Websites:
http://www.nbmedicine.org
http://www.sciencepub.net/nurse

Emails:
editor@sciencepub.net
nbmeditor@gmail.com

Biomedicine and Nursing

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Occurrence of *Escherichia Coli* O157:H7 in lactating Cows and dairy farm environment and its antimicrobial susceptibility pattern at Adami Tulu Jido Kombolcha district, mid Rift Valley, Ethiopia

Frehiwot Mesele[1], Kebede Amenu[2], Samson Leta[2] and Fufa Abunna[2]

[1]Adami Tulu Agricultural Research Center, P.O. Box: 35, Ziway, Ethiopia
[2]Addis Ababa University, College of Veterinary Medicine and Agriculture, P.O. Box 34, Bishoftu, Oromia, Ethiopia
*Corresponding author: e-mail: fufa.abunna@aau.edu.et, Mobile: +251-911899435

Abstract: **Objectives**: **A**ssessing the occurrence of *Escherichia coli* O157:H7 in lactating cows and in dairy environment and to determine its antimicrobial resistance pattern. **Methods**: A cross sectional study was conducted from December 2017 to June 2018 on apparently healthy lactating cows at Adami Tulu Jido Kombolcha district. A total of 408 samples (water, milk, manure and feces) were collected and processed according to OIE terrestrial manual 2016. **Results**: From 408 samples collected and processed, 19 samples were positive for *E. coli* O157:H7. The overall prevalence of *E. coli* O157:H7 was 4.7% (95% CI: 2.6; 6.7). Of 19 *E.coli* O157:H7 isolates, water samples (4/50), milk samples (7/154), manure samples (2/50) and fecal samples (6/154). The multivariable logistic regression analysis indicated that, the prevalence of *E. coli* O157:H7 was significantly ($p < 0.05$) higher for areas (urban, rural), floor type, cleaning of pens, milking location and hand washing during the time of milking. On the contrary, breed, herd size, use of towel and detergent, and history of mastitis did not show any significant difference ($p > 0.05$). All 19 *E.coli* O157:H7 isolates were subjected to *in vitro* antimicrobial sensitivity test to ten commonly used antimicrobials. Accordingly, a varying degree of resistance; 100% resistance was observed for ampicillin, cephalothin and rifampin and 100% susceptibility was observed for chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline. With regard to streptomycin, 63.15% of the isolates were susceptible and 36.8% were intermediate. All 19 *E.coli* O157:H7 isolates showed the presence of multidrug resistance. **Conclusion**: The occurrence of *E. coli* O157:H7 was observed both in lactating cows (milk and feces) and dairy farm environment (manure and water) sustaining a continuous transmission of the bacteria. The development of multidrug resistance could hamper the control and prevention effort. Therefore, strict control measures such as treatment of positive cases using effective drugs and prevention measures such as strict hygiene practices should be established, including cleaning of floor, pens and milking barns as well as proper hand cleaning and use of clean towel.

[Frehiwot Mesele**,** Kebede Amenu, Samson Leta and Fufa Abunna. **Occurrence of *Escherichia Coli* O157:H7 in la ctating Cows and dairy farm environment and its antimicrobial susceptibility pattern at Adami Tulu Jido Ko mbolcha district, mid Rift Valley, Ethiopia.** *Biomedicine and Nursing* 2023;9(3):44-55]. ISSN 2379-8211 (print); I SSN 2379-8203 (online). http://www.nbmedicine.org 07. doi:10.7537/marsbnj090323.07.

Keywords: *Lactating cows, E. coli O157:H7, Dairy farms, Antimicrobial susceptibility test, Ethiopia*

1. Introduction

Escherichia coli is a Gram-negative, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of most mammalian species (Tenaillon et al., 2010). Most *E. coli* are commensal and harmless, but small proportions are an important cause of disease worldwide. The pathogenic *E. coli* are classified into different strains based on the production of virulence factors and on the type pf clinical disease they cause (Caprioli *et al.*, 2005; Fairbrother and Nadeau, 2006; Pennington, 2010). The category of *E. coli* strains producing Shiga toxins (Stx) is referred to as verotoxinogenic *E. coli* and Stx producing *E. coli* (STEC). Shiga toxin-producing *E. coli* (STEC), also called verotoxinogenic E. coli, usually do not cause clinical disease in animals but may cause a life threatening disease in humans. Zoonotic STEC include *E. coli* O157:H7 strains and these strains are increasing being reported globally (Fairbrother and Nadeau, 2006; Preussel *et al*., 2013).

Strains of *E. coli* O157:H7 are commonly found in a wide range of farm animals. The primary reservoir host of *E. coli* O157:H7 is cattle, in the cattle gut, the bacteria lives in symbiosis with its host (Fairbrother and Nadeau, 2006; Money *et al*., 2010). The bacteria can sometimes be found in other mammals including pigs, rabbits, horses, dogs, domestic, and wild birds (Caprioli *et al*., 2005; Faith *et al*., 1996; Rice and Johnson, 2000; Saeedi *et al*., 2017; Wallace *et al*., 1997). The bacteria are transmitted to humans through the ingestion of foods or water contaminated with animal feces, or through direct contact with the infected animals or contaminated farm

environment. The main sources of *E. coli* O157:H7 infections for cattle are contaminated feed and water, and the immediate environment of the animal (Caprioli *et al*., 2005; Fairbrother and Nadeau, 2006). Risk factors that have been identified for infection of animals with *E. coli* O157:H7 include age, weaning, movement of the animals, season, feed composition, and the ability of the bacteria to persist in the environment (Fairbrother and Nadeau, 2006; Saeedi *et al*., 2017).

Farm environment is the main factor when it comes to sustaining a population of viable bacteria. The bacteria have been shown to survive in feces, manure, on pen surfaces, bedding and flooring and water (Awadallah *et al*., 2016; Davis *et al*., 2005; LeJeune *et al*., 2001; LeJeune and Wetzel, 2007; Money *et al*., 2010; Sargeant *et al*., 2004). Combinations of urine and bedding have been found to enhance the growth of *E.coli* O157:H7 (Davis *et al*., 2005; LeJeune and Wetzel, 2007) further emphasizing the importance of clean pens and beds. Cleaning, change of bedding material and frequent removal of feces could ensure that populations do not buildup and colonize the space. Bedding moistened with bovine urine was found to more frequently contain higher levels of *E.coli* O157:H7. Manure could allow prolonged survival of the bacterium outside the host (Kudva *et al*., 1998) and should therefore be removed as frequently as possible. Contaminated animal drinking water may contribute to the dissemination and/or maintenance of *E. coli* O157:H7 on farms (LeJeune *et al*., 2001).

Antibiotics are often used for therapy of infected humans and animals as well as for prophylaxis. Inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult (Kolar, 2001). Antimicrobial resistance in E. coli has been reported worldwide. Treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (Sabate, 2008) including ampicillin, amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, methicillin, tetracycline and vancomycin (Constable *et al*., 2017; Vijayarani *et al*., 2010). The development of antimicrobial resistance in *E. coli* O157:H7 is the matter of increase concern and generate new public health challenge (Newell *et al*., 2010).

The safety of food with respect to *E. coli* O157:H7 is one of great concern around the world. This is especially true in developing countries like Ethiopia, where production of food often takes place under unsanitary conditions. Consumptions of different food items particularly animal origin like milk which can be contaminated by feces, manure and contaminated water in dairy farms are the major source of infection for humans. Different research works have been undertaken in Ethiopia on the prevalence of *E.coli* O157:H7 and antimicrobial susceptibility test were also performed on *E.coli* O157:H7 isolated from samples such as carcass, meat and environmental samples but additional studies is needed to verify gap on epidemiological linkages on raw milk, feces, water and manure in dairy farms particularly in the study area. Thus, this study aimed to investigate the occurrence of *E.coli* O157:H7 from dairy cattle and dairy environment. In addition, risk factors associated with *E.coli* O157:H7 occurrence and antimicrobial profile of the isolates were evaluated. The general objective of this study was to assess the occurrence of *E. coli* O157:H7 in lactating cows and dairy farm environment and its antimicrobial susceptibility profile at Adami Tulu Jido Kombolcha district.

2. Materials and Methods

3.1. Study area

Adami Tulu Jido Kombolcha district is found in the mid-Rift Valley at 7° 9‘N latitude and 38° 7 ‘E longitude. The altitude of the district is ranges 1500-2300 m above sea level. The average annual rainfall of the area is 760.9 mm. The main climate type is semi-arid. Minimum and maximum annual mean temperatures are 14 and 27 ^{0}C respectively. Livestock production is the dominant farming system and crop production is not common. Dairy cattle are mostly reared in small-scale dairy operations, in which animals are managed both intensively and extensively. It has a light texture soil class, which is vulnerable to both wind and soil erosion. Fishing and horticulture are the major basis of economy in the area (Abdissa *et al*., 2011; Jergefa *et al*., 2009).

The district is characterized by bimodal pattern of rainfall; with short rainy season running from February to April and long rainy season from June to September. However, the pattern of rainfall is usually erratic with fluctuations in the start and end of the season, in addition to the total absence of rainfall at times (Shiferaw, 2008).

3.2. Study population

The study population was lactating dairy animals in ATJK district. There is an estimated 68 small and 22 medium scale dairy farms in the district. The study population comprises exotic, cross and local breeds in small and medium scale dairy farms managed under intensive and extensive management conditions. They are often provided with some supplementary diet in addition to the natural pasture and agricultural by products.

3.3. Study design

A cross-sectional study was conducted from December 2017 to June 2018 to estimate the occurrence of *E. coli* O157:H7 and to assess the antimicrobial

susceptibility profile of isolates. Raw milk and feces samples were collected from teat and rectum of selected dairy cows, respectively. From dairy farm environment, manure and water samples were also collected. Semi structured questionnaire was used to collect risk factors associated with the occurrence of *E. coli* O157:H7 in lactating cows and dairy farm environment.

3.4. Sample size determination

Sample size were determined by using the formula given by (Thrusfield, 2005) with assumption of 95% confidence interval and 5% precision.

$$n = \frac{z^2 * P_{exp}(1 - P_{exp})}{d^2} = \frac{1.96^2 * 0.104(1 - 0.104)}{0.05^2} = 143$$

Where, n= required sample size

Z= alpha value of 95% confidence interval

d=desired absolute precision

The expected prevalence was set at 10.4% based on previous study conducted by (Abebe et al., 2014). Additional 11 samples were incorporated and the sample size became 154. Thus, 154 dairy cows in 50 dairy farms were sampled. The total sample size then became 408 (154 milk sample, 154 feces sample, 50 water and 50 manure samples).

3.5. Sampling methods

Stratified random sampling method were undertaken to categorize farms based on their herd size in to three stratums (small scale, medium scale and large scale commercial farms) using the classification made by Megersa *et al.* (2011) as a reference, small scale (<10 animals), medium (10 to 50 animals) and large (>50 animals). Based on the data obtained from the district livestock and fishery agency, there are no large scale dairy farms in the district. The district has 22 medium and 68 small scale dairy farms. A simple random sampling method was used to select farms from each stratum and to select individual animals in the selected farm. The farm owners were then interviewed using a semi structured questionnaire.

3.6. Sample collection

All collected samples were tagged by sample ID, date of sampling and sample type. The samples were transported to the veterinary bacteriology laboratory, College of Veterinary Medicine and Agriculture of the Addis Ababa University using ice box in cold chain for microbiological analysis. Up on arrival, the samples were stored in refrigerator at 4°C for 24 hours until being processed for isolation as described by (Quinn *et al.*, 2004).

3.6.1. *Milk sample*

Milk samples were collected directly from teats. The udder and teats were thoroughly cleaned and dried before sampling; each teat was rubbed gently with cotton swabs moisturized with 70% ethyl alcohol. The first 3–4 streams of milk were discarded, and approximately 5ml of milk was collected aseptically by sterile screw topped universal bottle and the sample were transported using an ice box (4^{o}C) for further processing and microbiological analysis. Isolation and identification of *E. coli* O157:H7 from milk samples were passed on the basis of colony morphology in different media, staining characteristics and biochemical properties (ISO 18593, 2004).

3.6.2. Fecal sample

The fecal samples were collected using sterile stomacher bag aseptically directly from rectum and stored in ice box and ice pack until analysis (within 24 hours). Milk and feces samples were collected from the same dairy animals.

3.6.3. Water sample

Water samples (10ml) were collected using sterile capped universal bottle from the selected dairy farms pipe, and wide mouthed containers such as barrels, plastic buckets and jugs that was used for animal drinking, hand and udder washing.

3.6.4. Manure sample

Pooled manure samples were also collected from the selected dairy farms using sterile stomacher bag from different points including pen, floor surface and dung storage area.

3.7. Questionnaire survey

A pre-tested semi structured questionnaire was used to collect additional data on demographic characteristics, milking system, milking and hygienic practices (washing of milkers' hand, milk utensils and udder before milking), farmers' awareness of cattle and milk-borne zoonoses, transmission routes, sources of farm water, housing management. The interview was made in local language (Afaan Oromo/Amharic). All 50 farm owners were interviewed through face to face conversation.

3.8. Laboratory work

Nonselective pre-enrichment is necessary for the effective recovery of low levels of stressed *E. coli* O157. Then enrichment broths were pre-warmed to prevent cold-shocking the organisms and slowing their initial growth. Milk and water samples were enriched using 1ml/9ml buffered peptone water and incubated at 37°C and 41.5°C, respectively for 24 hours. Similarly

feces and manure samples were enriched with 25g/225ml BPW and incubated at 37°C for 24 hours to increase recovery of the organisms (OIE, 2016.). All enriched samples were cultured on sterilized Sorbitol MacConkey agar plate, (CM0813, Oxoid Basingstoke, England). Then the plates were incubated at 37°C for 24 hours. The SMAC agar plates were examined for the presence of non-sorbitol fermenting (colorless) colonies. Then, up to six colorless colonies (non-Sorbitol fermenters) on SMAC agar were taken and sub-cultured separately on MacConkey agar (Oxoid) and incubated for 24 hours at 37°C for clarification. Then the confirmed pure cultures considered as *E. coli O157:H7* positive was transferred to nutrient agar to be used for additional biochemical and serological confirmation (Quinn *et al.,* 2004). The sub-cultured and purified colonies were tested for hydrogen sulphide and indole production using Triple Sugar Iron agar (TSI) slant (Oxoid) and Indole production test. The isolates giving a result of yellow slant and butt with gas but no hydrogen sulfide (Y/Y/ H2S -) production on TSI slant agar after incubation of the media at 37°C for 24 hours were kept with tubes capped loosely to maintain aerobic conditions. Indole test was carried out using one pure colony inoculated into 4 ml of tryptone soya broth (Oxoid) with a straight inoculation wire. Incubation was done for overnight at 37°C. Then one drop of Indole (Kovac's) reagent was added to the tryptone soya broth culture to test for indole production (formation of red ring indicating positive reaction). Tryptone soya broth and indole positive colonies were then confirmed using Oxoid Dryspot *E. coli* O157 latex test kit. The Dry spot *E. coli* O157 latex test confirmed by agglutination of *Escherichia* strains possessing the O157 serogroup antigen. One drop of saline was dispensed to the small ring (at the bottom of each oval) in both the test and control reaction areas ensuring that the liquid did not mixed with the dried latex reagents. Using a sterile single use plastic loop, a portion of the colony to be tested was selected and carefully mixed in the saline drop until the suspension was smooth. Then, using paddle the suspension was mixed into the dry latex spots until completely suspended and spread to cover the reaction area. The test card picked up and stirred for up to 60 seconds and looked for agglutination under normal lighting conditions. A result was considered positive if agglutination of the latex particles occurs within 1 minute. This indicates the presence of *E. coli* serogroup O157. A negative result is obtained if no agglutination occurs and a smooth blue suspension remains after 60 seconds in the test area.

Antimicrobial susceptibility tests were performed by standard disc diffusion technique using commercially available antimicrobial disks and recommended from the guideline of antimicrobial susceptibility testing from CLSI (2015). Antimicrobial disks containing Ampicillin (10µg), Cephalothin (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Gentamicin (10µg), kanamycin (30µg), Nalidixic acid (30µg), Rifampin (5 µg), Streptomycin (10µg) and tetracycline (30µg) (HI media, India) were used.

Serologically confirmed colony from pure fresh culture was transferred in to a test tube of 5 ml tryptone soya broth (TSB) (Oxid, England) and incubated at 37ºC for 6 hours. Then turbidity of the culture broth was adjusted using sterile saline solution or by addition of more isolated colonies to get turbidity analogous with that of 0.5 McFarland standards (approximately 3x108 CFU per ml). A Mueller-Hinton agar (Oxid, England) plate was ready according the manufacturer. Then after sterile cotton swab was immersed into the suspension and revolved against the side of the tube to remove the excess fluid and then swabbed in three directions homogeneously on the surface of the plates. After the inoculated plates dried, antibiotic disks were placed by the help of sterile forceps. The disks were slowly pressed onto the agar to ensure stable contact with the agar surface, and incubated at 37ºC for 24 hours. Subsequent the diameter of inhibition zone created around each disk was measured using digital caliper by laying it over the plates. The results were classified as sensitive, intermediate and resistant according to the standardized table supplied by the manufacturer CLIS (2015).

3.9. Data management and statistical analysis

Data were entered to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Descriptive statistics (determination of proportions) were used to summarize the data. The overall prevalence of *E. coli* O157: H7 in milk, feces and environmental samples were estimated using standard formula. The number of positive samples were divided by the total number of samples examined multiplied by 100. R statistical software Version 3.3.2 (R Core Team, 2016) was used to analyze the data. Pearson chi-square, Pearson's Chi-squared test with Yates' continuity correction and fisher exact tests were used to association of different risk factors with the occurrence of *E. coli* O157:H7. Univariable and multivariable logistic regression analysis were performed to quantify crude and adjusted effect of the risk factors on the occurrence of *E. coli* O157: H7. The stepAIC function in 'MASS' package was used to select the final multivariable logistic regression model. P-value less than 5% ($P<0.05$) was considered statistically significant. Conditional logistic regression was used to assess the presence stratification and matching in the data using 'clogit' function in 'survival' package, but there is no difference between the binary and conditional regression models and thus, the binary logistic regression results were used. In cases of estimating the effect of different risk factors

in terms of Odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its value. For antimicrobial susceptibility test the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2015) 'interpretive criteria for *Enterobacteriaceae*.

3.10. Ethical consideration

Before collection of samples undertaken the protocol approved by Addis Ababa University, collage of veterinary medicine animal research ethical committ ee with reference number VM/ERC/28/05/10/2018.

4. Results

4.1. Occurrence of *E.coli* O157:H7

Out of 408 samples collected and processed, 19 were positive for *E. coli* O157:H7. The overall prevalence of *E. coli* O157:H7 was 4.7% (95% CI: 2.6; 6.7). Of these positive cases, the isolation of *E.coli* O157:H7 was the highest in water sample 4(8%), followed by milk samples 7 (4.54%), in manure 2(4%) and 6 (3.89%) (Table 1).

Table (1): Occurrence of *E.coli* O157:H7 in different sample types

Sample type	Total sample examined	*E. coli* isolates	*E. coli* O157:H7 strains
Milk	154	15(9.74)	7(4.54)
Feces	154	16(10.4)	6(3.9)
Water	50	6(12)	4(8)
Manure	50	5(10)	2(4)
Overall	408	42(10.3)	19(4.7)

4.2. Univariable analysis of the association of *E. coli* O157:H7 with different risk factors

The effect of potential risk factors on the occurrence of *E.coli* O157:H7 was assessed and from the risk factors considered, cleaning of pens, milking location, use of towel and hand washing during the time of milking had a statistically significant impact on the occurrence of *E. coli* O157:H7 ($P< 0.05$) using univariable logistic regression analysis. On the contrary, factors such as breed of the animal, herd size, area, floor type, use of detergent and history of mastitis did not show significant difference ($p > 0.05$) (Table 2).

Table (2): Univariable logistic regression analysis of *E.coli* O157:H7 occurrence with various risk factors

Risk factors	*E. coli* O157:H7					
	No. examined	No. of positive	X^2	P-value	Crude OR	95% CI OR
Area						
Rural	120	8	0.61	-	-	-
Urban	269	11		0.306	0.61	0.24 - 1.6
Breed						
Exotic	101	1	*	-	-	-
Cross	183	13		0.059	7.17	0.93 - 55.65
Local	105	5		0.155	4.81	0.55 - 41.89
Cleaning of pens						
No stay overnight	133	13	7.81#	-	-	-
Stay overnight	256	6		0.005**	4.17	1.61 - 12.5
Herd size (Farm scale)						
Medium scale	137	3	2.23	-	-	-
Small scale	252	16		0.095	2.90	0.94 - 12.62
Milking location						
In barn	151	13	5.43#	-	-	-
Anywhere	238	6		0.005**	3.45	1.32 – 10.0
Hand wash						
Before and after milking	377	15	*			
Only before milking	12	4		0.000***	8.37	2.15- 27.49
Floor type						
Earthen	214	8	0.75#	-	-	-
Concrete	175	11		0.275	1.68	0.67 - 4.42

Use of towel						
No use of towel	96	8	*	-	-	-
Before milking	174	9		0.310	0.6	0.22 -1.61
Before and after milking	138	2		0.023	0.16	0.03- 0.78
Use of detergent						
No	52	2	*	-	-	-
Yes	356	17		0.767	1.25	0.35-8.06
History of mastitis						
No	104	8	*	-	-	-
Yes	304	11		0.096	0.45	0.18-0.19

Keys: *** Significant level (P< 0.001), ******Significant level (P< 0.01), ***C**omputed using fisher exact test, - reference, # Pearson's Chi-squared test with Yates' continuity correction

4.3. Multivariable analysis of the association of *E. coli* O157:H7 with different risk factors

From potential risk factors considered in this study (Table 3), area, floor type, cleaning of pens, milking location and hand washing during the time of milking were significantly associated (P< 0.05) with the occurrence of *E. coli* O157:H7. As shown in Table 3, the odds ratio of *E. coli* O157:H7 occurrence of was 9.32 times higher in urban areas than rural areas. In pens where the feces stay overnight, the odds ratio of *E. coli* O157:H7 occurrence is 50 times higher. Animals which were milked anywhere in the farm had 16.67 times at higher risk compared to animals which are milked in a milking barn. Hand washing practice had also a significant impact on the occurrence of *E. coli* O157:H7, the odds ratio of *E. coli* O157:H7 occurrence in farms where hand washing is practiced only before milking were 8.51 times higher when compared with farms where before and after milking hand wash is practiced. Farms with concrete floor were 48.74 times at higher risk when compared with farms with ordinary floor type (earthen floor).

Table (3): Multivariable logistic regression analysis of *E.coli* O157:H7 occurrence with various risk factors

Risk factors	Adjusted OR	95% CI OR	p-value
Area			
Rural	-	-	-
Urban	9.32	1.79 - 48.60	0.008**
Cleaning of pens			
No stay overnight	-	-	-
Stay overnight	50	7.69-500	0.000***
Milking location			
In barn	-	-	-
Anywhere	16.67	3.03-83.33	0.001**
Hand wash			
Before and after milking	-	-	-
Only before milking	8.51	1.88 - 38.49	0.005**
Floor type			
Earthen	-	-	-
Concrete	48.74	3.49 - 680.66	0.004**

Keys: *** Significant level (P< 0.001), ****** Significant level (P< 0.01), ***** computed using fisher exact test, - reference

4.4. Antimicrobial susceptibility pattern of isolates

All isolates were subjected to *in vitro* antimicrobial sensitivity test to ten commonly used antimicrobials. The test result indicates (Figure 1) varying degree of resistance; 100% resistance was observed for Ampicillin, Cephalothin and Rifampin and 100% susceptibility was observed for chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline. With regard to streptomycin, 63.15% of the isolates were susceptible and 36.8% were intermediate. All of them showed the presence of multidrug resistance. Multidrug resistance refers to resistance of single isolate against more than two drugs).

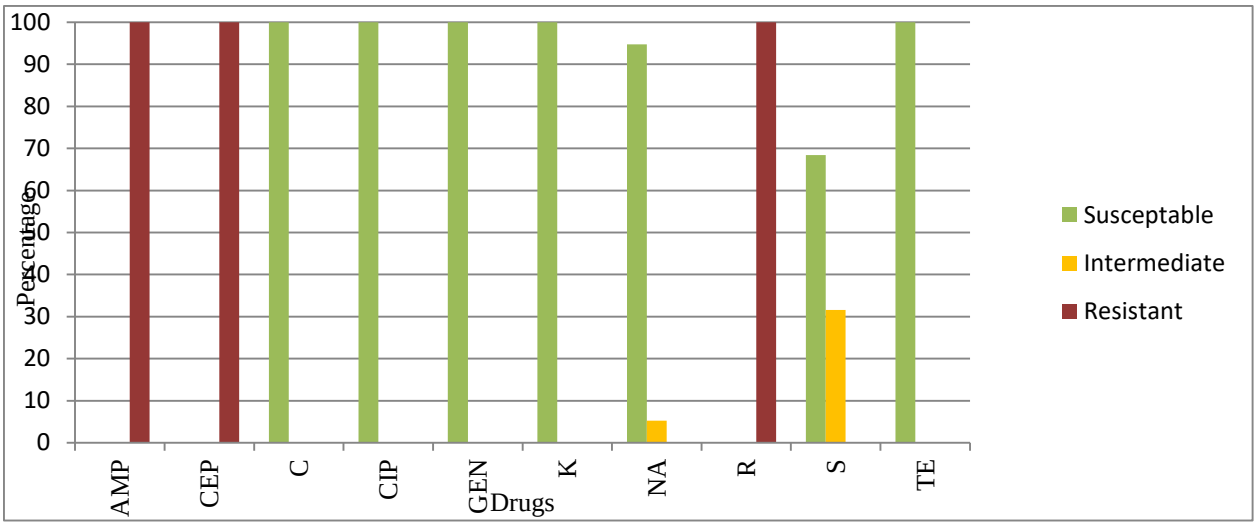

Figure 1: Antimicrobial susceptibility pattern of *E. coli* O157:H7 to ten antimicrobials
Key: AMP: ampicillin, C: chloramphenicol, CIP: ciprofloxacin, K: kanamycin, NA: nalidixic acid, S: streptomycin, TE: tetracycline.

5. Discussion

In Ethiopia, of *E. coli* O157:H7 is considered to be an important challenge for the dairy development and public health development. This study also indicates *E. coli* O157:H7 to be the major dairy development challenge in the study area. The prevalence of *E. coli* O157:H7 in lactating cows (milk and feces) and dairy farm environment (water and manure) at ATJK district found to 4.7%. The result is in line with the result reported by Bedasa et al. (2018) who reported a prevalence of 3.5% in food of animal origin in Bishoftu town, Central Ethiopia. This result also supports previous evidence (Fairbrother and Nadeau, 2006; Ferens and Hovde, 2011; Hancock *et al.*, 1998; Sami and Firouzi, 2007; Wells *et al*., 1991) that cattle are asymptomatic animal carriers a major reservoir of *E. coli* serotype O157:H7. Carrier animal excretes the organism occasionally and in low numbers in feces (Rahn et al., 1997). Cattle and their feces have been considered as the primary source of *E. coli* O157:H7 (Shere *et al*., 2002; Venegas-Vargas *et al*., 2016).

Raw milk can be a vehicle of transmission for *E. coli* O157:H7 (USDA, 1997). Risk of *E. coli* O157:H7 infection related to consumption of raw milk is high, indicating that there is risk of *E.coli* O157:H7 infection even though the prevalence detected is relatively low (Lye *et al*., 2013). Isolation rate of *E. coli* O157:H7 from raw milk samples similar to that recorded in the current study (4.54%) was slightly in agreement with the report of 2.9% by Disassa *et al*. (2017). But, the prevalence is far lower when compared to the reports of (Bedasa *et al*., 2018) who reported 12% and (Abebe *et al*., 2014) who reported 10.4% from Bishoftu town and Tigray, Ethiopia. In the result of the present study was also comparable with the 8.75% result reported by Lye *et al*. (2013) from Malaysia. The highest occurrence of *E. coli* O157:H7 was reported by Chye *et al*. (2004) who reported 33.5% from Malaysia and Msolo *et al*. (2016) who reported 55% from South Africa. The differences might be attributed to the differences in animal management, milking systems, and milk hygiene and handling practices among different farms in different countries. The detection of *E. coli* O157:H7 in milk is not only a reliable indicator of fecal contamination, but is also an indicator of poor hygiene and sanitary conditions during milking and handling.

In the present study, 3.9% isolation rate of *E. coli* O157:H7 was recorded from feces sample. This is in agreement with prevalence reported by Atnafie *et al.* (2017) and Mersha *et al*., (2010), both reported 4.7% in Hawasa and Modjo, respectively. The result is slightly lower than the 7.26% report by Nazareth (2017) and 9% report by Lupindu *et al.* (2014) from USA and Tanzania, respectively. The result of the present study, is higher than the prevalence reported by (Sami and Firouzi, 2007; Hancock *et al*., 1994; Swirski *et al*., 2014; Faith *et al*., 1996 and Hancock *et al.*, 1998) who reported a prevalence of 0.51, 0.71, 1 and 1.8 and 2.3%, respectively. The difference could be attributed to difference in bacterial isolation and identification method used and differences in farm management and hygienic practices. Isolation of *E. coli* O157:H7 from feces regarded as an important epidemiological information. Infected cattle could shade 10^1 to 10^7 cfu of *E. coli* O157:H7 per gram of feces. Given that typical cattle excrete 20 to 50 kg of feces per day, this provides a large inoculum of *E. coli* O157:H7 for the farm environment and could contaminate dairy products in the presence of poor hygienic practices (Mathhews *et al*., 2014).

From 50 water samples, 4 (8%) were *E. coli* O157:H7 positive. Four of the positive samples were from animal drinking water. The presence of *E. coli* O157:H7 in the drinking water may contribute to the prevalence of infection in cattle, a factor directly related to the contamination of dairy products and the environment. Contaminated water can serve as a vehicle of *E. coli* O157:H7 transmission in cattle, although there was variation among animals in the doses necessary to initiate shedding (Shere *et al*., 2002). Animal drinking water was identified as one source of *E. coli* O157:H7 in the farm (Faith *et al.*, 1996).

In this study, 4% isolation rate of *E. coli* O157:H7 was recorded from manure sample. Farm manure may disseminate, transmit, or propagate *E. coli* O157:H7. Manure is a good vehicle of *E. coli* O157:H7. Manure sewages from cattle houses could result in contamination of the surrounding land, with cattle keepers and their household members being at increased the risk. The survival of *E. coli* O157:H7 in manure depends on many variables, including the level of pathogen shedding by animals, conditions, and duration of manure storage, extraneous microbial interactions within stored manure, and interactions with water (Ziemer *et al*., 2010). A number of researchers have investigated the survival of *E. coli* O157:H7 in manure from various animals, under different conditions such as temperature or aeration, presence of different manure amendments, and at a range of manure-to-soil ratios (Duffy, 2003). Kudva *et al.* (1998) found that *E. coli* O157:H7 survived for more than 21 months in ovine manure at levels ranging up to 106 cfu/g manure. Experiments with artificially inoculated bovine feces have also confirmed the survival of *E. coli* O157:H7 for greater than 40 days, dependent on initial inoculum and holding temperature (Wang *et al*., 1996).

The perceived dissimilarity in the outcome of the current study from other studies could be the differences in husbandry practices and climate usual climatic conditions which may account for the varied prevalence of *E.coli* O157:H7 for different geographical locations. The method and techniques used for identification of bacteria in this study could accountable; immunomagnetic separation (IMS) technique with supplemented enrichment in broth culture has been reported to improve the identification of *E.coli* O157:H7 from samples with low concentration (Ojo *et al*., 2010). In present study buffered peptone water without supplement and IMS was used for enrichment of the samples as described in OIE (2016) that gives good recovery of stressed organism. Enrichment before plating on selective media agar might increase E.coli O157:H7 isolation compared to direct plating of the test samples on selective agar (Hashemi *et al*., 2010).

Many factors were tested for associations with E. coli O157:H7, yet relatively few were significant in the final model. Factors such as area (urban, rural), floor type, cleaning of pens, milking location and hand washing during the time of milking was found to be significantly associated with the occurrence of E. coli O157:H7. E. coli O157:H7 shedding in cattle and its survival in the environment could be multifactorial. No single factor could stands out as the major risk factor for shedding (USDA, 1997). But, factors related to poor hygienic practices were found to affect the occurrence of the bacteria which is consistent with observations in the literature. The multivariable analysis demonstrated a significant association between the presence of *E. coli* in and cleaning pens (OR, 50; 95% CI, 7.69–500; P = 0.000) in farms where the feces stay overnight, the odds ratio of *E. coli* O157:H7 occurrence is 50 times higher. Milking location and hand wash practice were also significantly associated with the occurrence of *E. coli* O157:H7 and animals which were milked anywhere in the farm and hand washing practice 'only before milking' being a risk for high occurrence. Floor type was also significantly associated the occurrence of *E. coli* O157:H7. Farms with concrete floor were significantly at higher risk when compared with earthen floor (OR, 48.74; 95% CI, 3.49–680; P = 0.004). It is important to note that, the use of towel and detergent and history of mastitis were not significantly associated with the occurrence of *E. coli* O157:H7. This suggests that use of towel and detergent alone is unlikely to prevent the presence of E. coli O157: H7 while the other hygienic are poorly practiced. Thus, general hygienic practices might represent a critical control point for reducing transmission of E. coli O157 in dairy farms.

In this study all isolates were resistant to ampicillin, cephalothin and rifampin. Previous study reported high degrees of resistance for *E.coli* O157:H7 originating from cattle (Um *et al.*, 2018). Cabal *et al*., (2013) compared the resistance of *E. coli* O157:H7 strains versus non-O157:H7 *E. coli* isolated from cattle feces. These authors reported a significantly higher proportion of resistant isolates among *E. coli* O157:H7 isolates than in non-O157:H7. The appearance and distribution of multidrug resistance in *E.coli* O157:H7 can serve as a reservoir for different antimicrobial resistance genes (Srinivasan et al., 2007). It is important to note that the isolates are susceptible to most commonly used antibiotics including chloramphenicol, ciprofloxacin, gentamicin (Kibret and Abera, 2011) and tetracycline. Um *et al*. (2018) reported resitant of *E.coli* O157:H7 to tetracycline from France.

6. Conclusion

The current study revealed a substantial occurrence of *E. coli* O157:H7 in lactating cows and dairy farm environment at Adami Tulu Jido Kombolcha district. *E. coli* O157:H7 was isolated from feces, manure, milk and water designating a sustaining transmission of the

bacteria. The occurrence of *E. coli* O157:H7 in milk samples suggests a potential zoonotic risk of raw milk consumption in the area. Factors related to poor hygienic practices such as cleaning of pens, milking location and hand washing were the main factors that backed the occurrence of *E. coli* O157:H7 in the dairy farms. *E. coli* O157:H7 isolates manifested a multi-drug resistance; 100% resistance to Ampicillin, Cephalothin and Rifampin was observed. Antibiotics such as chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline could be considered as first choice drugs as the isolates are susceptible to these drugs.

Recommendations

Strict animal and environment level hygienic practice should be practiced to break a sustained transmission of the bacteria in the farms and physicians in the area should consider chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline as first choice drugs in the treatment of clinical diseases associated with *E. coli* O157:H7.

7. Reference

[1]. Abdissa R, Haile W, Fite AT, Beyi AF, Agga GE, Edao BM, Tadesse F, Korsa MG, Beyene T, Beyene TJ (2017). Prevalence of *Escherichia coli* O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *BMC infectious diseases* **17**: 277.

[2]. Abdissa T, Chali A, Tolessa K, Tadese F, Awas G (2011). Yield and yield components of sweet potato as influenced by plant density: In Adami Tulu Jido Kombolcha District, Central Rift Valley of Ethiopia. *American Journal of Experimental Agriculture* **1**: 40.

[3]. Abebe M, Hailelule A, Abrha B, Nigus A, Birhanu M, Adane H, Genene T, Getachew G, Merga G, Haftay A (2014). Antibiogram of *Escherichia coli* strains isolated from food of bovine origin in selected Woredas of Tigray, Ethiopia. *African Journal of Bacteriology Research* **6**: 17-22.

[4]. Adam, M. R. and Moss, M. O. (2008). Food Microbiology. 3rd Edition. *Royal Society of Chemistry, Cambridge.* Pp. 216-224.

[5]. Atnafie B, Paulos D, Abera M, Tefera G, Hailu D, Kasaye S, Amenu K (2017). Occurrence of *Escherichia coli* O157: H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. *BMC microbiology* **17**: 24.

[6]. Awadallah, M.A., Ahmed, H.A., Merwad, A.M., Selim, M.A., 2016. Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. *Veterinary. Journal*. 217, 83-88.

[7]. Bedasa S, Shiferaw D, Abraha A, Moges T (2018). Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157: H7 from food of animal origin in Bishoftu town, Central Ethiopia. *International Journal of Food Contamination* **5**: 2.

[8]. Beyi AF, Fite AT, Tora E, Tafese A, Genu T, Kaba T, Beyene TJ, Beyene T, Korsa MG, Tadesse F (2017). Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 in beef at butcher shops and restaurants in central Ethiopia. *BMC microbiology* **17**: 49.

[9]. Calder wood, S.B., Acheson, D.W.K., Keusch, G.T., Barrett, T.J., Griffin, P.M., Strockbine, N.A., Swaminathan, B., Kaper, J.B., Levine, M.M., Kaplan, B.S., Karch, H., O'Brien A.D., Obrig, T.G., Takeda, Y., Tarr, P.I. and Wachsmuth, I.K. (1996). Proposed new nomenclature for SLT (VT) family. *ASM News*, **62:**118–119.

[10]. Caprioli, A., Morabito, S., Brugere, H., Oswald, E.,(2005). Enterohaemorrhagic *Escherichia coli* emerging issues on virulence and modes of transmission. *Veterinary. Research*. 36, 289-311.

[11]. Chye FY, Abdullah A, Ayob MK (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food microbiology* **21**: 535-541.

[12]. CLSI (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI) document Wayne, *PA, M100-S25*. Vol. 35 No. 3.

[13]. Cabal, A., S. Gomez-Barrero, C. Porrero, C. Barcena, G. Lopez, R. Canton, C. Gortazar, L. Dominguez, and J. Alvarez. (2013). Assessment of virulence factors characteristic of human *Escherichia coli* pathotypes and antimicrobial resistance in O157:H7 and non-O157:H7 isolates from livestock in Spain. *Applied Environmental Microbiology*. 79:4170–4172.

[14]. Constable, P. D., Hinchcliff, K. W., Done, S. H., and Grundberg, W. (2017). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. UK: *Elsevier* 1591 p.

[15]. Davis, M.A., Cloud-Hansen, K.A., Carpenter, J., Hovde, C.J., (2005). *Escherichia coli* O157:H7 in environments of culture-positive cattle. *Applied Environmental Microbiology*. 71, 6816-6822.

[16]. Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D (2017). Prevalence and Antimicrobial Susceptibility Pattern of *E. coli* O157: H7 Isolated from Traditionally Marketed Raw Cow

Milk in and around Asosa Town, Western Ethiopia. *Veterinary medicine international* .
[17]. Duffy, G., (2003). Verocytoxigenic *Escherichia coli* in animal feces, manures and slurries. *Journal Applied Microbiology* (Suppl.), 94–103.
[18]. Fairbrother, J.M., Nadeau, E., (2006). *Escherichia coli*: on-farm contamination of animals. *Rev Sci Tech* 25, 555-569.
[19]. Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.S., Luchansky, J.B., Kaspar, C.W., (1996). Prevalence and clonal nature of *Escherichia coli* O157: H7 on dairy farms in Wisconsin. *Applied and Environmental Microbiology* 62, 1519-1525.
[20]. Ferens WA, Hovde CJ (2011). *Escherichia coli* O157: H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease* **8**: 465-487.
[21]. Haile, W (2017). Prevalence and Sources of Contamination of Cattle Meat at Municipal Abattoir and Butcheries as well as its Public Health Importance in Addis Ababa, Ethiopia. MSc Thesis. Addis Ababa University College of Veterinary Medicine and Agriculture. Bishoftu, Ethiopia [dissertation].
[22]. Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE, Carpenter LV (1998). Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. *Preventive veterinary medicine* **35**: 11-19.
[23]. Hashemi, M., Khanzadi, S., and Jamshidi. (2010). Identification of *Escherichia coli* O157:H7 isolated from cattle carcasses in Mashhad Abattoir by multiplex PCR. *World Applied Sciences Journal*, **10**: 703-708.
[24]. ISO 18593 (2004). (International Organization for Standardization): Microbiology of food and animal feeding stuffs: Horizontal methods for sampling techniques from surfaces using contact plates and swabs: 1st edition, 2004-06-01. *ISO*, Geneva.
[25]. Islam Md., Musekiwa A., Islam K., Ahmed Sh., Chowdhury Sh., Ahad A. and Biswas P. (2014). Regional variation in the prevalence of *E. coli* O157 in cattle: A meta-analysis and meta-regression. *PloS one* **9:** 4, e93299.
[26]. Jergefa,T., Kelay,B., Bekana,M.; Teshale,S., Gustafson,H., Kindahl, H (2009). Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Revue scientifique et technique* **28:** 933
[27]. Kibret M, Abera B (2011). Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. *African health sciences* **11**: 40-45.
[28]. Kolar, M., Urbanek, K., Latal, T., (2001). Antibiotic selective pressure and development of bacterial resistance. *Int. J. Antimicrob. Agents* 17, 357-363
[29]. Kudva, I.T., Blanch, K., Hovde, C.J., (1998). Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64, 3166-3174.
[30]. LeJeune, J.T., Besser, T.E., Rice, D.H., Hancock, D.D., (2001). Methods for the isolation of water-borne *Escherichia coli* O157. *Lett. Appl. Microbiol*. 32, 316-320.
[31]. LeJeune, J.T., Wetzel, A.N., (2007). Preharvest control of *Escherichia coli* O157 in cattle. *J. Anim Sci.* 85, E73-E80.
[32]. Lim JY, Yoon JW, Hovde CJ (2010). A brief overview of *Escherichia coli* O157: H7 and its plasmid O157. *Journal of microbiology and biotechnology* **20**: 5.
[33]. Lu Z, Breidt F (2015). *Escherichia coli* O157: H7 bacteriophage +a241 isolated from an industrial cucumber fermentation at high acidity and salinity. *Frontiers in microbiology* **6**.
[34]. Lupindu AM, Olsen JE, Ngowi HA, Msoffe PL, Mtambo MM, Scheutz F, Dalsgaard A (2014). Occurrence and characterization of Shiga toxin-producing *Escherichia coli* O157: H7 and other non-sorbitolGÇôfermenting E. coli in cattle and humans in urban areas of Morogoro, Tanzania. *Vector-Borne and Zoonotic Diseases* **14**: 503-510.
[35]. Lye YL, Afsah-Hejri L, Chang WS, Loo YY, Puspanadan S, Kuan CH, Goh SG, Shahril N, Rukayadi Y, Khatib A (2013). Risk of *Escherichia coli* O157: H7 transmission linked to the consumption of raw milk. *International Food Research Journal* **20**.
[36]. Megersa M.; FeyisaA.; WondimuA.; Jibat T. (2011). Herd composition and characteristics of dairy production in Bishoftu Town, Ethiopia. *Journal of Agricultural Extension and Rural Development* **3**: 113-117
[37]. Mersha G, Asrat D, Zewde BM, Kyule M (2010). Occurrence of *Escherichia coli* O157: H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Letters in applied microbiology* **50**: 71-76.
[38]. Money, P., Kelly, A.F., Gould, S.W., Denholm-Price, J., Threlfall, E.J., Fielder, M.D., (2010). Cattle, weather and water: mapping *Escherichia coli* O157:H7 infections in humans in England and Scotland. *Environ. Microbiol*. 12, 2633-2644.
[39]. Msolo L, Igbinosa EO, Okoh AI (2016). Prevalence and antibiogram profiles of

Escherichia coli O157: H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa. *Asian Pacific Journal of Tropical Disease* **6**: 990-995.

[40]. Nazareth JR (2017). Prevalence of Salmonella species and *Escherichia coli* O157: H7 in organically managed cattle and food safety status of selected meat products.

[41]. Newell D., Koopmans M., Verhoef L., Duizer E. and Aidara-kane k. (2010). Food-borne diseases The challenges of 20 years ago still persist while new ones continue to emerge. *International journal of microbiology* **139:** 3-15.

[42]. OIE, (2016)**.** World Organization for Animal Health. Manual of diagnostic tests and vaccines for terrestrial animals. Verocytotoxigenic *Escherichiacoli*. Available at http://www.oie.int/eng/normes/mmanual/2016/pdf/2.09.11_VERO_E_COLI.pdf.

[43]. Ojo, O. E., Ajuwape, A. T. P., Otesile, E. B., Owoade, A. A. , Oyekunle, M. A. and Adetosoye, A. I. (2010). Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the feces and meat of food-producing animals in Ibadan, Nigeria. *International Journal of Food Microbiology*, **142**:214-21.

[44]. Pennington, H., (2010). Escherichia coli O157. Lancet 376, 1428-1435.

[45]. Preussel, K., Hohle, M., Stark, K., Werber, D., (2013). Shiga toxin-producing *Escherichia coli* O157 is more likely to lead to hospitalization and death than non-O157 serogroups--except O104. *PLoS. One*. 8, e78180.

[46]. Quinn, P., Carter, M., Markey, B., and Carter, G. (2004). Clinical Veterinary Microbiology. London, UK.: *Wild life Publisher*. 101 p.

[47]. Rahn K, Renwick SA, Johnson RP, Wilson JB, Clarke RC, Alves D, McEwen S, Lior H, Spika J (1997). Persistence of *Escherichia coli* O157 [ratio] H7 in dairy cattle and the dairy farm environment. *Epidemiology & Infection* **119**: 251-259.

[48]. R Core team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. url = https://www.R-project.org/

[49]. Rice, E.W., Johnson, C.H., (2000). Survival of *Escherichia coli* O157: H7 in dairy cattle drinking water. *Journal of dairy science* 83, 2021-2023.

[50]. Sabate, M., Prats, G., Moreno, E., Balleste, E., Blanch, A.R., Andreu, A., (2008). Virulence and antimicrobial resistance profiles among *Escherichia coli* strains isolated from human and animal wastewater. *Res. Microbiol*. 159, 288-293.

[51]. Saeedi, P., Yazdanparast, M., Behzadi, E., Salmanian, A.H., Mousavi, S.L., Nazarian, S., Amani, J., (2017). A review on strategies for decreasing *E. coli* O157:H7 risk in animals. *Microb. Pathog*. 103, 186-195.

[52]. Sami M, Firouzi R (2007). Prevalence of *Escherichia coli* O157: H7 on dairy farms in Shiraz, Iran by immunomagnetic separation and multiplex PCR. *Iranian Journal of Veterinary Research* **8**: 319-324.

[53]. Sancak YC, Sancak H, Isleyici O (2015). Presence of *Escherichia coli* O157 and O157: H7 in raw milk and Van herby cheese. *Bulletin of the Veterinary Institute in Pulawy* **59**: 511-514.

[54]. Sargeant, J.M, Gillespie. J.R. Oberst, R.D, Phebus, R.K, Hyatt, D.R, Bohra, L.K and Galland J.C. (2000). Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. *J Am Vet Med Assoc* **61(11)**:1375-1379.

[55]. Sargeant, J.M., Sanderson, M.W., Smith, R.A., Griffin, D.D., (2004): Associations between management, climate, and *Escherichia coli* O157 in the faeces of feedlot cattle in the Midwestern USA. *Prev. Vet. Med*. 66, 175-206.

[56]. Shere JA, Kaspar CW, Bartlett KJ, Linden SE, Norell B, Francey S, Schaefer DM (2002). Shedding of *Escherichia coli* O157: H7 in dairy cattle housed in a confined environment following waterborne inoculation. *Applied and Environmental Microbiology* **68**: 1947-1954.

[57]. Shiferaw, T., (2008). Socio-ecological functioning and economic performance of rain-fed farming systems in Adami Tulu Jido Kombolcha district, Ethiopia. *Agroecology MasterGÇÖs Program Norwegian University of Life Sciences* .

[58]. Smith D. (2014). Vaccination of Cattle against *Escherichia coli* O157:H7. *Microbiology Spectration* **2:** 6.

[59]. Srinivasan V, Nguyen LT, Headrick SI, Murinda SE, Oliver SP (2007). Antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* O157: H7 and O157: H7GêÆ from different origins. *Microbial Drug Resistance* **13**: 44-51.

[60]. Swirski AL, Pearl DL, Williams ML, Homan HJ, Linz GM, Cernicchiaro N, LeJeune JT (2014). Spatial epidemiology of *Escherichia coli* O157: H7 in dairy cattle in relation to night roosts of Sturnus vulgaris (European starling) in Ohio, USA (2007GÇô2009). *Zoonoses and public health* **61**: 427-435.

[61]. Tassew, Asmelash (2015). Isolation, Identification, Antimicrobial Profile and Molecular Characterization of Enterohaemorrhagic *E. Coli* O157: H7 Isolated

From Ruminants Slaughtered at Debre Zeit ELFORA Export Abattoir and Addis Ababa Abattoirs Enterprise [dissertation].

[62]. Tenaillon, O., Skurnik, D., Picard, B., Denamur, E., (2010). The population genetics of commensal *Escherichia coli*. *Nat. Rev. Microbiol*. 8, 207-217.

[63]. Thrusfield M. (2005). Veterinary Epidemiology. Blackwell Science Ltd,.UK, 233 - 250.

[64]. USDA APHI (1997). An update: Escherichia coli O157: H7 in humans and cattle. Centers for Epidemiology and Animal Health.

[65]. Venegas-Vargas C, Henderson S, Khare A, Mosci RE, Lehnert JD, Singh P, Ouellette LM, Norby B, Funk JA, Rust S (2016). Factors associated with Shiga toxin-producing *Escherichia coli* shedding by dairy and beef cattle. *Applied and Environmental Microbiology* **82**: 5049-5056.

[66]. Vijayarani K., Parthiban M., Raja A. and Kumanan K. (2010). Occurrence and characterization of *Escherichia coli* 0157:H7 and other serotypes in goat and sheep meat in India. *Indian Journal of Animal Sciences* **80:** 1019-1021.

[67]. Wallace, J.S., Cheasty, T., Jones, K., (1997). Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *J. Appl. Microbiol*. 82, 399-404.

[68]. Wang, G., Zhao, T., Doyle, M.P., (1996). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Appl. Environ. Microbiol*. 62, 2567–2570.

[69]. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, Downes FP, Martin ML, Griffin PM, Ostroff SM (1991). Isolation of *Escherichia coli* serotype O157: H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. *Journal of Clinical Microbiology* **29**: 985-989.

[70]. Ziemer, C.J., Bonner, J.M., Cole, D., Vinje, J. (2010). Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application. *J. Anim. Sci*. 88, E84–94.

8/22/2023