Short communication

Distribution of *E. coli* biotypes shed by dairy calves in selected dairy farms in Bishoftu, Ethiopia

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Abstract

A longitudinal study was undertaken to investigate *E. coli* using standard biochemical and sugar fermentation tests. Faecal samples were taken from calves purposively from three selected dairy farms in Bishoftu Ethiopia. Four different sampling times were used to observe the detection rate of *E. coli*. The overall detection of *E. coli* was 84/104 (80.70%). The detection of *E. coli* isolates in different sampling points ranged from 16.34% to 25.00% in which the occurrence of *E.coli* has a significant association. All *E. coli* isolated showed different sugar fermentation patterns. *E. coli* was biotyped into 14 biotypes and variation occurred for samples taken during the first to fourth sampling points, the pattern ranging from 20.20% to 31.00%. Among 14 biotypes, biotypes VI and III dominate with 55.95% and 16.67% respectively. *E. coli* biotype (predominantly group VI) distribution concerning sampling time points have a significant association (p=0.039).Diverse natures and variations of *E. coli* were observed in calves with different sampling points as the main determinant. Farm management practice could reduce the occurrence of the pathogen in farm animals, particularly neonatal calves.

Keywords: Biotype; Calf diarrhea; *Escherichia coli*; Ethiopia, Risk factors.

Introduction

Calf mortality due to diarrheagenic *Escherichia coli* (*E. coli*) in neonatal calves is a major problem on dairy farms. Different determinants including seasons, age, hygiene, husbandry practices, and production systems could be potential

risk factors for the occurrence of disease caused by *E. coli* (Radostits *et al.,* 2007; Berber *et al.,* 2021).

Escherichia coli, an enteric Gram-negative bacillus from the family *Enterobacteriaceae*, is a noninvasive commensal that grows in mass culture in human and animal gut lumen. They can be also out-competent and inhibitory activities for other pathogenic bacteria (Zihler *et al*., 2009). However, pathogenic *E. coli* strains are related to gastroenteric diseases such as diarrhea, and hemorrhagic colitis, and extra intestinal infections in humans and animals. Diarrhea caused by this pathogen affects newborn calves, lambs, and children resulting in significant losses (Boschi-Pinto *et al*., 2008; Malik *et al*., 2011).

Biochemical reactions and sugar fermentation studies have conventionally been used for the identification of bacterial distributions either in human or animal subjects (Krishnan *et al*., 1987). Having information on biochemical characterization and sugar fermentation tests of *E. coli* is important to suggest the distribution of the pathogen. In Ethiopia, no sufficient biotype identification studies have been conducted on *E. coli*. Identification of the diversity of *E. coli* biotypes and its potential deriving factors in different ecological settings is very important to characterize the distribution of the bacterium in a specific area. Therefore, the objective of this study was the isolation and biotype characterization of *E. coli* from both diarrheic and healthy calves' feces in association with determinants for the occurrence of calf diarrhea in dairy farms.

Materials and methods

Study area

The study was conducted on dairy farms in Bishoftu and its surroundings in Ethiopia. The area is located 47 km Southeast of Addis Ababa at 9°N and 39°E at an altitude of about 1900m above sea level. The area receives a mean annual rainfall of 865 mm with mean temperatures in the range of 15°C to 28°C. The mean relative humidity of the area is 61.3% (CSA, 2009). There are many small-scale and large-scale exotic and cross-breeding dairy farms in these areas.

Study population

Calves aged from 1 week to 4 months old that did not get treated with antibiotics were the study populations.

Study design and sampling methodology

A longitudinal study on three dairy farms in four different sampling times in the winter and spring seasons was conducted. Lists of farms were purposively selected and a total of 104 calves, 45 with diarrhea and 59 apparently healthy, were included. Fecal materials were collected and were transported in the icebox in sterile tubes to Veterinary Microbiology Laboratory, Addis Ababa University. Samples were stored at 4°C, for a maximum of overnight, until the time of processing.

Isolation and identification procedures

Isolation of *E. coli*

The isolation and identification of *E. coli* were undertaken according to the techniques recommended by Quinn *et al.* (2002) and the International Organizations for Standardization (ISO 16654:2001). Fecal samples were inoculated on MacConkey agar and incubated at 37° C overnight. From each plate, two isolated lactose fermenting colonies that were inoculated on an Eosin-methylene blue (EMB) agar medium having a characteristic metallic sheen were considered presumptive *E. coli.* All the isolates were stained by Gram's staining method to determine the Gram negativity of the isolates.

Primary and secondary identification

Oxidase test with dark purple colors along the streak line and catalase test having an appearance of oxygen gas (bubble) indicated the positive reaction was conducted as primary identification for *E. coli. Escherichia coli* isolates were preliminarily identified by IMViC biochemical tests (Sagar, 2017).

Biotyping of isolated *E. coli*

Sugar fermentation reactions were used for biochemical typing/ identification of *E. coli.* The isolates were characterized by dulcitol, raffinose, rhamnose, salicin, sucrose, inositol, lactose, maltose, and xylose. The isolates were inoculated into 1% of each sugar medium after being cultured in phenol red broth. The tubes were incubated at 37 °C for 7 days and measured every 24 hrs. The production of yellow color was seen as a favorable reaction, and appropriate controls were maintained (Quinn *et al.*, 2002; Aklilu *et al.,* 2013).

Data management and analysis

Data were classified, filtered, and coded using Microsoft Excel 2007 and exported to SPSS version 20 for appropriate statistical analysis. Descriptive statistics and Chi-square ($\chi 2$) test were used to measure the association between determinants. A p-value of ≤ 0.05 was considered statistically significant.

Results

Isolation and identification of *E. coli* isolates

From 104 diarrheic and apparently healthy calves, 84 (80.70%) samples were found to be positive for *E. coli*. The isolation rate in the diarrheic and healthy calves categories was recorded at 37/45 (82.20%) and 47/59 (79.60%) respectively. Some of the sick calves were diarrheic at the beginning of the study while others became diarrheic during the second, third, and fourth sampling point in the study period.

Association of *E. coli* positivity with different factors

The association of *E. coli* positivity was assessed concerning several factors relevant to diarrhea and the study design as presented in (Table 1). *E. coli* positivity was found to be variable among the four points of sampling. *E. coli* was isolated at the highest rate at the first time point (25%), followed by the second time point (23.07%) and the third and fourth time points with a similar isolation rate (16.34%). This variation was found to be statistically significant (p=0.031).

Table1: Association of *E. coli* positivity in diarrheic calves with different factors

Variable		No. tested *E. coli*			*P*- value
		Positive (%)	Negative (%)		
Age at occurrence *E.coli*	7 days	1	1 (100)	0	0.560
	8-15 days	11	8 (72.70)	3 (27.30)	
	16-30 days	21	21 (100)	0	
	31-60 days	39	31 (79.50)	8 (20.50)	
	61-90 days	21	13 (61.90)	8 (38.10)	
	90-120 days	11	10 (90.90)	1 (9.10)	
Sex	Male	39	35 (89.70)	4 (10.30)	0.582
	Female	65	49 (75.40)	16 (24.60)	
Health status	Diarrheic	45	37 (82.20)	8 (17.70)	0.428
	Healthy	59	47 (79.70)	12 (20.30)	
Farm	1	76	63 (82.90)	13 (17.10)	0.916
	2	12	8 (66.60)	4 (33.40)	
	3	16	13 (81.25)	3 (18.75)	
Sampling time points	1st	26	25 (96.20)	1 (3.80)	0.031
	2nd	26	23 (88.50)	3 (11.50)	
	3rd	26	16 (61.54)	10 (38.50)	
	4th	26	16 (61.54)	10 (38.50)	

M=Male; F=Female; P=Probability

Dynamics of *E. coli* biotypes in calves

A total of 14 different biotypes were identified throughout the study time points. Overall, biotype VI (47 isolates, 55.95%) was the most dominant, followed by biotypes III (14 isolates, 16.67%), V and VII (each with 5 isolates, 5.95%), I (3 isolates, 3.57%), IX (2 isolates, 2.38%), and II, IV, XII, VIII, X,XI, XIII and XIV (each accounting for 1 isolate, 1.19%) (Table 2).

Table2: Designation of biotypes of *E. coli* isolates based on fermentation reactions

Biotype No.	Isolates	The proportion of isolates No (%)	Fermented sugars
I	295D, 4515D, 4514C	3 (3.60)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose and xylose
II	3345B	1 (1.19)	Dulcitol, raffinose, salcine, sucrose. lactose, maltose, inositol and xylose
III	4503A, 295B, 21B, 4511B, 4515A, 20B. 4505B, 4503C, 369B, 360A, 363C, 20A, 364C, 3346B	14 (16.67)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose, inositol and xylose
IV	295A	1 (1.19)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose and inositol
V	4518B, 4506B, 4512A, 3346A, 4511A	5 (5.59)	Raffinose, rhaminose, sucrose. lactose, maltose, xylose and inositol
VI	4509C, 4513A, 4509A, 364A. 369A, 364B, 4515B, 4509B. 4510C,4503B, 368D, 4507D, 295C, 4518A, 4505D, 22D, 4512D, 361D, 4503D, 4510D, 4511D, 364D, 21D, 22B, 3345C, 4513D, 363D, 362D, 360D, 4504A, 21A, 368B, 362A, 4507A, 4510A, 4504C, 366C, 4506A, 22C, 4507B, 363B, 4514B, 4504B, 368C, 360B, 4512C, 366B,	47 (55.95)	Dulcitol, raffinose, rhaminose, salcine, sucrose. lactose, maltose, xylose and inositol
VII	4510B, 4513B, 366A, 4514A, 361A	5 (5.59)	Raffinose, rhaminose, salcine, sucrose. lactose, maltose, inositol and xylose
VIII	361C	1 (1.19)	Dulcitol, rhaminose, salcine, lactose, maltose, xylose and inositol
IX	4506C, 4518C	2 (2.38)	Raffinose, rhaminose, salcine, sucrose. lactose, maltose and inositol
X	4512B	1 (1.19)	Dulcitol, rhaminose, sucrose. lactose, maltose, xylose and inositol
XI	4505A	1 (1.19)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose and xylose

Biotype No.	Isolates	The proportion of isolates No (%)	Fermented sugars
XII	22A	1 (1.19)	Dulcitol, raffinose, rhaminose, sucrose. lactose, maltose and inositol
XIII	21C	1 (1.19)	Rhaminose, salcine, xylose and inositol
XIV	362C	1 (1.19)	Rhaminose, salcine, lactose, maltose, xylose and inositol
Total		84 (100)	

Biotype diversity was found to be variable among the four points of sampling, and this variation was found to be statistically significant (p=0.039) (Table 3).

Table 3: Association of biotypes in diarrheic calves with different factors

Variable	Categories	Number of Biotypes (%)	*P- value*
Age at occurrence *E.coli*	7 days	1 (1.20)	
	8-15 days	8 (9.60)	0.225
	16-30 days	21 (25.10)	
	31-60 days	31 (37.00)	
	61-90 days	13 (15.50)	
	90-120 days	10 (11.90)	
Sex	Male	35 (41.70)	0.499
	Female	49 (58.30)	
Health status	Diarrheic	37 (44.00)	0.135
	Healthy	47 (56.00)	

Variable	Categories	Number of Biotypes (%)	*P- value*
Farm	1	63 (75.00)	0.358
	2	8 (9.50)	
	3	13 (15.50)	
Sampling time points	1st	26 (31.00)	
	2nd	24 (28.60)	0.039
	3rd	17 (20.20)	
	4th	17 (20.20)	

The distribution of the biotypes along the sampling time points was observed as indicated in Table 4 below.

Table4: Distribution of *E. coli* biotypes and variables

Variable		The abundance *of E. coli* biotypes in fecal samples, Number (%)													
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**	**XIV**
Sampling time points	1st	0 (0)	0 (0)	5(6)	1(1.2)	3(3.6)	11(13.1)	4(4.8)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	1(1.2)
	2nd	0(0)	1(1.2)	7(8.3)	0 (0)	2(2.4)	11 (13.1)	1(1.2)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	3rd	1 (1.2)	0 (0)	2(2.4)	0 (0)	0 (0)	9 (10.7)	0(0)	0(0)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	0(0)	0(0)
	4th	2 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	14 (16.7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Age at the occurrence of diarrhea	7 d	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)
	8-15d	0(0)	0(0)	2(2.4)	0(0)	2(2.4)	3(3.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	0(0)
	16-30d	0(0)	1(1.2)	6(7.2)	0(0)	2(2.4)	9(10.8)	2(2.4)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	31-60d	2(2.4)	0(0)	4(4.8)	1(1.2)	1(1.2)	17(20.2)	3(3.6)	0(0)	0(0)	2(2.4)	1(1.2)	0(0)	0(0)	0(0)
	61-90d	1(1.2)	0(0)	2(2.4)	0(0)	0(0)	8(9.6)	0(0)	0(0)	1(1.2)	0(0)	0(0)	1(1.2)	0(0)	0(0)
	91-120d	0(0)	0(0)	0(0)	0(0)	0(0)	10(11.9)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Health status	Diarrheic	3(3.6)	1(1.2)	10(11.9)	0(0)	3(3.6)	17(20.2)	2(2.4)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)	0(0)
	Healthy	0(0)	0(0)	4(4.8)	1(1.2)	2(2.4)	30(35.7)	3(3.6)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	1(1.2)	0(0)	1(1.2)
Farms	1	1(1.2)	1(1.2)	10(11.9)	1(1.2)	4(4.8)	37(44)	3(3.6)	0(0)	1(1.2)	2(2.4)	1(1.2)	1(1.2)	1(1.2)	0(0)
	2	1(1.2)	0(0)	2(2.4)	0(0)	0(0)	2(2.4)	1(1.2)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1.2)
	3	1(1.2)	0(0)	2(2.4)	0(0)	1(1.2)	8(9.5)	1(1.2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

d=day(s)

Discussion

Different pathogenic strains of *E. coli* cause diarrhea in animals and humans. Diarrhea is the most significant cause of morbidity and mortality in neonatal dairy calves throughout the world (Uhde *et al*., 2008). In the present study, 84(80.70%) *E. coli* isolates were obtained from 104 calves' fecal samples based on the biochemical and sugar fermentation tests. The isolation rate of *E. coli* from calves in this study was higher than the previous isolation studies in different countries, which ranged from 8.10% (Mebrhatu and Kebede, 2017) to 73.90% (Adesiyum *et al*., 2001). In another case, the present study could show a lower isolation rate than that of 84.30% of *E. coli* from diarrheic calves (Aklilu *et al*., 2013). The sampling point is a significantly different factor that indicates *E. coli* positivity decrease at the sampling point increase within increased age of the calves. That implies the pathogen is potentially risky at the early age of neonates. In addition to this, the result of the current study varies from the reports in different areas, which might be due to the variations in the management conditions of the farms including hygienic conditions and housing system of the farms (Bekele *et al*., 2009; Charles and Lani, 2010).

Biochemical and sugar fermentation reactions have conventionally been used for the identification of bacteria. In the present study, the fermentation reactions made by all *E. coli* isolates were variable. The identified 14 biotypes were similar to previous findings of Aklilu *et al.* (2013) in diarrheic lamb in Debre Birhan, Ethiopia. Whereas, the number of biotypes is different from a study that found 12 *E. coli* biotypes (Gargan *et al*., 2013). The distribution of the isolates into different biotypes indicates the diversity of the bacterium due to the presence of enzymes that ferments a given sugar. Sampling points are the only significantly different variable for the association of *E. coli* distribution in neonatal calves in the study area that suggests that seasonal variation could be an important determinant for the occurrence of the disease in calves.

Conclusions

The isolation rate and biotype diversity of *E. coli* in calves were found to be high in the study area. The distribution of the pathogen based on sugar fermentation is also grouped into 14 different biotypes. Both *E. coli* positivity and biotype distribution were significantly associated with sampling points and sampling times during the study. The high isolation rate and diverse nature of the study organism that caused diarrhea in calves could suggest that calve

diarrhea due to *E. coli* could be an economically important health problem in dairy farms. Determinants need to be identified and farm management practices should be improved to improve the health status of farm animals in general and calves in particular.

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