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REGULAR ARTICLES

Characterization of *Escherichia coli* isolated from calf diarrhea in and around Kombolcha, South Wollo, Amhara Region, Ethiopia

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Abstract This study was carried out from October 2012 to end of February 2013 in and around Kombolcha, Amhara regional state, Ethiopia, using a total of 201 neonatal calves aged 1 day to 4 months and suffering from diarrhea. The objectives of the study were to isolate *Escherichia coli* from diarrheic calves, and to determine *E. coli* biotypes and risk factors associated with its isolation. The fecal samples were collected, transported, and processed following standard microbiological procedures. Seventy-four isolates of *E. coli* were identified. Yellowish diarrhea, younger age, and low-colostrum feeding were significantly associated with rate of *E. coli* isolation ($P<0.05$). Then the 74 isolates of *E. coli* were biotyped using fermentation of 9 sugars and grouped into 12 biotypes; the most dominant was biotype III (36.8 %). Finally, by comparing with studies elsewhere, from the 12 isolated biotypes, 3 of them were suggested to be enteropathogenic *E. coli* (EPEC), entherotoxigenic *E. coli* (ETEC), and adhesion and effacing *E.* coli (AEEC) pathogenic strains. The present study showed that *E. coli* accounted for 37 % of calf diarrhea, with very diverse biotypes.

Keywords Biotype · Calf diarrhea · Calves · *Escherichia coli* · Kombolcha · Sugar fermentation · Risk factors

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Introduction

Newly born calves represent an important source of animal production for either meat or breeding worldwide. Diarrhea is one of the very common disease syndromes in neonatal calves in different countries, and this can have severe impacts both economically and in terms of animal welfare (Africa Union 2008). Calves are at greatest risk of developing diarrhea within the first month of life, and the incidence of diarrhea decreases with age (Meganck et al. 2014).

Neonatal calf diarrhea is a multifactorial disease which despite decades of research in the topic remains the most common cause of neonatal calf mortalities (Uhde et al. 2008). There is a multitude of interactions of the noninfectious causes (predisposing factors) (flaws or gaps in management—inadequate nutrition, exposure to severe environment, insufficient attention to the newborn calf, or a combination of these) and infectious causes (Blanchard 2012; Cho and Yoon 2014).

Several enteropathogens are implicated in neonatal calf diarrhea, and their relative prevalence varies geographically but the most common prevalent infections in most areas are *Escherichia coli*, *Rotavirus*, and *Corona virus*, *Clostridium perfringens*, *Salmonella*, and *Cryptosporidium*. Causes of neonatal calf diarrhea are commonly associated with more than one of these agents, and the causes of most outbreaks are usually multifactorial (Foster and Smith 2009; Blanchard 2012; Cho and Yoon 2014). *E. coli* strains are the most common primary agents causing calf diarrhea and septicemia (Kolenda et al. 2015).

Escherichia coli is a motile Gram-negative bacilli that falls within the family Enterobacteriaceae. It colonizes the infant gastrointestinal tract within hours of life, and thereafter *E. coli* and the host derive mutual benefit for decades (Welch 2006; Foster and Smith 2009). Many strains of the bacterium are harmless to the calf, but certain strains that acquire virulence

genes can cause moderate to severe scours and even death (Wani et al. 2004). Two of the more prominent virulence factors identified for Enterotoxigenic *E. coli* strains are (i) expression of fimbrial (pilli) antigens that enable the bacteria to adhere to and to colonize the luminal surface of the small bowel and (ii) elaboration of one or more enterotoxins that influence intestinal secretion of fluids (Chen and Franke 2005; Welch 2006).

Severity of *E. coli* symptoms varies from mild transient diarrhea to severe sustained diarrhea resulting in death. Typical presentation is in calves less than 7 days of age and as early as 12 h of life. *E. coli* typically produces a secretory diarrhea switching the intestinal epithelial cells from an absorption mode to a secretion mode. Diarrhea is typically whitish to yellowish in color (Ata et al. 2013; Cho and Yoon 2014).

E. coli are able to ferment a variety of carbohydrate substrates, generally by converting them to glucose or to a substrate on the fermentative chain of the breakdown of glucose. The ability to ferment a given sugar of the types described above by a strain of *E. coli* is dependent on the strain having the requisite enzymes to convert it to glucose or to a substance on the degradative chain from glucose (Aklilu et al. 2013). This is the basis of biotyping of *E. coli*. These tests are also easy to perform, by determining, whether a strain of *E. coli* will produce acid following growth in the presence of the carbohydrate. Of the various typing systems available, study of the organisms' biological properties ("biotyping") appears to be a useful method of identification (Aklilu et al. 2013). In this regard, Chattopadhyay et al. (2003) classified six different biotypes of verotoxin-positive *E. coli* on the basis of sugar fermentation reactions of three sugars, viz., sorbitol, raffinose, dulcitol, and decarboxylase test with lysine, arginine, and ornithine. In addition, Murinda et al. (2004) reported diagnostic significance of rhamnose fermentation test; they recorded rhamnose non-fermenters belonging to *E. coli* O26 were 100 % STEC producers.

There were no previous studies conducted on calf diarrhea as well as on diversity of *E. coli* biotypes in the study area. However, there are frequent cases of calf diarrhea in the study area leading to morbidity and mortality and often seeking professional intervention. Therefore, this work was conducted to determine the diversity of *E. coli* using standard sugar fermentation tests and identify risk factors associated with its isolation from diarrheic calf feces in the area. We believe the data in this work contributes to diagnosis and control of calf diarrhea in Ethiopia, in particular, and worldwide, in general.

Materials and methods

Study area

This work was carried out from October 2012 to end of February 2013 using a total of 201 neonatal calves of different ages (1 day–4 months old) examined clinically for diarrhea on dairy farms in and around Kombolcha, South Wollo, Amhara Region, Ethiopia. Kombolcha is located 380 km northeast of Addis Ababa at a latitude of 11° 4′ N 39° 44′ E, longitude of 11.067° N 39.733° E, and elevation between 1842 and 1915 m above sea level (Fig. 1). The woreda has an annual mean temperature of 11.7–24.9 °C (CSA 2009) and receives the shortest rainfall from March to May and the longest rainfall from June to September (750–900 mm). There are many small-scale and large-scale dairy farms in this area that supply milk and milk products to consumers of the town and surrounding urban areas. These dairy farms contain either local or exotic breeds depending on the scale of production.

Study population

Animals included in this study were calves under 4 months of age that were clinically affected with diarrhea and exhibiting signs of systemic disease (e.g., poor appetite, fever, dehydration, decreased mentation, and reduced suckle reflex) and had pasty–watery feces with different colors. All of these calves were found suffering from different degrees of diarrhea, dehydration, emaciation, and weakness.

The calves were born in 85 different farms located in the area, 60 dairy farms in Kombolcha, and 25 dairy farms around Kombolcha; in all of the dairy farms, the management system was similar, i.e., animals were kept under intensive system. The calves were kept in isolated pens for the whole day except for few hours when the calves were released in the compound for exercise. In some farms, the calves were released for suckling their dam. In all of the farms, the floors were concrete and cleaning of the calves' pens was practiced daily when calves were out. In addition, in all of the farms, calves were fed on colostrum three times per day from their respective dams with different amount and for different periods of time. The weight of each calf was determined by the heart girth system.

Study design and sampling methodology

The study was purposive type, i.e., samples were collected from calves that show clinical signs of diarrhea. Approximately 10 ml of fecal samples were collected from non-treated diarrheic calves directly from the rectum by using disposable plastic gloves and transferred immediately to sterile universal bottles. The samples were then transported in ice box to the Kombolcha veterinary diagnostic laboratory, Kombolcha, for processing. Feces were stored at 4 °C until the time of processing.

Isolation and identification of *E. coli*

Fecal samples were inoculated onto blood and MacConkey agar and incubated at 37 °C overnight. From each MacConkey agar plate, lactose-fermenting colonies were inoculated on Eosin

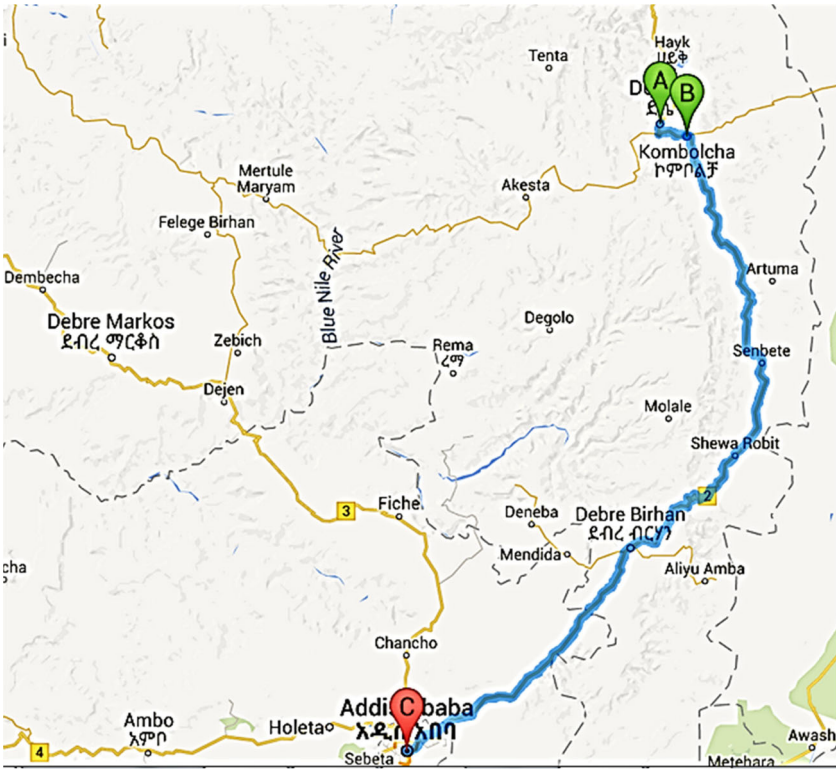

Fig. 1 Location of the study area, Kombolcha town, in Ethiopia (source: Google)

Methylene Blue (EMB) agar medium, which selectively grows members of the *Enterobacteriaceae* and permit differentiation of enteric bacteria on the basis of morphology. Colonies showing characteristic metallic sheen on EMB agar were then picked up and considered as presumptive *E. coli*. The purified cultures of *E. coli* were stored temporarily as nutrient broth cultures for further identification by biochemical tests. All the isolates were stained by Gram stain to determine the cell morphology, Gram reaction and purity of the isolates under the oil immersion lens (×100). Identification of suspected *E. coli* colonies was conducted following standard bacteriological procedures described in Quinn et al. (2002). Thus, *E. coli* isolates were preliminarily characterized by IMViC tests, viz., indole, methyl red, Voges-Proskauer, and citrate utilization. The isolates which exhibited the IMViC pattern of +, +, −, and −, respectively, were presumed as *E. coli* isolates.

Biotyping of *E. coli* isolates

The identified isolates were then further characterized for their sugar fermentation reactions on nine sugars, viz., dulcitol, raffinose, rhamnose, salicin, sucrose, inositol, lactose, maltose, and xylose according to Aklilu et al. (2013). The isolates grown in phenol red broth were inoculated into 1 % of each sugar medium. Tubes were incubated at 37 °C for 7 days, and readings were then recorded after every 24 h. Production of yellow color was considered as positive reaction and proper controls were kept for each of the sugar tests performed.

Furthermore, literature was searched for previous studies that utilized similar sugars like ours in biotyping of *E. coli* and conducted strain identification in parallel. Comparison was made between our biotype data and those published works; whenever a pathotype strain fermenting similar sugars with any of our biotypes was encountered, this strain was suggested as a possible pathotype for our biotype.

Data collection, management and analysis

Data describing the diarrhogenic conditions suggestive of *E. coli* infection observed on calves along with the amount of colostrum given, sex, weight, diarrhea type, and age were classified, filtered, and coded using Microsoft Excel® 2007. The data were then exported to SPSS windows version 18.0 (SPSS INC. Chicago, IL) for appropriate statistical analysis. The detection rate of *E. coli* and the abundance of identified biotypes were determined by using descriptive statistics. Chi-square (χ^2) was used to measure associations between the detection rates of *E. coli* and their biotypes with relevant factors. Associations were reported as statistically significant if *P* value is less than 5 %.

Results

During the present study, 74 (36.8 %) out of the 201 diarrheic calves that showed calf diarrhea were *E. coli* positive.

Table 1 Types of calf diarrhea and their association with *E. coli* isolation

Type of diarrhea	No. of samples	No.+ve for *E. coli*	Proportion Within diarrhea type (%)	Proportion Within total samples (%)
Yellowish	118	43	36.4	21.4
Blood-tinged	13	10	76.9	5
Bloody	14	9	64.3	4.5
Greenish	48	9	18.8	4.5
Watery, colorless	3	2	66.7	1
Mucoid, colorless	5	0	0	0
Total	201	74	-	36.8

Calf diarrhea and *E. coli* isolation associated with host and management factors

Among the 201 diarrheic fecal samples, six types of diarrhea, namely, yellowish, blood-tinged, pure bloody, greenish, watery, and mucoid, were observed at different proportions. There was a statistically significant association between rates of *E. coli* isolation and color type of diarrhea (P=0.001), isolation rates being highest in blood-tinged diarrhea and lowest in greenish diarrhea. In contrast, there was no *E. coli* isolation in calves with mucoid type of diarrhea (Table 1). The majority of the isolates were originated from calves with yellowish diarrhea due to the large number of calves with yellowish diarrhea in the area.

The diarrheic calves examined were classified into four age groups which yield different proportions of *E. coli* isolates. There is a high occurrence of diarrhea on 1 week old calves, followed by 8–15 days old group, and then by 16–30 days old group. There was no direct association between age of calves and occurrence of diarrhea; however, the isolation rate of *E. coli* was highest at the earlier age groups, decreasing as the age of diarrheic calf increases. The *E. coli* isolates that were recovered from the age groups of 1–7 days and 8–15 days constitute 85 % of the total isolates. This difference in isolation rate of *E. coli* was statistically significant (P=0.000) (Table 2).

It was also shown that isolation rate of *E. coli* was not statistically associated with either sex (P=0.703) or body weight (P=0.45) of the calves studied. Similarly, higher amount of colostrum feeding does not reduce significantly isolation rate of *E. coli* (P=0.09) (Table 2). Bloody diarrhea was observed only in the youngest and oldest age groups; whereas, the other types of diarrhea were observed in all age groups. Distribution of the diarrheal types among the different age categories of calves showed no significant association (Table 3).

Diversity of *E. coli* biotypes isolated from calf diarrhea

Types and relative abundance of E. coli biotypes in calves

All the 74 *E. coli* isolates were studied for their fermentation activities on 9 sugars; 72 (97.3 %) isolates showed the ability to utilize one or more sugars while 2 (2.7 %) isolates failed to utilize any of the 9 sugars tested. The most abundant *E. coli* biotypes from calf diarrhea, namely, biotypes III, VIII, V, IV, and I, constituted the majority of the isolates (87.7 %). Furthermore, literature search showed that Gargan et al. (1982), Levine et al. (1983) and Johan et al. (1988) have used similar sugars like this study for biotyping; they also reported the pathotype strains of their biotypes. Accordingly, by comparison with their data, the biotypes III, IV and VII, and VIII of the current study could possibly be entherotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and adhesion and effacing *E. coli* (AEEC) strains (Table 4).

Distribution of E. coli biotypes among different age groups of calves and types of diarrhea

The present study showed variation on the pattern of distribution of biotypes among calf age groups; the predominant biotypes in the age groups of 1–days, 8–15 days, 16–21 days, 22–30 days, and 31–60 days were III, IV, IV, V, and VIII, respectively. There was a statistically significant association between the different age groups and occurrence of the different biotypes (P<0.05). Overall, biotype III was the most predominant

Table 2 Occurrence of *E. coli* based on age, weight, and sex of the calves and amount of colostrum fed

Variable	Category	No. of samples	No positive (proportion in %)
Age (days)	1–7	62	46 (22.9)
	8–15	27	17 (8.5)
	16–30	29	5 (2.5)
	31–60	29	5 (2.5)
	61–90	54	1 (0.5)
Weight (Kg)	12–15	62	22 (10.9)
	16–20	103	41 (20.4)
	21–26	36	11 (5.5)
Sex	Male	29	7 (3.5)
	Female	172	67 (33.2)
Colostrum fed (liters)	2–3	146	62 (42.7)
	>3	55	12 (21.8)

Table 3 Distribution of types of diarrhea among age groups of calves

	Types of diarrhea				
Age	Yellowish	Blood-tinged	Bloody	Greenish	Total
1–7	41 (67.2 %)	6 (9.8 %)	5 (8.1 %)	10 (16.1 %)	62
8–15	19 (70.4 %)	4 (14.8 %)	0 (0.0 %)	4 (14.8 %)	27
16–30	13 (44.8 %)	2 (6.9 %)	0 (0.0 %)	14 (48.3 %)	29
31–60	20 (69 %)	2 (6.9 %)	0 (0.0 %)	7 (24.13 %)	29
61–90	33 (61.1 %)	4 (7.4 %)	4 (7.4 %)	13 (24.1 %)	54
Total	126	18	9	48	201

biotype. It was also observed that as age increased, the diversity of biotypes observed decreased, the highest numbers of biotypes observed were in the youngest age (1–7 days); only one and two biotype variants were detected in the age groups of 61–90 days and 31–60 days, respectively (Table 5).

Furthermore, associations on the distribution of the different biotypes among diarrhea types were assessed; the predominant biotypes in the diarrhea types of yellowish, blood-tinged, pure bloody, and greenish were III; III, IV, and V; IV, and VIII, respectively (Table 6).

Discussion

Neonatal diarrhea is a major health problem in dairy farms, leading to high mortality and is hindering sustainable development of the dairy sector; *E. coli* is the most important cause of bacterial scours in calves (Charles et al. 2003; Razzaque et al. 2006; Kolenda et al. 2015). The involvement of *E. coli* in calf diarrhea has not been well studied in Ethiopia, with the exception of a recent study by Dawit (2012).

In the present study, *E. coli* was isolated from 36.8 % of calf diarrhea cases which is in agreement with the report by Razzaque et al. (2006) and Bekele et al. (2009) who reported 24 and 37 %, respectively. This finding is also consistent with the work of Amoki (2001) in the central high land of Ethiopia. The higher calf diarrhea due to *E. coli* in the present study might be attributed to the variations in age groups examined as well as environmental and management conditions of the farms such as insufficient and/or poor-quality colostrum intake by the calves as stated by Charles et al. (2003). In addition to that poor hygiene often allows buildup of pathogenic strains in the young animal's environment. Besides, a large dose of pathogenic *E. coli* may overcome colostral immunity (Quinn et al. 2005).

The present study shows much lower isolation rate than Achá et al. (2004) (76 %), Qais et al. (2011) (64 %), and Dawit (2012) (64 %). On the other hand, lower (13.5 %) percentages of calf diarrhea caused by *E. coli* were reported by Darsema (2008), 13.5 %, and Uhde et al. (2008), 5.5 %. The reason why the result of the current study varies from the reports in other areas might be due to variations in farm management conditions. As documented in Charles et al. (2003) and Radostits et al. (2007), gaps in management specifically calf handling practices including inadequate nutrition, exposure to severe environment, insufficient attention to the newborn calf, or a combination of these, qualitative and

Table 4 Diversity of *E. coli* biotypes isolated from calf diarrhea on the basis of fermentation reactions on nine sugars

Biotype no.	Isolates	Proportion of isolates no. (%)	Fermented sugars	Comparison with literature strain (sugars fermented)	Source
I	023,033,035,067,073,75	6 (8.1)	Only dulcitol		
II	030,062,084,	3 (4.1)	Dulcitol and inositol		
III	057,060,071,080,082,083,091,096, 097,098,099,102,103,104,106, 108,112,115,125	19 (25.7)	Dulcitol, inositol and rhamnose	ETEC (dulcitol, rhamnose and inositol)	Gargan et al. 1982
IV	002,028,029,031,032,034,036,038, 039,040,156	11 (14.9)	Dulcitol, inositol[a], rhamnose and sucrose	AEEC (dulcitol, rhamnose and sucrose)	Johan et al. (1988)
V	119,133,135,137,138,139,167,168, 171,176,190,195	12 (16.2)	Dulcitol and lactose		
VI	159	1 (1.4)	Dulicitol and maltose		
VII	121	1 (1.4)	Dulcitol and rhamnose	AEEC (dulcitol, rhamnose)	Johan et al. (1988)
VIII	003,006,009,021,022,024,025,026, 027,066,068,078,160,184,194	15 (20.3)	Dulcitol, rhamnose and xylose	EPEC (dulcitol, rhamnose and xylose)	Levine et al. (1983)
IX	011, 081	2 (2.7)	Dulcitol and sucrose		
X	061	1 (1.4)	Inositol		
XI	105	1 (1.4)	Rhamnose		
XII	157,158	2 (2.7)	None		
Total		74 (100)			

[a] The sugar inositol has not been used by Johan et al. 1988

Table 5 Distribution of *E. coli* biotypes among different age groups of diarrheic calves

Age (days)	*E. coli* biotypes, no. (%)												Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
1–7	4 (5.4)	2 (2.7)	16 (21.6)	4 (5.4)	6 (8.1)	0	1 (1.4)	8 (10.8)	2 (2.7)	1 (1.4)	1 (1.4)	1 (1.4)	46 (62.2)
8–15	1 (1.4)	1 (1.4)	3 (4)	5 (6.7)	1 (1.4)	1 (1.4)	0	4 (5.4)	0	0	0	1 (1.4)	17 (23)
16–30	1 (1.4)	0	0	2 (2.7)	1 (1.4)	0	0	1 (1.4)	0	0	0	0	5 (6.7)
31–60	0	0	0	0	4 (5.4)	0	0	1 (1.4)	0	0	0		5 (6.7)
61–90	0	0	0	0	0	0	0	1 (1.4)	0	0	0		1 (1.4)
Total	6 (8.1)	3 (4.1)	19 (25.7)	11 (15)	12 (16.2)	1 (1.4)	1 (1.4)	15 (20.3)	2 (2.7)	1 (1.4)	1 (1.4)	2 (2.7)	74 (100)

quantitative conditions of the colostrum, are often involved in scours outbreaks.

The color and consistency of feces in *E. coli* scours have their own value in making a diagnosis of any type of diarrhea (Ata et al. 2013; Cho and Yoon 2014). In the present study, the color of diarrhea in relation to involvement of *E. coli* in calf diarrhea was also considered. Accordingly, four types of diarrheal color were identified, the most dominant being the yellowish one (62.7 %) from which 22.9 % were positive for *E. coli*. In comparison, Bartels et al. (2010) reported that among the 23.8 % of the yellowish-colored calves' diarrhea, 2.6 % was caused by *E. coli*. Ata et al. (2013) also reviewed that in calf scours due to *E. coli*, the small intestine may be filled with fluid and the large intestine may contain yellowish feces. Factors that may contribute to these differences between studies could be related to presence or absence of mixed infections, variations on management conditions of the farms, and the age of the calf as also supported by the present study. In support of this, Charles et al. (2003) stated that onset of *E. coli* diarrhea varies in color and consistency that could be consistent with intestinal overload possibly due to high-volume colostrum feeding.

There were agreements and contradictions on isolation rates of *E. coli* from the other diarrhea types in the present study and previous studies conducted elsewhere. Thus, the isolation rate of *E. coli* from blood-tinged diarrhea in the present study (9.0 %) was inconsistent with that of James and James (2003), who isolated *E. coli* from 22 % of blood-tinged calf diarrhea. In support of this, Naylor and Smith (2002) reported that diarrhea due to *E. coli* is characterized by profuse, pasty to watery, foul smelling, and occasionally flecked with blood (blood-tinged). The isolation rate of *E. coli* from pure bloody diarrhea in the present study (4.5 %) was consistent with the 7 % reported by James and James (2003). The overall variations in color of feces may be as a result of differences on the basis of pathogenic features and mechanisms of the disease. Exudative diarrhea is observed in inflammatory diseases when mucosal inflammation and ulceration cause outpouring of plasma, mucus, and blood into the stool. Diarrhea of the large intestine is associated with frequent small-volume stools with the presence of blood (Naylor and Smith 2002; Foster and Smith 2009). Involvement of mixed infections could also complicate the appearance of feces (Ata et al. 2013; Cho and Yoon 2014).

When we look at sex variation, female calves (33.3 %) yielded higher *E. coli* isolation rate compared to male calves (3.5 %). In contrary, male animals do generally get less attention and management care as their role in the farms is considered irrelevant especially as the replacement stock.

Furthermore, the association between the different age groups with the occurrence of diarrhea due to *E. coli* was studied. The highest percentage (22.9 %) of *E. coli*-positive

Table 6 Distribution of *E. coli* biotypes among different types of diarrhea

Type of diarrhea	*E. coli* biotypes												Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Yellowish	4	1	14	4	6	1	1	9	1	1	1	2 (2.7)	46
Blood-tinged	0	2	3	3	3	0	0	2	0	0	0	0	13
Bloody	1	0	1	2	1	0	0	1	0	0	0	0	6
Greenish	0	0	1	2	2	0	0	3	1	0	0	0	9
Watery, colorless													
Mucoid, colorless													
Total	5	3	19	11	12	1	1	15	2	1	1	2 (2.7)	74 (100)

samples were identified in those calves found in the 1–7 days age group; this result is inconsistent with reports of Achá et al. (2004) (76 %). The causes of variation might be as a result of variations in management of the farms and immunity of the animals. Most newborn calves are exposed to *E. coli* from the environment, particularly when sanitation is marginal (Charles et al. 2003). The author stated that young neonates under 1 week of age are particularly susceptible because the normal flora of the intestine is not fully established. In addition to that, they have a naive immune system and also receptors for the adhesions of *E. coli* are present on the first week of life of the calves (Villarroel 2009). The result of this study is in agreement with the above idea. In addition, other authors reported that this is the age group most commonly affected clinically (Maddox-Hyttel et al. 2006; Santin et al. 2004).

The isolation rate of *E. coli* from the age group of 8–15 days (8.5 %) was consistent with the literature (Villarroel 2009) where it is said that *E. coli* affects calves within the first 10–14 days of age. The isolation rates of the bacteria in the subsequent age groups were shown to decrease as supported by the reports of Villarroel (2009). In support of this, calf diarrhea was noted to decrease as the age of the calves increased. This could be due to the poorly developed immune system of the days old calves as compared to older ones to fight against the disease-causing agents (Darsema 2008; Bekele et al. 2009). Scott et al. (2011) and Radostits et al. (2007) also documented that neonatal infection is higher during their early stage of life because of the stress and infection pressure encountered shortly after birth.

The cause of high isolation rate in the calves receiving less amount of colostrum could be for various reasons. An inadequate, in quality and quantity, supply of colostrum and delay in first colostrum feeding, which leads to failure of transfer of passive immunity (FPT) is an important reason. Calves with inadequate colostral immunoglobulin concentration within 24 h of birth were at greater risk of neonatal morbidity and mortality. In addition, FPT could be due to bacterial contamination of the fed colostrum (Keith et al. 2010). Colostrum feeding practices also have effect in that allowing calves to nurse their dam may predispose them to FPT since they consume late and small amount (Meganck et al. 2014).

The present study also investigated the identity of different types of *E. coli* biotypes in calf diarrhea. Biotyping based on sugar fermentation tests has been utilized by various workers for identifying diversity within a bacterial spp. The most dominant biotype was biotype III (25.7 %) which fermented dulcitol, rhamnose, and inositol. This result was consistent with a report by Gargan et al. (1982) who determined *E. coli* biotyping using the same type of sugars. They assigned biotype I as an isolate fermenting the above three sugars, which accounted 66.6 % of the isolates, and based on the API 20E system, they concluded that the identified biotype I confirmed with serotyping was *ETEC* containing O-antigen. So according to their data, biotype III of the present study could be *ETEC* (entherotoxigenic *E. coli*). Chattopadhyay et al. (2003) classified six different biotypes verotoxin-positive *E. coli* on the basis of sugar fermentation reactions in three sugars, viz., sorbitol, raffinose, dulcitol, and decarboxylase test with lysine, arginine, and ornithine. In addition, Murinda et al. (2004) reported diagnostic significance of rhamnose fermentation test; they recorded rhamnose non-fermenters belonging to *E. coli* O26 were 100 % STEC producers. The current study identified 12 different biotypes. Dawit (2012) reported 15 biotypes based on 6 sugars (dulcitol, raffinose, rhamnose, salicin, starch, and sucrose) in the central part of Ethiopia. Their data showed that the biotype that fermented dulcitol was the most dominant one (20 %), followed by the biotypes that ferment dulcitol, raffinose, and rhamnose (13.3 %).

One of the limitations of this study was the lack of pathotype strain identification of the *E. coli* isolates due to lack of resource. Literature was searched for similarity in sugars used and relation of biotypes and strains; then comparison was made with the biotypes of this study. Johan et al. (1988) also reported 11 different biotypes, using four carbohydrates, from healthy and diarrheic rabbits; biotype 3 (35.5 %) (dulcitol, rhamnose, and sucrose fermenters) and biotype 8 (7.1 %) (dulcitol and rhamnose fermenters) induced lesions characteristic of attaching and effacing *E. coli* (*AEEC*). Serotyping showed a close relationship between biotype and serotype of the *AEEC* examined. Even though the proportions of biotype 3 of the above result was dissimilar to the present study of biotype IV (16.2 %) (fermented dulcitol, inositol (not used by the above author), rhamnose, and sucrose), their fermentation activity was identical. But the result and fermentation activity of the present study of biotype VII (1.4 %) (dulcitol and rhamnose fermenter) was consistent with the result of biotype 8 of Johan et al. (1988). In reference to their work, biotypes IV and VII of the present study could be *AEEC*. Variation in results of biotype IV of the present study and biotype 3 of Johan et al. (1988) might be the result of differences in sample size and animal species studied. These results were also in agreement with Levine et al. (1983) who stated that a given strain from diarrheic rabbit (that fermented dulcitol, rhamnose and xylose and assigned as biotype 6) isolated at the rate of 17.6 % was able to induce watery diarrhea and high mortality after experimental infection. This was in consistent with biotype VIII (20.3 %) of the present study. Similar observations have been made with other strains in England (Varga and Pesti 1982), Belgium and the Netherlands (Peeters et al. 1984), and France (Camguilhem et al. 1986). None of these strains produced heat-labile or heat-stable enterotoxins, nor were they enteroinvasive. Histology and electron microscopy has also showed their tight adherence to the brush border of intestinal epithelial cells. So they are

considered to be enteropathogenic *E. coli* (EPEC). So, biotype VIII of the present study could be suggested to be EPEC.

In the present study, associations between age groups and the different biotypes were also assessed. Hence, young calves in the first age group (1–7 days) and in the second age group (8–16 days) were at a significantly high risk of being affected with diarrhea ($P<0.05$). This finding is also comparable with the result obtained from the biochemical test results which indicated these groups of calves were at a significantly high risk of being affected with *E. coli*-caused diarrhea than the older age groups ($P<0.05$).

In conclusion, during the present study, calf diarrhea was investigated at Kombolcha town in order to isolate *E. coli* and determine the biotypes of the isolates as well as identify risk factors associated with calf diarrhea. Using 9 sugars, 12 biotypes were identified, among which, biotype III was the most dominant one affecting calves in the first week of age. Finally, by comparing with studies elsewhere, from the 12 isolated biotypes, 3 of them were enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and adhesion and effacing *E. coli*. Biotyping is relatively cheaper and can be conducted by less-experienced personnel. Compared to molecular and serotyping techniques, the technique would be valuable for resource poor countries. However, further studies involving comparison of biotyping with PCR and serotyping should be conducted in order to study the applicability of the method for strain identification among pathogenic *E. coli* strains.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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