The Immunological Study of Salmonella Infantis in white Mice Immunized with Killed Antigen

Basimraihan Sahar[1], Khalied Yassenzakairalzamily[1], Alaa SalimHamzah[2]

[1]Department of Medical Laboratory Techniques,Kut Technical Institute, Middle Technical University , Baghdad, Iraq, [2]Department of Nursing, Institute of Medical Technology, Middle Technical University, Baghdad, Iraq.

Abstract

The present study was carried out to investigate the effect of formalin-killed antigen for Sallmonellainfantis in vivo with the study of cellular immune response through the study of cytokines.Sallmonellainfantis were isolated and diagnosed from local milk products (milk and cheese). 74 milk samples and 53 cheese samples were taken.The media were usedgeneral and special culture media as well as biochemical tests were used to diagnose the isolates and the diagnosis was confirmed by using API 20.

Sallmonellainfantis antigens were used systemically. 50 Swiss white mice of both sexes were randomly divided into three groups. The first group prevented (20 mice) with the bacterial antigen killed by formalin in a dose (0.3) I.P for each animal. The group was injected with a booster dose and the same first immunization dose two weeks after the first dose. The second group (20 mice) and the third group (10 mice) were counted as positive and negative control groups respectively.

The first group and the second group received a dose of 0.3 ml of stuck S.infantis containing (1×108 live cells / ml) I.P, while the third group injected 0.3 ml of neutral phosphate buffer solution under the skin. The results showed that 4 animals from the first group were killed and all the remaining animals from the first group were killed 20 days after the challenge dose and serum was taken to measure cellular immunity. The results of serological tests showed that the level of (IL-4 and IL-6) were (38.48 ± 2.1 and 31.81, 3.01) respectively, as well as the results of measurement of concentration (INF gamma and TNF-α) were (401 ± 2.12 and 173.421 ± 3.11) respectively.We conclude from the study that there was a high percentage of S.infantis isolated from milk products as well as we conclude that the formalinS.infantiskilledantigen partially protected against the infection.

***Key Words**:Sallmonellainfantis, killed Antigen ,IL-4 , IL-6, INF gamma , TNF-α.*

Introduction

Non-typhoidal Salmonella enterica (NTS) are considered to be one of the main common causes food borne infection worldwide that effect on humans (1). Salmonella Infantis is the most recurrence strain in numerous countries, involving Asian countries. detection and isolate of S. Infantis from animals , humans and vegetables (2,3) . when salmonellosis causes infect of animals, due to increase in death -rate in infected animal leads to economic loss for countries, these Infected animals act as a source of infection that during to direct or indirect contact injury to humans) 4). The humans and animals infected with salmonella contaminate the environment and nutrition at most by feces (5) also Salmonella colonizes at most in the intestinal tract (6) . The dairy products and Milk, especially poorly pasteurized, are among the possible causes of the transmission of many pathogens to humans through food (7). The feces of infected cattle, infected udder, milking equipment, contaminated skin, feed and from milkers considered as main sources of contaminate raw milk ,causes salmonellosis and other pathogen (8, 9, 10).

The present study was carried out to investigate theprevalence of Salmonella infant in milk and cheese, and revealout the serotypes of the Salmonella isolates.

Materials and Method

Sample collection:

Dairy products (milk and cheese) samples were collected local supermarkets in kut city. The samples collected during the period from January to April 2019. Samples were rapidly put in selenite broth containing sterile tubes and transmitted by ice box during 2 hour to the laboratory of bacteriology in pathological analysis department in kut technical institute.

Bacterial Isolation:

All samples collected were cultured onto MacConky agar and incubated at $37c_o$ for 24-48 hour. The growing colones were examined by naked eye concering their color , shape and size , sub-cultured onto selective media such as Eosin Metheline blue , SS and XLD agar and incubated at $37c_o$ for 24hour. Method of culturing was done according to(11).

Bacterial Identifecation:

The colonies were examined by eye according to their color, shape , size, the Gram stain was done in addition to biochemical tests including :Catalase test, Oxidase test, Lactose fermentation, Urase test, Indole test, Citrate utilization test these test were done according to (12) , then confirm diagnosis of the isolates by APi 20E according to (13).

Challenge dose

The Salmonella Infantiswas cultured ,growth and purification on the media of this study , the live bacterial cell counted according to (14).

Antigens(killed Salmonella Infantis antigen):

The killed S.Infantis antigens were prepared according to (15).

Cytokine assessment:

Interleukin-4, Interleukin-6, INF gamma and TNFα Assay Procedure, The procedure is performed at room temperature according to manufacturer's instructions (Boster´s –Korea).

Experimental design:

Fifty white mice were used (male and female), the ages (8-10weeks) and their weight ranged between (25-30gram), obtained from institute of sera and vaccines \ ministry of health, and reared in cages furnished clean sawdust , and fed concentrate feed during the duration of the experiment. The white mice were divided into three groups were first group includes 20 mice immunized inoculated with 0.3ml of formalin killed antigen,two dose ,2weeks intervals at day 30 post immunization, and it was inoculated I/P with 1X108cfu /ML.The second group includes 20 mice was considered as positive control.it was inoculated I/P with as 1st group. Third group includes 10 mice was inoculated I/P with 0.3ml of sterile normal saline andservedas control negative group. At day 30 post infection ,all animals were sacrificed and blood samples were collected for determine cell mediated immune response by measurement of cytokines including IL 4 , IL 6, INF gammaandTNF-α.

Results

Bacterial isolation:

Theresults were revealed the percentage of bacterial isolation from dairy product (milk and cheese) as in table 1:

Table 1: The table reveal the percentage bacterial isolation as per source of dairy products.

Type of sample	No. of sample	Positive isolation	Percentage of positive isolates
Milk	74	46	62.61%
Cheese	53	31	58.49%
Total	127	77	60.62%

Immune response

The cellular immune response

The results were showed that serum levels of IL-4 and IL-6 were 38.48±2.1and 31.81±3.01respectively as shown in table 2.

Table 2: Cytokine profile of Salmonella infantis in immunized mice post infection

Groups	IL-4(pg/ml) mean±SE	IL-6(pg/ml) mean±SE
G1immunized group	38.48±2.1	31.81±3.01
G3 negative group	3.5±0.36	2.1±0.45

The results were showed that serum levels of INF gamma (pg/ml) at 30 days and TNF-α (pg/ml) at 30 days were 401 ±2.12 and173.421±3.11. respectively as shown in table 3.

Table 3: Mean values of serum levels of INF gamma in immunized mice post infection

Groups	INF gamma (pg/ml) mean±SE at 30 days	TNF-α (pg/ml) mean±SE at 30 days
G1immunized group	401 ±2.12	173.421±3.11
G3 negative group	48 ±0.81	25.7±0.601

Discussion

The present study recorded highly percentage of bacterial isolated in milk and cheese (62,16 and 58,49)% respectively.This result could indicatedthat the food product (milk and cheese) were thoughtof a supply a microorganism infectionofhuman once dangerous handling, particularly in Asiancountry, the individuals consumed great amount of diary product, these results agreement with The farm product and Milk, particularly poorly change integrity, are among the attainablecauses of the transmission of the many pathogens to humans through food(7).The current study showed the immunized group with virulence salmonella infantiswhen infected with lethal dose the death only 4 mice from this group (20 mice) during post 48 hr lethal dose infection, these may be, the killed antigen give partially protection, these results agreement with (16,17)who showed this might be indicate that antigens stimulate response with partial protection potential thatdestroyedsome microorganism at website of immunisation however sizable amount of those organism reach to internal organs and proliferation but the body tried to localize of this organism by neoplasm reaction.While positive Control (non immunized group) when infected these animals with lethal dose, the death of all animals during 24 hr post lethal doseinfectionmayindicatedthat,theS. infantis ishighlyvirulence bacterium thatovercome traditional defensemechanisms ofthehostand spread tointernalorganscause bacteriaemia and septicaemi that cause high mortality, this results are agreement with (18) UN agency showed that, non infectious disease enterobacteria is major causes of morbidity and mortality post dissemination infections.

These results of present study were revealed that serum levels of IL4 and IL6 in immunized group were (38.4±2.1 and 31.81±3.01) respectively. The cytokine profile in immunized mice were showed the killed bacteria as a good immunogenic antigen which stimulate immune response with an intrinsic immunologic adjuvant which can lead to enhancement off immune response and at the same time can immunosuppressed. The cells which affecting their function might be through regulation T-cell (19).At sites of microbial invasion, leukocyte and other cells send out distress signals by releasing cytokines or chemokines. These proinflammatory signals activate local cardio vascular endothelium (IL1, TNFα) to express selection molecules, increase expression of chemotaxic molecules (IL1 and IL8) and activate leukocyte (IL1, IL6, IL8, IL12 and TNFα), these results agreement with (20,21).

The result of current study may be due to bacterial component which stimulate TLRs that mediated pro-inflammatory cytokines production such as TNFα, IL8 and IL6 which considered attraction of neutrophil, these results are agreement with (22).who showed that salmonella infection in animal models characterized by abundant neutrophils infiltration.

The our results of immunized mice were showed that serum levels of IFN gamma and TNFα were (401±2.12 and 173.421±3.11) respectively.These results showed decreasing bacterial load in the present study with stimulated cell mediated immune response may indicated that both innate and acquired immune response may indicated play important role in eradication of s. infantis infection, these results are agreement with (23) who recorded that innate immune system which included phagocytic cells and cytokines can controlled the low virulence of these pathogen required both innate and acquired immune response including TNFα, IFN gamma, IL12, NK cells, humeral and cellular immune response particularly in the previous immunized or exposure to sub lethal infection showed that both B and T cell play important role in the protection against salmonella infection (24).

These was few bacterial isolated from examined organs of immunized infection mice which all of them were death during first 24hr post infection with sever bacterial load in all examined organs, these results agreement with (25) who recorded that vaccinated mice provided Th1-cytokines (TNFα, IFN gamma and IL12) as well as salmonella specific antibodies that controlled challenge with virulence salmonella infection.

Conflict of Interest: Non

Source of findings: Self findings.

Ethical Clearance: Non

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