EVALUATION OF VACCINE AGAINST ESCHERICHIA COLI MASTITIS IN DAIRY CATTLE

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ABSTRACT

The present study was conducted to evaluate the immune response of dairy cows to inactivated E. coli oil adjuvant vaccine. E. coli was isolated from cases of clinical and subclinical mastitis and characterized on the basis of morphological, cultural, biochemical and serological tests. Cows were vaccinated with the prepared vaccine at 8 weeks before parturition and received booster dose three weeks later. Control cows were not immunized. Serum samples were subjected to ELISA technique to detect anti-E. coli IgG titers. The results revealed that the antibody titers were higher at calving for vaccinated cows than for unvaccinated controls. The prevalence of E. coli in quarters milk samples taken after calving and the incidence of mastitis were lower significantly among the vaccinated group in comparison with unvaccinated controls. These results suggest that the locally prepared E. coli vaccine elicits a non- specific health improvement of the udder in addition to specific protection against udder infection with E. coli.

INTRODUCTION

Mastitis is recognized world wide as the most important and costly disease of dairy animals. Field surveys of major livestock disease in Egypt have indicated that mastitis is one of the most important health hazards in the country (**Seleim et al., 2002; Abdel-Rady and Sayed, 2009**).

Economical losses are due to loss in milk production, discarding abnormal milk and milk withheld from cows treated with antibiotics, degrading of milk quality and price due to high bacterial or somatic cell count (SCC), costs of drugs for treatment, veterinary services and increased labor costs, increased risk of subsequent mastitis, herd replacement, and problems relate to antibiotics residues in milk and its products (**Bramley et al., 1996**).

The World Health Organization (**WHO, 2000**) stated that 30% of people in industrial countries and hundreds of millions in developing countries are suffering from diarr-

heal diseases caused by coliforms. Mastitis results in a marked reduction in the amount of milk and changes in levels of specific milk components, reducing the overall milk quality (**Harmon, 1994**).

The disease is caused by interaction of various factors associated with host, pathogens and environment. Infectious agents like bacteria, viruses and fungi are mostly the primary cause of the disease (**Quinn et al., 1994**). Coliform mastitis is one of the most difficult diseases to treat in the modern dairy industry. The incidence of clinical mastitis caused by environmental pathogens such E. coli is a concern of the dairy industry. Teat dip treatment and dry cow therapy for mastitis control are ineffective in controlling coliform mastitis, because of continuous exposure of teat to coliform pathogens (**Smith et al., 1985**).

The control of subclinical mastitis with a subsequent reduction in milk Somatic Cell Count (SCC) does not appear to decrease the incidence of clinical mastitis and may increase the susceptibility of cows to clinical mastitis caused by coliforms (**Scott et al., 1998**).

Chemotherapeutic agents remain only moderately effective and depend on the stage at which the diseases are treated. The most successful strategies of combating coliform mastitis appear to be prevention by hygienic measures and/or prophylactic immunization (**Dosogne et al., 2002**). Since E. coli is among the most common mastitis pathogens, the present study was conducted to study the efficacy of a locally prepared E. coli vaccine with Montanide ISA 206 as an oil adjuvant.

MATERIAL AND METHOD

Animals:

A total of (200) dairy cows (Frisian breed) were examined in a private lactating farm. All animals were subjected to clinical and physical examination with special interest toward the udder and teats to determine incidence of clinical and subclinical mastitis. A total of twenty dairy cows with no prior history of coliform mastitis were used to evaluate potency, another 5 cows for control test and 5 cows for safety test.

Milk samples:

Milk samples were collected under a septic condition. About 15 ml of the fore milk were discarded and the next 15ml were collected into screw- capped bottles. All samples were stored at 4°C.

California Mastitis Test (CMT) (Schalm et al., 1971)

All milk samples positive with CMT were cultured for microbial examination.

Isolation and identification of E. coli field isolate

All samples were centrifuged for 15 minutes at 3000 rpm and a loopfull was taken from sediment and inoculated separately onto

10% sheep blood agar and Mac-Conkey agar media. The inoculated plates were incubated at 37°C for 24 hours. Selected colonies were identified morphologically, biochemically and serologically according to **Quinn et al. (1994) and Waage et al. (1999)**.

Preparation of E. coli vaccine:

E. coli (O_{18}) was seeded into tryptic soy broth medium containing 0.05% yeast extract and incubated for 24 hours at 37°C. The culture was adjusted at a concentration 1 x 10^9 colony forming unite (CFU) per 1ml. The broth cultures were taken to check purity, before the inactivation by 0.5% formalin at 37°C for 24 hours according to **Acres et al (1979).** After completion of inactivation, the oil adjuvant vaccine was prepared using mineral oil (SEPPIC Montanide ISA 206 SEPPIC Division Cosmetique-Pharmacie, Paris, France). The inactivated culture was mixed with equal volume of Montanide ISA 206 as recommended by manufacturer and preserved with 0.01% thiomersal.

Quality control of prepared vaccine:

1. Sterility test:

Samples of inactivated vaccine were cultured for detection of aerobic, anaerobic bacteria, mycoplasma and fungal contamination according to **Code of American Federal Regulation (2005).**

2. Safety test:

Intra muscular injection of 10 ml of the prepared vaccine in each of 5 cattle and kept under observation for 10 days according to **Code of American Federal Regulation (2005).**

3. Immunization of dairy cows:

Mastitis caused by coliforms is highly associated with the 2-week period prior to calving and early lactation (**Smith, et al., 1985**). The high rate of coliform mastitis at calving coincides with a period of reported immunosuppression (**Craven and Williams, 1985**). An effective vaccine that would increase resistance to coliform infections during these periods would reduce significantly the losses caused by this disease.

Twenty cows were injected intra muscular with 5 ml of the prepared E. coli vaccine at 8 weeks before parturition and received booster dose three weeks later. Five cows served as unimmunized controls. Cows were observed for adverse reaction at the injection site, and rectal temperature were taken at 0, 12, 24, 48 and 72 hours post immunization. Serum Samples from all cows were collected monthly. Milk samples were collected monthly after parturition for recovery of E. coli and measure antibody level in whey.

ELISA:

Samples of serum were tested by indirect ELISA (**Ziegler et**

al., 1982) to estimate anti-E. coli IgG antibodies.

Results and Discussion

This study was undertaken to prepare and evaluate the immune response of dairy cows to formalin inactivated E. coli vaccine. Bacteriological culture of milk is the gold slandered method for determining the cause of clinical mastitis (**National Mastitis Council, 1999**) and **Milne et al (2003**). The E. coli isolate was selected on the basis of its morphological, biochemical and serological characterization. Morphologically E. coli isolate was gram negative rods and colony culture was transparent (pinkish) on MacConkey agar. Biochemically, E.coli isolate was catalase positive, lactose fermenter and indole positive. Serologically E. coli isolate belonged to serotype O_{18}.

The incidence of mastitis as presented in table (1) showed higher subclinical mastitis incidence (23%) than the clinical one (11%) in examined samples .these support the previous findings by **Mohamed et al. (1993)** who estimating 17.29 % and 3.67 % for subclinical and clinical mastitis respectively. This could be due to the results of in efficiency of treatment or development of drug resistance.

Proper isolation and identification of the causative agents are still one of the most efficient processes for diagnosis the diseases and for efficient vaccination method.

The safety test in cows of the prepared vaccine was tolerated and no general reactions observed. Rectal temperatures did not differ among the vaccinated and control group. Prepared vaccine did not cause any adverse reactions at injection site. Minimal swelling of the injection site (<2.5 cm) was disappeared within 48 h. These observations revealed that the prepared vaccine was non pyrogenic and safe at use. Local reactions in the form of swelling were limited so this adjuvant can be considered safe to use. **Mozen et al. (1996)** reported that the use of mineral oil alone in bacterin used in food animals still poses potential problems to carcass quality and animal discomfort.

Serum antibody titers to E. coli antigen in vaccinated dairy cows are shown in table (2). Immunized cows had higher IgG antibody titers than non-vaccinated controls.

A similar study was conducted by **Vangroenweeghe et al. (2004)** who evaluate the dynamic of immunological response of dairy cows to different immunizing E. coli bacterin.

Whey antibody titers to E. coli antigen in vaccinated dairy cows are shown in table (3). Immunized cows had higher antibody titers than non-vaccinated controls which agree

with results obtained by **Hogan et al., (2005).**

The concentration of recognized antibodies may affect the rate of bacterial clearance from infected quarters (**Hill et al., 1983**). Rapidly Increased the rate of antibodies to E. coli was associated with reduce the rate and severity of coliform mastitis (table 4). These results were similarly reported by **Hogan et al. (1992)**. The presence of IgG after primary and secondary immunization is an indication of the involvement of helper T-cells in immune response and the induction of IgG producing B-cells **Dresser and Popham (1979)** which is critical for rapid clearance of infectious agents from mastitis udders with long-lasting protection.

In conclusion, locally prepared E. coli vaccine elicits a non-specific health improvement of the udder in addition to specific protection against udder infection with E. coli.

Table (1): Incidence of clinical and subclinical mastitis in examined dairy cows

No of examined cows	Clinical mastitis*				Subclinical mastitis**			
	Milk samples from clinically infected cows		Isolation of E. coli		Milk samples from apparently healthy cows		Isolation of E. coli	
	No	%	No	%	No	%	No	%
200	22	11	17	77	41	23	26	63

*** Hot, hard sensitive udder that is acute painful to the animal with changes in composition.**

**** No visible changes in appearance of udder and/or the milk but the milk production decreased by 10% and positive for California Mastitis Test (CMT).**

Table (2): The mean serum OD values in dairy cows vaccinated with inactivated E. coli vaccine as measured by ELISA test

Group	First dose	Booster 3WPV	8 WPV	12 WPV	16 WPV	20 WPV	24 WPV	2	32 WPV
Vaccinated	0. 042	0.115	0.436	0.765	0.666	0.507	0.471	0.342	0.061
Controls	0.053	0.056	0.059	0.056	0.063	0.056	0.044	0.058	0.032

Wpv = weeks post vaccination

Table (3): The mean whey OD values in dairy cows vaccinated with inactivated E. coli vaccine as measured by ELISA test

Group	First dose	Booster 3WPV	8 WPV	12 WPV	16 PV	20 WPV	24 WPV	28 WPV	32 WPV
Vaccinated	dry	dry	dry	0.692	0.523	0.471	0.448	0.386	0.048
Controls	dry	dry	dry	0.068	0.058	0.048	0.061	0.057	0.039

Wpv = weeks post vaccination

Table (4): Incidence of clinical and subclinical mastitis in vaccinated dairy cows post parturition

No. of examined cows	Clinical mastitis				Subclinical mastitis			
	Milk samples from clinically infected cows		Recovery of E. coli		Milk samples from apparently healthy cows		Recovery of E. coli	
	No	%	No	%	No	%	No	%
20	1	5	0	0	2	11	0	0

REFERENCES

Abdel-Rady, A. and Sayed, M. (2009): Epidemiological studies on subclinical mastitis in dairy Cows in Assuit Governorate. Veterinary World, Vol. 2 (10) 373-380

Acres, S.D; Isaacson, R.E; Babiuk, L.A. and Kapitany, R.A. (1979): Immunization of calves against Enterotoxigenic Colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins. Infection and Immunity 25(1): 121-126.

Bramley, A.J; Cullor, J.S; Erskine, R.J; Fox, L.K; Harmon, R.J; Hogan, J.S; Nickerson, S.C; Oliver, S.P; Smith, K.L. and Sordillo, L.M. (1996): Current Concepts of Bovine Mastitis. 4th Edition National Mastitis Council.

Craven, N., and Williams, M. R. (1985): Defenses of the bovine mammary gland against infection and prospects for their enhancements. Vet. Immunol. Immunopathol.2:71.

Code of American Federal Regulation (2005): Published by: The office of the Federal Register National Archives Records Service. General Services Administration, 2005

Dosogne, H; Vangroenweghe, F. and Burvenich, C. (2002): Poten-

tial mechanism of action of J5 vaccine in Protection against severs bovine coliform mastitis. Vet. Res. 33(1):1-12. 19.

Dresser, D.W and Popham, A.M. (1979): The influence of T-cells on the initiation and expression of the immunological memory. Immunology 38: 265

Harmon, R, J (1994): Physiology of mastitis and factor affecting somatic cell count. J. dairy Science, 77:2013-21129.

Hill, A.W; Heneghan, D.J.S; Field, T.R. and Williams, M.R. (1983): Increase in specific opsonic activity in bovine milk following experimental E. coli mastitis. Res. Vet Sci 35: 222.

Hogan, J.S; Smith, K.L; Todhunter, D.A, and Schoenburger, P.S (1992): Field trial to determine efficacy of an E. coli J5 mastitis Vaccine. J. dairy Science, 84: 75-78.

Hogan, J.S; Cannon, V.B.; Smith K.L; Rinehart, C. and Miller, S.(2005): Effect of adjuvant on safety and efficacy of an Escherichia coli J5 Bacterin. J. dairy Science, 88: 534:542.

Milne, M.H; Biggs, A.M; Fitzpatrick, J.K and Barett, C (2003): Use of clinical information to predict the characteristics of bacteria isolated from clinical cases of bovine mastitis. Vet. Rec. May 17,152 (20):615-617.

Mohamed, Ibtisam, E.G.E and Elowni, O.A.O (1993): A study on the incidence and etiology of bovine mastitis in Sudan .second Sic. Cong., Egyptian Society for cattle Diseases, 5-7 Dec. Assuit, Egypt.326-336.

Mozen, B.M., Villacres, E.A., and Bengtsson, K.L. (1996): Novel adjuvant and vaccines delivery system.Vet. Immunol. Immunopath. 51: 373-348

National Mastitis Council (1999): Laboratory Handbook on Bovine Mastitis, received ed. Madison, Wisconsin: Natl. Mastitis Counc. Inc. 1-30

Quinn, P.J; Carter, M.E; Markey, B .K. and Carter, G.R (1994): Clinical veterinary Microbiology .Wolf Publishing Mosby.

Schalm, O.W; Carroll, E. and Jain N.C. (1971): Bovine mastitis 1st Ed. Lea and Febiger, Philadiphia, USA.

Scott, H.M; Sargeant, J.M; Ireland, M.J; Lissemore, K.D; Leslie, K.E; Kelton, D.F. and Mallard, B.A. (1998): Effect of a core antigen vaccine against gram negative bacteria on physiologic and yield parameters of dairy cows during late lactation and dry period. J. dairy Science, 81: 1928-1935.

Seleim, S.R; Rashed, Amany, Y.M and Fahmy, B.G.A. (2002): Mastitis pathogens; attachment–related Virulence feature, whey protien-

markers and antibiotics Efficacy in cows. Vet. Med. J.Giza, 50:405.

Smith, K.L; Todhunter, D.A. and Schoenburger, P.S. (1985): Environmental mastitis: cause, prevalence and Protection. J. dairy Science, 68:1531

The World Health Organization (WHO 2000): Control of food borne infection and intoxication in Europe. Newsletter, No 63 March 2ooo

Vangroenweeghe, F., Rainard, M., Poope, L., and Burvenich, C (2004): Increase of E.coli inoculum doses induce faster innate Response in prim porous cows. J. dairy Science, 87(12): 4132-4144.

Waage, S; Mork, T; Rroros, A; Hanshamar, A. and Odegaard, S.A. (1999): Bacteria associated with dairy Hifers. J. dairy Science, 82: 712.

Ziegler, E.J, McCuthan, J.A., Fierier. J. and Braude, A.I.(1982): Treatment of gram negative bacterimia and Shock with human antiserum to a mutant E.coli. New England J. Med., 307: 1225.

الملخص العربى

تقييم كفاءة لقاح ضد ميكروب الايشيريشيا كولاى المسبب لالتهاب الضرع فى الأبقار الحلابة

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أجريت هذه الدراسة لتقييم الاستجابة المناعية للأبقار الحلابة للقاح الايشيريشيا كولاى الزيتى الميت. وقد تم تحضير هذا القاح من عترة معزولة من حالات مصابة بالتهاب الضرع وذلك بعد تصنيف هذه العترة المعزولة على أساس شكل الميكروب والتصنيف الكيميائى والتجارب السيرولوجية. وقد تم تحصين الأبقار باللقاح المحضرعند ثمانية أسابيع قبل الولادة ثم حقن جرعة ثانية بعد 3 أسابيع من الجرعةالأولى ولم يتم تحصين مجموعة الأبقارالضابطة. تم تقييم مستوى الاجسام المناعية بواسطة اختبار الاليزا الذى أظهرارتفاع معدل مستوى المناعة عندالأبقارعند الولادة عن مجموعة الأبقارالضابطة. وجد أن نسبة وجود الايشيريشيا كولاى فى عينات اللبن بعد للولادةونسبةحدوث التهاب الضرع فىالأبقارالمحصنة أقل من الأبقارالضابطة. أظهرت النتائج الحالية بأن اللقاح المحضر يعطى مناعة متخصصة ضد الاصابة بالايشيريشيا كولاى.