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IMMUNOPOTENTIATED K_{99} VACCINE FOR BUFFALO DAMS TO PROTECT NEONATES AGAINST E.COLI ENTEROTOXICOSIS

By

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SUMMARY

The present study was conducted on the neonates of 40 pregnant buffalo dams kept in a large dairy herd in Kalubia province. A comparison of passive immunity of newly born buffalo calves especially against Enterotoxic colibacillosis by vaccinating dams with commercially available (Nobi-vac K99) and immunopotentiated K99 vaccine (Ultracorn accociated) is presented here with a general disscussion of immunomodulating effect of ultracorn administration.

Calves were assigned into four comparable groups according to K99 vaccination and/or ultracorn treatment of their dams precalving.

Clinical observation of neonates of test and control groups clearly demonstrated that K99 vaccination and/or ultracorn treatment induced a pronounced decrease in incidence of diarrhoea, preumonia and other early neonatal troubles, culling and mortality rates.

The result of immune response to K99 vaccine revealed that there was a striking increase in the anti-K99 titres of serum of calves born to dams vaccinated with ultracorn associated K99 vaccine than those born to dams receiving the vaccine alone, throughout the test periods post colostral. The difference in K99 titres between the two groups can be explained as a result of immunopotentiating effect of ultracorn.

It is important to anounce that the simultaneous adminstration of K99 and ultracorn preparation to dams had the most ameliorating effect on the immunity, health and vitality of neonatal buffalo calves.

It is highly recommended that a prograr depending on the vaccination of buffalo dam prior to calving with ultracorn associated K9 vaccine could be instituted and successfully use to protect neonates especially against *E. c* enterotoxicosis.

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INTRODUCTION

Several studies have been conducted to demonstrate that vacciantion of pregnant dams with *E.coli* bacterins to ensure production of colostral specific antibodies ralsed against K_{99} antigen and protect calves during the crucial period of the first days of life. Moon et al., 1978; Acres, et al., 1982; Collins et al., 1988; and Valente et al., 1988.

Recently, non-specific immunostimulants are gaiining increasing attention in recent days to counteract the effect of environmental immunosuppressive factors and help the animal in its struggle against disease agents and potentiation of its immune response to applied vaccines. A variety of non-specific immunostimulants were suggested e.g. B.C.G. (Safwat et al., 1984; El-Garhy, 1987 and Saleh et al., 1989). Levamisole (Babluk and Misra, 1981, Nair and Rojan, 1983 and Armanious et al., 1991), Pind-Avi (Mayr, 1978), Vitamin E (Reddy et al., 1987 and Armanious et al., 1991), Vitamin C (Blair and Cummins, 1984), Vitamin A (Sterm et al., 1981), synthetic drugs such as sodium diethyldithio carbonate DTC (Renoux and Renoux, 1984) Corynebacterium parvum (Gelencser et al., 1980; Malhorta et al., 1984; Givergea and Copprean, 1985, Archamboult et al., 1989, Soliman et al., 1990 and Soliman et al., 1991).

Attention is directed in this work to the possible beneficial effects of incorporating ultra-corn (non-specific immunostimulart of Virbac) to promote the immunogenic properties of K_{99} vaccine given to dams prior to parturition.

It might therefore, be advantageous at this time to study the effectiveness and comparative efficiency of buffalo dams incoulation with comercially available and immunopotentiated K99 vaccine in addition to ultracorn alone to provide passive protection of their neonates against *E. coli* enterotoxicosis.

It is hoped that an improved bacterin for vaccinating dams and prevention of enterotoxigenic colibacillosis of calves would be valuable and instituted.

MATERIAL AND METHODS

Buffalo Dams

40 pregnant buffalo dams were used, 4-8 years old, clinically healthy belonging to larg dairy herd of Kalubia province and fed grain, grass and concentrates.

Buffalo Calves

40 buffalo calves born at an organized Kalubia buffalo breading farm were used in this study. The calves remained with their dams for four days post partum obtaining colostrum by suckling before being transferred to the rearing unit. Every parturition was supervised as soon as possible in order to help the calf to suckle its dam soon after birth. At 5 days of age, the calves were fed milk according to appetite until 30 days of age and kept

under continuous observation. All the subjects (calves and dams) under went a direct and continuous clinical control.

The calves were assigned into four comparable groups as follows:

First group:

10 buffalo calves delivered from pregnant dams mainiated as control, they received neither vaccine nor ultracorn.

Second group:

10 buffalo calves delivered from pregnant dams vaccinated with two doses of Nobi-Vac K99 Vaccine,

Third group:

10 buffalo calves delivered from pregnant dams injected by ultracorn simultaneously with the booster dose of Nobivac K99 vaccine.

Fourth group:

10 buffalo calves delivered from pregnant dams received ultracorn 1/M one month before calving.

Ultracorn:

A complete lysate bacterial extract which is produced by ultrasound treatment of Corynbacterium cutis strain, the drug was obtained from Virbac Co. France.

Nobi-vac K99:

It is a vaccine containing a high amount of purified K99 antigen of *E. coli* in an oil adjuvant emulsion. the vaccine was obtained from Intervet Co. Holland.

Vaccination:

Twenty pregnant buffalo dams were vacinated 3 months pre-calving with Nobi-vac K99 (Intervet) (2 ml, i.m.) and given a booster appoximatelly 4 weeks before calving. It appeared that 2 vaccinations with an interval of 8 weeks guarantee high titre of antibodies of new born calves.

Inoculation of ultracorn: 10 ml of ultracorn (Virbac) were injected intramuscularly to pregnant dams one month before delivery. Ultracorn was given alone in fourth group or simultaneously with the booster dose of K99 vaccine in third group.

Blood samples:

Blood samples were collected from buffalo calves of control & vaccinated dams. Samples were drawn from jugular vein at intervals of 24 hr. 1st, 2nd, 3rd and 4th week post partum. Serum was separated off.

Passive haemagglutination test:

The test was carried out according to the metho of Neter (1956) as outlined by Sojka (1965) determine the anti K99 antibody level in sera buffalo calves of control and vaccinated dams.

RESULTS

The vaccinated and ultracorn treated pregnant buffalo dams, including the controls did not have general clinical changes. The gestation and parturition were normal, followed by birth at the end of term and the milk production was within the average of the herd. Calves were born healthy and showed no signs of illness any general health hazards.

Clinical observations of newly born buffalo calves in test and control groups:

The data given in Table (1) represent morbidity and mortality rates among the 4 test groups-each of 10 buffalo calves according to dam vaccination and/or ultracorn treatment.

Clinical sign scores were recorded on a daily basis for calves in each treatment and control group.

Control calves born from unvaccined dams:

From the 10 calves in this group, 2 died with rapid deterioration in condition. 4 calves began to scour shortly after birth and once diarrhoea was established, two of those developed pneumonia. The remaining 4 control calves, 2 showed signs of cullig one had arthritis and one had ampholophlibitis. Calves that became ill improved by treatment measures and were still surviving at the end of the experiment.

Calves born from dams given primary and booster dose of Nobi-Vac K99:

All 10 calves of this group survived, 2 of those showed signs of dairrhoea and pneumonia while still receiving colostrum, but subsequent daily treatment alleviated the symtoms and improved throughout the experiment.

Calves born from dams given ultracorn injection simultaneously with Nobi-Vac K99 boostering:

All calves born from dams of this group survived. The occurrence of diarrhoea, and pneumonia and other symptoms was stopped while at the same time there was no need to any preventive treatment.

Calves born from dams given I/M injection of ultracorn

All calves of this group survived, one calf began to scour shortly after birth and improved and one showed sign of culling.

Anti K99 *E. coli* titres in serum of new born buffalo calves of test and control groups:

Data given in Table (2) and Fig (1) represents the titre range and mean value of the k99 antibody titres in sera of calves from different 4 experimental groups.

The highest mean value was present in calves from dams vaccinated with k99 and treated with ultracorn followed by calves from dams

Table (1)
Clinical observations of buffalo calves in each treatment and control groups.

* Syndrome	New born buffalo calves of			
	Control dams G I	2 dose K99 vaccinated dams G II	2 dose K99 vaccinated & ultracorn treated dams G III	Ultracorn treated dams G IV
Diarrhaea	** 4/10	1/10	0/10	1/10
Pneumonia	2/10	1/10	0/10	0/10
Arthritis	1/10	0/10	0/10	0/10
Amphalophlibitis	1/10	0/10	0/10	0/10
Tympany	1/10	0/10	0/10	0/10
Culling	2/10	1/10	0/10	1/10
Mortality	2/10	0/10	0/10	0/10

* Identified clinically.
** Calves sick or dead/ calves tested.

Table (2)
Mean serum K-99 antibody titres in
new born buffalo calves of test and control dams.

Time of calf serum collection (post partum)	New born buffalo calves of							
	Control dams G I		2 dose K99 vaccinated dams G II		2 dose K99 vaccinated and ultracorn treated dams G III		Ultracorn treated dams G IV	
	Titre range	Mean	Titre range	Mean	Titre range	Mean	Titre range	Mean
24 hours	0 to 80*	16	20 to 640	176	40 to 640	184	10 to 40	20
1st week	0 to 80	16	40 to 320	152	40 to 1280	488	20 to 80	32
2nd week	0 to 160	32	80 to 640	400	320 to 1280	768	40 to 160	72
3rd week	0 to 40	16	80 to 320	208	80 to 1280	384	20 to 160	76
4th week	0 to 320	68	40 to 320	200	40 to 1280	472	10 to 20	12

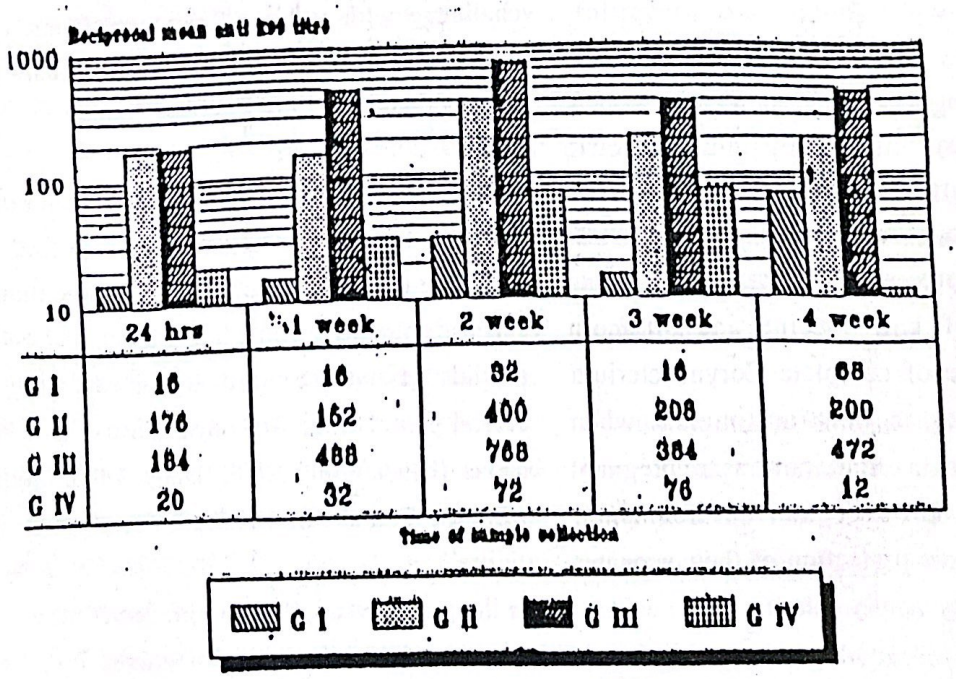
* Receprocal Anti K99 titre.

Table (3)
Comparison of K99 E. coli antibody titres in buffalo calves groups II and III during the test period

Time of calf serum collection (Post partum)	New born buffalo values of			
	G II		G III	
	Titre range	Mean	Titre range	mean
24 hours	20 to 640 *	176	40 to 640	184
1 week	40 to 320	152	40 to 1280	488
2 weeks	80 to 640	400	320 to 1280	768
3 weeks	80 to 320	208	80 to 1280	384
4 weeks	40 to 320	200	40 to 1280	472

* Reciprocal anti K99 titre!

Fig (1):
Mean serum K99 titres in new born buffalo calves of test & control dams



vaccinated with kgg vaccine then calves from dams treated with ultracorn (expect the value obtained in the 4th week). The lowest mean values were in calves of control dams.

Comparison of k99 *E. coli* antibody titres in buffalo calue groups II and III during the test period:

Data given in table (3) in-dicated that the mean serum anti k99 titre was markedly higher in calves delivered from dams received ultracorn associated k99 vaccine (GIII) than those born from dams that had the vaccine alone (GII) throughout the course of study. Calf sera collected from the former group (III) at 24 hrs, lst week, 2nd week, 3rd week and 4th week post colostral had a mean titre of 1/184, 1/488, 1, 1/768, 1/384 and 1/472 versus to 1/176, 1/ 152, 1/400, 1/208 and 1/200 in the latter group II respectively.

DISCUSSION

The possibility of using non specific immunostimulants to rise the animal resistance against enterotoxic colibacillosis is suggested. The aim of the present study was to investigate the potential of k99 vaccine and ultracorn (ultrasonic lysate of complete Corynbacterium cutis bacteria) as an immunostimulant when applied alone or in combination to pregnant bufaloes kept under Egyptian environmental condition to provide protection of their neonates against infection especially enteritis.

In the present study, It is quite clear that k99 vaccination and/or ultracorn treatment of pregnant buffaloes induced an ameliorating effect on morbidity, culling and mortality rates of their neonates, Table (1).

The difference between the test and control calf groups was evident in death, incidence of diarrhaea, pneumonia and other neonatal hazards.

It is intersting to note that the incidence of diarrhoea and other neonatal troubles, culling and mortality rates were minimized among new born buffalo calves by k99 vaccination or ultracorn treatment of their pregnant dams and a significant and pronouncing effect was observed when ultracorn was given simultaneously with k99 vaccine. This result coincides with that obtained in other animal species by Gelencser et al., (1980). Corrier and Wagner (1984) and Soliman et al., (1991) who observed that ultracorn enhanced the degree of protection against challenge with virulent microorganisms when ultracorn was administrated simulataneously with a specific vaccine.

Enterotoxigenic *E. coli* vaccination of pregnant dams has been recommended since long time ago to ensure the production of colostrum containing specific antiodies against k99 antigen and able to provide passive immunity to calves during the crucial period of the first days of life of new born calves (Moon et al., 1978, Nagy, 1980, Mettias, 1987 and Collins et al., 1988)

In the present study Anti k99 *E. coli* titres were determined in blood sera of calves born from control, k99 vaccinated, k99 vaccinated k99 vaccinated ultracorn treated dams and ultracorn

treated dams (Table 2). It was obvious that calves delivered from k99 vaccinated dams showed higher mean titre (1/176) than calves delivered from non-vaccinated ones (1/16) during the first 24 hours after birth. Maximum mean titres (1/400) were detected at the 2nd week of life in calves of vaccinated dams Table (2) which confirm earlier reports by (Kaechenbeek et al., 1961, Kaechenbeek and Schoenaers, 1964, Reisinger, 1965).

As shown in Table (3). Treatment of pregnant buffaloes with ultracorn improved markedly the immune response developed against k99 vaccine. The highest mean anti k99 titres were recorded in sera of calves born from vaccinated ultracorn treated dams throughout the test periods as compard to calf group of dams that had the k99 vaccine alone. A peak level of mean titre (MT) (768) was detected at the 2nd week post colostral. These results coincide with that obtained by Padany et al., (1980) and Soliman et al., (1991) who reported that inactivated corynbacterium suspension or ultracorn immunomodulator given to animals immunized with specific vaccine had increased the vaccine potency several times when given simultaneously with such vaccine. It can be concluded that pre-calving treatment of buffalo dams with ultracorn preparation, a complete lysate of corynbacterium cutis, as nonspecific immunostimulant with k99 vaccine, potentiated the immune response developed against k99 vaccine and enhanced the health state and activity of neonatal calves delivered from immunized dams. This conclusion agrees the earlier reports of Scott (1979).

Although the precise mechanism of ultracorn and nature of its immunopotentiating factors remains to be elucidated, yet it has been shown by various workers to augment host defenses by modulating cell mediated immune responses, enhancement of phagocytic activity of macrophages (Soliman et al 1991) and T. cell functions (Verbac circular). These functions may explain the protection and better resistance of calves born from dams treated with ultracorn alone (GIV). However, more definitive studies are needed to clarify dosage, duration and time of treatment in relation to immunization. Ultracorn perhaps through its effect on the immune system may be more effective and protective.

As the humoral and clinical investigations demonstrated conclusively that new born buffalo calves receiving colostrum from their dams received immunoptentiated k99 *E. coli* vaccine (ultracorn associated) showed pronounced decrease, in the incidence of diarrhaea and mortality with an apparent higher anti k99 titer, vaccination associated with ultracorn treatment programme to dams 3 months precalving should be started soon to protect neonates from *E. coli* enterotoxicosis in addition to increase their performance.

It could be concluded from the present study that it is econmically important to apply both specific (k99 vaccine) and dnon-specific (ultracorn) immunostimulants on pregnant buffalo dams to achieve less enteric, respiratory and death loses among Egyptian born buffalo calves by stimulation of their humoral and cell mediated immune response. It is also important to anounce

that the simultaneous administration of k99 vaccine and ultracorn had the most ameliorating effect on the immune response of neonatal buffalo calves.

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