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EVALUATION OF THE EFFICACY OF *Escherichia Coli* (K99) VACCINE ON THE INCIDENCE OF *E. Coli* AND IMMUNITY IN BUFFALOES

A. FARID*; G. E. EID* ; Y. R. ABDEL-MAWLA** and NAGAT A. SALEH**

* Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Cairo University.

** Department of Buffalo Research, Animal Health Research Institute, Agriculture Research Center, Dokki.

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SUMMARY

Bacteriological examination was carried out on faeces of 119 buffalo-calves delivered from 99 vaccinated and 20 unvaccinated buffalo-dams. The fecal samples were taken by rectal swabs from apparently healthy and diarrhoeic calves of different ages from 1 to 30 days. *E. coli* was the predominant organism in comparison with other Gram negative enteric bacterial pathogens. The results of the determination of K99 adhesive antigen among *E. coli* isolated from apparently healthy calves showed 0 % and 20 % in calves delivered from vaccinated and unvaccinated dams, respectively while in case of diarrhoeic calves the percentages were 50 % and 80 % in calves born from vaccinated and unvaccinated dams respectively. Regarding the detection of enterotoxin production in relation to the presence of K99 adhesive antigen, all the K99+ *E. coli* strains that were recovered from diarrhoeic calves delivered from vaccinated and unvaccinated dams were positive for enterotoxigenic effect. The results of field application of Nobi-vac vaccine containing K99 adhesive antigen denoted significant decrease in the percentage of diarrhoea to 5.05 % in calves born from vaccinated dams, as compared to 40 % in calves delivered from unvaccinated dams. Serum samples were collected from the vaccinated and unvaccinated pregnant buffalo-dams and from their newly born calves as well as colostrum for detection and evaluation of K99 antibodies by the use of solid phase ELISA. The K99 antibody titers in the sera of vaccinated and unvaccinated pregnant buffalo-dams showed a significant difference at $P < 0.05$. The highest mean titers were 5670, 5167, 10791 and 7946 at 2 week and 4 week after the 1st dose, 2 week post-boostering and 48 hours after parturition in the vaccinated buffalo-dams while it reached to 1342, 1267, 1426 and 1262 in the unvaccinated buffalo-dams, respectively. The mean colostral K99 anti-

body titers of buffalo-dams during 24 hours after parturition reached to 16291 in vaccinated dams while it reached to 3051 in the unvaccinated dams. Furthermore, the K99 antibody titers in the sera of newly-born calves were differed significantly at $P < 0.05$ and the mean antibody titers increased from 0 to 9250 and 7160 after 48 hours and one week, respectively. On the other hand, the K99 antibody levels appeared higher in the serum of calves delivered from vaccinated dams than in calves delivered from unvaccinated dams.

INTRODUCTION

Diarrhoea of young calves is the main cause of economic losses through poor growth and mortality. Morbidity and mortality rates of neonatal calves with diarrhoea have been related to low post colostrum serum immunoglobulin concentration. The disease is considered as one of the major problems facing livestock production not only in Egypt, but also all over the world. Dirrhoea is a complex disease, which can be caused by various infective agents proliferating in the intestinal tract alone or in combination with other microorganisms of which enterotoxigenic *E. coli* (ETEC) possessing the K99 antigen is the most incriminated in addition to Rota virus, Corona virus, Bovine Viral Diarrhoea and Salmonella species (Acres et al., 1975). Enterotoxigenic *E. coli* is considered as the major cause of diarrhoea and colibacillosis in newborn calves. This is due to its ability to produce toxin and to colonize in the intestinal epithelium. Colibacillosis in young calves often affects over 50 % of newborn calves in herd with death rates of 10 to 20 % (Newman et al., 1973).

In Egypt, diarrhoea continues to be the first cause of mortality, which ranges between 27.4 % to 55.5 % of the total deaths in young calves (Ahmed, 1980). ETC exotoxin cause great losses of fluid and electrolytes leading to severe dehydration, and metabolic acidosis. Treatment of colibacillosis in young calves is often ineffective due to the presence of drug resistant strains of *E. coli*. Therefore, vaccination of buffalo-dams prior to calving with *E. coli* K99 vaccine could be useful for protection of neonates against colibacillosis (Salem, 1992). Previous reports indicated that vaccination of dams prior to calving with *E. coli* bacterin containing the K99 antigen reduced the infection with enterotoxigenic *E. coli* in young calves (Daniel et al., 1979). The main objectives of this work were to study the incidence of *E. coli* infection among newly born buffalo-calves, to evaluate the role of *E. coli* K99+ strain as a cause of diarrhoea and to evaluate the immunopotential of E. coli K99+ improved vaccine for protection of newly born calves from diarrhoea.

MATERIAL AND METHODS

MATERIALS: Faecal samples: A total of 119

rectal faecal samples were collected from newborn buffalo calves (Bubulus bubalis) at different ages (from 1 to 30 days old). Out of these, ninty-nine calves were born from *E. coli* (K99 Nobi-vac vaccine) vaccinated dams and the rest (20) were born from unvaccinated dams. All these animals belonged to two Governmental Farms (Nattaf farm and Mehallat Moussa farm). Infant mice: A total of 24 mice 1 to 3 days old were used to study the enterotoxigenic activity of the isolated K99+ *E. coli* strains.

Media: Nutrient broth and tryptone soy broth (Oxoid); MacConkey agar medium (Oxoid); Blood agar medium (Oxoid); Eosin methylene blue agar (Oxoid) and Minca isovitalex agar media (Minca-IS); Media used for biochemical reactions and sugar fermentation: Peptone water (1.0 %) (Oxoid); Triple sugar iron agar (Oxoid); Simmon s citrate (Difco); Nitrate broth (Difco); Urea agar base (Difco); Methyl red; Voges Proskauer medium (Difco); Christensen s medium (Christensen); Motility test medium (Mac Faddin); Phenylalanine deaminase (Difco); and Decarboxylase Moeller base broth (Biolife).

Biochemical tests: Catalase test (Oxoid); Indole test (Oxoid); Methyl red reagent (Oxoid); Andrade s solution indicator; Voges Proskauer (Oxoid); Kovac s reagent; Nitrate reduction test (Difco); Urease test: (40 % urea solution) (Oxoid); Phenylalanine deaminase test; Arginine dehydrolase test; Lysine decarboxylase test and Ornithine decarboxylase test.

Stains: Gram's stain (Cruickshank et al., 1975).

Diagnostic reagent: Latex agglutination reagent. It was kindly provided by Dr. K.N. Mettias, Animal Reproduction Research Institute, Giza, Egypt. Vaccine: Imported *E. coli* vaccine (Nobi-vac oil-adjuvant vaccine, Intervet Netherlands).

Animals for vaccination program: Animals: 119 pregnant buffalo-dams (99 vaccinated and 20 unvaccinated) of 3 to 4 years old at late stage age of pregnancy (6 weeks before the expected calving) as well as their off springs after parturition were used to study the immune response of *E. coli* K99+ vaccine.

Blood samples were collected from buffalo-dams and their newly born calves for separation of serum at different intervals as follows: directly before and 15 days after the first dose of vaccination; directly before and 15 days after the booster dose of vaccination and 48 hour after delivery. Blood samples from calves: collected directly before colostrum feeding; 48 hours and 7 days post colostrum feeding. Colostrum samples were also collected from vaccinated and non-vaccinated buffalo-dams within 24 hours of parturation to determine antibody levels against *E. coli* K99.

Material used for solid phase ELISA: Carbonate-bicarbonate coating buffer (Mettias et al.,

1994); 0.02 M Phosphate buffer saline (PBS); Washing buffer: (0.05 % Tween 20 in PBS); Block-ing buffer: (0.05 % Tween 20 and Bovine albumin in PBS pH 7.4); Rabbit antibovine globulin (IgG) conjugated with horse raddish peroxidase (Sigma); Substrate solution (ABTS): 2,2-azino-bis (3-ethyl-benzthiazoline - 6 - sulphonic acid); Ammonium salt (Sigma).

METHODS: Cultivation of samples: The rectal swabs were inoculated into tryptone soya broth medium and incubated at 37°C for 72 hours. A loopfull from inoculated tryptone soya broth medium was then subcultured onto the surface of MacConkey agar, sheep blood agar, nutrient agar and Minca Isovitalex plates. The plates were incubated at 37°C for 24-48 hours.

Identification of isolates by: Smears from selected bacterial colonies were prepared and stained with Gram s method and examined microscopically for detection of morphological appearance, arrangement and staining reaction of the isolates. Smears were examined to observe Gram negative, medium sized stained evenly and no sporing rods. Isolated colonies were identified biochemically according to Finegold and Martin (1982).

Biotyping of the isolates: It was determined according to Burrows (1985) by using of carbohydrate fermentation, decarboxylation of lysine, ornithine and hydrolysis of esculin. Detection of K99 adhesive antigen in *E. coli* isolates using slide agglutination test by picking up 2 colonies from isovitalex agar medium, suspended in physiological saline, then added one drop of monoclonal K99+ *E. coli* antiserum. An isolate was designated positive for the presence of K99 antigen.

Haemagglutination (HA) test for E. coli: It was carried out according to Burrows et al., (1976). Equal volumes of 2 % guinea pig erythrocytes in 1 % D-mannase and bacterial suspension were mixed together for 5 to 8 minutes. Results were recorded and considered as mannose resistant (MR) when HA occurred with 1 % D-mannose suspension. On the other hand, bacteria were considered, manose sensitive (MS) when HA did not occur with 1 % D-mannose suspension.

Haemolytic activity of *E. coli*: It was done on 5 % sheep blood agar plates.

Latex agglutination test for the detection of E. coli K99 antigen: It was carried out in accordance with method of Mettias et al. (1996).

Detection of Enterotoxigencity: This test was carried out to detect the enterotoxigenic activity of the isolated K99+ *E. coli* strains. For this, 24 baby suckling mice and 6 *E. coli* strains possessing K99 adhesive antigen were included. The method used was that recommended by Giannella (1976) and El-Shennawy et al., (1982).

Colostrum whey separation: Colostrum whey

samples were prepared and separated using 1 % rennet by the method described by Selman et al. (1973).

Solid phase ELISA for the quantitative estimation of E. coli K99 bacterin antibodies: It was done according to Nagy et al., (1984) with a slight modification. Briefly, microtitre ELISA plate was coated with 100 ul of purified E. coli K99 antigen at dilution 1:400. Following incubation, washing and fixation the fixed dried wells were then blocked with 100 ul of the blocking buffer and left at room temperature for 2 hours. The blocking buffer was decanted and 100 ul of diluted unknown serum (1:100) as well as positive and negative control sera were added to each well and left at room temperature for 2 hours. The plates were then washed three times then 100 ul of the antibovine IgG conjugated with horse-reddish peroxidase were added to each well at dilution 1:20000 for 2 hours at room temperature. The conjugate was decanted and washed three times; then 100 ul of the substrate working solution (ABTS) were added to each well and the plates were left at oom temperature in a dark place for 15 minutes. The enzymatic reaction was rapidly stopped by adding 50 ul of 5 % SDS per well. The developed colour was read at 405 nm using Bio-Tek microplate ELISA reader. The average positive and negative control serum absorbance was calculated. The average negative control absorbance was substracted from the average positive absorbance and the difference representing the corrected positive control absorbance. A sample to positive (SP) ratio was calculated according to the following equation formula (Snyder et al., 1984):-

$$Sp = \frac{\text{(Sample Absorbance)- Average negative control absorbance}}{\text{Corrected positive control absorbance}}$$

The titre was calculated as follows:-Log 10 titre = (1.464 x Log 10 Sp) + 3.197

Titre = antilog of log 10 titre

Statistical analysis: The differences between vaccinated and unvaccinated animals were tested by the calculated least significant difference (LSD) at 5 % level according to Steel and Torrie, (1960).

RESULTS

Effect of Nobi-vac (*E. coli* K99) Vaccine on Morbidity Rates of Buffalo Calves: This study was done to reveal the effect of vaccination on the control of diarrhoea among 1 to 30 days old buffalo calves. The results of 3 years study are shown in Table (1); it can be seen that the incidence of diarrhoea decreased from 40 % among calves delivered from unvaccinated dams to 5.05 % in calves delivered from vaccinated dams.

Incidence and Identification of *E. coli* from Buffalo Calves: The results of *E. coli* isolated from faecal samples collected from 119 calves

born from vaccinated and unvaccinated dams are illustrated in Table (1). It was found that 46 (38.6 %) *E. coli* isolates were recovered from 119 calves, in which 34 and 2 isolates were isolated from non-diarrhoeic and diarrhoeic calves born from vaccinated dams with percentages of 36.2 and 40, respectively. In addition to, 10 (5+5) *E. coli* isolates from diarrhoeic and non-diarrhoeic calves born from unvaccinated dams with percentages of 62.5 and 41.7, respectively.

Identification of the isolated *E. coli* isolates using haemagglutination (HA) test: It was revealed that 7 out of 46 *E. coli* (15.22%) isolates were HA positive. None of the 34 *E. coli* strains recovered from non-diarrhoeic calves born from vaccinated dams showed any HA positivity, however, 4 isolates from the 5 isolated *E. coli* (80%) from diarrhoeic calves born from unvaccinated dams showed HA positivity. Moreover, 32 isolates out of the total number of isolated *E. coli* (46) were haemolytic onto 5 % sheep blood agar media with percentage of 69.7.

The presence of K99 adhesive antigen in the isolated *E. coli* was determined using monoclonal antibody against K99 E. coli using latex agglutination test. Table (1) shows the presence of K99 adhesive antigen in one out of 2 *E. coli* isolates that were recovered from 5 diarrhoeic calves born from vaccinated buffaloes. While *E. coli* K99 adhesive antigen did not detect in the 34 *E. coli* isolates that were recovered from 94 apparently healthy calves born from vaccinated dams. Furthermore, the presence of K99 adhesive antigen in 4 out of 5 *E. coli* isolates (80 %) related to 8 diarrhoeic calves born from unvaccinated dams. On the other hand, one out of 5 strains isolated from 12 apparently healthy calves born from unvaccinated dams was only positive for K99 antigen.

Identification of Enterotoxin Production in K99$^+$ *E. coli* Isolates: Infant mouse bioassay was used to evaluate the heat stable-enterotoxin (ST) production by enterotoxigenic *E. coli* (ETEC). Only the organisms which were K99 positive by latex agglutination test were tested for enterotoxigenicity and the results are recorded in table (1). It is obvious that no enterotoxigenic *E. coli* K99$^+$ (0 %) was recovered from apparently healthy (non-diarrhoeic) calves born from unvaccinated or vaccinated dams, while all *E. coli* K99$^+$ (with percentage of 100 %) isolated from diarrhoeic calves born from both vaccinated as well as unvaccinated dams had enterotoxigenic effect in baby suckling mice (Table 1).

Immune Response of Pregnant Buffalo-dams to Nobi-vac (*E. coli* K99) Vaccine: Table (2) shows the mean levels of antibody against *E. coli* K99 in the sera collected from pregnant buffaloes pre- and post-vaccination. The mean levels of *E. coli* K99 antibodies in serum samples collected from

Table (1): Incidence and identification of *E. coli* isolated from diarrhoeic and non-diarrhoeic calves born from vaccinated and unvaccinated buffaloes-dams.

Calves delivered from	Calves status	Calves		Incidence of E. coli		Positive HA		Positive LA (K99)		Positive ET K99+	
		No.	%	No.	%	No.	%	No.	%	No.	%
Vaccinated dams (99)	Non-diarrhoeic	94	-	34	36.2	00	00	00	00	00	00
	Diarrhoeic	5	5.05	2	40	1	50	1	50	1	100
Unvaccinated dams (20)	Non-diarrhoeic	12	-	5	41.7	2	40	1	20	0	00
	Diarrhoeic	8	40	5	62.5	4	80	4	80	4	100
Total results		119	-	46	38.6	7	15.22	6	13.04	5	83.3

HA = Haemagglutination
LA = Latex agglutination
ET = Enterotoxigenic

Table (2): Mean values of serum and colostrum anti-K99 antibody titers of Buffalo-dams unvaccinated and vaccinated with Nobi-vac vaccine

Animals	Mean serum anti-K99 antibody titers					Mean colostrum anti-K99 titers
	Pre-Vaccination	2 weeks post-1st dose	4 weeks post-1st dose	2 weeks post-boostering	48 hours post-parturition	24 hours post-parturition
Vaccinated dam	1119 ±0.18	5670 ±0.2	5167 ±0.15	1079 ±0.26	7946 ±0.27	16291 ±0.32
Unvaccinated dams	1119 ±0.18	1342 ±0.22	1267 ±0.20	1426 ±0.23	1262 ±0.24	3051 ±0.24
L.S.D.	0.05	0.05	0.05	0.05	0.05	0.05

vaccinated dams were found to be ranging from 5162 to 10791, while in the unvaccinated buffaloes were ranged from 1262 to 1426.

E. coli K99 antibody Titers in Colostral Whey: Colostral samples were collected 24 hours post-parturation, from previously vaccinated and unvaccinated buffalo-dams; and the antibody titers against *E. coli* K99 were estimated using ELISA. It is indicated that the mean titres were 16291 and 3051 in the vaccinated and unvaccinated dams, respectively (Table 2).

Levels of Maternal Immunity in Delivered Calves: Passive transfer of K99 antibody levels in the serum samples of newly born calves delivered from unvaccinated and vaccinated dams with Nobi-vac (K99) vaccine were measured. Table (3) shows the level of *E. coli* K99 antibody in sera of calves derived from vaccinated and unvaccinated buffaloes before and after colostrum feeding. It is evident that the level of antibody against E. coli K99 in the sera of calves was zero before colostrum feeding. While the mean levels of antibody against *E. coli* K99 in sera of calves delivered from vaccinated dams at 48 hours and 7 days post-colostral feeding were 9250 and 7160 respectively, and were 2920 and 2060 for calves born from non-vaccinated dams, respectively.

DISCUSSION

Neonatal calf diarrhoea is a major problem facing livestock production especially calves under 30 days old. *E. coli*, while being, a normal inhibitant

Table (3):Mean values of anti-K99 antibody titers in serum of buffalo-calves delivered from non-vaccinated and Nobi-vac vaccinated dams

Types of group	No. of calves	Mean serum anti-K99 antibody titers		
		Pre-colostral feeding	48 hours post-colostral feeding	7 days post-colostral feeding
Calves born from vaccinated dams	99	0	9250 ±0.42	7160 ±0.25
Calves born from non-vaccinated dams	20	0	2920 ±0.24	2060 ±0.38
L.S.D.	-	-	0.05	0.05

Mean ± Standard error
L.S.D.= Least significance difference between groups at P<0.05.

of the intestinal tract, can be also associated with a variety of pathological conditions in man and animals (Gay, 1965). However, enterotoxigenic *E. coli* (ETEC) is considered to be the main cause of diarrhoea affecting calves less than one week old (Jayappa et al., 1984). Pathogenic *E. coli* possess number of virulence factors (Zeman et al., 1989) that are the main cause of diarrhoea in newly born animals. In many studies, the K99 pili have been found to be the main antigen in the colonization of *E. coli* to the calf intestine, after colonization in the small intestine, ETEC elaborate toxin. The majority of ETEC isolated from calves were found to produce K99 antigen (Moon and McDonald, 1983). The presence of fimbriae on the surface of many strains of *E. coli* has been found to correlate significantly with pathogencity. Moreover, they are strongly immunogenic, so that vaccine based on *E. coli* fimbriae have proved extremely successful in veterinary medicine (Klemm, 1985).

In the present study, a trial was achieved to control the morbidity and mortality of newly born buffalo-calves in Egypt due to *E. coli*. For this purpose the monovalent vaccine; Nobi-vac, K99 was used. From the obtained results, it could be noticed that the incidence of diarrhoea was decreased from 40 % to 5.05 % in calves delivered from unvaccinated and vaccinated dams, respectively. This finding may be attributed to the effect of vaccination; this comes in complete agreement with the result mentioned by Latinovic et al., (1990).

The results of bacteriological examination of faecal samples collected from apparently normal calves born from vaccinated and unvaccinated buffaloes were recorded. It could be revealed that *E. coli* is the predominate pathogens with an incidence of 36.2 % and 41.7 % in diarrhoeic and non-diarrhoeic calves born from vaccinated dams, respectively. This is almost similar to the results obtained by Osman (1972) who isolated *E. coli* with an incidence 35.4 % from apparently normal calves. The fact that the high ratio of isolation of *E. coli*, comes in agreement with *E. coli* appeared to be a normal inhabitant of the intestinal tract and it may become pathogenic when the animal is exposed to various harmful factors as deprivation of colostrum and bad hygienic conditions. Concerning the results of isolation of *E. coli* from faecal samples collected from 5 diarrhoeic calves born from vaccinated dams and 8 diarrhoeic calves born from un-vaccinated dams, the percentages of *E. coli* isolation were 40 % and 62.5 %, respectively. The incidence of *E. coli* isolation was lower in diarrhoea calves delivered from vaccinated dams than other diarrhoeic calves. This revealed that the cause of dirrhoea may be due to other agents other than *E. coli*. This is supported by Brenner et al., (1993) who mentioned that most common enteropathogens causing diarrhoea in young calves were enterotoxigen-

ic *E. coli* K99+, Salmonella spp., Cryptosporidium and rotavirus. The contribution of haemolysin production of *E. coli* isolates was found, that 69.7 % of the procured *E. coli* isolates were haemolytic. This comes in an approximate agreement with the results obtained by El-Gannam and Sidorov (1986) who reported that among 44 *E. coli* strains isolated from calves in USSR, 34 (77.3 %) were haemolytic and 29 (65.9 %) produced enterotoxin. The significance of the recovery of K99 antigen from *E. coli* isolates incriminated in calf diarrhoea was mentioned by Brenner et al., (1993) who stated that the most common enteropathogens causing diarrhoea in the neonatal calves were enteropathogenic *E. coli* K99 positive. Therefore, the detection of K99 antigen among the isolated E. coli in the present investigation was of great value. This was based on the hypothesis of Hadad and Gyles (1978) who pointed out that the K99 antiserum was of potential value in identifying bovine enterotoxigenic *E. coli* since the K99 antigen was found on a large percentage of the isolates among diarrhoeic calves. The result of the haemagglutination (HA) activity of cultured *E. coli* is illustrated in table (1), which indicate that the majority of *E. coli* isolates that had HA activity with percentages of 50 % and 80 %, were from diarrhoeic calves born from vaccinated and unvaccinated buffaloes, respectively. This may agree with Varga (1991) who found that out of thirteen *E. coli* strains isolated from calves with diarrhoea, most of the strains agglutinated red blood cells of horse, ox, guinea pig and chickens. On the other hand, some strains of *E. coli* isolated from apparently healthy calves born from vaccinated and un-vaccinated dams agglutinated guinea pig RBCs with percentages of 0 % and 40 %, respectively.

Serological studies on 2 *E. coli* isolates that were recovered from 5 diarrhoeic calves delivered from vaccinated dams, revealed that one isolate was positive for the presence of K99 adhesive antigen. Moreover, this K99 isolate was positive for enterotoxigenic test. This disagrees with Gmelch (1983) who mentioned that no enterotoxigenic *E. coli* was detected in the faeces from calves with diarrhoea born from seventy cows previously vaccinated with K99 antigen, inactivated Rota virus and different adjuvants. Also, Eichhborn et al., (1983) mentioned that K99 positive enterotoxigenic *E. coli* could not be detected in any of the faeces tested from diarrhoeic calves born from cows and heifers vaccinated with combined Rota virus-enterotoxigenic *E. coli* K99. It was noticed that vaccination of pregnant cows and heifers reduced morbidity diarrhoea among newborns. In addition, K99 positive *E. coli* was not detected in any of the faecal samples taken from diarrhoeic calves, whereas K99 negative enteropathogenic *E. coli* was found in some of them. In the present study, isolation of one K99 enterotoxigenic *E. coli* from diarrhoeic calves delivered from vaccinated dams may be due to the calf was fed on an unadequate amount of colostrum. It may be attributed also to any error in the vaccina-

tion process in some buffalo-dams. While, concerning diarrhoeic calves born from unvaccinated dams, a serological confirmation was applied on 5 *E. coli* isolates that were recovered from 8 diarrhoeic calves. Four isolates were positive for the presence of K99 adhesive antigen. This agrees with Valente et al., (1983) who reported that 47.62 % of the *E. coli* isolated from newborn calves with diarrhoea were positive for K99.

A logical finding was obtained with healthy calves delivered from vaccinated dams, where no enterotoxigenic *E. coli* could be isolated from those calves. While among healthy calves delivered from unvaccinated dams, 4 K99+ *E. coli* were isolated from 12 clinically normal newborn calves. This finding is in agreement with Martel et al., (1981) who showed that *E. coli* K99 was detected from faecal samples in 8.2 % of clinically healthy calves. It could be concluded that a positive correlation between the presence of K99 adhesive antigen and the enterotoxigenicity of *E. coli* especially among diarrhoeic calves delivered from unvaccinated dams. This may explains the important role played by these two attributes in induction of calf scours. Also, Kaeckenbeek et al., (1977) reported that 99 % of the calf enterotoxigenic strains possessed the K99 adhesive antigen, while this antigen was only detected in 21 % of non-enterotoxigenic strains. Moreover, Taku et al., (1991) reported that all the K99+ *E. coli* strains isolated from diarrhoeic calves produced heat stable enterotoxin as detected by suckling mice test.

Regarding the levels of antibody titer post-vaccination, it was noticed that the antibody titers at 15 days after the booster dose of vaccination was almost as twice as that obtained at 15 days and 30 days after the 1st dose. This may prove once more the necessity of the booster vaccination as mentioned by Rodriguez et al., (1987) who reported an increase of blood and colostrum antibody levels of dams revaccinated with K99 vaccine compared with single vaccinated ones. The booster dose vaccination was also recommended by Acres et al. (1982). A significant increase was also detected in the serum of vaccinated buffaloes than unvaccinated ones. This was also mentioned by Rodriguez et al. (1987) who noticed an increase of the blood and colostral antibody levels against *E. coli* in vaccinated animals if compared with unvaccinated control. Titers were detected in the serum and colostrum of dams 48 and 24 hours after parturition. A significant increase in the antibody titer was detected in the serum and colostrum of the vaccinated group than unvaccinated one. This agrees with Waltner-Toews et al., (1985). Also, Bodourova (1973) mentioned that specific immunization of pregnant cows with E. coli produces high titres of antibody in the colostrum. He also found that agglutinin titres and gammaglobulin contents of the blood and colostrum of dams as well as the blood of calves were generally higher in the affected farms than in the healthy ones. It seems that vaccination of dams

prior to delivery is the best way to improve the colostral quality as a passive immunization tool for the newborn calves. These findings are in agreement with those reported by Mettias (1987). The K99 antibodies are withdrawn from the blood at the time of delivery to be concentrated in the colostrum of vaccinated dams as stated by Schipper et al., (1984). In this study, the maximum titres of antibody in the colostrum of vaccinated animals were higher than those obtained from the colostrum of unvaccinated animals. For the same pregnant individual buffaloes, the antibody titer against *E. coli* was much more higher in the colostrum than in the serum of vaccinated animals just before delivery.

Concerning newly born calves, a significant increase in the antibody titers against E. coli was detected in calves which received hyperimmune colostrum from vaccinated buffalo-dams if compared with calves that received colostrum from non-vaccinated buffalo dams. On the other hand, in calves, which did not receive colostrum within 2 days the serum antibody level was (0). This finding is in agreement with Deutsch and Smith (1957) who concluded that the bovine calf in a case like this will be born agammaglobulinaemic.

The transfer of antibodies from vaccinated dams to their offspring via colostrum was suggested to provide passive immunity to calves during the crucial period of calves life (Nagy, 1980 and Mettias, 1987). In this investigation, it was found that the maximum antibody titers towards E. coli were detected in newly born calf serum at 48 hours post-parturition. This finding agrees with Ali (1983) who reported that maximum titers of antibodies, were detected in serum samples of newborn calves 48 hours after birth.

It is recommended that in buffalo farms suffering from problems due to neonatal calf diarrhoea, the pregnant females should be vaccinated with Nobivac or another K99+ *E. coli* vaccine. The vaccination protocol could be twice vaccinations one at 2 months before parturition and the second dose after 4 weeks later.

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