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Short communication

Prevalence and Characterization of *Escherichia coli* from Rectal Swab of Apparently Healthy Cattle in Mymensingh, Bangladesh

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ABSTRACT

Cattle are considered as one of the sources of pathogenic *E. coli* worldwide. The present study was designed to determine the prevalence and identification of the *E. coli* isolated from rectal swab of apparently healthy cattle in Mymensingh, Bangladesh. A total of 128 rectal swab samples were assessed by cultural, morphological and biochemical examination followed by Polymerase Chain Reaction (PCR) using primers ECO-1 and ECO-2 that are specific for *E. coli* 16S rRNA gene. Data obtained from this study were analyzed based on the age, sex, breed and management systems of cattle. This study revealed a 75% prevalence of *E. coli* in the rectal swab of cattle. Higher prevalence was found in female cattle of unorganized farming systems, and in cattle ≥3 years of age. From this study, it may be concluded that, irrespective of age, sex, breed and management system, *E. coli* is prevailing in the rectal swab of apparently healthy cattle.

Key Words: Prevalence, Characterization, *E. coli*, Rectal swab, Apparently healthy cattle.

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Introduction

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. Most of the *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (CDC, 2012; Vogt and Dippold, 2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Bentley and Meganathan, 1982) and by preventing the establishment of pathogenic bacteria within intestine (Hudault *et al*., 2001; Reid *et al*., 2001).

Ruminant livestock such as cattle, deer, goats and sheep naturally carry *E. coli* O157:H7 in their systems. The cattle, however, are considered to be one of the primary sources of *E. coli* O157:H7 worldwide. Numerous studies have shown that *E. coli* O157:H7 prevalence is widespread in dairy and beef animals, and can be found in, on and around cattle in most parts of the world without causing any disease symptoms (Hazarika *et al*., 2007, Arthur *et al*., 2002; Asakura *et al*., 2001; Elder *et al*., 2000; Griffin and Tauxe, 1991). Peoples of dairy farm families could be at high risk of infection because of their close contact with animals, manure and unpasteurized milk (Wilson *et al*., 1996).

The bacterium can be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. Remarkable works has been done throughout the world (Alexa *et al*., 2011; Kesava *et al*., 2011; Cookson *et al*., 2006; Fratamico *et al*., 2004; Chapman *et al*., 1994). In Bangladesh, several reports have been published on isolation and characterization of *E. coli* from different sources including rectum of different animals (Singh *et al*., 2012, Paul *et al*., 2010, Islam *et al*., 2007, 2008; Zinnah *et al*., 2007; Ali *et al*., 1998; Amin *et al*., 1988). In these cases, prevalence of pathogenic *E. coli* was investigated mainly focusing on diseased or slaughtered animals, poultry and human being. However, there are very few reports on the prevalence study of *E. coli* in apparently healthy cattle in Bangladesh. The present study was thus designed to investigate the prevalence of *E. coli* in the rectal swab of apparently healthy cattle reared in Bangladesh Agricultural University (BAU) dairy farm and visiting BAU veterinary clinic, Mymensingh, Bangladesh.

Materials and Methods

Sample collection

The rectal swab samples (n=128) were collected randomly from apparently healthy cattle comprising 35 samples from the Veterinary Clinic, and 93 samples from Dairy Farm, Bangladesh Agricultural University (BAU), Bangladesh following a convenience sampling method without repetition of animals. Sterile cotton buds were used for the collection of swab samples, and the swab was transferred to nutrient broth instantly. The swab samples were transported to the Bacteriology Laboratory at the Department of Microbiology and Hygiene, BAU. During sample collection information regarding the age, sex, breed and management systems of the animals were also recorded.

Cultural and biochemical examination

The nutrient broth containing swab samples were incubated overnight at 37°C. After overnight incubation samples from the nutrient broth were cultured on to EMB agar, MacConkey agar and Cefixime Tellurite – Sorbitol MacConkey (CT-SMAC) medium (Zadik *et al*., 1993). Isolated organisms with supporting growth characteristics of *E. coli* were subjected to sugar (dextrose, fructose, maltose, lactose and sucrose) fermentation, MR-VP and indole production test following the procedure mentioned by Cheesbrough (1985).

DNA Extraction

Crude DNA was obtained from the isolates using boiling method (Queipo-Ortun *et al*., 2008) with little modification. Briefly, the organisms were cultured onto EMB agar at 37°C. After overnight incubation a medium sized colony was picked up with sterile tips and mixed in 200µl of deionized water. The mixture was then heated in boiling water for 10 minutes followed by dipping into ice for 10 minutes and centrifugation at 10,000rpm for 10 minutes. The supernatant was collected which contains DNA. The DNA sample was kept in -20°C until use.

PCR for the confirmation of the isolates as *E. coli*

The isolated organisms that were preliminarily identified as *E. coli* were confirmed by PCR using primers specific to *E. coli* 16S rRNA gene (Table-1). PCR was performed following the procedure described by Schippa *et al*., 2010, with slight modification. 25µl

Table 1. Primers used in this study

Target gene	Primer name	Sequence	Product size	Tm (^{o}C)	Reference
16SrRNA gene	ECO-1	GACCTCGGTTTAGTTCACAGA	585bp	58^{o}C	Schippa*et al*., 2010
	ECO-2	CACACGCTGACGCTGACCA			

Table 2. Prevalence of *E. coli* in the rectal swab of cattle

Parameters		Positive	Negative	Total	P value(χ2 test)*
Age	0-3 yrs	45 (69.2%)	20 (30.8%)	65	0.126
	3-above	51 (81.0%)	12 (19.0%)	63	
Sex	Male	30 (69.8%)	13 (30.2%)	43	0.331
	Female	66 (77.6%)	19 (22.4%)	85	
Breed	Local	12 (66.7%)	6 (33.3%)	18	0.378
	Cross	84 (76.4%)	26 (23.6%)	110	
Management systems	Organized	71 (76.3%)	22 (23.7%)	93	0.567
	Unorganized	25 (71.4%)	10 (28.6%)	35	

*Data were analyzed based on the age, sex, breed and management systems of the animals. P values were calculated using Pearson's Chi-Square test. P values below 0.05 were considered as significant.

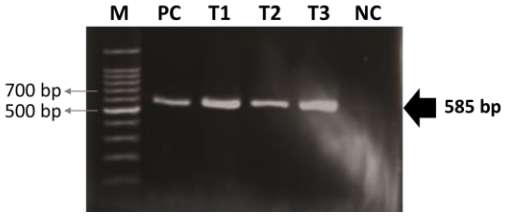

Fig 1. Representative photograph of the PCR of *E. coli* using the primer ECO-1 and ECO-2 targeting 16S rRNA gene. M = 100 bp DNA Ladder, PC = Positive control, T1-T3 = Test samples, NC = Negative control.

reaction containing 1X Taq Polymerase PCR master mix and 10pmol of primer was prepared. After initial incubation at 95^{o}C for 3 min, a 30-cycle amplification protocol was followed as 94^{o}C for 45 s, 58^{o}C for 45 s and 72^{o}C for 60 s, and a final extension step of 72^{o}C for 3 min. Electrophoresis of the PCR products was done using 2% agarose gel. After electrophoresis, the gel was stained for 10 minutes in ethidium bromide for visualization.

Data analysis

Data were entered and statistical analyses were performed using SPSS version 17. Using Pearson's Chi-square (χ2) test difference among the variables was calculated. *P* values less than 0.05 were considered as significant.

Results

Prevalence of *E. coli*

A total of 128 rectal swab samples were examined where 96 (75%) were positive for *E. coli* following cultural, morphological, biochemical and molecular examination. The study was also focused on the prevalence of *E. coli* in different age, sex, breed and management systems. According to this study the prevalence of *E. coli* was higher in the age group above 3 years, female and unorganized management (farming) systems but the difference was not statistically significant among different age, sex, breed and management systems (Table-2).

Cultural and biochemical examination

All the isolates upon overnight incubation at 37^{o}C produced purple black colored colonies with characteristic metallic sheen on EMB agar and purple colored (sorbitol fermenting) colonies on Cefixime Tellurite-Sorbitol MacConkey (CT-SMAC) medium. All the isolates fermented 5 basic sugars with the production of both acid and gas. The isolates were positive to MR and indole production test but negative to VP test.

PCR for *E. coli* 16S rRNA gene

The isolated *E. coli* were subjected to PCR using *E. coli* specific ECO-1 and ECO-2 primers. All the cultural, morphological and biochemically characterized *E. coli* were found positive by PCR examination (Fig. 1).

Discussion

Isolation and characterization of *E. coli* from diseased animal become a common practice for diagnostic purpose but very limited work is done on the prevalence and characterization of *E. coli* from apparently healthy cattle in Bangladesh. This study revealed the presence of *E. coli* in 75% in the rectal swab collected from cattle, as well as the prevalence of *E. coli* in different age group, sex, breed and management systems. According to this study prevalence of *E. coli* was high in cattle ≥3 years of age, female and unorganized farming systems, but the differences were not significant. The overall prevalence as obtained in this study support the findings of Ogunleye *et al*. (2013), who described a prevalence of 80% in the apparently healthy cattle of Nigeria but higher than the prevalence described by the Masud *et al*. (2012). According to Masud *et al*. (2012), the prevalence of *E. coli* in the rectal swab of apparently healthy cattle of 2-3 years of age is 23.21% in another geographic location in Bangladesh. These differences might be due to the differences in methodology employed in these studies. Ogunleye *et al*. (2013) and Masud *et al*. (2012) described their findings based on cultural and biochemical examination. In this study, in addition to traditional techniques *i.e.*, morphology, staining, cultural and biochemical test, PCR was employed for the confirmatory identification of *E. coli* from rectal swab of apparently health cattle. To the best of our knowledge, this is the first report on prevalence study of *E. coli* from rectal swabs of apparently healthy cattle in Bangladesh. Besides, as a base line study, we are reporting for the first time the prevalence of *E. coli* relating to age, sex, breed and management systems of apparently healthy cattle in Bangladesh.

Morphological, staining, cultural, biochemical characteristics and result of PCR examination of the isolates is in consent with the description of other authors (Schippa *et al*., 2010; Nazir *et al*., 2007, Hasina, 2006; Beutin *et al*., 1997; Mckee *et al*., 1995; Zadik *et al*., 1993; Buxton and Fraser, 1987; Cheesbrough, 1985). However, further studies are necessary to reveal out the complete characteristics of the *E. coli* isolates in Bangladesh.

Conclusions

Prevalence study of commensal *E. coli* in the rectal swab of apparently healthy animals is essential to reveal out the epidemiology of disease outbreaks and development of antibiotic resistance by *E. coli*. Though there are reports on the prevalence of pathogenic *E. coli*, information on the commensal *E. coli* are very few in Bangladesh. This study will provide a base line data on the prevalence of *E. coli* in the rectal swab of different groups of cattle in the study area. However, the area selected and sample examined in this study is very few, a study comprising larger population size origination from other part of the country will reveal the actual figure of prevalence *E. coli* in the rectal Swab of apparently healthy cattle in Bangladesh.

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