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IMMUNIZATION OF MICE WITH KILLED *E. coli* K99 VACCINE FOR PROTECTION AGAINST COLIBACCILLOSIS

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Abstract-

Background- Enterotoxigenic *Escherichia coli* (ETEC) continue to be problematic for both humans and animals. This disease primarily affects adult travelers to areas where it is endemic. The pathogenesis of ETEC infections has been well understood for two decades, and there is good evidence for immunity after infection. This study aimed to prepare and evaluate *E. coli* possess K99 vaccine to induce humoral and cellular immunity against challenge with homologues strain of *E. coli* in mice.

Materials and Methods- *E. coli* posses K99 was isolated from diarrheal fecal samples of horses and used for preparation of killed vaccine. Two groups of mice (twenty in each) were used, first group was vaccinated with 0.5 ml of killed *E. coli* K99 vaccine containing 1×10^8 C.F.U, twice at two weeks intervals subcutaneously (S/C), second group was injected S/C with phosphate buffer saline (PBS). ELISA test was used to detect humoral immunity at 2, 4 and 6 weeks post booster dose, while cellular immunity detected by delayed type hypersensitivity test (DTH-skin test) after 21 days of immunization, the vaccinated and control mice groups were challenged with (1×10^{10}) of virulent *E. coli* Post six weeks of vaccination.

Results- Antibody titers (IgG) was increased significantly (p <0.05) at 2, 4 and 6 weeks in the immunized group and the maximum increase was determined at fourth week to reach (687.1±56.9) in comparison with the control group which remained within the normal value in all times of the experiment. Immunized groups revealed a significant increase in foot-pad thickness after 24 & 48 hrs. post inoculation with soluble antigen in comparison with control group in delayed type hypersensitivity test (DTH test).

A significant protection was observed in immunized groups challenged with $4LD_{50}$ (4×10^{10} cells) compared with control group of mice which died within 1-3 days.

Conclusion- Vaccination of mice with killed *E. coli* vaccine was induced humoral and cellular immune responses against challenge with virulent *E. coli*. This vaccine of *E. coli* [K99] proved efficacy to use against colibaccillosis.

Keywords- *Escherichia coli*, Immunity to *E. coli* K99, Skin test, Elisa test, Enterotoxigenic *E. coli*

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Introduction

E. coli are a remarkably versatile and diverse genus of bacteria, which includes both commensally and pathogens. Despite more than 100 years of investigate we still do not understand all of the pathogenic mechanisms utilized by the different *E. coli* [1].

Enterotoxigenic *E. coli* is the most common enteropathogen that causes diarrhea in newborn farm animals. The bacteria cause diarrhea by adhering to colonizing and producing enterotoxins in the small intestine they are not invasive [2]. Enterotoxigenic *E. coli* (ETEC) strains as the important cause of sever diarrhea in newborn calves carry fimbriae (such as F5 (K99) and F41) mediating binding of the bacteria to microvilli of enterocytes of the small intestine [3,4].

Osman, et al [5] report that the large and small ruminants could be a potential source of infection with enterovirulent *Escherichia coli* pathotypes in humans in Egypt.

Different vaccines have been developed using ETEC strains producing K99 and F41. These vaccines may consist of bacterin [6,7] crude K99 and F41 extracts [8] or purified fimbriae [9].

Diarrhea-causing *Escherichia coli (E. coli*) possess colonization antigens or adhesins that enable the bacteria to colonize the small intestines [10]. The K99 (F5) fimbrial, antigen has been reported to be associated with a majority of enterotoxigenic *E. coli* (ETEC) isolated from cases of diarrhea in neonatal calves [11]. Methods for treatment and control of ETEC diarrhea are still a matter of debate

among Veterinarians, livestock producers. The use of small therapeutic doses of antibiotics may help protect animals from some, but not all, of these bacterial strains. Moreover, the use of antimicrobials at sub-therapeutic levels has been linked to the problem of emerging antibiotic resistance among several bacterial species, including ETEC strains. Therefore the use of immune- based therapy is considered promising approach for combating against ETEC. [12].

In this paper, we describe the preparation and evaluation of killed *E. coli* K99 vaccine in inducing of immune responses in mice against challenge with virulent strain of *E. coli*.

Material and Methods

Bacterial Strain

E. coli strain was isolated from fecal samples of horses at equestrian club location in Iraq, these samples was conducted initially to culturing on different selective media (MacConkey agar and, Hicrome rajhans modified medium, and Eosine methylene blue), biochemical (SIM, TSI, SC, Urease, MR/VP) [13] and KB003 Hi25^TM (Enterobacteriaceae identification Kit).

Latex Test (K99 Pili test)

Latex test (Mariel company) was used for detection of K99 antigen in isolated *E. coli* [13].

Preparation of *E. coli* Killed Vaccine for Immunization

Isolated *E. coli* possess (K99) bacteria was used for preparation of killed vaccine. The vaccine prepared as follows: The cultured bacteria was inoculated into brain heart infusion broth at 37°C for 14 hrs. and harvested with phosphate buffered saline. Cells were washed in PBS by centrifugation at 500 X g for 10 min at 4°C and then suspended to the appropriate density in PBS. Colony counts were performed to verify the number of bacteria at $1X10^8$ CFU/ml. Bacteria were killed by heating suspensions to 60°C for 1 hr. [14]. The vaccine was tested for sterility before use according to OIE [15].

Immunization of Mice

Forty healthy mice aged 4 to 6 weeks were selected. All mice had negative fecal bacteriological culture for *E. coli.* They were reared in separate cages in the animal house of Veterinary College, University of Baghdad. The mice were divided equally into two groups. The first group was immunized subcutaneously with *E. coli* K99 vaccine twice at two weeks intervals at a dose of 0.5 ml containing $1^{\times}10^8$ CFU/ml. The second group was injected S/C with 0.5 ml of PBS. Sera were collected at 2nd, 4th and 6th week post injection with booster immunization. Sera were stored at -20ºC until use for analysis by ELISA.

Post immunization the immunity was detected by Enzyme-Linked Immunosorbent Assay (Elisa). Elisa and its reagents were prepared according to manufacturer (immunological consultant's laboratory, Inc.). This test used to follow up humoral immunity by detection of IgG in the serum at 2, 4, 6 weeks of immunization.

Delayed type hypersensitivity test (DTH) skin test was done according to Mitov, et al [16] after 21 days of immunization by Injection of 0.1 ml of soluble antigen of *E. coli* (K99) which prepared according to Hundson & Hay [17] and Yousif, et al [18] intradermally in right footpad of the mouse while the left side was injected by 0.1 ml of sterile PBS (pH = 7.2). The thickness of skin was measured by vernier caliper before injection and at 24, 48 and 72 hrs. after injection of soluble antigen.

Challenge of Immunized Mice

Virulent strain of *E. coli* which possesses (K99) was used for challenge. The viable count of this bacteria was done by bacterial plate count method in eight fold dilution ($10^{-}1$, $10^{-}2$, $10^{-}3$, $10^{-}4$, $10^{-}5$, $10^{-}6$, $10^{-}7$, $10^{-}8$) according to Quinn, et al [13]. The LD_{50} was estimated according to Reed and Muench [19]. Forty eight healthy mice of both sexes were divided into (8) groups (6 mice in each group). Seven groups of mice were injected subcutaneously with 0.5 ml calculated CFU diluents, and the eighth group was considered as a control group which received PBS. All groups were monitored for 30 days to calculate total live and dead mice.

At 6th weeks after the first immunization, all mice were challenged intraperitonially with 5 LD50 of virulent *E. coli* (K99) in 0.5 ml PBS. The relative degree of protection afforded by antigens was assessed by the number of mice surviving 30 days after infection.

Ethical Approved

This study was approved by the ethical and research committee of Veterinary Medicine of College /University of Baghdad/ Minstery of High Education and scientific research.

Statistical Analysis

Statistical analysis were coffenducted to determine the statistical differences among different groups using ready - made statistical de -sign statistical package for social science (SPSS).

Results

Identification of *E. coli*

Isolation and identification of *E. coli* were confirmed in different media, biochemical tests and KB003 Hi25 Enterobacteriaceae. The isolates which give +ve results for agglutination Latex K99 test [Fig-1] was used for preparation of vaccine.

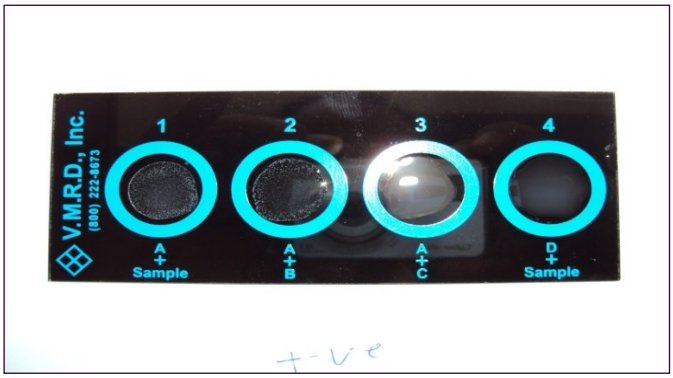

Fig. 1- Show results to slide latex agglutination test of K99
1-Positive result to isolated bacterial reaction. 2- positive control reaction. 3- Negative control reaction. 4-Control reaction.

Elisa Test

Immunized mice showed IgG levels with a mean titer (430.4±19.6) post 2nd week of immunization. After four weeks, the serum IgG titers reach the peak ranged (687.1±56.9). The results showed significant increase of antibody titers at (p <0.05) after (4 and 6) weeks post immunization with booster dose, as compared with the zero time and control group [Table-1], [Fig-2].

Delayed Type Hypersensitivity Test (Skin Test)

The results of delayed type hypersensitivity have showed increases in the thickness of the foot pad skin of the immunized mice, and the highest means of the thickness arise after 24 hrs. (4.250±0.250)

post injection with soluble antigen. Then decline slightly after 48 and 72 hrs.

DTH tests indicated that the values were significantly high at (p <0.05) in the immunized groups compared to the control group [Table-2] [Fig-3].

Table 1- Antibody titers in the immunized and control groups in Elisa test

Time (weeks)	Immunized group with *E. coli* K99 vaccine Mean ± SE	Control group Mean ± SE
0 time	195±11.2A	189.9±12.4A
2nd	430.4±19.6A	203±11.1B
4th	687.1±56.9A	189.1±12.1B
6th	561.7±2.95A	191±11.3B

Means with different capital letters in the same row differ significantly (P < 0.05)

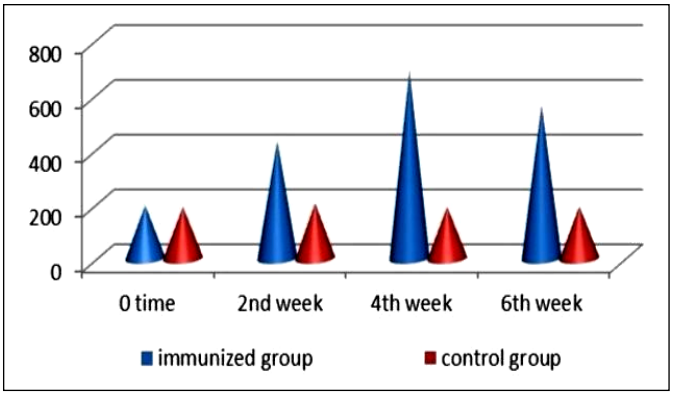

Fig. 2- Antibody titers in the immunized and control groups in Elisa test.

Table 2- Skin thickness (millimeters) of immunized and control groups in DTH test

Times (Hours)	Immunized group with *E. coli* K99 vaccine	Mean ± SE second group
0 hours	2.00±0.119A	2.05±0.0597A
24 hours	4.250±0.250A	2.1±0.0640B
48 hours	4.050±0.210A	2.15±0.0611B
72 hours	2.825±0.312A	2.00±0.0611B

Means with different capital letters in the same row differ significantley (P < 0.05)

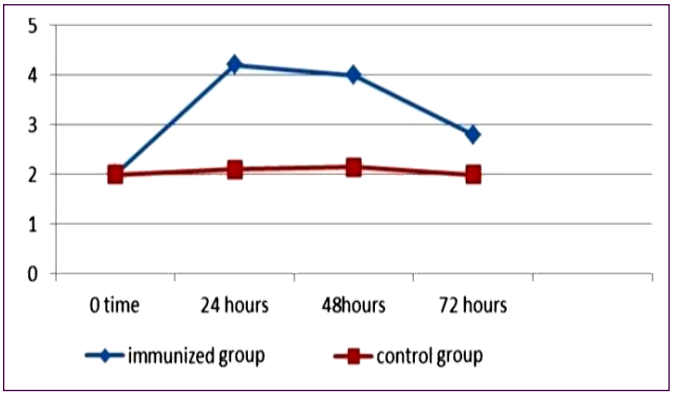

Fig. 3- Skin thickness (millimeters) of immunized and control groups in DTH test

Results of Experimental Challenge

According to the dose resulted in experiment of Estimating the LD50 ($1X10^{10}$), Experimental challenge was done by administration all groups intraperitonially with 5 LD50 ($5 X 10^{10}$) of *E. coli* (K99).

The control group showed different clinical sings of colibaccillosis post challenge, the infected mice died within 3-4 days. While the immunized mice showed mild signs of illness and depression for 2-3 days without signs of diarrhea and returned normal within 7 days without mortality.

Discussion

Enterotoxigenic *Escherichia coli* (ETEC) strains colonize the small intestine, secrete enterotoxins causing diarrhea and Colonization is facilitated by pili (fimbriae). Pili facilitate adherence of ETEC to intestinal mucosa [20]. Pilus adhesions that are known to be important in ETEC infections of neonatal animals are K88, K99, 987P, FY and F41 [21].

In the current study, immunization of mice with dose of *E. coli* vaccines possesses K99 resulted in stimulation of immunity in the immunized group compared with control group. This is in agreement with study of Dhasarathan, et al [22] who found estimation of antibody levels in theserum after an antigenic challenge will expose the functioning of humoral immune systems. In immunodeficient animal antibody production was affected and there byhumoral response against a disease causing antigen was less But the immune complex treated animals enhance the production of antibody, pathogen and heat killed pathogen treated mice showed anantibody suppressive effect. The suppression of antibody reflects on the reduction of humoral immune response and this state subject the mice to easy infection. Immunomodulation of whole pathogen with antiserum and heat killed pathogen with antiserum treated mice showed moderate change in antibody production.

It is obvious that *E. coli* vaccines containing (K99) antigens are able to induce cellular immune response as detected by DTH-skin test in this study is in agreement with Kshash, et al [23] who used purified LPS of *E. coli* 0111:B4 in immunization of mice and record a highly significant increase of skin thickness of right footpad after 24 hrs and became at peak after 48 hrs. when compared with control group.

Our study revealed that the vaccination with *E. coli* vaccines protected mice against challenge with intraperitonially rout, this incompatible with study of Acres, et al [24] and Isaacson, et al [25], they found that the Purified pilus vaccines protect against challenge with ETEC strains that produce the same pilus antigen, and there are two lines of evidence that K99+ vaccines protect against challenge with ETEC strains that produce both K99 and F41 (K99+ F41+). A study of Asco`n, et al [26] showed that retention of K99 fimbrial subunit as native fimbriae or with the deletion of *fanGH* is sufficient to confer protection.

Conclusion

The results demonstrate the potential for the use of *E. coli* possess plus K99 as a vaccine to induce cellular and humoral immune response. Therefore, this vaccine protect mice against challenge with virulent strain of *E. coli* possess K99.

References

[1] Clements A., Young J.C., Constantinou N. and Frankel G. (2010) *Gut Microbes*, 3(2), 71-87.

[2] Debroy C. and Maddox C.W. (2001) *Anim. Health Res. Rev.*, 1, 129-140.

[3] Nagy B. and Fekete P.Z. (2005) *Int. J. Med. Microbiol.,* 295, 443 -454.

[4] Van Gerven N., De Greve H. and Hernalsteens J.P. (2008) *Antonie. Van. Leeuwenhoek*, 93, 219-226.

[5] Osman K.M., Mustafa A.M., Elhariri M., Abdelhamed G.S. (2013) *Transbound Emerg Dis*., 60(1), 69-78.

[6] Contrepois M.H.C., Girardeau H.D., Gouet P. (1985) *Annal. Rech. Vet*., 16, 41-46.

[7] Cornaglia E.M., Fernadez F.M., Gottschalk M. (1992) *Vet. Microbiol*., 30, 191-202.

[8] Nagy B. (1980) *Infect. Immun*., 27, 21-24.

[9] Yano T., Leite D.S., Pestana-Decastro A.F. (1995) *Braz. J. Med. Biol. Res*., 28, 651-654.

[10] Zhao X., Li Y., Wang L., You L., Xu Z., Li L., He X., Liu Y., Wang J. and Yang L. (2010) *Mol. Biol. Rep.*, 37, 2183-2188.

[11] Jay C.M., Bhaskaran S., Rathore K.S, Waghela S.D. (2004) *Vet. Microbiol.*, 101, 153-160.

[12] Aref N.E.M. and Saeed A.M. (2010) *J. Immunol. Methods.*, 366 (1-2), 100-105.

[13] Quinn P.J., Carter M.E., Markey B. and Carter G.R. (2004) *Clinical Veterinary Microbiology*, 6th ed., Mosby-Wolfe, London.

[14] Lim S.Y., Bauermeister A., Kjonaas R.A. and Ghosh S.K. (2006) *J. Immune Based Ther. and Vaccines*, 4(5).

[15] OIE (2004) *Manual of Diagnostic Tests & Vaccines fur Terrestrial Animal*, 5th ed., 1018.

[16] Mitov I., Denchen V. and Linde K. (1992) *Vaccine*, 10, 61-66.

[17] Hundson L. and Hay F. (1980) *Practical Immunology*, 3rd ed., Black-Well Scientific Publications, Oxford, London.

[18] Yousif A.A., AL-Taai N.A. AND Mahmood N.M. (2013) *International Journal of Immunology Research*, 3(1), 17-20.

[19] Reed L.J. and Muench H. (1938) *Am. J. Hyg*., 27(16), 8739-8744.

[20] Moon H.W., Isaacson R.E., Pohlenz J. (1979) *Am. J. Clin. Nutr.*, 32, 119-127.

[21] Contrepois M. and Girardeau J.P. (1985) *Infect. Immun*., 50, 947-949.

[22] Dhasarathan P., Saravanan S. and Perianayagasamy A. (2010) *African Journal of Pharmacy and Pharmacology*, 4(3), 119-122.

[23] Kshash Q.H., Habasha F.G. and Al-Rammahi A.K. (2009) *Iraqi Journal of Veterinary Sciences*, 23(II), 223-230.

[24] Acres S.D., Isaacson R.W., Babiuk L.A. and Kapitany R.A. (1979) *Infect. Immun*., 25, 121-126.

[25] Isaacson R.E., Dean E.A., Morgan R.L. and Moon H.W.(1980) *Infect. Immun*., 29, 824-826.

[26] Asco´n M.A., Ochoa-Repa´ra J.O., Walter N. and Pascual D.W. (2005) *Infection and Immunity*, 73(11), 7274-7280.