

## EFFECT OF TETRAVALENT VACCINE AS A CONTROL MEASURE FOR CALF DIARRHEA THROUGH DAM VACCINATION

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### SUMMARY

The present study was conducted on 80 pregnant buffalo dams and their neonates, of which 20 pregnant dam were injected s/c two times, at 8 weeks before the expected time of calving and again 4 weeks later with tetravalent vaccine (Lactovac vet.) to control neonatal calf diarrhea. The rest of dams were used as a control group. Immunoglobulin (IgM and IgA) concentration levels and some biochemical parameters (total protein, albumin and globulin) were measured in serum of buffalo dams prior to vaccination and after calving and compared with those of non vaccinated dams. The means of serum immunoglobulins and (total protein, albumin and globulin) of vaccinated dams after calving were increased when compared to those of control non vaccinated dams after calving.

A high significant difference was noticed between serum IgM of vaccinated and non vaccinated dams after calving. High significant differences were observed between 36-48 h old calves of both groups with regard to serum IgG, IgM and IgA. High significant differences were observed between colostrum IgG, IgM and IgA of vaccinated and non vaccinated dams. High significant reverse correlations were noticed between serum IgM of vaccinated dams and their calves as well as between colostrum IgG of non vaccinated dams and serum of their calves. A significant positive correlation between colostrum IgA of vaccinated dams and serum of IgA of their calves was observed. The previous results of immunization of pregnant buffalo dams with (lactovac vet) revealed that there was a striking increase in the level of both serum and colostrum immunoglobulins of vaccinated dams in

comparison with non vaccinated dams as well as in serum of 36-48 h old calves born to vaccinated dams in comparison to those born to non vaccinated dams. Clinical observations of neonates (test and control groups) clearly demonstrated that (Lactovac vet.) induced a pronounced decrease in the incidence of diarrhea, pneumonia and mortality rates. It is highly recommended that a program depending on vaccination of dams during late gestation with (Lactovac-vet.) could successfully used to protect neonates against the most prevalent diarrhoeogenic agents and minimize a great economic loss.

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## INTRODUCTION

Diseases of the newborn and neonatal mortality are a major cause of economic loss in livestock production and every practical economic effort should be made to minimize disease and mortality (Radostitis et al., 1994). Morbidity and mortality due to neonatal calf diarrhea are of significant economic concern to beef and dairy producers in Egypt. Economic losses to producers include the cost of the calf and investments in feed, housing, health care and labor. The application of various hygienic measures, good management techniques and investments in feed, housing, health care and labor. The application of various hygienic measures, good management techniques and strict

sanitary precautions to minimize the risk of scours among calves are recommended.

Coronavirus, enterotoxigenic *E.coli* (ETEC) with pilus antigen K99 and rotavirus are the most important agents implicated in the etiology of calf diarrhea in Egypt (Abou-Hassan et al., 1995). These etiologic agents are known to be highly spread under bad hygienic measures.

It is generally accepted that transplacental passage of immunoglobulin molecules is totally prevented in ruminants (Tizard, 1987). The newborn calf enters an environment which is more antigenic and pathogenic than its previously sheltered life in uterus. Although the bovine fetus develops immunocompetence during gestation, full immunological reactivity is only reached during postnatal life (Solomon, 1971). Since, it has been shown that ingested milk antibodies protect calves against enteric infections, control measures for diarrhea during the neonatal phase concentrate more and more on vaccination of dams (Acres et al., 1979; Gastra and De Graaf, 1982; Bachman, 1984).

The aim of dam vaccination with enteric agents is to improve lactogenic passive immunity, i.e. increase colostrum and milk antibody titers and prolong antibody secretion with colostrum and milk. Vaccination is an important part in the control of many animal diseases, but immunity is a relative thing. It may be overcome by massive



exposure to germs. Moderate exposure to highly virulent strain of the infectious agent or stress, such as poor hygienic measures or nutritional conditions may overcome the response of the vaccines. Vaccination should not be considered as the final answer in disease control. It should always be combined with proper nutrition, management practices to reduce stress and sanitary measures designed to prevent the introduction and spread of infection (Baker and Greer, 1981).

In the present study the immune responses and some biochemical parameters in sera and colostrum of buffalo dams vaccinated with tetravalent vaccine containing inactivated viral agents (rota, corona and parvoviruses) and inactivated E.coli-K99-pilus antigen were investigated in comparison with those of control non vaccinated buffalo dams after (1st day after calving). The immune responses of buffalo calves from immunized dams were also monitored and compared with those of calves from non immunized buffalo dams in relation to their clinical data.

## MATERIAL AND METHODS

### Vaccine:

A liquid commercial tetravalent inactivated vaccine (Lactovac vet., Hoechst Vetrinar GmbH, Germany) was used. The vaccine represented a liquid preparation of inactivated rotavirus (strain

VI005/ 78) and (strain Holland), inactivated coronavirus (strain 800), inactivated parvovirus (strain Haden) propagated on calf cell culture and inactivated E.coli K99-pilus antigen. The preparation contains aluminum hydroxide and saponine as adjuvants.

### Animals:

- a. Dams: Pregnant buffaloes from the farm of the Faculty of Veterinary Medicine, Suez Canal University were assigned alternately to either a vaccinated (20 buffalo dams) or control non vaccinated (60 buffalo dams) group during the calving season from November 1997 - April 1998. Both dam groups were kept on an open, fronted shed house with access to free yard and freely watered. They were managed and reared under good hygienic conditions. Pregnancy diagnosis was done by rectal palpation. Heavy pregnant buffaloes were injected twice in 5 ml quantities S/C, 6-8 and 2-3 weeks before calving. None of the pregnant buffaloes had been immunized before with either E.coli K99-pilus antigen or vaccines containing inactivated rota, corona and parvoviruses.
- b. Calves: Calves which were delivered from the vaccinated and non vaccinated dams put under investigation to follow up their clinical condition and to test their sera.

Every calf included in the experiment was born

at full term, healthy and supplied exclusively with colostrum and milk, respectively of its mother for the whole observation period lasting 1 month

Serum samples were collected from immunized buffalo dams just prior to immunization and after calving and from control dams at the corresponding times of sampling in immunized group. Likewise, serum samples were collected from delivered calves at 36-48 hours post colostrum feeding.

Colostrum was collected from vaccinated and non vaccinated dams just after parturition and immediately frozen at  $-20^{\circ}\text{C}$  together with serum samples until assays were performed. After thawing colostrum samples, they were clarified by centrifugation (18.000 RPM X 20 min.) at  $4^{\circ}\text{C}$ . Using long needle, the clear supernatant fluid under the fatty layer was collected and used.

IgG, IgG and IgA concentration levels in serum and colostrum were determined by double sandwich ELISA assay according to Erhard et al., (1992). Microtiterplates were coated overnight 200  $\mu\text{g}/\text{ml}$  PBS, pH (7.2) at  $4^{\circ}\text{C}$  and then blocked with 200  $\mu\text{l}$ / well of 0.5% gelatin in PBS (pH 7.2) for 60 min. at  $37^{\circ}\text{C}$ . The wells were then filled with the standard sera prediluted to 1:5000 for IgG (Immunotec, USA), 1:2000 for IgM (Sigma, USA) and 1:250 for IgA analysis

(Pasture Inst., France) and incubated at  $37^{\circ}\text{C}$  for 90 min. The secondary marked antibovine peroxidase conjugate (100 $\mu\text{l}$ / well) was added to all wells of the plate after being diluted 1:4000 for IgG, 1:10000 for IgM and 1:1000 for IgA and incubated for 90 min at  $37^{\circ}\text{C}$ . The enzyme activities in the individual wells were measured by adding 100 $\mu\text{l}$ / well of the peroxidase substrate solution containing citrate - phosphate buffer and H2O2. After 10 min., the reaction was stopped with 5 $\mu\text{l}$ / well And 2 mol/L HCl and the developed colors have been read in ELISA reader (Dynatec MR 7000) at an OD 492 nm. Between each individual incubation step, the ELISA plate was washed 3 times with PBS-tween 20 (0.5% tween).

Total protein concentration in serum and colostrum was determined by biuret method (Pardawill and David, 1949).

Albumin concentration in serum and colostrum was determined by bromocresol green method (Doumas, 1971).

Globulin concentration in serum and colostrum was determined by subtracting albumin concentration from total protein concentration.

#### **Clinical observations:**

A careful clinical record of calf's health during the first month of their life including the incidence of diseased conditions and the mortality rates was conducted. Statistical



analysis of results by T-test and correlation coefficient was preformed according to Sendecor (1960), while Chi square value was determined according to Danies (1947).

## RESULTS

The data given in table 1 represent the values of immunoglobulins (IgG, Igm and IgA) and some biochemical parameters ( total protein, albumin and globulin) in serum of buffalo dams prior to vaccination as well as after calving in comparison to those of non vaccinated dams after calving. Serum IgG, IgM and IgA levels of dams prior to vaccination were  $1618.4 \pm 39.3$ ,  $312.77 \pm 4.72$  and  $35.21 \pm 1.59$  (mg/dl), respectively, while serum total protein, albumin and globulin levels were  $6.59 \pm 0.66$ ,  $3.31 \pm 0.28$  and  $3.28 \pm 0.58$  (g/L), respectively. After calving, IgG, IgM and IgA levels were  $1876.77 \pm 39.74$ ,  $397.92 \pm 8.53$  and  $39.60 \pm 1.62$  (mg/dl), respectively in serum of vaccinated buffalo dams as compared with  $1799.12 \pm 18.69$ ,  $267.81 \pm 9.93$  and  $35.41 \pm 1.19$  (mg/dl), respectively in serum of non vaccinated buffalo dams. Total protein, albumin and globulin concentrations were also increased in serum of vaccinated buffalo dams after calving to  $8.56 \pm 0.56$ ,  $3.28 \pm 0.61$  and  $5.28 \pm 0.78$  (g/L), respectively as compared to  $7.21 \pm 0.64$ ,  $2.87 \pm 0.255$  and  $4.64 \pm 0.75$  (g/L), respectively in serum of control non vaccinated dams after calving . High significant differences were computed between serum IgG and IgM of

dams prior to vaccination and each of serum IgG and IgM of non vaccinated dams after calving ( $t=4.15^{**}$  and  $4.09^{**}$ ) and those of vaccinated dams after calving ( $t=4.62^{**}$  and  $8.73^{**}$ ) (table 2). A significant difference between serum total protein of dams before vaccination and that of vaccinated dams after calving ( $t=3.08^*$ ). A highly significant difference between serum IgM of non vaccinated and vaccinated dams after calving ( $t=9.94^{**}$ ) was observed . 36-48 h old calves delived from vaccinated dams gave high serum immunoglobulin levels of  $2054.74 \pm 45.51$ ,  $357.3 \pm 7.88$  and  $51.75 \pm 1.41$  (mg/dl) for IgG, IgM, and IgA, respectively when compared to levels given by 36-48h old calves delivered from non vaccinated dams  $1809.68 \pm 28.63$ ,  $257.91 \pm 10.32$  and  $36.87 \pm 2.02$  (mg/dl) for IgG, IgM and IgA, respectively (table 1).

Concerning the biochemical parameters, 36-48h old calves delivered from vaccinated dams had the highest values of  $8.01 \pm 1.09$ ,  $3.08 \pm 0.39$  and  $4.92 \pm 1.22$  (g/L) for serum total protein, albumin and globulin, respectively comparatively with those of 36-48h old calves delivered from non vaccinated dams  $7.45 \pm 0.98$ ,  $2.87 \pm 0.15$  and  $4.57 \pm 0.96$  (g/L), respectively. There were high significant differences between serum of 36-48h old calves delivered from non vaccinated dams and those delivered from vaccinated dams with regard to IgG ( $t=4.56^{**}$ ), IgM ( $t=7.65^{**}$ ) and IgA ( $t=6.02^*$ ) [Table 2]. a strong linear reverse correlation ( $r=-0.740^{**}$ ) was noticed with regard



**Table (1):** Levels of serum and colostral immunoglobulins and some biochemical parameters of buffalo dams and their calves.

Item	IgG mg/dl Mean±SE	IgM mg/dl Mean±SE	IgA mg/dl Mean±SE	Total Protein g% Mean±SE	Albumin g% Mean±SE	Globulin g% Mean±SE
Serum of dams before vaccination	1618.4±39.3	31.77±4.72	35.21±1.59	6.59±0.66	3.31±0.28	3.28±0.587
Serum of non vaccinated dams after calving	1799.12±18.69	267.81±9.93	35.41±1.19	7.21±0.64	2.87±0.259	4.64±0.751
Serum of vaccinated dams after calving	1876.77±39.74	397.92±8.53	39.60±1.62	8.568±0.562	3.28±0.611	5.286±0.788
Serum of 36-48h old calves from non vaccinated dams	1809.68±28.63	257.91±10.32	36.87±2.02	7.45±0.98	2.87±0.152	4.578±0.965
Serum of 36-48h old calves from non vaccinated dams	2054.74±45.51	357.3±7.88	51.75±1.41	8.01±1.09	3.08±0.391	4.924±1.22
Colostrum of non vaccinated dams	6052.95±74.38	858.97±92.86	366.82±13.34	6.52±1.67	1.62±0.507	4.89±1.08
Colostrum of vaccinated dams	8135.1±92.72	1366.5±66.22	703.77±26.41	6.84±1.36	0.83±0.184	4.82±1.53

**Table (2):** T-Values of serum and colostral immunoglobulins and some biochemical parameters of buffalo dams and calves.

	IgG	IgM	IgA	Total Protein	Albumin	Globulin.
Serum of dams before vaccination x serum of non vaccinated dams after calving	4.15**	4.09	0.012	1.68	0.351	1.42
Serum of dams before vaccination x serum of vaccinated dams after calving	4.62**	8.73**	1.93	3.08*	0.396	2.04
Serum of nonvaccinated dams x serum of vaccinated dams after calving	1.56	9.94**	2.07	1.36	0.62	0.593
Serum of 36-48h old calves from non vaccinated dams x serum of calves from vaccinated dams	4.56**	7.65**	6.02**	0.36	0.49	0.22
Colostrum of non vaccinated dams x Colostrum of non vaccinated dams	6.42**	6.58**	11.75**	0.164	1.60	0.54

\*\* High significant difference at 0.05 and 0.01 levels of probability.

**Table (3):** Mortality rates and incidence of some clinical disorders among buffalo calves

Buffalo calves	Total	Mortality No.%	Enteritis No. %	Pneumonia No.%	Chi square Value
From vaccinated dams	20	2 (10)	2 (10)	3 (15)	18.1 <sup>a</sup>
From non vaccinated dams	60	14 (23)	15 (25)	13 (21.5)	0.93 <sup>b</sup>

a = Significant at 0.1 level of probability

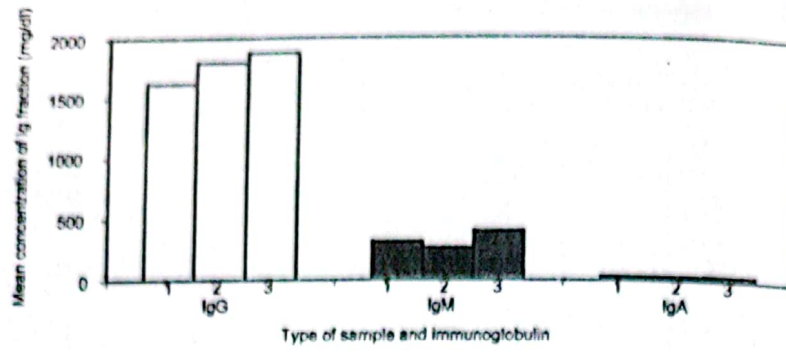
b = Non significant at 0.90-0.95 level of probability.

**Table (4):** Correlation coefficients of serum and colostral immunoglobulins and some biochemical parameters of buffalo dams with those in serum of their calves.

	IgG	IgM	IgA	Total Protein	Albumin	Globulin
Serum of vaccination dams x their calves	-0.213	-0.740**	-0.458	-0.323	0.580	0.255
Serum of non vaccination dams x their calves	-0.151	-0.155	0.494	0.532*	-0.276	-0.025
Colostrum of non vaccinated dams x serum of their calves	-0.752**	0.632	0.347	-0.591	-0.097	-0.168
Colostrum of vaccinated dams x serum of their calves.	-0.540	-0.603**	0.627**	0.40	-0.105	0.663

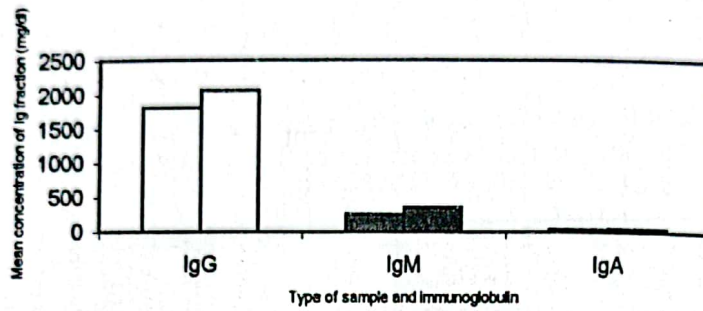
\*\* High significant difference at 0.05 and 0.01 levels of probability.





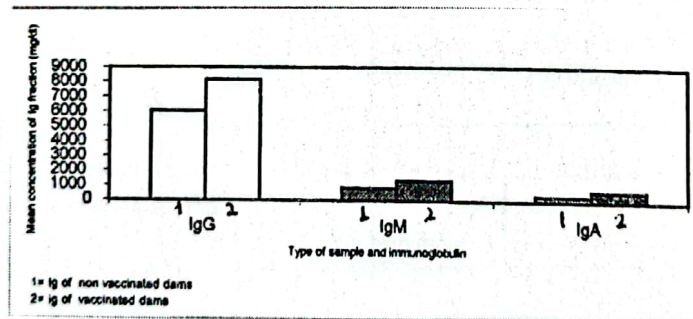
1= Ig of dams before vaccination  
 2= Ig of non vaccinated dams after calving  
 3= Ig of vaccinated dams after calving

Fig. (1): Comparative distribution of immunoglobulin in sera of dams before vaccination, non vaccinated and vaccinated dams after calving.



1= Ig of 1 day old calves from non vaccinated dams  
 2= Ig of 1 day old calves from vaccinated dams

Fig. (2): Comparative distribution of immunoglobulin delivered from non vaccination, and vaccinated dams.



1= Ig of non vaccinated dams  
 2= Ig of vaccinated dams

Fig. (3): Comparative distribution of immunoglobulin in colostrum of non vaccination, and vaccinated dams.



to IgM in serum of vaccinated dams after calving and their calves, while a positive linear correlation ( $r=0.532^*$ ) was noticed between non vaccinated dams after calving and their calves in concern to serum total protein [Table 4].

A clear rise in colostral immunoglobulins of vaccinated dams was noticed  $8135.1\pm 92.27$ ,  $1366.59\pm 66.22$ ,  $703.77\pm 26.41$  (mg/dl) for IgG, IgM and IgA, respectively in comparison to those of non vaccinated dams  $6052.95\pm 74.38$ ,  $848.97\pm 29.86$  and  $366.82\pm 13.34$  (mg/dl), respectively. On the other hand, a slight rise of total protein ( $6.84\pm 1.36$ g/L) and globulin ( $5.82\pm 1.53$ g/L) were observed in colostrum of vaccinated dams in comparison to colostral total protein ( $6.52\pm 1.67$ g/L) and globulin ( $4.89\pm 1.08$ g/L) of non vaccinated dams [table 1]. High significant differences were computed between colostral IgG ( $t=6.42^{**}$ ), IgM ( $t=6.58^{**}$ ) and IgA ( $t=11.75^{**}$ ) of vaccinated and non vaccinated dams [table 2]. A strong linear reverse correlation ( $r=0.752^{**}$ ) was noticed between colostrum of non vaccinated dams and serum of their calves with regard to IgG [table 4]. A reverse linear correlation ( $r=-0.603^*$ ) was observed between colostrum of vaccinated dams and serum of their calves in concern to IgM, while a positive linear correlation ( $r=0.627^*$ ) was noticed between colostrum of vaccinated dams and serum of their calves regarding IgA [table 4].

Table 3 demonstrates the pronounced decrease in mortality rates, diarrhea and pneumonia among buffalo calves delivered from vaccinated dams which were 2 (10%), 2 (10%) and 3 (15%), respectively, while were 14 (23%), 15 (25%) and 13 (21.5%), respectively among calves delivered from non vaccinated dams.

## DISCUSSION

The acquisition and absorption of adequate amounts of colostral immunoglobulins are essential to the health of the neonate as it is born virtually devoid of circulating immunoglobulin and relies on antibodies acquired from colostrum for protection against common environmental pathogens. Passive maternal immunoglobulin transfer to newborn calves via colostrum is critically important aspects of neonatal calf immunity and disease prevention. The newborn calf requires a sufficient volume of colostrum with high immunoglobulin concentration to ensure availability of a high immunoglobulin mass necessary to achieve adequate passive immunity (Stott et al., 1979).

The experimental field study which has been done in this work describes the successful application of tetravalent vaccine which included rota, corona and parvoviruses and E.coli K99-pilus antigen. Serum immunoglobulin concentration means of buffalo dams prior to



vaccination were  $1618.4 \pm 39.3$ ,  $312.77 \pm 4.72$ ,  $35.21 \pm 1.59$  (mg/dl) for IgG, IgM and IgA, respectively. These results are in agreement with those reported by Amina et al., (1994) who found the plasma immunoglobulin concentration levels of pregnant cows in farm 1 prior to vaccination with (NOBI Vac K99) 19.0, 2.013 and 0.348 (mg/ml) for IgG, Igm and IgA, respectively.

Serum immunoglobulin levels of vaccinated buffalo dams after calving were  $1876.77 \pm 39.74$ ,  $397.92 \pm 8.53$ ,  $39.6 \pm 1.62$  (mg/dl) for IgG, Igm and IgA, respectively compared to serum levels of non vaccinated dams after calving which were  $1799.12 \pm 18.69$ ,  $267.81 \pm 9.93$ ,  $35.41 \pm 1.19$  (mg/dl) for IgG, IgM and IgA, respectively. A highly significant difference was noticed in serum IgM between vaccinated and non vaccinated dams after calving. Nearly similar results were obtained by Amina et al., (1994) who recorded the increase in plasma immunoglobulin levels of vaccinated cows at parturition in farm 1 as 21.00, 2.48, 0.87 (mg/ml) and in farm 2 as 37.5, 2.77, 0.196 (mg/dl) for IgG, IgM and IgA, respectively. Our results are slightly lower than those obtained by Mohamed (1995) with regard to serum IgG from buffalo dams (24 hours post partum)  $38.1 \pm 2.01$  (mg/ml) and are in accordance with his results concerning serum IgM and IgA ( $3.49 \pm 0.51$  and  $0.38 \pm 0.26$  mg/ml), respectively. High significant differences were observed between serum IgG and IgM levels of pregnant dams before

vaccination and each of serum IgG and IgM of vaccinated ( $t=4.62^{**}$  and  $8.73^{**}$ ) and non vaccinated dams ( $t=4.15^{**}$  and  $4.09^{**}$ ) after calving. The mean values of serum immunoglobulins of buffalo calves born to vaccinated dams \*36-48h post colostrum feeding) were  $2054.74 \pm 45.51$ ,  $357.3 \pm 7.88$ ,  $51.75 \pm 1.41$  (mg/dl) for IgG, IgM and IgA, respectively, while were  $1809.68 \pm 68.28$ ,  $257.91 \pm 10.32$  and  $36.87 \pm 2.02$  (mg/dl) for IgG, IgM and IgA, respectively for buffalo calves born to non vaccinated dams. High significant differences were monitored between both calf groups ( $t = 4.56^{**}$ ,  $7.65^{**}$ , and  $6.02^{**}$ ) for IgG, IgM and IgA, respectively.

Garry et al. (1996) obtained nearly similar results concerning serum IgG concentrations from calves fed natural bovine colostrum (12.4-31.6 mg/ml) at 24 hours after birth. On the contrary, Mohamed (1995) reported serum IgG levels of  $6.16 \pm 0.74$  and  $8.8 \pm 2.5$  mg/ml from healthy and diarrhoeic Holstein calves in France, respectively which were obtained from dams vaccinated by attenuated vaccine against colibacillosis at late stage of pregnancy, while serum IgG of buffalo calves (24 hours post colostrum feeding) was  $33.95 \pm 5.40$  (mg/ml).

The difference between our results and others regarding IgG may be attributed to the fact that the amount of immunoglobulin (Ig) absorbed from the intestine of the depends on the amount



of ingested colostrum, the concentration of Ig in colostrum and the absorption efficiency of the gut (Rajala and Castern, 1995). Concerning serum IgM levels are nearly in accordance with those obtained by Mohamed (1995)  $2.91 \pm 0.65$  mg/ml from buffalo calves 24 hours post colostrum feeding and by Quigley, III et al. (1995) from Jersey calves (3.4-3.5 g/L), while were higher than those obtained from healthy ( $0.75 \pm 0.22$  mg/ml) and diarrhoeic Holstein calves ( $0.36 \pm 0.004$  mg/ml) in France (Mohamed, 1995). Our mean results of serum IgA from calves born to vaccinated and non vaccinated dams are lower than those reported by Logan et al. (1974) in serum of newborn calves  $2.96 \pm 0.29$  mg/ml and by Mohamed (1995) in serum of buffalo calves 24 hours post colostrum feeding  $1.95 \pm 0.40$  (mg/ml), but higher than those of healthy and diarrhoeic. Holstein calves born to dams vaccinated against colibacillosis  $0.18 \pm 0.01$  and  $0.18 \pm 0.009$  (mg/ml), respectively. Young animals that suckle soon after birth take colostrum into their intestinal tracts. Colostral proteins are not degraded and used as a food source but instead reach the small intestine intact. In the ileum they are actively taken up by epithelial cells through cells through pinocytosis and passed through these cells into lacteals and possibly the intestinal capillaries. Eventually the absorbed immunoglobulins reach the systemic circulation and newborn animals thus obtain a massive transfusion of maternal immunoglobulins. The successful absorption of

colostral immunoglobulins immediately supplies them with serum immunoglobulins particularly (IgG) at a level approaching that found in adults. Peak serum Ig levels are normally reached between 12-24 hours after birth (Tizard, 1987). The only fraction of immunoglobulin which is responsible for protection of the calves differ from one to another.

Mohamed (1995) recorded that serum IgG and IgA of buffalo calves played an important role in protection of calves against colibacillosis.

Tizard (1987) stated that secretory IgA present in the intestine of young animals is the most important factor protects them against enteric infection. On the other hand, Otteridge (1985) reported that it was vital for neonatal ruminants to ingest colostral IgM during the first 48 hours of life to receive its complement of maternal antibodies protective against potential pathogens causing enteritis. Tizard (1987) stated that IgG is the predominant immunoglobulin in colostrum and milk of ruminants which may account for 65-90% of its total immunoglobulin content; IgA and other immunoglobulins are usually minor but significant components. This notion agreed with our results concerning either colostrum of non vaccinated or vaccinated dams. The obtained results, were  $8135.10 \pm 92.27$ ,  $1366.59 \pm 66.22$ ,  $703.77 \pm 26.41$  (mg/dl) for IgG, IgM and IgA, respectively in colostrum of vaccinated dams and  $6052.95 \pm 74.38$ ,  $858.97 \pm 29.86$ ,  $366.82 \pm 13.34$  (mg/dl) for IgG, IgM and IgA, respectively in



colostrum of non vaccinated dams. These results are supported by Tizard (1987) who recorded the bovine colostrum immunoglobulin levels as 3400-8000, 300-1300 and 100-700 (mg/100ml) for IgG, IgM and IgA, respectively.

The level of total serum protein (Tsp) concentration in serum of buffalo dams before vaccination was  $6.59 \pm 0.66$  (g%), while was  $7.21 \pm 0.64$  and  $8.56 \pm 0.65$  (g%) in serum of non vaccinated and vaccinated dams after calving, respectively. A significant difference in Tsp was observed between vaccinated dams and dams before vaccination ( $t = 3.08^*$ ). These results are supported by the findings of (Pyne and Maitra, 1979) who recorded Tsp levels of Harina, Sahiwal and Gir heifers in India of 7.45, 7.45 and 7.61 (mg/100ml), respectively. The level of Tsp in calves is a good indicator for immunoglobulins (Igs) levels (McBeath et al., 1971). The mean of Tsp concentration in 36-48 h old buffalo calves born to vaccinated dams was  $8.01 \pm 1.09$  (g%), while was  $7.45 \pm 0.98$  (g%) in serum of 36-48h old buffalo calves born to non vaccinated dams. These results coincide with those reported by Awad et al., (1984) who found the mean Tsp level of 1 day old calves after colostrum feeding as 7.3 (g/100ml) and Salwa El-Sayed (1992) who recorded that the highest means Tsp (7.74 and 7.72g%) were observed among calves born to ultracorn treated and K99 Vaccinated treated dams respectively, followed by that of calves delivered from K99 vaccinated

dams (7.08g%), while the lowest mean Tsp (6.95 g%) was observed among control group. It is of interest to note that there was no significant difference between mean Tsp in calves born to vaccinated dams and those born to non vaccinated dams. Similar findings were obtained by Mettias (1987) and Salwa El-Sayed (1992). Mean total protein (Tp) in colostrum of vaccinated and non vaccinated buffalo dams were  $6.84 \pm 1.36$  and  $6.52 \pm 1.67$  (g%), respectively. These values were lower than those recorded by Mohamed (1991) who recorded a mean colostrum Tp of 13.8 (g%) at zero time before colostrum intake.

This difference may be attributed to changes in total protein (Tp) levels following colostrum feeding. The mean serum albumin levels of buffalo dams prior to vaccination, vaccinated and non vaccinated dams after calving were  $3.31 \pm 0.28$ ,  $2.87 \pm 0.25$  and  $3.28 \pm 0.61$  (g%), respectively. These values were higher than those recorded by Single et al., (1979) 0.94, 0.93, 0.45 and 1.004 (g%) in  $3/8$ ,  $1/2$ ,  $5/8$  and  $3/4$  Brown Swiss X Sahiwal lactating cows in India.

This difference may be referred to the variation of dietary intake and milk secretion between these animals. The mean albumin serum levels of 36-48h old buffalo calves born to vaccinated and non vaccinated dams were  $3.08 \pm 0.39$  and  $2.87 \pm 0.15$  (g%), respectively.



These values were similar to those obtained by Salwa El-Sayed (1992) who reported serum albumin concentration of 3.27 (g%) and 2.95 (g%) in 24 hours old calves delivered from ultracorn treated dams and control dams, respectively.

The means of albumin concentration in colostrum of vaccinated and non vaccinated buffalo dams were  $0.83 \pm 0.18$  and  $1.62 \pm 0.507$  (g%) , respectively. Similar result was obtained by Mohamed (1991) who recorded the mean albumin content of 7 buffalo colostrum whey samples as 0.8 (g%). The total globulin values were  $3.28 \pm 0.58$ ,  $4.64 \pm 0.75$  and  $5.28 \pm 0.78$  (g%) in sera of buffalo dams before vaccination, non vaccinated and vaccinated dams after calving, respectively. In the same concern, Ibrahim (1981) showed that the normal total globulin values in serum of non pregnant cows and buffaloes were 4.13 and 4.06 (g%), respectively. The total globulin values were  $4.57 \pm 0.96$  and  $4.92 \pm 1.22$  (g%) in serum of 36-48h old calves born to non vaccinated and vaccinated dams, respectively. These results are confirmed by those obtained by Salwa El-Sayed (1992) who recorded values of 4.09, 5.67, 6.0 and 3.48 (g%) after colostrum feeding in calves delivered from control dams, K99 vaccinated dams K99 vaccinated ultracorn treated dams and ultracorn treated dams, respectively. The means of colostral globulin levels non vaccinated and vaccinated dams were  $4.89 \pm 1.08$  and  $5.82 \pm 1.53$  (g%), respectively. These results are lower than

those obtained by Mohamed (1991) and Quigley III, et al., (1995) who reported higher values, but this variation may be attributed to the fact that the differences in total globulin levels came parallel to the changes in total protein (Eid, 1986).

Concerning the clinical data, calves from vaccinated buffalo dams had much better general health, lower mortality and a reduction of scours as compared to calves from unvaccinated control. These data were confirmed by earlier reports of many investigators (Lentesch and Brodt, 1983; Youssef et al., 1994 and Gad El-Said et al., 1996) and also by statistical analysis using Chi square test which revealed a significant value of 18.1 at 0.1 level of probability for calves from vaccinated dams and a non significant value of 0.93 at 0.90-0.95 levels of probability for calves from non vaccinated dams . In this study assessing of immune status of vaccinated buffalo dams and their calves is indicated by increasing levels of immunoglobulin classes.

Paying greater attention to the practice of giving the colostrum to the newly born calves immediately after birth and improvement of the standard of housing and hygiene are critical. It could be concluded that it is economically essential to vaccinate pregnant buffalo with (Lactovac. Vet.) because these vaccines containing inactivated rota, corona and parvo



viruses, in addition to enterotoxigenic E.coli (ETEC) with pilus antigen K99 given to pregnant buffalo provides a good protection and against diarrhea in calves caused by these enteropathogens and achieved less enteric, respiratory and death among their neonates.

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## REFERENCES

- Abou El-Hassan, D.G., Salem, S.A.H. and Fayed, A.A. (1995): Neonatal calf diarrhea in Egypt. 3rd Sci. Cong., Egyptian Society for cattle diseases, Assuit, Egypt.
- Acres, S.D., Isaacson, R.E. and Babiuk, L.A. (1979): Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins . *Infec. Immun.* 25:121-126.
- Amina, A.M. Nawar, A.F., Farid, A.F. and Abdel- Gawad, A.F. (1994): Quantitative studies of immunoglobulins in vaccinated cows with a K99 vaccine. *Vet. Med. J. Giza*, 42: 11-15.
- Awad, M.M., Gergis, S.M., Al-Mola, A., Ahmed S.A. and Aly, S.M. (1984): Serum immunoglobulin variations associated with the transfer of passive immunity and the onset of active immunity in Egyptian buffalo calves. *Agric. Res. Rev.*, 62:277-284.
- Bachmann, P.A., Baljer, G., Gmelch, X., Eichhorn, W., Plank, P. and Mayer, A. (1984): Vaccination of cows with K99 and rotavirus antigen: Potency of K99 antigen combined with different adjuvants in stimulating milk antibody secretion. *Zbl. Vet.Med. B.*, 31:660-668.
- Baker, J.K and Greer, W.J. (1981): *Animal Health A Layman's guide to disease control. The interstate printers & publishers, Inc. 2<sup>nd</sup> printing.*
- Cornell, J.A.G., Pardawill, C.S. and David, M.M. (1949): Spectrophotometric determination of total protein by buiret method . *J. Biol. Chemistry*, 177:751.
- Danies, O.L. (1947): *Statistical Methods in Research and production with special reference to the chemical industry. Imperial Chemical Industries Limited. London.*
- Doumas, P.T. (1971): Spectrophotometric determination of albumin by bromocresol green method. *Clin. Chemist. Acta.* 31: 87-96.
- Eif, G. El-Said (1986): Immune status of newly born buffalo calves M.V.Sc. Thesis, Cairo University.
- Erhard, M.H., Von Quistrop, I., Schrunner, I., Juengling, A., Kasers, B., Schmidt, P. and Kuehmann, R. (1992). Development of specific enzyme linked immunosorbent antibody assay for the detection of chicken immunoglobulins G<sub>1</sub>M<sub>1</sub>A using monoclonal antibodies. *J. Poult. Sci.*, 71:302-310.
- Gaastra, W. and DE Graaf, F.D. (1982): Host specific fimbrial adhesion of non invasive enterotoxigenic



- Escherichia coli* strains. *Microbiol. Rev.*, 46: 129-161.
- Gad El-Said, W.A. Salem, S.E., El-Garhy, M.M. Mettias, K.N., El-Rashidy, A.A., and Tawfik, M.S. (1996): Immunopotiated K99 vaccine for buffalo dams to protect neonates against *E.coli* enterotoxigenesis. *Vet.Med. J. Giza*. 44: 749-759.
- Carty, F.B. Adams, R., Cattle, M.B. and Dinsmroe, R.P. (1996): Comparison of passive immunoglobulin transfer to dairy calves fed colostrum or commercially available colostrum-supplement products. *J.A.V.M.A.*, 208: 107-110.
- Gay, C.C. (1983): Failure of Passive transfer of colostrum immunoglobulins and neonatal disease in calves: A review. Page 346 in *Proc. 4th Int. Symp. Neonatal Dis. Vet. Infect. Dis. Org., Sakatoon, SK, Canada.*
- Ibrahim, I.A. (1981): Some biochemical studies on blood of cattle during pregnancy, M.V.Sc. Thesis, Zagazig University.
- Lentsch, R.H. and Brodt, D.E. (1983): Vaccination against enterotoxigenic *E.coli* infection: Protection of calves by inoculation of dams. *Modern, Vet. Practice*, 64: 729-731.
- Logan, E.F. (1974): Colostral immunity to colibacillosis in neonatal calf. *Br. Vet. J.* 30: 405-412.
- McBeath, D.G., Penhal, W.J. and Logan, E.F. (1971) : An examination of the influence of husbandary on the plasma immunoglobulin level refractometer test for assessing immunoglobulin content. *Vet. Rec.*, 88:266-270.
- Mettias, K.N. (1987): Studies in enteritis on buffalo calves and its control by application on some vaccines. Ph. D. Vet. Thesis, Cairo University.
- Mohamed, A.N. (1991): Evaluation and development of the immune status in buffalo calves in Egypt. M. Some V.Sc. Thesis, Cairo University.
- Mohamed, G.R. (1995): Some biochemical studies on blood of calves suffering from colibacillosis during suckling period Ph.D. Vet. Thesis. Suez Canal University.
- Outteridge, P.M. (1985): *Vet. Immunology*. Academic Press, INC. harcourt Brace Jovanovich, Publisher.
- Pyne, A.K. and Maitra, D.N. (1979): The biochemical constituents of blood of Hariana, Sahiwal and Gir heifers. *Indian. Animal. Sci.*, 49: 397-399.
- Radostitis, O.M.; Blood, D.C.; and Gay, C.C. (1994): "Veterinary Medicine: A Textbook of the disease of cattle sheep, pigs goats and horses". 3th Edition. Baillier Tindally, England
- Rajala, P. and Castren, H. (1995): Serum immunoglobulin concentrations and health of dairy calves in two management system from birth to 12 weeks of age. *J. Dairy Sci.* 78: 2737-2744.
- Salwa El-Sayed (1992): Comparison of passive immunity of buffalo calves following vaccination with commercial immunopotiated K99 vaccine. M.V.Sc., Cairo University.
- Singla, S.K., Ludri, R.S., Balakrishman S. and Nair, K.G.S. (1979): Major serum proteins fractions in different graded of Brown swiss x Sahiwal cows. *Indian. J. Anim. Sci.*, 49: 401-402.
- Snedecor, G.W.(1960): *Statistical methods* . 5th ed. Ames. Iowa State Collected press.
- Solomon, J.B. (1971): Foetal and neonatal immunology. IN *Frontiers of biology series*, Vol, 20 , North Holland Amstradam.

Stott, G.H., Marx, D.B., Meneffe, B.E. et al. (1979): Clostral immunoglobulin transfer in calves 1. period of absorption J.Dairy Sci., 62: 1632-1638.

Tizard, Ian (1987): An introduction to Veterinary immunology, W.B. Saunders Company.

Youssef, N.M.A., El-Sanousi, A. Fathia, M.M. Shalaby, M.A., Mousa, A.A., Salem, S.A.H. Saber, M.S and Saleh, S.M. (1994): Estimation of rotavirus antibodies in vaccinated cattle and their neonates Vet. Med. J. Giza, 42: 59-65.