MANNOSE SENSITIVITY AND MANNOSE RESISTANT HAEMAGGLUTINATON BY *ESCHERICHIA COLI* STRAINS ISOLATED FROM DIARRHOEIC CALVES

Aniruddha Udaykar and Rakesh Sharda
Department of Microbiology

College of Veterinary Science and Animal Husbandry, Mhow, (M.P.)

ABSTRACT

Sixty two strains of E. coli were isolated from 116 diarrhoeic fecal samples out of which 20 (32.25%) and 25 (40.32%) exhibited mannose sensitive haemagglutination (MSHA) and mannose resistant haemagglutination (MRHA), respectively. The MSHA strains belonged to the somatic group O13, O14, O28, O69, O88, O106, O116 and O132, whereas MRHA strains belonged to somatic group O3, O13, O14, O28, O88, O106, 0116. Four untypable strains also showed MRHA. Six different MRHA patterns were recorded.

KEY WORDS : *E. coli,* Haemagglutination, MRHA, MSHA.

INTRODUCTION

Haemagglutination (HA) of *E.coli* was first described by Guyot (1908), who observed that some strains possessed the ability to agglutinate red blood cells of number of animal species. Duguid *et al*. (1979) classified this HA in two types viz., MSHA and MRHA, on the basis of its sensitivity or resistance to D-mannose. They recognized HA as an adhesive property of fimbriae observed under electron microscope. Thus, HA provides a mean for detecting and classifying specific adhesions of *E.coli* strains (Duguid and Old 1980; Gaastra and deGraff, 1982). In the present communication we report the mannose-sensitive (MS) and mannose-resistant (MR) Haemagglutination strains of *E. coli* isolated from diarrhoeic cow and buffalo calves.

MATERIALS AND METHODS

A total of 116 diarrhoeic fecal samples were collected from 63 buffalo and 53 cow calves reared in organized and unorganized farms of the Malwa region of Madhya Pradesh and processed for isolation of *E. coli*, following the method of Edwards and Ewing (1972). The isolated strains were identified as *E. coli* on the basis of biochemical and morphological characteristic as described by Barrow and Feltham (1993), and confirmed serologically by National *Escherichia* Typing Centre, CRI, Kasuli (H.P.).

The test for mannose sensitive and resistant haemagglutination ability of the *E. coli* isolates was carried out to characterize their colonization factor as per method described by Old (1985). Blood samples from cow calf, buffalo calf, goat, sheep, and poultry were collected aseptically in Alsever's solution. The red blood cells (RBC) were washed thrice by centrifugation with phosphate buffer saline (PBS; pH 7.4) and finally a 3% (v/v) solution of erythrocytes in saline was prepared and stored at 4^0C for maximum of 4 days. The isolates were subcultured for three consecutive days on brain heart infusion (BHI) agar at 37^0C and suspended in PBS (pH 7.4) to give a concentration of approximately 10^{10} organisms per ml.

For haemagglutination test, 20 µl of bacterial suspension was mixed with an equal volume of RBC suspension on a cavity glass slide and 20 µl of PBS was added. Reactions were recorded (+) for heamagglutinaton or (-) for no heamagglutination. A control consisting of RBC suspension and PBS was included in the test.

To demonstrate mannose sensitive Haemagglutination (MSHA) of the isolates, 1% (w/v) D (+) mannose solution was used. The effect of carbohydrate was assessed by adding 20µl of sugar solution to the bacteria-red blood cell suspension as described above. A positive control consisting of equal volumes of bacterial suspension and RBC suspension was included in each test. MSHA was recorded if previously positive haemagglutination turned negative.

RESULTS AND DISCUSSION

In the present study, 32.25 and 40.32 per cent of the isolated strains belonging to 8 and 7 different somatic groups showed MSHA and MRHA, respectively. Four strains belonging to serogroups O69 and O159

exhibited only MSHA (MS+/MR-), whereas 9 strains from somatic group O3, O88 and four untypable strains showed MRHA (MS-/MR+). Sixteen isolates from serogroups O13, O14, O28, O88, O106, O116, 0132, were positive for both MSHA and MRHA (MS+/MR+). Thirty two strains belonging to somatic groups O2, O12, O15, O20, O22, O60, O97, O98, O105, O108, O110, O127, O138, O149, O166, O171 and two rough strains, however, failed to exhibit MS/MR agglutination with red blood cells of any selected animal species (MS-/MR-). Kaura, *et al.* (1991) reported 18 types, Dubey *et al.* (2001) reported 3 types and Yadav and Sharda (2008) reported 6 types of MRHA patterns. The present findings are in partial agreement with other workers (Shome and Shome ,1996; Yadav and Sharda, 2008), who observed variable percentage of *E. coli* strains exhibiting MSHA and/ or MRHA.

Twenty five isolates comprising 8 'O' serogroups namely O3, O13, O14, O28, O88, O106, O116, O132 and four untypable strains were grouped into six different MRHA patterns based on their haemagglutination reactions with erythrocytes of one or more animal species. The strains belonging to somatic group O3, O14 and four untypable strains produce type I pattern. The strains belonging to somatic group O28 and O88 showed type III pattern, whereas strains belonging to O116, O132, O13, and O106 showed II, IV, V and VI pattern, respectively (Table 1).

TABLE 1 DIFFERENT PATTERNS OF MRHA IN *E. COLI* ISOLATES FROM DIARRHOEIC CALVES

S. No.	Serotype	No. of MRHA Isolate	Patterns of MRHA	Haemagglutination reaction with erythrocyte of				
				Cattle	Buffalo	Sheep	Goat	Poultry
1	O14	5	I	+	+	-	-	-
2	O3	2	I	+	+	-	-	-
3	UT	4	I	+	+	-	-	-
4	O116	3	II	+	+	+	+	-
5	O28	2	III	-	+	+	-	-
6	O88	34	III	-	+	+	-	-
7	O132	1	IV	-	-	+	+	+
8	O13	2	V	-	-	-	+	-
9	O106	2	VI	-	-	-	-	+

The pathogenecity of *E. coli* strains is related to the presence of adhesins on their surface, some of which promote MRHA of animal and human erythrocytes. Thus, MRHA can be considered as an indirect indicator of virulence of *E. coli* that should be investigated for their pathogenecity (Parry and Rooke, 1985). Moreover, HA typing can also be used as an epidemiological tool.

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