International Journal of Microbiological Research 3 (3): 227-237, 2012
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2012.3.3.71164

Phenotypic Characterization of *E. coli* Isolated from Farm Animals with Diarrhea: Use of Lectin as a Non-Specific Immunostimulant

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Abstract: Serogroups O26, O55:K59, O86, O91, O111, O118 and O145 of *E. coli* were prevalent among the diarrheic calves' and lambs' newborns which are of zoonotic impact posing a serious public health risk to humans. No single virulence factor was identified to be common to all isolates; nevertheless their virulence may be allied many and varied combinations of identified biological properties which work in concert with one another to produce a desired effect on the host cell. Lectin could overcome medication interference dilemma, since it improves host immunity which is the best way to overcome the worldwide threat of *E. coli* diarrhea in newborns as it significantly enhanced the phagocytic activity, phagocytic index and humeral immune response resulting in reduction of the death rate and the intense of infection evidenced by histopathological examination.

Key words: *E. coli* · Lectin · Erthyrophagocytosis · Phagocytic index

INTRODUCTION

Homeostasis of the mammalian intestinal epithelium is maintained by keeping a balance between tolerance and response to luminal microorganism. Infection with pathogens disrupts this balance, leading to intestinal inflammation [1].

Newborns diarrhea is considered as one of the most tenacious field problems, threatening their life, as some epidemiological studies have revealed that 30% of the new born calves are hypogamma globulinemic and highly susceptible to opportunistic *Escherichia coli* (*E. coli*) infection [2], which leads to great economic losses directly through need for treatment and potential death and indirectly through possible impact on subsequent growth performance posing a significant threat to the food animal industry worldwide.

E. coli are ubiquitous bacteria form a wide variety of ecosystems including the gastrointestinal tract of warm blooded animals and humans [3]. Few microorganisms are as versatile as *E. coli*, beside their presence as a gut normal flora; they could be frequently deadly pathogens [4].

E. coli serogroups frequently isolated from diarrheic calves and lambs are O26, O55, O86, O91, 103, O111, O118, O145 and O166 [5-8]. Most of them are clinically relevant to non *E. coli* O157 shiga toxigenic *Escherichia coli* (STEC) [9-11], which are more prominent than *E. coli* O157:H7 in many countries [4, 12-14].

Human infection with STEC can cause a wide spectrum of clinical manifestations from asymptomatic infection, mild to moderate diarrhea and hemorrhagic colitis to hemolytic uremic syndrome (HUS) [15, 16]. *E. coli* O157:H7 is a widely reported cause for human outbreaks, albeit other non *E. coli* O157 STEC can cause a similar disease spectrum [17]. Ruminants either healthy or diarrheic ones are the main reservoir of human infections [10, 18, 19] that are transmitted to humans either through direct contact [20] or through food chain [21].

The WHO endorsed that one attractive strategy to decrease morbidity and mortality due to infectious diarrhea is to improve the host resistance to infection ever since the cellular immune response to attaching-effacing (A/E) pathogens plays an important role in bacterial pathogenesis as it can clear the bacteria or modulate the inflammatory process [22].

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The aim of this study was to inspect the predominant serogroups of *E. coli* isolated from diarrheic claves and lambs, keep an eye on their virulence factors and assess lectin as a non-specific immune stimulant against gastrointestinal infection.

MATERIALS AND METHODS

Fecal Samples: A total of 89 fecal swabs was collected from 57 calves and 32 lambs suffering from diarrhea, with ages up to four months. Samples were collected from El-Fayoum Governorate from March 2010 up to October 2010. The collected swabs were submitted to the laboratory on ice packs in chest. Samples were processed directly after reception.

Isolation and Biochemical Identification of *E. coli*: Isolation and biochemical identification of *E. coli* were carried out as outlined by Collee *et al.* [23].

Serological Identification of *E. coli*: Serological identification of *E. coli* was carried out according to Ewing [24].

Detection of Virulence Traits: Congo red binding assay [25], hemolytic activity on 5% washed sheep blood agar medium [26], hemagglutination (HA) of *E. coli* isolates with human, cattle, buffalo, sheep, horse, camel, chicken and mice erythrocytes in the presence and absence of mannose [27], serum resistance test [28] and the ability to grow for 18 hrs in serum [29].

Experimental Challenge

Experimental Design: Ninety BALB/c mice (20-25 g, each) were purchased from Research Institute of Ophthalmology Financial Management (Giza, Egypt) to be used in this study. Mice were divided randomly into six groups, 15 each. Group C (was kept as negative control); group L (was given lectin "Lector, Micro biotic, 50 mg/ml" in drinking water in the recommended dose, 0.25 ml/L till the end of the experiment); group H (was given lectin in drinking water double the recommended dose, 0.5 ml/L till the end of the experiment); group I (each mouse of the group was orally inoculated with a single dose of 150 μL containing 10^{10} CFU of *E. coli*) [30] by pipette feeding method to imitate the natural route of exposure to the organism after 16 hrs starvation, as prolonged dietary restriction promotes enterohemorrhagic *E. coli* (EHEC) colonization [31]; group LI (was given lectin in drinking water in the recommended dose, 0.25 ml/L for 14 days and then each mouse of the group was orally inoculated with a single dose of 150 μL containing 10^{10} CFU of *E. coli* and then lectin administration with the same dose was continued till the end of experiment) and group HI (was given lectin in drinking water double the recommended dose, 0.5 ml/L for 14 days and then each mouse of the group was orally inoculated with a single dose of 150 μL containing 10^{10} CFU of *E. coli* and then lectin administration with the same dose was continued till the end of experiment).

Shedding Follow Up: It was done by direct plating of fecal samples obtained from all groups at 72 hrs before challenge, at the day of challenge and then at 1, 3, 5, 7, 10 and 14 days post challenge. A sweep of each plate was homogenized in 50 μL PBS and mixed with an equal volume of mice serum infected with the inoculated strain.

Assessment of Lectin Immunomodulating Effect on Phagocytic Capacity by Erythrophagocytosis: Erythrophagocytosis was done according to Zhang *et al.* [32], in addition the phagocytic activity and the phagocytic index (P.I) were calculated according to Bassøe *et al.* [33] in order to estimate the immunomodulating effect of lectin on phagocytes.

Assessment of Lectin Immunomodulating Effect on the Humeral Immune Response: Assessment of lectin immunomodulating effect on the humeral immune response was inspected by the plate agglutination test according to Bokermann *et al.* [34].

Histological Examination: Histological examination of kidneys and ceca was carried out according to Banchroft *et al.* [35].

RESULTS

Isolation and Serological Identification: *E. coli* isolates belonged to six O serogroups (O26, O86, O91, O111, O118 and O145) were detected.

Virulence Traits

Congo Red (CR) Binding Assay: All *E. coli* isolates were CR^{+}.

Hemolytic Activity: Out of the 99 *E. coli* isolates, 15 (15.2%) were ß-hemolytic while 9 (9.1%) were α-hemolytic.

Table 1: Hemagglutinating activities of *E. coli* isolates towards erythrocytes of various mammalian and avian species

	HA titer					HAI titer				
Erythrocyte	+	++	+++	++++	Total	+	++	+++	++++	Total
Human AB	19	9	0	6	34	0	11	8	3	22
Cow	10	14	0	10	34	3	8	5	3	19
Buffalo	13	7	5	0	25	0	7	0	0	7
Sheep	11	0	7	0	18	7	0	0	0	7
Horse	7	10	2	5	24	3	0	0	0	3
Camel	10	16	5	12	43	3	0	3	0	6
Mice	24	0	0	0	24	6	0	0	0	6
Chicken	23	5	0	6	34	0	0	0	0	0

HA: Hemagglutination

HAI: Hemagglutination inhibition with 1% mannose

Number of "+": Indicate the degree of agglutination

Hemagglutination Traits of *E. coli* Isolates: Table 1 reveals that out of the 99 *E. coli* isolates, 34(34.3%), 34(34.3%), 25(25.3%), 18(18.2%), 24(24.2%), 43(43.4%), 24(24.2%) and 34(34.3%) were hemagglutinating for human (AB Rh^+ blood group), cow, buffalo, sheep, horse, camel, mice and chicken erythrocytes respectively. While hemagglutination inhibition test was done with incorporation of mannose 1%, the results revealed that the mannose resistant hemagglutinating *E. coli* were, 22 (22.2%), 19(19.2%), 7(7.1%), 7(7.1%), 3(3%), 6(6.1%), 6(6.1%) and 0(0%) for human (AB Rh^+ blood group), cow, buffalo, sheep, horse, camel, mice and chicken erythrocytes respectively out of the 99 *E. coli* isolates.

Serum Resistance Traits of *E. coli* Isolates: Table 2 reveals the inhibitory effect of serum on the isolated *E. coli* and complete inhibition was reported in 1(1%) and 6(6.1%) after 3 and 6 hrs of serum incubation respectively out of the 99 *E. coli* tested isolates, while by expanding the incubation time to 18 hrs, the inhibitory effect increased to 23(23.2%) out of the 99 tested isolates.

Results of Experimental Challenge:

Influence of Lectin on Death Rate: The death rates of the groups received infection were 33.3, 6.7 and 6.7% for the groups I, LI and HI respectively.

Shedding of the Inoculated *E. coli* Strain among the Examined Groups: Table 3, shows the re-isolation of the inoculated *E. coli*, which was not recorded in C, L and/or H groups. The inoculated E. coli was re-isolated from I, LI and HI groups at the 1st, 3rd, 5th, 7th and 10th day, post challenge, while it is only isolated from I and LI at 14th day post challenge not from LH group.

Table 2: Serum resistance traits of *E. coli* isolates towards the homologous species serum

	Serum resistance				
Time	-	+	++	+++	++++
0 Hour	0	0	3	9	87
1 Hour	0	3	0	45	51
3 Hours	1	8	20	31	39
6 Hours	6	24	21	16	32
18 Hours	23	76			

"-": No growth

Number of "+": Indicate the degree of growth

Table 3: Shedding percentage of inoculated *E. coli* among the examined groups

Days	C	L	H	I	LI	HI
0	-	-	-	-	-	-
1	-	-	-	100%	33.3%	25%
3	-	-	-	100%	33.3%	25%
5	-	-	-	100%	33.3%	25%
7	-	-	-	100%	33.3%	25%
10	-	-	-	100%	33.3%	25%
14	-	-	-	100%	33.3%	-

C: Control which did not receive any treatment

I: Infected group which did not receive lectin

L: Low lectin dose group which did not receive infection

H: High lectin dose group which did not receive infection

LI: Low lectin dose group which received infection 14 day after beginning of lectin administration

HI: High lectin dose group which received infection 14 day after beginning of lectin administration

Assessment of Lectin Immunomodulating Effect on Phagocytic Capacity: Table 4 shows the phagocytic activities of the groups C, L and H which were 45.66, 66.47 and 70.73 % respectively and phagocytic activities of the groups I, LI and HI were respectively 74.73, 92.39 and 94.52% at the 7th day and 77.14, 90.77 and 85.54% at the 14th day post challenge.

Table 4: Phagocytic activities and phagocytic indices in all mice groups

				I		LI		HI	
	C	L	H	7th day	14th day	7th day	14th day	7th day	14th day
Phagocytic activity %	45.66	66.47	70.73	74.73	77.14	92.39	90.77	94.52	85.54
PI	1.17	1.39	1.45	1.91	2.41	3.23	3.13	3.28	2.14

PI: Phagocytic index

C: Control which did not receive any treatment

I: Infected group which did not receive lectin

L: Low lectin dose group which did not receive infection

H: High lectin dose group which did not receive infection

LI: Low lectin dose group which received infection 14 after beginning of lectin administration

HI: High lectin dose group which received infection 14 after beginning of lectin administration

Table 5: Comparison of humeral immune responses in all groups using plate agglutination test

Days	C	L	H	I	LI	HI
7th	-	-	-	1:4	1:64	1:64
14th	-	-	-	1:32	1:128	1:128
21st	-	-	-	1:64	1:256	1: 512

C: Control which did not receive any treatment

I: Infected group which did not receive lectin

L: Low lectin dose group which did not receive infection

H: High lectin dose group which did not receive infection

LI: Low lectin dose group which received infection 14 after beginning of lectin administration.

HI: High lectin dose group which received infection 14 after beginning of lectin administration

The same table blurts out that the phagocytic indices were 1.17, 1.39 and 1.45 for C, L and H groups respectively and the phagocytic indices of the groups I, LI and HI were respectively 1.91, 3.23 and 3.28 at the 7th day and 2.41, 3.13 and 2.14 at the 14th day post challenge.

Assessment of Lectin Immunomodulating Effect on the Humeral Immune Response: Table 5, presents the humeral immune response in all groups, no detectible antibody was observed in the groups that did not receive infection, while the end titer of the detectible antibodies in the groups that received infection I, LI and HI were respectively (1:4), (1:64) and (1:64) at the 7th day; (1:32); (1:128) and (1:128) at the 14th day and (1:64), (1:256) and (1:512) at the 21st day post challenge.

Histological Examination: Ceca and kidneys of groups C, I, LI and LH were examined. Histopathological examination of the group C revealed the normal histological structure of the glomeruli and tubules at the cortex (Fig. 1) and the normal histological structure of the mucosa, submucosal muscular and serosa (Fig. 2).

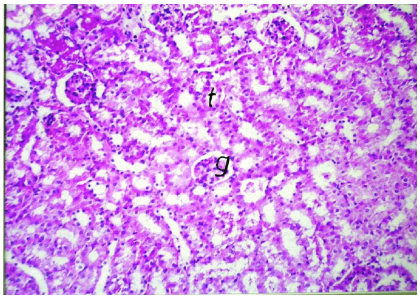

Fig. 1: Kidney of group C, showing normal histological structure of the tubules (t) and glomeruli in the cortical portion (H and E x40)

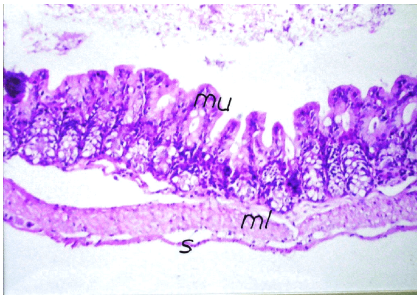

Fig. 2: Cecum of mice in group C showing normal histological structure of mucosal layer (mu), muscular and serosal one (s) H and E x40

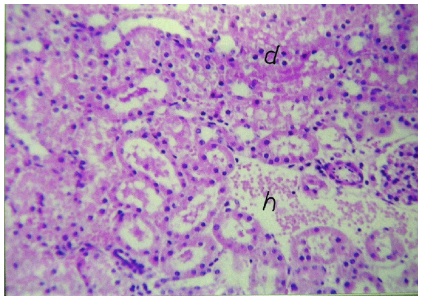

Fig. 3: Kidney of group I, showing extravasations of blood cells (hemorrhage) in between the degenerated tubules at the cortex (H and E x40)

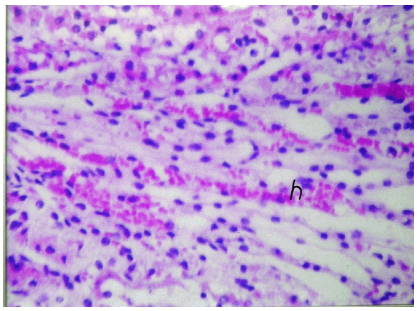

Fig. 4: Kidney of group I, showing focal hemorrhage in between the tubules in the corticomedullary portion (H and E x64)

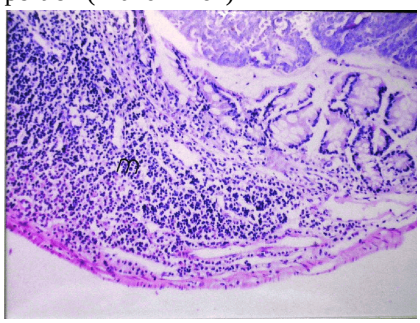

Fig. 5: Cecum of group I, showing focal lymphoid proliferation in the submucosal layer (H and E x40)

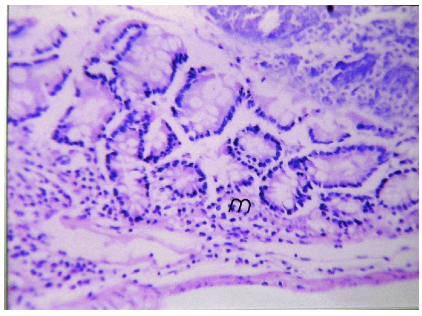

Fig. 6: Cecum of group I, showing inflammatory cells infiltration in the lamina propria of the mucosal layer extended to submucosa and serosal layer (H and E x64)

When kidney and cecum of group I were examined, focal hemorrhage was detected in between the degenerated tubules at the cortex associated with swelling and vacuolization in the endothelial cells lining the glomerular tufts (Fig. 3) as well as the corticomedullary portion showed focal hemorrhages also in between the tubules (Fig. 4), while the lesions reported were focal lymphoid cells proliferation in the submucosal layer (Fig. 5), associated with inflammatory cells infiltration in the lamina propria of the mucosal layer which was extended to the underlying submucosa (Fig. 6).

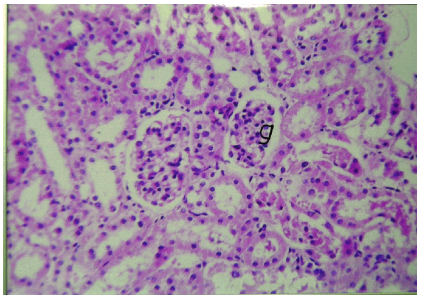

Fig. 7: Kidney of group LI, showing swelling of the endothelial cells lining the glomerular tuft (H and E x64)

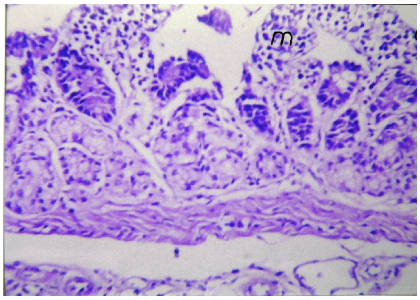

Fig. 8: Cecum of group LI, showing mucosal epithelial cells desquamation with inflammatory cells infiltration in lamina propria

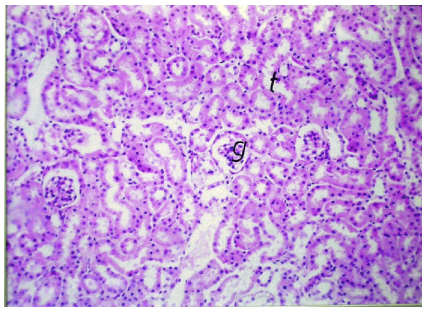

Fig. 9: Kidney of group HI showing intact normal histological structure (H and E x40)

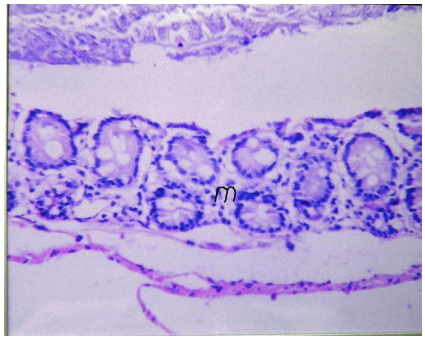

Fig. 10: Cecum of group HI showing few inflammatory cells infiltration in lamina propria of mucosal layer (H and E x64)

Group LI histopathological examination appeared in (Fig. 7), showing glomeruli with swelling in the lining endothelial cells and (Fig. 8), showing inflammatory cells infiltration in the lamina propria of the mucosal layer associated with desquamation in the lining epithelium.

Furthermore, kidney and cecum of group HI showed no histopathological alteration (Fig. 9) and few inflammatory cells infiltration was noticed in the lamina propria of the mucosal layer (Fig. 10).

DISCUSSION

Non-O157 STEC are worrisome emerging pathogens; escaping diagnosis by laboratories which mostly use sorbitol MacConkey agar to detect only *E. coli* O157 [36], that implicated as an etiological factor of diarrhea in calves [13, 14, 37-39] and lambs [5]. In addition, they are of zoonotic impact posing a serious public health risk to humans [40].

A global surveillance consortium of 35 countries that tracked enteric infectious diseases, showed that the number of human cases of illness caused by non-O157 EHEC, increased globally by 60.5% between 2000 and 2005, while at the same time the number of cases linked to EHEC O157 increased by only 13% [41]. In addition, Food and Drug Administration has recognized that non-O157 STEC serogroups are emerging as an important cause of food-borne disease [42].

Serological identification gives advantage to enlighten the physicochemical surface structures which give an over view of the virulence of such strain [43, 44] as described before that *E. coli* O55:K59 which has smooth hydrophilic O antigen properties shows resistance to phagocytosis [45], side by side serological identification is a valuable epidemiological and reference tool.

In our study, O26, O86, O111, O118 serogroups were isolated from calves with diarrhea, which were reported formerly to be responsible for digestive disorders in calves [7, 8, 46], while the O118 serogroup was reported to be the major group of STEC pathogens in calves [47]. Serogroups O91, O145, O55K59 and O111 were isolated from lambs with diarrhea and they were previously isolated from diarrheic lambs [5, 6].

Concerning host specificity of *E. coli*, there was no difference in the pathogenic potential in *E. coli* serogroups O26, O111 and O118 [7, 48, 49]. Large and small ruminants in Egypt could be a potential source of infection in humans [5].

Discrimination of pathogenic strains from the normal flora depends on the identification of the virulence traits of the isolated *E. coli*. All *E. coli* isolates were positive for the Congo red binding test, which is an easily identifiable phenotypic marker that has been used to assess virulence in enteropathogenic *E. coli* (EPEC) and EHEC [50] since it is associated with curli expression [51], the main factor responsible for the increased adherence to the human colonic cell line "Caco-2 cells", which could promote higher levels of colonization and inflammation in the intestine and consequently increase the chance of toxin exposure and the likelihood of progression to HUS [52].

ß-hemolysis phenotypic trait was reported in 15 (15.2%) out of the 99 tested isolates. Side by side α-hemolysin trait which could not be detected except on washed blood agar medium [53], was detected in 9 (9.1%) out of the 99 tested isolates. Enterohemolysins traits role in causing calf diarrhea has not been demonstrated [39], but it has suggested that these virulence factors may compliment the effect of Stx [54].

HA traits were observed in 34(34.3%), 34(34.3%), 25(25.3%), 18(18.2%), 24(24.2%), 43(43.4%), 24(24.2%) and 34(34.3%) out of the 99 *E. coli* isolates for human (AB, Rh^+ blood group), cow, buffalo, sheep, horse, camel, mice and chicken erythrocytes respectively. HA behavior of each isolate revealed that no single isolate was agglutinating for erythrocytes of the all tested species, furthermore the presence of a diversity of surface adhesion molecules indicating multiple adherence mechanisms to epithelium tissues [55], as well as the erythrocyte ligand structure differences of different species [27]. The diversity reported within the isolated serogroups implying a high degree of diversity in STEC group [56].

Common fimbrial adhesion expressed by both pathogenic and non-pathogenic *E. coli* causes a mannose-sensitive hemagglutination (MSHA) of erythrocytes from various animal species [55]. While, the mannose resistant hemagglutinating *E. coli* were, 22 (22.2%), 19(19.2%), 7(7.1%), 7(7.1%), 3(3%), 6(6.1%), 6(6.1%) and 0(0%) out of the 99 tested isolates for human (AB Rh^+ blood group), cow, buffalo, sheep, horse, camel, mice and chicken erythrocytes respectively; moreover, incorporation of mannose not only inhibit the agglutination but what's more it could only reduce the degree of agglutination in many cases which indicate the presence of both fimbrial and non-fimbrial adhesins within the same isolate. In mannose resistant hemagglutinating (MRHA) *E. coli*, MRHA capacity is related to non-pili low

molecular weight proteins [27, 57]. Moreover, MRHA *E. coli* did not adhere to rat peritoneal macrophages and human polymorphonuclear granulocytes [58] giving a virulent advantage for *E. coli* strains.

Complete inhibition of growth was reported in 1(1%) and 6(6.1%) out of the 99 *E. coli* tested isolates after 3 and 6 hrs of serum incubation respectively and the inhibitory effect increased to 23(23.2%), when the incubation time expanded to 18 hrs. Serum resistance is one of the important virulence traits in smooth strains of Gram negative bacteria [59] and it is often associated with the F17-G fimbrial adhesin subunits in pathogenic *E. coli* strains in domestic animals that recognize N-acetyl-D-glucosamine (GlcNAc) receptors present on bovine intestinal cells [60].

It seems noticeable that, no single virulence factor was identified to be common to all isolates; nevertheless their virulence may be allied many and varied combinations of identified biological properties which work in concert with one another to produce a desired effect on the host cell.

Lectins are widely distributed among plants and animals. They are proteins with multi-carbohydrate recognition domains which bind to cell surface sugars [61], competing for STEC adhesion sites putting a stop to their adhesion to the intestinal epithelium, the critical step for infection by this type of bacteria [62]. What is more, phagocytic cells could be cytotoxic to other cells in the presence of lectin [63] therefore lectin is considered as an opsonin, since it increases the phagocytic index *in vitro* [61]. Our study aimed to test lectin immunomodulating action *in vivo* in BALB/c mice.

Mouse infection model mirror various aspects of STEC pathogenesis or disease such as intestinal colonization, renal impairment and death [31]. Using mouse model with normal gut flora in order to better reflect the usual gastrointestinal environment to which STEC is exposed following ingestion with typical exposure to molecular signals through a quorum-sensing regulatory network of commensal bacteria that result in type three secretion system expressions [64] requisite for adhesion.

In our experiment, phagocytic capacity was measured by mouse intraperitoneal injection of chicken erythrocyte, in view of the fact that murine peritoneal macrophages phagocytose heterologous blood cells after their intraperitoneal administration [65].

We found that the phagocytic activities were 45.66, 66.47 and 70.73% for groups C, L, H respectively, while the phagocytic activities of the groups I, LI and LH were respectively 74.73, 92.39 and 94.52 at the 7th day and 77.14, 90.77 and 85.54 at the 14th day post challenge. In addition the phagocytic indices were 1.17, 1.39 and 1.45 for the C, L and H groups respectively, while the phagocytic indices for the groups I, LI and HI respectively were 1.91, 3.23 and 3.28 at the 7th day and 2.41, 3.13 and 2.14 at the 14th day post challenge. These results indicated a positive effect of lectin on the phagocytic activity and phagocytic index *in vivo* [66] and when compared with the re-isolation rate of the inoculated *E. coli* strain, group I shed the inoculated strain till the last monitoring time at the 14th day in 100% of the examined mice, but isolation rates from LI and HI groups 33.33 and 25% from the 2nd till the 10th day post challenge. At the 14th day post challenge; groups received lectin and inoculated with *E. coli* strain (LI and HI groups), the re-isolation rate was not only lower than those that did not receive lectin but moreover, the group HI which received lectin in a high dose (0.5 ml/L water) completely had got rid of the infection. The observed result is of entire harmony with the report of Liu and Zheng [67] who declared that the phagocytic capacity *in vivo* is an integrated performance of monocytes-macrophages to capture and digest the invading organisms that involve the secretion of cell adhesion molecules, exerting phagocytosis, lysosome activation, antigen process and presentation which is indicative of greater pathogen clearance [68].

In addition, lectin could compete with *E. coli* for its intestinal receptors, as *E. coli* adhesion to the host cells is mediated by lectin present on their surface that binds to complementary carbohydrates on the surface of the host tissues [69] which result in inhibition of bacterial adherence and subsequently colonization.

On the other hand, detection of humeral immune response with plate agglutination test revealed that there is not any detectable titer against the inoculated strain in the C, L and H groups, while the end titer for the I, LI and HI groups are respectively, 1:4, 1:64 and 1:64 at the 7th day; 1:32, 1:128 and 1:128 at the 14th day and 1:32, 1:256 and 1:512 at the 21st day post challenge and this reflects positive influence of the phagocytic activity on humeral immune response [68].

Formation of A/E lesions on the gut enterocytes is the central to the pathogenesis of EHEC [70], followed by Stx production which reach globotriaosyl ceramide (Gb3) rich organs like kidneys [71] through the circulation. Histopathological examination of kidney and cecum sections from groups C, I, LI and HI with the intention to correlate the death rate, immunological status and

shedding manner with the healthy of the cecum and kidney tissues the targets for STEC and their products [72] and they are of matching manner, the most prominent lesions were seen in group I, such as focal hemorrhage in between the degenerated tubules at the cortex associated with swelling and vacuolization in the endothelial cells lining the glomerular tufts (Fig. 3) moreover, focal hemorrhages in between the tubules of the corticomedullary portion (Fig. 4) in kidney section [31] While the cecum lesions reported were focal lymphoid cells proliferation in the submucosal layer (Fig. 5), associated with inflammatory cells infiltration (Fig. 6) in the lamina propria of the mucosal layer which was extended to the underlying submucosa [31].

The intense of the lesions were reduced in group LI, (Fig. 7) showing swelling in the lining endothelial cells of glomeruli and (Fig. 8), showing inflammatory cells infiltration in the lamina propria of the mucosal layer associated with desquamation in the lining epithelium. Furthermore, kidney and cecum of group HI showed no histopathological alteration (Fig. 9) and few inflammatory cells infiltration was noticed in the lamina propria of the mucosal layer (Fig. 10).

From this Study the Following Can Be Concluded: Serogroups O26, O55:K59, O86, O91, O111, O118 and O145 of *E. coli* were prevalent among the diarrheic newborns which are of zoonotic impact posing a serious public health risk to humans.

Lectin could overcome medication interference dilemma, since it improve host immunity which is the best way to overcome the worldwide threat of *E. coli* diarrhea in newborns.

Reduction of animal colonization with pathogenic *E. coli* will reduce contamination of food and environment and subsequently control *E. coli* infection in humans.

ACKNOWLEDGMENT

We wish to express our great gratefulness and from the bottom of our hearts thanks to Prof. Dr. Ismail Abd El-Hafeez Radwan, Prof. and head of Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University; Prof. Dr. Sabry Mohamed Tamam, Prof. of Virology, Virology Department, Faculty of Veterinary Medicine, Beni-Suef University and Dr. Samah Helmy, Researcher at Immunology Department, AHRI. Dokki, Giza, Egypt; for their fruitful efforts and support through out the work.

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