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"IMMUNOLOGICAL RESPONSE OF RABBITS TO DIFFERENT ANTIGENIC PREPARATIONS OF E. COLI O128:K67 "

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ABSTRACT In this present study the immunological response of rabbits to some bacterial antigens was studied. The pathogenicity of E. coli O128: K67 was studied by two methods, oral infection and ligated intestinal loop. Where, E.coli via oral infection cause depression, off food and diarrhoea with 60% mortality. The gross lesion showed severe catarrhal enteritis with grey necrotic patches on mucous membrane of caecum and colon. The toxigenicity of the organism was also proved by ligated intestinal loop. The immune response of rabbits to these bacterial antigens were also studied by two types of antigens formalized and aluminum hydroxide adjuvenated antigens which prepared from each strain, The antibody titre was measured by tube agglutination test (TAT) and Enzyme Linked Immunosorbent assay (ELISA) technique. ELISA was very sensitive as it showed reasonable titer even in non inoculated animals. So, TAT had the advantage of direct measuring antibody titer and more practical test for evaluation the injected antigen. The antibody response in rabbits injected with formalized antigen reached its titre at 6 weeks from the second dose. But, in rabbits injected with aluminum hydroxide adjuvenated antigen, the antibody titre was much higher in comparison with the formalized one.

INTRODUCTION

In Egypt, rabbits are considered a source of animal wealth, as it helps to a certain extent in solving the problem of meat shortage. Nevertheless rabbits have received very little, attention if compared with other specis of animals (***Zoher et al 1976***). The development of the rabbits industry is strongly associated with regular and dependable production, which required knowledage of the factors that influence the productivity of these animals. Disease is one of the most important problem, which faces rabbit breeders, and researchers where they generally observed that

important problem, which faces rabbit breeders, and researchers where they generally observed that high moralities were occured especially in young rabbits till 4 month *(Asdrubali et al 1977)*. E.coli is a common inhabitant of the intestinal tract of rabbits. In addition to their presence as normal fecal flora, E. coli is a world wide rabbits breeding problem as some strains under special circumstance causes high mortality in broiler and weaned rabbits (*Blanco et al 1994*). ***Cantey and Blake(1977)***stated that E. coli strain (RDEC) produced diarrhoea in rabbits by attachment of number of this bacteria on intestinal mucosa, ***Ali (1983)*** found that the four serogroups of E. coli O28, O126,O125, O119 were pathogenic at 4-6 weeks old of apparently health rabbits by oral inoculation. ***Milon et al (1989)*** tried to protect rabbits aganist enteritis due to of E. coli O103. The aim of this study was directed to make a complete bacteriological and immunological studies on E. coli O 128:K 67 which are known to be highly pathogenic to rabbits in Egypt.

MATERIAL AND METHODS

Animals: Five Baladi rabbits about one kgm were used to study the pathogenicity of E. coli by oral infection and two Baladi rabbits about 1.5 kgm were used to study the pathogenicity of E. coil by ligated intestinal technquic. Ten Baladi rabbits 6-12 months old were used to study the immune response to E. coli.

Bacterial strain: E. coli serotype O128: K67 was used to prepare two antigens.

Methods:

1- **Identification:** E. coli was grown aerobically on Mac Conkey agar plates for 24 hours at 37°C and identified according to ***Edwords and Ewing (1972).*** Suspect colonies were examined for morphological characteristics including staining reaction with gram stain. Colonies grown were picked on nutrient agar slope and incubated at 37°C for further biochemical and serological identification. Biochemical reaction was done according to ***Cruichshank et al (1975)*** . Also serological identification was carried according out to ***Hallmann and Burkhardt(1974).***

2- Studies on the pathogenicity of E. coli O128: K67 in rabbits.

2:a: By oral infection: Five rabbits 6-8 weeks old were infected orally with $2x10^7$ viable E. coli organism/animal ***Ali(1983).***

2: b: Studies on the pathogenicity of E. coli by ligated intestinal loop: this study was done according to ***Smith and Halls (1967)***

3: Preparation of E. coli antigens:Pure culture of tested strain was aerobically cultivated onto tryptic soya broth and incubated overnight at 37°C and used to prepare antigen.

3:a: Preparation of formalized E.coli antigen: Cultures were inactivated by addition of formalize

0.4% v/v and incubated at 37°C for 20 hours. The cells were harvested by centrifugation and were resuspended in formalin 0.3% ***Acres et al (1982).*** The antigen was adjusted to give optical density approximately $6x10^{10}$ organism/ml as recommended by ***Danieli et al (1979).***

3: b: Preparation of aluminum hydroxide adjuvenated E. coli antigen: It was carried out according to ***Myers (1980).*** E. coli strain was grown in tryptic soya broth at 37°C for approximately 20 hours. Cells were harvested by centrifugation and the sediment were suspended in formalized (0.3% formalin) isotonic saline solution.The cells was adjusted to give optical density of approximately $6x10^{10}$ organism /mL. Sufficient 3% aluminum hydroxide adjuvenated was added to the formalized antigen and mixed.

3:c: Preparation of E. coli antigen for tube agglutination test: It was done according to ***Stipkovits (1964).*** Culture of E. coli serotypes O 128:K67 was cultured on surface of nutrient agar which incubated at 37°C overnight. Growth was harvested aseptically was then centrifugated for 2 hours at 3000 rpm and the supernatant fluid was removed and the precipitate was resuspended in sterile saline. The process of washing bacteria was repeated 3 times. The bacterial suspension was killed by boiled for 2 hours at water bath. 0.5% phenolized normal saline was added to give optical density of approximately $5X10^8$ organism/ml diluted boiled suspension

3:d: Preparation of E. coli antigen for ELISA technique: This test was done according to ***Isaacsan(1977).*** E. coli was inoculated on tryptic soya agar and incubated at 37°C for 24 hours. Culture was harvested aseptically by adding sterile normal saline and the suspension was then centrifuged for 2 hours at 3000 rpm. The supernatant fluid was removed and the precipitate was resuspended in about 10ml sterile saline and heating at 56°C for 30 minutes.

4: The immune response of formalized E. coli antigen in rabbits: Five Baladi rabbits were injected S/C with 0.5 ml of antigen contain $6X10^6$ bacterial cell/ml. Serum samples were collected before infection and on weekly interval after injection for three weeks. Booster dose was injected three weeks after the first one. Serum samples were also collected biweekly interval after the second dose for 3 months.

5: The immune response of rabbits to aluminum hydroxide adjuvenated antigen: Five Baladi rabbits were injection S/C with 0.5 ml of antigen contain 6 $x10^{10}$ cell/ml. Serum samples were collected as before in formalized antigen.

6: The immune response: It was evaluated by measuring the antibody titre using:

6:a: Tube agglutination tests (TAT): This was done according to ***Mettias (1987).***

6:b: Enzyme linked immunosorbent assay (ELISA): the test was applied according to ***Mettias et al (1994)*** for estimation of antibody titre present in serum samples.

RESULTS

Bacteriological identification of E. coli O 128 K67: The strain was completely identified bacteriological by studying the morphology appearance, culture character, and biochemical reaction and typed serologically. The strain was proved to be E. coli O128: K67.

Pathogenicity of E. coli O128: K67 in rabbits:

1- by oral infection:

All inoculated rabbits showed depression, dullness, off food and diarrhoea after 2-4 days. About 60% of the rabbits was died within 3 weeks post oral infection. Gross lesions showed severe catarrhal enteritis with increase in the fluid of bowel contents. Whitish grey necrotic patches were observed on mucous membrane of caecum and colon with congestion and enlargment of the liver. The organism was reisolated from some internal organs of newly dead rabbits.

2- By ligated intestinal loop:

E. coli O128: K67 was tested for production of entrotoxins in ligated intestinal loop rabbits. The injected intestinal loop with E. coli toxin showed distension of the loop and increase in the fluid inside the loop to 5 ml.

3- Evaluation of immune response of rabbits injected with formalized E. coli antigen:

From data obtained in table (1) it can be seen that in tube agglutination test, the mean antibody titre increased form zero at zero time (Pre injeetion) to 28 at the first week post injection and reached 64 at the third week. Then the antibody titre showed an abrupt increase to 88 at 5 weeks (2 weeks post second dose) and reached a maximum 208 at (6 weeks post second dose). Then it began to decrease gradually till reach 36 at the end of 15 weeks. The agglutination antibody titre were measured as the reciprocal of the highest dilution showing positive agglutination. The ELISA antibody titre were calculated by S/P ratio as mentioned before. In ELLISA technique antibody titre began from 80 befpre injection and increased gradually until it reached 1100.4 at 3 weeks post injection, after second injection it increased until it reached the peak (2010.8) at 6 weeks post injection, then it decrese until it reached 786.6 at the end of 15 weeks.

Table (1): Titers of antibodies in sera of rabbits vaccinated with formalin killed antigen of E. coli O128: K67 measured by the tube agglutination test and ELISA.

Week post vaccinations	Titter of antibodies By	
	TAT	ELISA By S/P
Prevaccination	0	80
1st week Post –vaccination	28	198
2 week Post -vaccination	36	630.5
3 week then the 2nd dose	64	1100.4
5 weeks	88	1401.9
7 weeks	160	1834.5
9 weeks	208	2010.8
11 weeks	128	1712.9
13 weeks	72	1375.5
15 weeks	36	786.6

4- Evaluation of immune response of rabbits injected with aluminium hydroxide adjuvenated E. coli antigen:

From data in table "2" it can be seen that antibodies titre by tube agglutination test increased gradually till it reached (72) at 3 weeks post first injection then after second injection it highly increased till it reached (448) at 6 weeks post second injection after that it decreased gradually until it reached (96) at 12 weeks post second injection. By ELISA technique the antibody titre were calculated by S/P ratio. Antibody titre as shown in table 2 began from 80 then to increased gradually until reached (1650.5) at 3 weeks post 1st injection then after second dose it abrupt increased till it reached the peak (3400.4) at 6 weeks post second injection and after that it decreased gradually till it reached (1580.2) at 12 weeks post second injection.

Table(2): Antibodies titre in sera of rabbits injected with aluminum hydroxide E. coli O128: k 67 antigen measured by TAT and ELISA.

Serum collected after	Titter of antibodies measured By	
	TAT	ELISA By S/P
Prevaccinated	0	80
After 1st vaccine one week	20	147.5
2 weeks	32	899.6
3 week then the 2nd dose	72	1650.5
5 weeks	176	2420.6
7 weeks	256	2880.4
9 weeks	448	3400.4
11 weeks	352	2190.8
13 weeks	192	1980.2
15 weeks	96	1580.2

5- Comparison between immune response of rabbits injected with formalized and adjuvenated E.coli antiqen:

The results illustrated in table 3 showed the mean antibody titre in serum of rabbits injected with formalized and adjuvenated E. coli antigens measured by tube agglutination test and ELISA technique. It is cleared that there were significant difference in increasing of antibody titre in case of 2 antigens. In case of formalized E. coli antibody titre is increased gradually till it reached the peak which is (208) by tube agglutination and (2010.8) by ELISA technique at 6 weeks post second injection then it abrupt decreased till it reached (36) by tube agglutination and (786.6) by ELISA at 15 weeks. While in case of adjuvenated E. coli antigen antibody titre was abrupt increased and the peak was more than formalized E. coli antigen. The peak was (448) by tube at 9 weeks and (3400.4) by ELISA and then decreased gradually till reached (96) at 15 weeks by tube agglutination test and (1580.2) by ELISA techinque

Table(3): Antibodies titer in sera of rabbits vaccinated with formalized and adjuvenated E. coli O 128: K67 antigens using tube agglutination and ELISA technique.

Injected'	Weekly immune response																	
	TAT'									ELISA technique								
	1	2	3	5	7	9	11	13	15	1	2	3	5	7	9	11	13	15
Formalized E. coli antigen	28	36	64	88	160	208	128	72	36	630.5	1100.4	1401.4	1834.5	2010.8	2010.8	1712.9	1375.5	786.6
E., coli aluminum hydroxide antigen	20	32	72	176	256	448	352	192	96	147.8	899.6	1850.5	2420.6	2880.4	3400.4	2290.8	1980.2	1580.2

DISCUSSION

E. coli infection is one of the most important problem in rabbits. Where it cause diarrhoea and high mortality in broiler and weaned rabbits. ***Katoch et al (1993)*** examined 44 diseased rabbits and 71 dead rabbits of various ages for bacterial infection and found that the main cause of digestive diseases in rabbits was E. Coli. In Egypt, E. coli has attracted attention from some researchers as it causes an important problem for rabbits breeders. ***Abdel-Gwad(1988)*** isolated 39 strains of E. Coli from heart, liver, blood and spleen of 100 freshly dead rabbits of various ages. So a complete bacteriological and immunological study was done on one of the most prevalent E. coli strain O128: k67 causing severe losses in rabbits ***(Camguilhem and Milon 1989)***. The pathogenicity of the strain was studies in rabbits by two methods, oral infection and by ligated intestinal loops where all inoculated rabbits via oral infection showed depression, dullness, off food and diarrhoea after 2-4 weeks and about 60% of morbid rabbits were died within 21 days and the gross lesions showed severe catarrhal enteritis with increase in fluid of bowel contain congestion and enlargement of liver. The causative organism was reisolated from some internal organs of newly dead rabbits This results were agreed with ***Ali (1983)*** who isolated E. coli strain O 128 from different organs of 100 rabbits of various ages with enteritis and proved that the isolated organism was pathogenic to rabbits causing the same symptoms. Another rapid method to study enterotoxigenicity of E. coli strain O128: K67 by ligated intestinal loop ***Smith and Halls (1967)*** where the intestinal loops with suspecting material showed distention of the loops and increased in the fluid inside the loops to 5 ml while there is no change in loops injected with control negative material.

This results was confirmed by ***Peeter et al (1984 a)*** who stated that E. coli were found on the luminal intestinal border of 40% of diarrhoae rabbits examined E. coli strain O 128:K67 was proved in our study to be highly pathogenic to rabbits so two antigens were prepared from the organism, the formalized E. coli antigen and aluminum hydroxide adjuvenated E. coli antigen. The immune response of the formalized antigen was studied in rabbits by injection the 2nd dose at the 3 weeks and the immune response was evaluated by measuring the antibody titre using tube agglutination and ELISA technique biweekly interval for 3 months. The result revealed the mean antibody titre by tube agglutination was increase from zero at prevaccination to 28 at first week post vaccination and reached 64 at third weeks, then the antibody titre abruptly increased to 88 two weeks post the second dose and reached maximum to 208 at six weeks post second dose then the titre decrease gradually till the end of the experiment. The ELISA antibody titre was calculated also by S/P ratio ***Mohamed(1998).*** The titre began

from 80 before vaccination and increased gradually until reached 1100 three weeks after first week injection. After second dose the titre increased to reached its peak 201 at 6 weeks post injection then it decreased until reached the end of experiment. This results agreed by ***Forman et al (1982)*** who stated that subcutaneous injection of E. coli whole cell bacterin was immunogenic rather than the oral route ***Salib (1995)*** who showed that immune response to E. coli formalized bacterin in Buscat rabbit was much higher than our results in Baladi rabbits. Injection of aluminum hydroxide adjuvenated of E. coli antigen in rabbits showed the same results as formalized E. coli antigen but increase was more prominent. Six weeks after second dose, the titre reached it maximum 448 with 115% increase more than the titre in formalized bacterin and the titre at the end of the experiment was three times more than the titre in case of formalized bacterin. ***Lentch and Bord (1983)*** obtained the same results using ELISA technique. The difference in the immune response of formalized and aluminum hydroxide adjuvenated bacterin may be attributed to the adjuvenated effect of aluminum hydroxide incorporated, (***Bunn et al 1984***). From the present work, it was concluded that E. coli O128:K67 was highly pathogenic for Baladi rabbits showing high morbidity and high mortality. ELISA technique is very sensitive test that it showed reasonable titre even in non inoculated animals. So TAT has the advantage of direct measuring antibody titre and more practical test for evaluation injected antigen. Rabbits respond well to antigen prepared from E. coli formalized or adjuvenated antigen. Adjuvenated antigen induced a good antibody response as it slowly released to the immmune system so it prolong the immune response.

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