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ISOLATION AND CHARACTERIZATION OF *ESCHERICHIA COLI* FROM BUFFALO CALVES IN SOME SELECTED AREAS OF BANGLADESH

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ABSTRACT

The research works was conducted with a view to isolate and identify the *Escherichia coli (E. coli)* organism from diarrhoeic cases of buffalo reared in selected areas of Bangladesh as well the prevalence and antibiotic sensitivity pattern of the isolated *E. coli* in the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh-2202 during the period from April 2008 to May 2009. A total of 50 rectal swab samples were collected from 4 different places namely Haluaghat and Boira of Mymensingh, Madupur of Tangail and Kazipur of Sirajgonj districts. The samples were aseptically carried to the laboratory of the Department of Microbiology and Hygiene and subjected to different cultural, morphological and biochemical examinations. Upon cultural, morphological and biochemical examinations 23 (45%) samples were found to be positive for *E. coli*. The highest prevalence was found in Haluaghat, Mymensingh (53.33%) and the lowest (40.00%) in Boira, Mymensingh and Kazipur, Sirajganj. Antibiogram study revealed that the isolated *E. coli* was highly sensitive to Enrofloxacin and Ciprofloxacin, moderately sensitive to Cefalexin and Amoxicillin, and resistant to Nalidixic acid and Erythromycin.

Key words: *Escherichia coli*, isolation, identification, prevalence, antibiotic sensitivity

INTRODUCTION

The livestock sector in Bangladesh plays an important role in the national economy with the direct contribution of 2.79% in 2007-2008 to the agricultural GDP1. It generates more than 6 percent of the total foreign exchange earning through export of hide and skins, leather products, bones, hooves, edible offal. The estimated buffalo population is 1.26 million in 2007-2008. It provides meat and milk for human consumption as high quality protein, hides and draft power for ploughing, rural road and farm power transport, threshing, oil seed crushing, dung as fuel and manure (BER, 2008).

Buffalo calves are vulnerable to *E. coli* infection. Two age groups appear to be susceptible, buffalo calves of 1-2 days of age and buffalo calves of 3-8 weeks old. Symptoms include diarrhoea, a rise in temperature, weakness and lack of appetite. This is soon followed by coma and death within a few hours. In older animals there is a tendency of infection to localize itself in the joints of survivors. Lesions include enlarged, haemorrhagic spleens, and the accumulation of synovial fluid and sometimes pus in affected joints (Blood *et al.*, 1968).

Escherichia coli (*E. coli*) is Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobe and is classified under the family Enterobacteriaceae (Buxton and Fraser, 1977). *E. coli* is genetically the most versatile bacteria and is the source of many plasmid and phage mediated genes (Saylers and Whitt, 2002). *E. coli* produces septicemia and diarrhoea in a wide range of hosts including man, avian and animals such as cattle, piglets, kids, foals, lambs and buffaloes.

Pathogenicity of *E. coli* strains are due to the presence of one or more virulence factors including invasiveness factors like invasins, heat labile, heat stable enterotoxins, verotoxins and colonization factors or adhesins (Smith, 1967). Pathogenic *E. coli* are divided into two types- Enteropathogenic *E. coli* and Uropathogenic *E. coli*. Further pathogenic *E. coli* are grouped into enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), enterohemorrhagic *E. coli* (EHEC). Uropathogenic *E. coli* cause 90% of the urinary tract infection. The bacteria colonize from the faeces or perineal region and ascend the urinary tract to the bladder (Carter, 1986). The ETEC strains are known to produce two types of enterotoxins (Smith and Gyles, 1970). One type is characterized by having larger molecular weight, heat-labile (LT) and possesses immunogenic quality. This type causes delayed onset of secretory response on intestinal mucosa which persists for a longer duration. The second type is of smaller molecular weight, heat-stable (ST) toxin that is apparently nonimmunogenic (Smith and Gyles, 1970).

E. coli in buffalo is isolated, identified and characterized using cultural, staining, biochemical, serological and molecular techniques in the whole world. In Bangladesh *E. coli* organisms were isolated identified and characterized from various hosts like Chicken, Duck, Sheep, Goat, and Cattle by Choudhury *et al*., 1967; Rahman, 1987; Amin *et al*., 1988; Hossain, 2002 and Nazir, 2004. Isolation, identification and characterization of *E. coli* of Buffalo in Bangladesh still remain untouched. The objective of this study was to determine the prevalence of E. coli in faecal samples of diarrhoeic buffalo calves and establish their antibiotic sensitivity profile.

MATERIAL AND METHODS

The research work was conducted in the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh-2202 during the period of April 2008 to May 2009.

Collection and transportation of samples

A total number of 50 field samples comprising rectal swabs from diarrhoeic buffalo calves (1week to 1 year of age) were aseptically collected into nutrient broth (NB) from different areas and carried to the Department of Microbiology and Hygiene, BAU in ice box. The samples were collected from three different places in Bangladesh, namely, Haluaghat and Boira of Mymensingh district, Madupur of Tangail district and Kazipur of Sirajgonj district.

Cultural and biochemical examination of samples

The collected samples were cultured onto different cultural media like nutrient agar (NA), Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, MacConkey (MC) agar, Brilliant Green (BGA) agar and blood agar (BA) following procedure described by Cheesbrough, 1984; Carter, 1986 and Cowan, 1985. Upon cultural examination the isolated organism was subjected to different biochemical tests - sugar fermentation test, indole production test, Methyl Red –Voges Proskauer (MR-VP) test, Citrate utilization test following the method described by Chessbrough, 1984.

Preservation of *E. coli* isolates

The pure culture was stored in sterilie 80% glycerin and was used as stock culture. The equal volume of 80% glycerin and bacterial culture were mixed and capped tightly and stored at -20^0C. The isolated organisms were given code name for convenience.

Comparative antimicrobial sensitivity pattern of *Escherichia coli* isolates

Susceptibility and resistance of different antibiotics was measured *in vitro* by employing the modified Kirby-Bauer method (Bauer *et al*., 1966). The antimicrobial agent used with their disc concentration is mentioned in Table 1.

Table 1. Antimicrobial agents and their disc concentrations

Antimicrobial agent	Disc concentrations (µg/disc)
Amoxicillin	10 µg
Cephalexin	30 µg
Enrofloxacine	30 µg
Ciprofloxacin	5 µg
Cloxacillin	5 µg
Erythromycin	15 µg
Gentamicin	30 µg
Nalidixic acid	30 µg

Legend: µg = Microgram

RESULTS AND DISCUSSION

Isolation and identification results of the study indicated that the field sample contained Gram negative, rod shape and motile organism with various colony characteristics (large, smooth, round and sticky) in different bacteriological media. The isolate was able to produce characteristic black metallic sheen colonies on EMB agar, pink colony on MC agar, pinkish colony on SS agar, circular, raised, smooth, colorless colony on NA and greenish yellowish colonies in BG agar as well. The colony characteristics of the isolated *E. coli* in different media resemble the colony characteristics of *E. coli* as stated by Escherich, 1885 and Ali *et al.,* 1998. They reported that the faecal isolates showed various colony characteristics and biochemical reactions in different bacteriological agar media.

The pattern of fermentation reaction by the isolated *E. coli* with five basic sugars was observed. The organism was able to ferment lactose, dextrose and mannitol, sucrose and maltoses completely. The results of sugar fermentation partially agree with the findings of Beutin *et al.*, 1993 and Sandhu *et al*., 1996. They reported that although *E. coli* ferments all five basic sugars but it partially ferments sucrose and maltose. Variation of the results may be due to genetic factors and nature of inhabitant of the organisms. All the isolates were found to be positive in catalase, methyl-red positive and Indole but negative to VP test which supports the findings of Beutin *et al*., 1993.

Among the isolated organisms, the highest prevalence was found in Haluaghat, Mymensingh (53.33%) and the lowest (40.00%) in Boira, Mymensingh and Kazipur, Sirajganj (Table 2). The prevalence of *E. coli* partially coincides with the findings of Islam *et al.* 2008. They reported the prevalence of *E. coli* in buffalo is 37.9%.

Table 2. Overall prevalence percentage of *Escherichia coli* in diarrhoeic buffaloes

SI. No.	Sources and Location	Total no. of rectal swab samples collected	Number of buffalo calves positive for E. coli	Percentage of isolated E .coli
1	Madhupur, Tangail	15	7	46.66
2	Boira, Mymensingh	5	2	40.00
3	Haluaghat, Mymensingh	15	8	53.33
4	Kazipur, Sirajganj	15	6	40.00
Total		50	23	46.00

From antibiogram study it was found that *E. coli* was highly sensitive to Enrofloxacin, Ciprofloxacin, moderately sensitive to Cefalexin and Amoxicillin, and resistant to Nalidixic acid and Erythromycin (Table 3) which supports the findings of Nazir *et al.*, 2004.

Finally, from the findings of different cultural, staining, biochemical examinations it may be concluded that the isolated organism was *E. coli*.

Table 3. Antibiotic sensitivity pattern in percentage

Name of organisms	Sensitivity pattern	% of isolated strains sensitive to various antibiotic							
		CIP	ENR	AMO-X	Gn	E	CI	OB	NA
E. coli	Resistance	0	12.17	22.51	0	33.33	0	4	36.67
	Less sensitive	28.17	43.49	51.22	33.33	66.67	66.67	0	33.33
	Moderately sensitive	45.00	21.17	26.17	66.67	0	33.33	0	0
	Highly sensitive	26.83	23.17	0	0	0	0	0	0

Legends: AMOX = Amoxicillin, ENRO = Enrofloxacin, CIP = Ciprofloxacin, Cl = Cephalexin, E = Erythromycin, Gentamicin, NA = Nalidixic acid, OB = Cloxacillin

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