to disposal outside the backyard. Results reported from previous studies were variable and sometimes contradictory. According to Cardinale *et al*. (2004) an elevated risk of *Campylobacter* colonization was associated with manure spread inside the farm compared to disposal outside the farm, presumably through continual contamination of the environment. In contrast, a longitudinal study carried out by Guerin *et al*. (2007) revealed that the practice of spread of manure on the farm was associated with a decreased risk of colonization. Although *Campylobacter* tends to die out rather than multiply in an environment under normal conditions, the transmission of the organism to subsequent flocks can occur if the old manure is cross-contaminated and the growth conditions are favorable (high humidity and temperature) (Cardinale *et al*., 2004).

Limitations of the study

This study was a cross-sectional study therefore seasonal variation could not be addressed. In addition, drug susceptibility was not. Despite, these limitations objectives of the study were achieved and discussed.

Chapter 6. CONCLUSION AND RECOMMENDATIONS

Campylobacter species are the most common bacterial causes of human gastroenteritis around the world. Our study identified that *C. jejuni, C. coli, C. lari*, and unidentified *Campylobacter species* were prevalent in the study area. The relatively high proportion of *Campylobacter* species in chicken fecal samples posed potential risks for human campylobacteriosis. In addition the present study indicated that keeping chickens with other animals, disposal of manure inside the backyard and absence of working latrine increased the risk of *Campylobacter* colonization in chickens**.** Since the available evidence shows that chickens are the major reservoir for these organisms**,** reduction in human campylobacteriosis should be pursued by reducing *Campylobacter* colonization in chickens.

Thus based on the above conclusion the following recommendations are forwarded:

- Backyard chicken owners must separate chickens from human as well as other domestic animal environment
- Backyard chicken owners should use latrine to reduce colonization of chickens by Campylobacter species
- Backyard chicken owners should have good hygienic practice while rearing chickens to reduce the incidence of human campylobacteriosis
- Further detailed epidemiological and molecular studies should be carried out on *Campylobacter* species in different species of animals in the country
- Future research questions should be broadened to identify *Campylobacter* species other than thermophilic *Campylobacter* species
- A One Health approach should be employed to investigate *Campylobacter* species infections in livestock-owning society

Chapter 7. REFERENCES

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CHAPTER 8. ANNEXES

Annex 1:- Questionnaire format in English and Amharic version

Date: _____ Household ID: _____ Chicken ID: _____

1. Location of the household A). Urban B). Rural
2. Number of chickens in the household?
3. Age of chicken?
4. Sex of chicken A). Female B). Male
5. Breed of chicken A). Local B). Cross C). Exotic
6. Did you keep chickens during the day in your house? A). Yes, B). No
7. Did you keep chickens during the night in your house? A). Yes, B). No
8. Did you have other animal species than chickens in the household?

A). Yes

B). No

9. If the answer is yes for question number 8 which type of animal species do you have? If there is more than one species you can circle it

A). Cattle

B). Sheep

C). Goat

D). Dog

E). Cat

10. Did chickens have contact with other animal species in the household?

A). Yes

B). No

11. Where did you get the water for the chickens?

A). from the river

B). Wall

C). Tap

12. Water treated with chlorine? A). Yes B). No
13. Where do you dispose of the manure of animals?

A). Inside the backyard

B). Outside the backyard

14. Did you use additional bedding material for chickens?

A). Only earth

B). use additional materials like hay, perch

15. Is the house of chickens clean (Annex 4.) ? A). Yes B). No

16. Did you have a working latrine? A). Yes B). No

Amharic version

1. ቤቱ የሚገኝበት ቦታ? ሀ). ከተማ ለ). ገጠር

2. በቤት ውስጥ የሚገኙ የዶሮ ብዛት?

3. የዶሮው/ዋ እድሜ?

4. ጾታ ሀ).ወንድ ለ). ሴት

5. የዶሮው/ዋ ዝርያ? ሀ). ሐገር በቀል ለ) ድቅል መ) የውጭ

6. ዶሮወቹ ቀን ቀን ቤት ይውላሉ? ሀ). አወ ለ). አይ

7. ዶሮወቹ ማታ ማታ ቤት ያድራሉ? ሀ). አወ ለ). አይ

8. ሌሎች የእንሰሳት ዝርያወች በግቢያቹህ ውስጥ አሉ? ሀ). አወ ለ). አይ

9. አወ ከሆነ መልስወ የትኞቹ እንሰሶች ናቸው ያሉት ከአንድ በላይ ካለ ያክብቡት?

ሀ). ከብት

ለ). በግ

ሐ).ፍየል

መ).ውሻ

ሠ).ድመት

10. ዶሮወቹ ከእነዚህ እንሰሳት ጋረ ንክኪ አላቸው? ሀ). አወ ለ). አይ

11. ለዶሮወቹ ውሃ ከየት ነው የምታገኙት? ሀ). ከወንዝ ለ). ከጉድጓድ መ). ከቧንቧ

12. ውሃው በክሎሪን የታከመ ነው? ሀ). አወ ለ). አይ

13. የእንሰሳትን ጽዳጅ የት ነው የምታሰወግዱት? ሀ). ከጓሮ ለ). ከጓሮ ውጭ

14. ለደሮወች መተኛ ተጨማሪ የምትጠቀሙት ነገር አለ? ሀ). አወ ለ). አይ

15. የዶሮወች ቤት ንጹህ ነው? ሀ). አወ ለ). አይ

16. የሚሰራ መጸዳጃ ቤት አለወት? ሀ). አወ ለ). አይ

Annex 2. Preparation of culture media

2.1 Preparation of mCCDA Code**:** CM0739

The media was prepared according to the manufacturer's instructions (Oxoid CM 0739). 22.75gm of *Campylobacter* blood-free selective agar base was suspended in 500 ml of distilled water and brought to boiling point to dissolve the solids. Then it was sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 50°C in the water bath and poured into sterile Petri dishes.

2.2. Preparation of Mueller Hinton (Techno Pharmchem, India, Delhi)

Direction: 38g of the powder was suspended in 1000 ml distilled water; mixed thoroughly and heat to boiling with frequent agitation to dissolve the powder completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled down to 45-50°C in the water bath. 50 ml of sheep blood was added aseptically. Then it was mixed well and poured into sterile Petri dishes.

Annex 3. Biochemical identification

3. 1. Gram staining

Using a sterile loop a light suspension of organisms in sterile distilled water on a clean microscope slide was prepared. It was air-dried and fixed by heat by passing the slide twice through a gas flame and allowed to cool. Then the slide was placed on a staining rack and flooded with crystal violet solution and left for 30 seconds before being washed off with running tap water. Again the slide was flooded with iodine solution and left for 30 seconds before being washed off with running tap water. To decolorize, ethanol was run over on the film and washed off immediately with running tap water. After that safranin solution was flooded over the slide and left for 1 minute before washed off with running tap water. Then it was gently blotted and allowed to air dry. Finally, an oil immersion was added to the film and examined under the microscope using the 100 lens. Microorganisms that appear dark purple are Gram-positive; those that are pink are Gram-negative. *Campylobacter* species are gram-negative.

3. 2. Catalase test

The colony of the organism was emulsified in a drop of 3% Hydrogen peroxide on a glass slide. A positive test was indicated by the immediate formation of bubbles. *Campylobacter* species are catalase positive

3. 3. Oxidase test

About 3 drops of freshly prepared oxidase reagent (10g/l solution tetramethyl-p-phenylenediamine dihydrochloride) were added to a piece of filter paper. With a piece of match stick, a colony of the organism was smeared on the filter paper. A positive test was indicated by the development of a blue-purple color within a few seconds. *Campylobacter* species is oxidase positive.

3.4. Preparation of 3.5% ninhydrine and 1% hippurate solution (Eastman)

- 3.5 gram of ninhydrine was dissolved in 100ml 1:1 mixture of acetone and butanol and used and kept away from sun light.
- 0.5 g sodium hippurate was dissolved in 50 ml water and sterilized through a 0.2 μm membrane filter. Then the solution was dispensed aseptically into srew-cap tubes (0.4 ml/tube). The solution was frozen until used.

3. 5. Cephalothin and Nalidixic acid susceptibility test

Cephalothin and nalidixic acid susceptibility test of the strains isolated was determined by using Kirby-Bauer disk diffusion technique. Mullen-Hinton agar was prepared According to annex 2.2. Suspension was prepared by inoculation of large colony with the loop into 5ml of a sterile saline and adjustment of density to 0.5 McFarland was made. Cephalothin (30 μg) and nalidixic acid (30 μg) (Himedia) disks were applied and the plate was incubated at 37°C in microaerophilic conditions achieved by anaerobic jar with candle for 48 hours. After 48 hours of incubation, the inhibition zones were measured to the nearest millimeter using a ruler. Since, the size of inhibition zone and break-points for *Campylobacter* are not yet standardized the diameters of inhibition zones were measured and interpreted on the bases of EUCAST, 2019 interpretive criteria for Enterobacteriaceae to classify as sensitive and

resistant. *E.coli* (ATCC 25922) was used as control strains. Interpretive criteria for Nalidixic acid: sensitive ≥19mm; resistance ≤17mm. For cephalotin: sensitive ≥17mm; resistance ≤17mm.

Annex 4. Score for cleanness of the house according to Carron *et al*., 2018

Cleaning method for pen *Reference*: *full cleaning (defined as removing litter*,and *cleaning*

- The presence of litter on the ground
- Litter removed+ cleaned
- Litter removed+ cleaned + disinfected
- However we only take the first two for our study

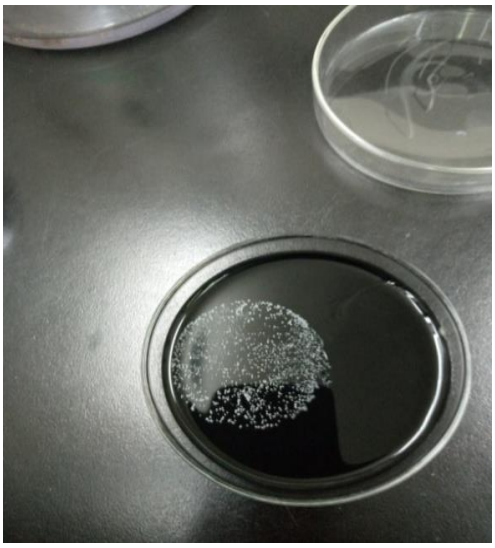
Annex 5. Result record form

Sample code	Colony morphology		Gram staining		Oxidase test		Catalase test		Hippurate test		Nalidixic acid		Cephalothin	
	Campy.	Not *C.*	P	**N**	P	N	P	N	P	N	S	R	S	R

Key:-P = Positive, N =Negative, S= Sensitive, R= Resistance, *Campy*. = *Campylobacter*, Not *C.* = Not *Campylobacter*

Annex 6. Images of laboratory works

Fecal suspension on filter on mCCDA

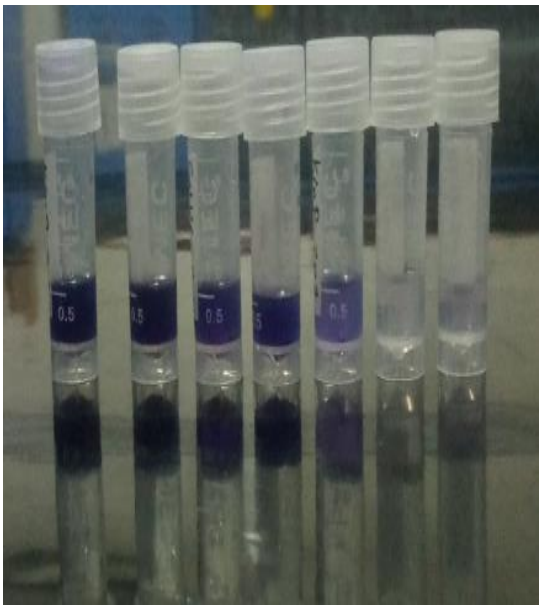


Colony of *Campylobacter* species

Growth of subcultured *Campylobacter* species

Observation of gram stained smears

Microscopic image of *Campylobacter* spp



Identification *C. jejuni*

Plates with lighting candle in anaerobic jar

Journey to sample collection