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Immunological Study for The Changes that Associated with Infection of Mice with Shiga Toxin Producing *Escherichia coli* (STEC) *O157:H7*

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Abstract

Present study discussed the immunological effect of STEC E. coli O157:H7 on mice. Sixty Albino mice, both sexes with age between(6–8) weeks of old were divided into two groups: "infected group" (30) were inoculated with infectious dose orally , and "control group" (30) were given phosphate buffer saline orally. The immunological feature, mice of the infected group revealed both of the humoral and cellular immune response. The HI response was detected by the passive haemagglutination test and the results revealed the higher titer of antibodies is about (253.0±21.70) at 28 day after infection. The CI response was detected by the skin test, the mean thickning of the right footpad was (3.03±0.15 mm) after about one day then dropped to reach (1.80 ±0.09 mm) after 3 days in mice with infectious dose. The cytokines level was measured by Sandwich ELISA (Interleukin 10 (IL10) and interferon gamma (IFN- γ)) which showed a major increase ($P<0.05$) in the comparison with control group at all experiment period (14, 21 and 28) days post infection. In conclusion, The investigational infection with STEC O157:H7 activated both CI and HI response with raised of inflammation cytokines (IFN- gamma and IL-10).

Key words: E. coli, CI, HI, IL10, INFγ

Introduction

Escherichia coli (*E.coli)* is a member of *Escherichia* genus in the Enterobacteria-ceae family. It is a Gram -ve, facultative anaerobic, non-spore forming organism which is originate into the nature, also in intestine and out of the intestine of mammals (Kaper *et al*., 2004 and Zainab *et al*., 2013). The STEC were classified depending on the importance of serotype O157:H7 in human disease, into two major categories, STEC O157 and non-O157 STEC (Bettelheim, 2007). E. coli O157:H7 is an developing public health problem in the world. It is the important cause of food-borne disease (Schlundt, 2001) and is transmitted through the faecal-oral route. The main site for O157:H7 is intestine of healthy animals. Specific animals are rapidly inhabited and shed E. coli in their stools (Bach *et al*, 2002). The source of E. coli O157:H7, which inhabit the animal, are little known, and little most was known about epidemiology of E. coli O157:H7 in environment (Bach *et al*, 2002). In addition, high variability in occurrence of the E. coli O157:H7 among the animals refers to the likelihood of reservoir of E. coli O157:H7 out to it. Nevertheless, other than the discovery of E. coli O157:H7 in other animals, like dogs, horses, sheep, and wild birds (Bach *et al*,

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2002), epidemiology of the bacterium has not widely known. One of the potential styles of the distribution of this bacteria into the surroundings is by the insects that are related with the animal manure, chiefly houseflies. The larvae develops into feces. So, HF make a so large inhabitants on the animal farmhouses and other animal facilities. Beforehand, a laboratory built observation showed that E. coli O157:H7 that is swallowed by HF stayed viable inside the fly excretion and that the HF were capable of carrying and dissemination E. coli for several days (Kobayashi et al, 1999).

Current detection and methods of isolation include culture-based, immunoassays and PCR-based, for Shiga toxin or major STEC strains.

Methodology

Estimating lethal dose LD50 and infectious dose (ID)

This was carried out according to the method of Yousif *et al*. (2013).

Bacteria:

Five colonies of more common isolated STEC O157 were inoculated on (10 ml) of tryptone soy broths at about37˚ for (24) hours & centrifuged in cooling centrefuge (5009 *g*) for about (15) minutes & residue (pellet) was washed 3 times with PBS (pH = 7.2) then it was kept by using (9) ml of PBS(pH=7.2) and tenfold delution (10^-1 ,10^-2 .10^-3 ,10^-4 ,10^-5 ,10^- 6 ,10^-7 ,10^-8 ,10^-9 & 10^-10) was ended.

1- Counting bacteria: practical counting of bacteria in all of diluent was done due to surface spread (Ogiehor and Ikenebomeh , 2006). Two of Petri dishes for each one diluting to be used was set off & were labeled with suitable dishes, utilizing new pipettes for all of dilutions , 100 ul of the samples were aseptically placed at center of all of Petri dishes and surface spread on the nutrient agar then all dishes were reversed & then incubated at 37º for about 24 hrs.

The viable counting of the bacterial colonies were done . Colony counting were considered via the multiply of averages numbers of the colony per the counting dish by mutual of dilution & was described as colony founding item for each ml of sample and diluent that have that concentration have been selected to inculate mouse:(1.5 × 10^5 cells) , (1.5 × 10^6 cells),(1.5 × 10^7 cells),(1.5 × 10^8 cells),(1.5 × 10^9 cells),(1.5 × 10^10 cells)&(1.5 × 10^11 cells) .

2- mouse drenching: seven groups, each contain 5 mice; six of these groups were drenched with 1 of considered(C. F. U. / ml) diluents by using stomach tubes about(1)ml each orally and last group was drenched with PBS(pH = 7.2)& was measured as a control group. All groups were observed for 10 days to estimate living & dead mice & to know LD50 according to(Reed & Muench, 1938).The infectious dose (ID) was estimated by selecting group of mice that showed clinical signs manifested by diarrhea with no mortality.

Mice infection with O157:H7 orally

Depending on (Krystle and Alison, 2011): Sixty Albino mice of both sexes with age between(6–8) weeks were modified to about two weeks before the test. Water which was heated to boiling degree& then cooled. Streptomycin was added (6mg/ml). After 1 day of streptomycin treatment, mice were hungry for about 18 to 24hrs & they were separated into two groups:

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Infected group: Thirty mice were orally inoculated with infectious dose of E. coli O157:H7 ($2.5\times10^{\wedge5}$ cells) that is suggested by Haas et al (2000) and Strachan et al (2001).

Control group: Thirty mice were given phosphate buffer saline orally.

At days 7,14,21 and 28, blood samples were taken by making heart puncher to 10 mice from each group. The blood samples were used for measuring IFγ and IL10 as mentioned by Ahmed (2016).

Results

Humoral immunity (PHA) test

The Ab. titer against O157:H7 revealed a major surging ($P< 0.05$) in the diseased mice cluster in comparison with control at all the experiment period, while infected group shown the maximum surge ($P< 0.05$)in antibody titer at day 28 in comparison with the days 7 and 14.Table (4-7).

Table (4-4): Antibodys titers in contradiction of STEC O157.

Groups / Time	Infected group	Positive control group	Negative control group
Day7	14.0 ± 2.94 (8-32)* A d	0±0 B a	0±0 B a
Day14	57.2±11.66 (16-128)* A c	0±0 B a	0±0 B a
Day21	190.0±21.11 (128-256)* A b	0±0 B a	0±0 B a
Day 28	253.0±21.70 (240-320)* A a	0±0 B a	0±0 B a

- Values are expressed as mean ± SE
- n= 10/group,
- Capital letters denote significant difference (P<0.05) within a row,
-Small letters denote significant differences (P<0.05) within a column.
- * Range of antibody titter.

Cellular resistance (skin test response):

Outcomes of the late kind hypersensitivity indicated an important surges in depth(mm)of right footpad of mouse of infected group ($P< 0.05$). Maximum mean of thickness was showed afterward about 24 hrs post the infection and was dropped considerably($P< 0.05$) after about48 hrs was resumed nearly to the usual depth after about 72 hrs after inoculation of soluble Ags. Table (4-8).

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Table (4-5): Skin thickness (mm) of mouse right footpad of that were diseased with STEC O157:H7.

Times afterward the inoculation of the soluble antigen	The infected group	Control Positive group	Control Negative group
Hour 0	1.56±0.05 A a	1.53±0.03 A a	1.52±0.04 A a
24 hours	3.03±0.15 A d	1.54±0.02 B a	1.53±0.04 B a
48 hours	2.60±0.11 A c	1.54±0.02 B a	1.54±0.03 B a
72 hours	1.80 ±0.09 A b	1.54±0.01 B a	1.53± 0. 03 B a

- Values are expressed as mean ± SE
- n= 5/group,
- Capital letters denote significant difference (P<0.05) within a row,
-Small letters denote significant differences (P<0.05) within a column.

Interleukin – 10 (IL10)

The (IL-10) levels (pg /mL) revealed the major surge (P < 0.05)in the infected group in contrast with the control group at similar time when diseased cluster shown the important surge (P < 0.05) in the IL10 at the days 14 & 21 in comparison with day7. Table(4-9).

Table(4-6):serum stages of IL10 (pg /ml)in diseased & controller clusters

groups / Time	Infected group	Positive control group	Negative control group
Day 7	362.55±15.24 A b	81.53±4.22 B a	81.42±4.31 B a
Day14	472.88±15.44 A a	80.85±4.32 B a	80.66±5.43 B a
Day21	481.35±8.55 A a	82.77±1.96 B a	81.77±3.11 B a
Day28	495.33±7.91	82.80±2.53	80.91±5.25

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	A a	B a	B a

- Values are expressed as mean ± SE
- n= 10/group,
- Capital letters denote significant difference (P<0.05) within a row,
-Small letters denote significant differences (P<0.05) within a column.

Interferon Gamma IFN- γ

Cytokine(IFN- γ) level(pq /ml) revealed the major surge (P< 0.05)in the infected group in the contrast with control group at the similar period while outcomes of infected group showed the major surge (P < 0.05)in IFN- γ levels at the day14 &day21 after the infection in the comparison with outcome of day7. Table(4– 10).

Table(4-7):serum stages of IFNγ(pg / ml)in the diseased & controller clusters.

groups / Time	Infected group	Positive control group	Negative control group
Day 7	302.65±9.03 A b	32.70±1.11 B a	32.00±1.22 B a
Day14	572.42±22.44 A a	32.55±0. .88 B a	31.34±1.01 B a
Day21	602.15±7.11 A a	34.54±1.00 B a	32.35±1.12 B a
Day28	552.31±6.52 A a	33.81±2.01 B a	32.55±2.05 B a

- Values are expressed as mean ± SE
- n= 10/group,
- Capital letters denote significant difference (P<0.05) within a row.
-Small letters denote significant differences (P<0.05) within a column.

Discussion

The mice which were infected by infectious dose, after 7 day showed antibody titers with a mean (14.0 ± 2.94) but after 14 days, they showed a significant increase in antibody titers (57.2±11.66) reaching to the 28 days post infection, it searged to the peak significantly increased in the antibody titers to reach a mean (253.0±21.70). The antibody response to Tir, intimin, Esp A and EspB, proteins that are secreted by the type III secretion system after STEC infections and also by lipopolysaccharide (Cristancho *et al*., 2008; Vilte *et al*., 2008; Joris, 2012). Larrie-Bagha *et al*. (2013), showed the important role of antibody producing B cell in protection against *E.coli* O157:H7.

The result of skin test showed that STEC O157:H7 elicited a cell mediated immune response, since delayed type hypersensitivity is the principle pattern of cellular mediated immunity (Ramzi *et al*., 1994). T cells activated during the sensitization phase are CD4+ T cell primarily of the Th1 subgroup but in a few cases CD8+ T cells have also been showed to induce a DTH response (Mastroeni *et al*., 2001).

This cellular immune response induced by STEC O157:H7 is similar to that recorded by other researchers who found the same result of cellular immune response induced by challenged of mice with specific antigen of O157 (Eko *et al*., 2011; Mayr *et al*., 2012). Oral inoculation of EHEC in mice also showed similar results (Cai *et al*., 2010). The present study agreed with Kshash and Habasha, (2009), who used a purified lipopolysaccharide of *E. coli* O111 in immunization of mice and recorded a high significantce of right footpad skin thickness after 24 hrs with a peak level at 48 hrs as compared with control group (Kshash and Habasha, 2009).

This result agrees with Zamely and Falh (2011) who found that the experimental infection of male rats by *E. coli* resulted in increase in the number of leukocytes but it disagrees with Hoffman *et al*. (2006), who recorded that STEC infections could suppress the development of an antigen-specific cellular immune response in cattle (Hoffman *et al*., 2006).

Our rusults about IL10 is similar to that of By Rene de Waal et al., which domenstrate that monocytes activated by lipopolysaccharides (LPS) were able to produce high levels of IL10, previously designated cytokine synthesis inhibitory factor (CSIF), in a dose dependent fashion. IL10 was detectable 7 h after activation of the monocytes and maximal levels of IL10 production were observed after several days . (Rene *et al.,* 1991).

The significant increase of IFN- γ is in agreement with the result found by Hayashi *et al*. (2001); Mayr *et al*. (2005) and Gobert *et al*. (2007), in mice; also Vallance *et al*., 2002) who demonstrated that in mouse model infection with EHEC strains, they suggested that INF- γ plays an important role in defense mechanism against pathogens in vivo. Another researcher explained that the level of IFN- γ increased in humans following *E. coli* infection (Long *et al*., 2010), and in *E. coli* O157:H7 enteritis and HUS, had a significant increase of IFN- γ in infected children (Proulx *et al*., 2001).

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