4th Sci. Congr. of Egypt. Soc. For Anim. Manag. 25-28 Oct.,2010:100-111

PROTECTION AGAINST E.COLI INFECTION IN RABBITS BY CELL WALL EXTRACT VACCINE

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ABSTRACT

Cell wall extract of E.coli was found to be an efficient method for preparation of an effective vaccine against colibacillosis in rabbits. Challenge experiments revealed that this vaccine provided the best protection compared with whole cell formalinized inactivated vaccine. The degree of protection conferred by the vaccine was positively correlated with the results of histopathological examination and (IgA*)* as detected by indirect fluorescent antibody technique.

INTRODUCTION

Prevention of infectious diseases through vaccination is an efficient method of protecting rabbits. Effective prevention can be achieved through proper use of vaccine .Viral diseases such as rabbit hemorrhagic disease virus (RHDV) is prevented by vaccination with inactivated form of virus **(Arguello, 1991).**Bacterial diseases such as rabbit pasteurellosis can be controlled by using the inactivated or attenuated form of bacteria as a vaccine**(Lu and Pakeo, 1981),**

Rabbit colibacillosis is one of the predominant bacterial disease affecting rabbit industry .Enteropathogenic E.coli are only class of pathogenic E.coli responsible of sever enteric diseases in suckling and weaned rabbits, with considerable economical impact in industrial fattening farms **(Boullier et al., 2003).** The disease in suckling rabbits is characterized by yellow diarrhea with soiling of hind quarters, and the mortality reaches 100% while in weaned rabbits, the animal show watery to mucoid diarrhea withough blood. Mortality is mostly between 5%-50%% according to severity of the pathogenic strains, **(Peeters et al., 1988).**

The prevention of Escherichia coli through administration of antimicrobial agents is costly and not always effective method due to

incidence of resistant strains (**Formmer et al., 1994)**. This vaccination could be one of the major important concepts.

The potential success of vaccination against rabbit colibacillosis depend on the antigen used and the method of administration. Two studies demonstrated that immunization can prevent the disease. In the first study, an orally administrated vaccine from formalin killed E.coli protected weanling rabbits against challenge with homologous live organisms **(Camguilhem and Milon, 1990)**, while in the second study,oral administration of live non-pathogenic e.coli protected weanlings against heterologous challenge **(Milon et al., 1989)** It was found that, vaccination with the first vaccine did not completely protect against challenge, while vaccination with latter was not save

The objective of the present study is to evaluate the cell-wall extract of E.coli, as an efficient method for the preparing a new E.coli vaccine of a higher potency against rabbit colibacillosis.

MATERIAL AND METHODS

Bacterial strain

The present work include E.coli serogroups O26/2+ and O128/8+, isolated and identified locally by the aerobic bacterial vaccine department. Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These strains was found to be the most common cause of colibacillosis in rabbits .The relative pathogenicity of these strains was re-evaluated in one month old susceptible rabbits before vaccine preparation and pre-challenge

Experimental animals

A total of thirty white New Zealand rabbits to be (one month old) weighing about 350-450 grams were used. All rabbits were confirmed coccidian free. The rabbits were fed a daily maintenance ration and kept under strict hygienic measures in special cages

Vaccine preparation

Preparation of dense culture of E.coli

Each strain of *E.oli* (O26/8+ and O128/2+)was separately seeded into Minica broth medium and incubated aerobically for,24 hours at 37^0C

.The culture of each strain was adjusted at a concentration of $3.8X10^9$ Colony Forming Unite(CFU)/ml. Samples from E.coli cultures were streaked for sterility purity on MacConkey agar media **(Milon et al., 1989) .**

Cell wall Extract vaccine

The vaccine was prepared from the extract of the cell wall of E.coli serotype O26 andO128 According to **Henric (1994)**. The extract was concentrated to 1mg /ml representing the vaccine dose per rabbits.

Inactivated vaccine

A 37% formaldehyde solution was added to bacterial suspension (equal volume of E.coli O26 andO128) to a final concentration of 0.5% according to **Camguilhem and Milon (1990).**

Quality control of the prepared vaccine

The final prepared vaccine in present study was tested for purity, sterility and safety tests according to standard international protocols as described by **British Veterinary Codex (1970) and Code of American Federal Regulation (1985)**

Vaccination

The experimental rabbits were divided into three equal groups (10 animal of each group), the rabbits in the 1st group were orally inoculated on days 0, 7, 14. with (1mg/ml)/animal of cell wall extract E.coli vaccines, while the rabbits in the 2nd group were orally administrated with 2ml /animal with inactivated vaccine at the same intervals The 3ed group was kept as unvaccinated controls.

Evaluation of the immune response of vaccinated rabbits

Flurescent antibody technique

Specimens of ileum, caecum and colon were quick frozen in isopentanein dry ice, and then they were sectioned to a thickness of 6-8 um in a cryostat at -20°C .The sections were stained with labeled fluorescent antibody to secretory IgA.Then it washed with saline and examined under florescent microscope **(Cantey and Blake, 1977)**

Challenge procedure

On day 28 all vaccinated and non-vaccinated rabbits were subdivided into two groups (five for each) and each one were challenged by oral administration of 2ml a fresh broth culture containing $2x10^4$ virulent *E.coli* serotypes O26 and O128.All rabbits were observed for 10 days after challenge for signs of weakness, diarrhea, and death. Each dead animal was autopsied and subjected to postmortem examination for any characteristic lesions**.**

Reisolation

Attempts were made to re-isolate the challenge organisms from caecal contents of freshly dead or scarified diarrheic rabbits.

Histopathological examination

Specimens of ileum, caecum, and colon from both vaccinated and control rabbits were examined for detection of the local mucosal immune response according to the method described by **Culling (1976) .**The results were interpreted according to severity: (+++ =severe diarrhea and sever intestinal lesions, ++ = moderate diarrhea and moderate intestinal lesions, + =slight diarrhea and slight intestinal lesions, - = No diarrhea and no intestinal lesions).

Protective indexes (PIs)

Using the following formula, PIs were assessed according to the incidence of clinical signs (CS), mortality (M), and PM lesions (PML) **(Timms and Marshall, 1989)**.

$$PI = \frac{\%(\text{CS, M OR PML}) \text{ in control} - \% \text{ in vaccinates} \times 100}{\% \text{ in controls}}$$

RESULTS AND DISCUSSION

Vaccination, when available, is undoubtedly the most cost-effective means for preventing and controlling, and even eradicating, viral and bacterial infectious diseases**.** Vaccination of animals serves many different purposes, such as controlling animal infections and infestations, thus improving animal health and animal welfare (**Bernard Vallat, 2007)**

The results of sterility test of the prepared vaccines revealed that these vaccines were free from any contaminants (aerobic, anaerobic bacteria, fungus and mycoplasmas) Concerning safety of the prepared vaccines ,it was found that rabbits vaccinated even with double vaccine did not show any abnormalities or adverse reactions.

Immunity against Rabbit colibacillosis depends largely on activation of cell-mediated responses, and gamma interferon has been shown to play a crucial role in this process in rabbits. Since the intestine is normally the organ in which infection is initiated and is the major site of pathology, immune responses in the intestine play a significant role in restricting initial infection with E.coli. The aim of the present study is to stimulate efficient immunity in the intestine by targeting the gut mucosa (**Mark et al., 2002).**

It can be cleared from the results given in **photo (1)** that conducting of IFA for detection of IgA in the intestinal tissue sections that intensely fluorescent reaction (++++) could be noted in intestine of rabbits vaccinated with cell wall extract vaccine , meanwhile clearly fluorescent reaction (++) was noted in intestinal tissue sections of rabbits vaccinated with inactivated vaccine **(photo 2)** . Negative fluorescent reaction (-) was observed in intestinal tissue sections of control unvaccinated rabbits. These results were explained previously by **O'Hanley and Cantey (1981)** who elicited that The synthesis and secretion of secretory IgA antibody were major components of the immune response of the ileum after infection with an invasive bacterium.

The results of challenge test (table 1) revealed that rabbits vaccinated with cell wall extract vaccine showed a striking reduction in mortality , intestinal lesions with protection of percentage 80% ,while the rabbits vaccinated with inactivated whole culture vaccine showed a relative higher mortality , intestinal lesions with protection of 60%.The control group showed extensive sever intestinal lesions with high mortality . the results indicated that challenge procedure could be considered as a parameter for evaluation of E.coli vaccines a described by **Pangraphy (1983**).

Histopathological examination of duodenum of rabbits vaccinated with cell wall extract vaccine or vaccinated with inactivated vaccine showed an filtration of inflammatory cells, including macrophages, lymphocytes, with hyperplasia in sub mucosal gland and slight edema in sub mucosa (photo 3, 4&5). These results agreed with those reported by **Rott et al. (1996**) who stated that a lymphocyte population that efficiently circulates to sites of mucosal inflammation. The rapid induction of these cells appears to play a crucial role in acquired immunity at mucosal surfaces **(Feng et al., 2000).** The efficiency of cell wall E.coli vaccine in protecting rabbits against challenge suggested that, some of the important immunogenic determinants are expressed. Antibody to these determinants may provide effective protection to vaccinated rabbits, these suggestion agreed with those of **Syuto and Mastumoto (1982)**. In conclusion, locally prepared E.coli extract vaccine elicits a specific protection against rabbit colibacillosis infection with E.coli.

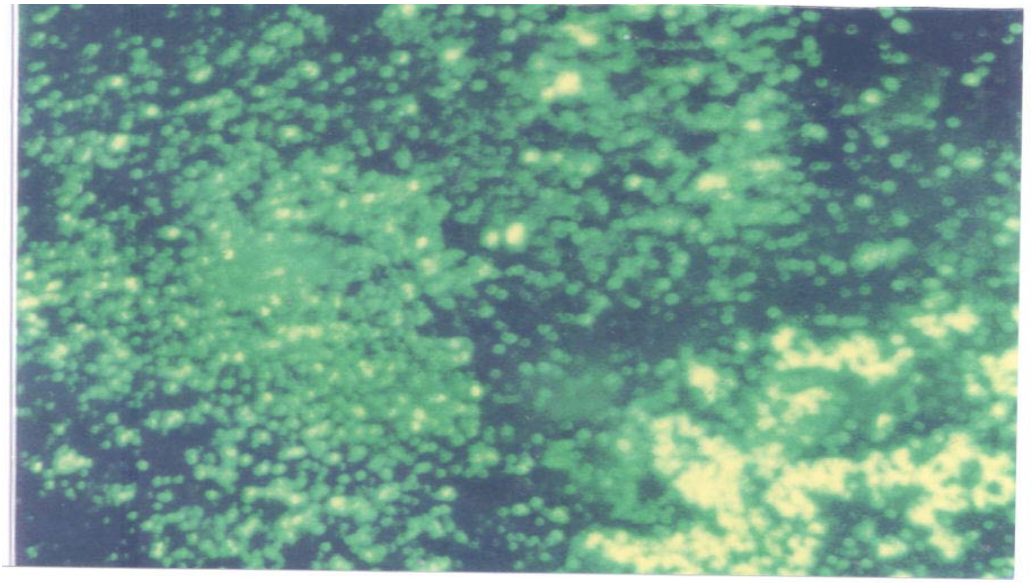

Photo (1) : intensely fluorescent reaction (++++)in intestine of rabbits vaccinated with cell wall extract vaccine

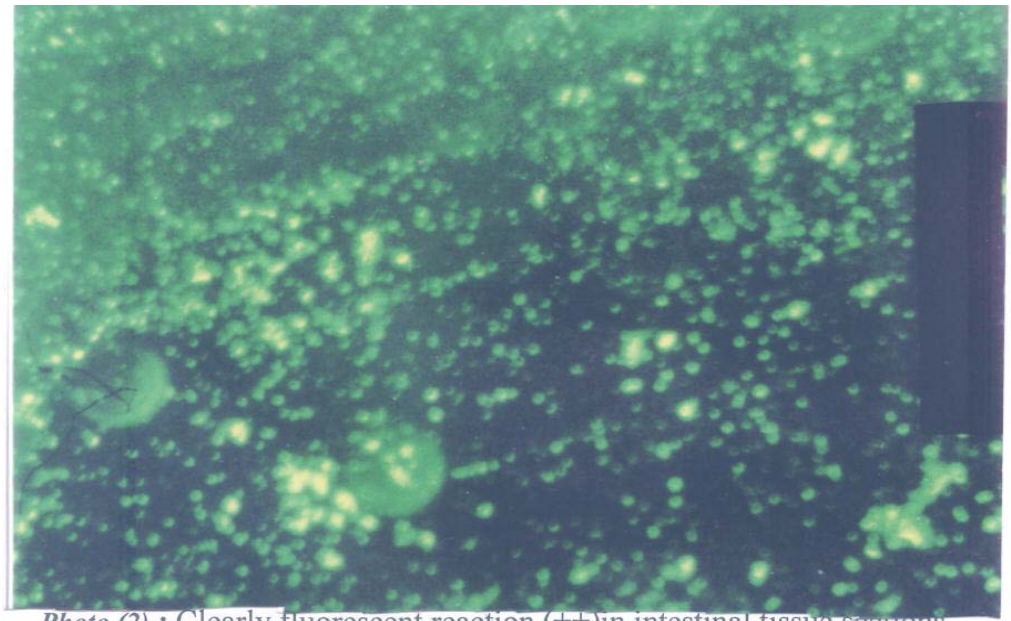

Photo (2) : Clearly fluorescent reaction (++)in intestinal tissue sections rabbits vaccinated with inactivated vaccine (photo 2)

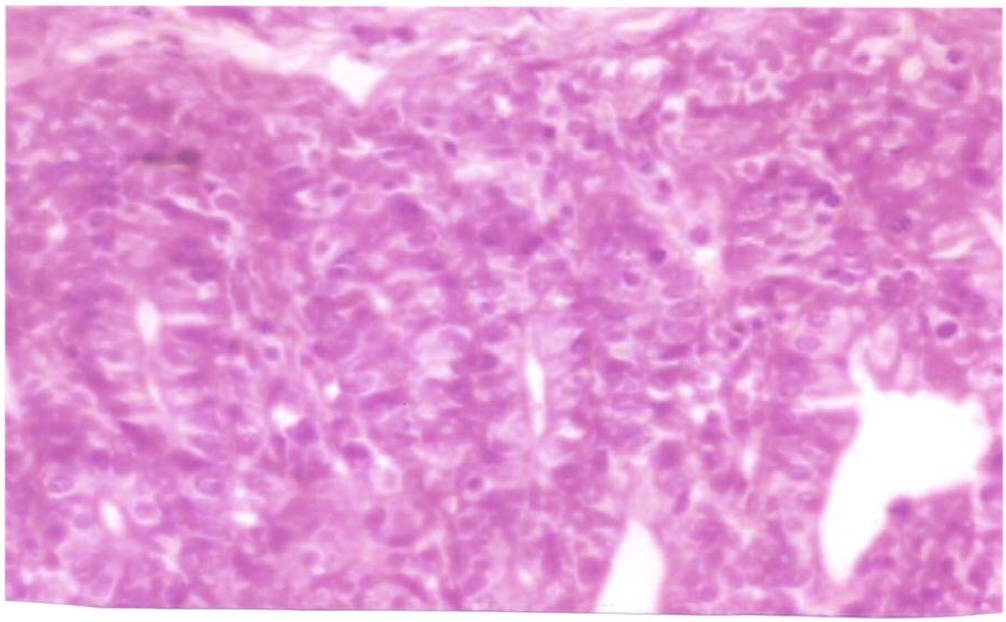

Photo (3): Intestine of rabbit vaccinated with cell wall extract vaccine showing leucocytic cell infiltration in lamina propia (H&E x 200)

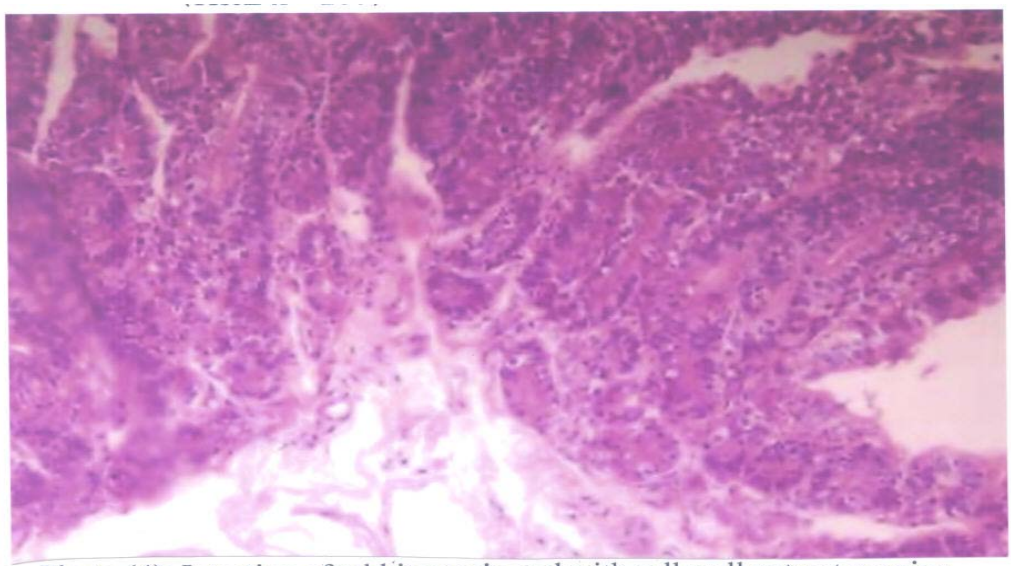

Photo (4): Intestine of rabbit vaccinated with cell wall extract vaccine showing submucosal edema . (H&E x 100)

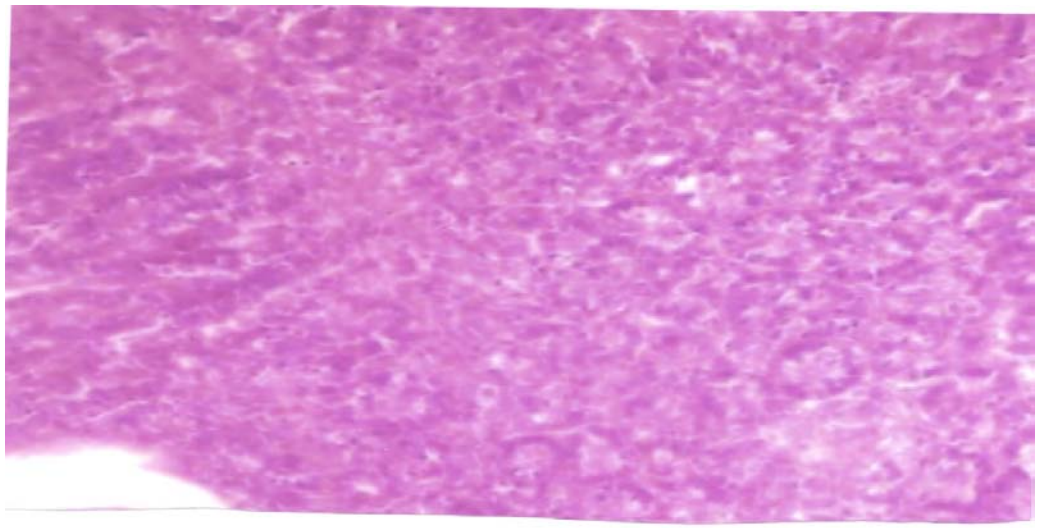

Photo (5): Intestine of rabbit vaccinated with cell wall extract vaccine Showing Hyperplasia of submucosal gland (H&E x 100)

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الحماية ضد عدوى الايشيريشياكولاى في الأرانب باستخدام لقاح مستخلص من الغلاف الخلوي

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تعتبر عدوى الايشيريشياكولاى في الأرانب من أهم المشا كل المرضية و التي تسبب خسائر اقتصادية كبيرة لما تسببه من حالات إسهال و التي يتبعها نسب نفوق عالية في الأرانب الرضيعة , ووقت الفطام .وقد أجريت هذه الدراسة بغرض تحضير ل قاح نوعى ضد هذا المرض باستخلاص الغلاف الخلوي لميكروب الايشيريشياكولاى المسبب للمرض كوسيلة متقدمة للسيطرة على هذه المشكلة المرضية.

وقد أوضحت الدراسة من خلال اختبار التحدي باستخدام العترة الضارية لميكروب الايشيريشياكولاى في الأرانب المحصنة باللقاح المست خلص من الغلاف الخلوي لميكروب الايشيريشياكولاى قد أعطى حماية عالية مقارنة باللقاح الخلوي المثبط بالفورمالين. جاءت نتائج الفحص النسيج ي لعينات الأمعاء من الأرانب المحصنة بكلا اللقاحين مطابقة لنتائج اختبار التحدي . أمكن باستخدام اختبار الفلوريسين الغير مباشر التعرف على الأجسام المناعية الموضعية IgA وكان التفاعل المناعي أقوى فى عينات الأرانب المحصنة باللقاح المستخلص من الغلاف الخلوي مقارنة بعينات الأرانب المحصنة باللقاح الخلوي المثبط بالفورمالين.

ومن هذا يمكن استنتاج أن اللقاح المحضرمن الغلاف الخلوي لميكروب الايشيريشياكولاى أنة امن في الاستخدام و ذو فاعلية قوية عن اللقاح المثبط بالفورمالين.