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ESTIMATION OF ROTAVIRUS ANTIBODIES IN VACCINATED CATTLE AND THEIR NEONATES

BY

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SUMMARY

Antibody response of pregnant cattle and their neonates to bovine rotavirus was estimated by ELISA. The dams have received two doses of commercial trivalent vaccine (Scour Guard 3"K", killed bovine Rota-Corona viruses and E.coli Bacterian) 8-10 weeks before delivery. Sera of immunized dams showed high antibody levels with titer ranges from 1,00 to 9,000 and from 2,000 to 27,000 at 14 days post vaccination and 14 days post boosting respectively.

Calves delivered from vaccinated dams gave a high antibody titer of 8515 if compared to a titer of 1042 given by calves delivered from non-vaccinated dams. Colostrum of vaccinated dams exhibited a good ELISA reactivities with a titer of 13254, while that of non-vaccinated dams gave a titer of 2491.

INTRODUCTION

Rotavirus has been incriminated as a cause of neonatal diarrhea in calves throughout the world. As the hazard of calf loss by rotavirus infection is usually awaited during the first two weeks of life (1,3). A correct protection program should be directed to active immunization of the pregnant dams during the last stage of pregnancy to increase the levels of the immunoglobulin against rotavirus in their colostrum and milk (8).

The intestinal absorption capacity of immunoglobulin molecules in calves is greatest at the first few hours after birth, therefore, calves should receive the colostrum with its high levles of immunoglobulins just after parturition to ensure a potent passive immunization (11).

The aim of this study is to investigate the efficacy of commercially available killed adjuvated trivatent vaccine in inducing the immune response of vaccinafed pregnant dams. The antibody levels in the sera of vaccinated and non-vaccinated dams and their neonates as well as in their colostrum against bovine rotavirus was estimated using indirect ELISA. This trivalent vaccine (Scour Guard 3"K") included killed bovine rota-, corona viruses and E.coli bacterin, was given to the pregnant cattle at 8-10 weeks before delivery.

MATERIAL AND METHODS

-ANIMALS:

a. Pregnant cows: A total number of 130 pregnant freisian cows from Dalla dairy farm at Nobaria district were used in this study. Two months before deliver, 98 out of 130 animals were immunized with the initial dose of commercial trivalent killed adjuvated vaccine (see down). One month just before delivery, these animals received a second booster dose of the same vaccine. The other 32 pregnant cows were left non-vaccinated and served as controls.

b. Calves: 130 calves were delivered from the

vaccinated and non-vaccinated dams and put under investigation to test their sera for the presence of specific antibodies to bovine rotavirus.

VACCOME:

A liquid commercial trivalent killed adjuvated vacine (Scour Guard 3"K", Smith Kline Beechman, USA) was used to vaccinate our pregnant cattle. This vaccine represented a liquid prepartation of inactivated bovine rota- and corona viruses propagated on established cell line and K99 E. coli bacterin). The vaccine was given intramuscular (I/M) in a dose of 2 ml/animal.

- VIRUS STRAIN:

Bovine rotavirus (Nebraska calf diarrhea virus strain) was used as antige for coating ELISA microplates. The virus has been primarily propagated in MA104 cell line using trypsin treatment to enhance virus infectivity (11). Propagated virus was harvested by subjecting the infected cells to 2 cycles of freezing and thawing, followed by clarification through spinning down at 3,000 rpm for 30 min. at 4°C. Original virus titer was estimated to be 10^7 $TCID_{50}$/ml Virus supermafant was then concentrated 10X using polyethylene glycol 6,000. This virus concentrate has been used as antigne for coating ELISA plates at a final dilution 1:50.

-SAMPLE COLLECTION AND PREPARATION:

a. Serum samples: Blood samples have been collected from all vaccinated and non-vaccinated dams for sera separation at the following fime intervals: just before vaccination, 2 weeks post vaccination and 2 weeks post boosting. Likewise, serum samples were collected from delivered calves at the following times: just precolostrum feeding, 24 hrs, 1 week, 2 weeks and 3 weeks post colstrum feeding.

b. Colostrum samples: Colostrum was collected from vaccinated and non-vaccinated dams just after parturition. These samples have been used for ELISA after being skimmed by removal of fats through centrifugation at 3,000 rpm for 45 min. at 4°C. Using a long needle, the clear supernatant under the fatty layer was collected and preserved at -20°C.

ELISA PROCEDURE:

96 wells flat bottomed ELISA microplates (Nunc, Denmark) were coated with the rotavirus concentrate at a final dilution of 1:50 in carbonate-bicarbonate buffer, pH 9.6. Adsorption of antigen was carried out at room temperature (RT) for 20 hours. ELISA procedures were done according to (13) with the following modification:

Coated ELISA microplates wer bolcked for 2 hrs at RT with PBS, pH 7.4 containing 10% skimmed milk and 0.2% tween 20 over a platform shaker. Tested serum and colostrum samples as well as positive and negative control sera were diluted 1:10 and dispensed in duplicate into the blocked ELISA microplates and incubated at RT for 1 hr over the platform skaker. Dilution buffer consisted of PBS,pH 7.4 containing 5% skimmed milk and 0.2% tween 20 (PBS-MT). After rmoval of the reacting sera microplates were washed 3 times (5min. each) with PBS, pH 7.4 containing 0.2% tween 20 (PBS-T). Secondary anti-bovine peroxidase conjugate was then added to all individual wells of the microplate after being diluted 1:2,000 in PBS-MT and left for reaction at RT over the shaker for 1 hr. The plates were finally wasked with PBS-T as above and enzyme activities in the individual wells were measured by adding 100 ul/well of the peroxidase substrate solution containing 5-aminosalicylic acid (5-ASA) and H_2O_2. Developed colors have been read in the SLT SPECTRA (USA) ELISA reader at an OD 492. Absorbances obtained from the individual tested samples were used to estimate the titers using the statistical calculation described by (10).

RESULTS

Effect of naturally existing rotavirus antibodies on the antibody response of vaccinated and boostered pregnant cattle:

The different ELISA readings showed two separate groups of reacting animals with respect to the natural existence of certain levels of anti-rotavirus antibodies (table 1). Those animals with pre vaccination titer > 1000 (38 animals) and those with titer < 1000 (60 animals). 14 days post vaccination 76% of vaccinated animals in the first group gave average antibody titers from 1721 to 4574 and 24% with average titers from 5181 to 8403. In the second group, 91% of vaccinated animals gave average titers from 1359 to 4046 and 9% gave average titers from 5511 to 8229.

14 days post boosting, table 2 shows clearly that 76% of vaccinated animals in the first group exhibited a higher antibody response with average

Table (1): Levels and distribution of antibodies to Bovine Rotavirus in pregnant cattle vaccinated by trivalent vaccine (Killed Rotavirus and Coronavirus + E. coli K99 bacterin) as measured by Indirect ELISA.

AB level in prevacc. cattle	14 days post vaccination			14 days post boostering	
	Titer range	No. of cattle	Average titer	Titer range	Average
* Titer > 1000	1000-2000	6	1721	5000-15000	9273
	2000-3000	5	2382	3000-23000	12219
	3000-4000	13	3435	6000-21000	13892
	4000-5000	5	4574	8000-23000	14423
	5000-6000	3	5181	11000-22000	16435
	6000-7000	0	0	0	0
	7000-8000	0	0	0	0
	8000-9000	6	8403	20000-26000	23372
@ Titer < 1000	1000-2000	25	1359	2000-15000	5739
	2000-3000	17	2385	3000-20000	8074
	3000-4000	11	3432	9000-27000	13261
	4000-5000	2	4046	6000-13000	9663
	5000-6000	1	5511	11000-12000	11473
	6000-7000	1	6121	10000-11000	10213
	7000-8000	0	0	0	0
	8000-9000	3	8229	11000-16000	11042

* Total Number of animals 38
@ Total Number of animals 60

titers from 3,000 to 23,000 if compared with the lower antibody response given by vaccianted animals in the second group, where 91% of which gave average iters from 2,000 to 27,000 and only 9% gave average titers from 10,000 to 16,000.

-Levels and distribution of atibodies to bovine rotavirus in colostrum and sra of calves delivered from vaccinated and non-vaccinated dams:

Table (2): The effect of naturally existing anitbodies on the immune response of pregnant cattle to bovine rotavirus after vaccination with trivalent (Scour Guard 3"K") vacine* as measured by indirect ELISA.

Ab Level in Pre-Vacinated Cattle	14 Days Post Vaccination		14 Days Post Boostering		% of Reacting Animals
	No.	Titer Range	No.	Titer Range	
Titer > 1,000 (38 Animals)	29	1,000-5,000	29	3,000-23,000	76
	9	5,000-9,000	9	11,000-26,000	24
Titer < 1000 (60 Animals)	55	1,000-5,000	55	2,000-27,000	91.5
	5	5,000-9,000	5	10,000-16,000	8.5

Ab: Antibody

Primary Antibody response to Bovine Rota virus in sera of cattle vaccinated with Trivalent vaccine (killed Rota & Corona viruses +E.coli K99 Bacterin in relation to the prevaccination sera titer as meausred by the indirect ELISA

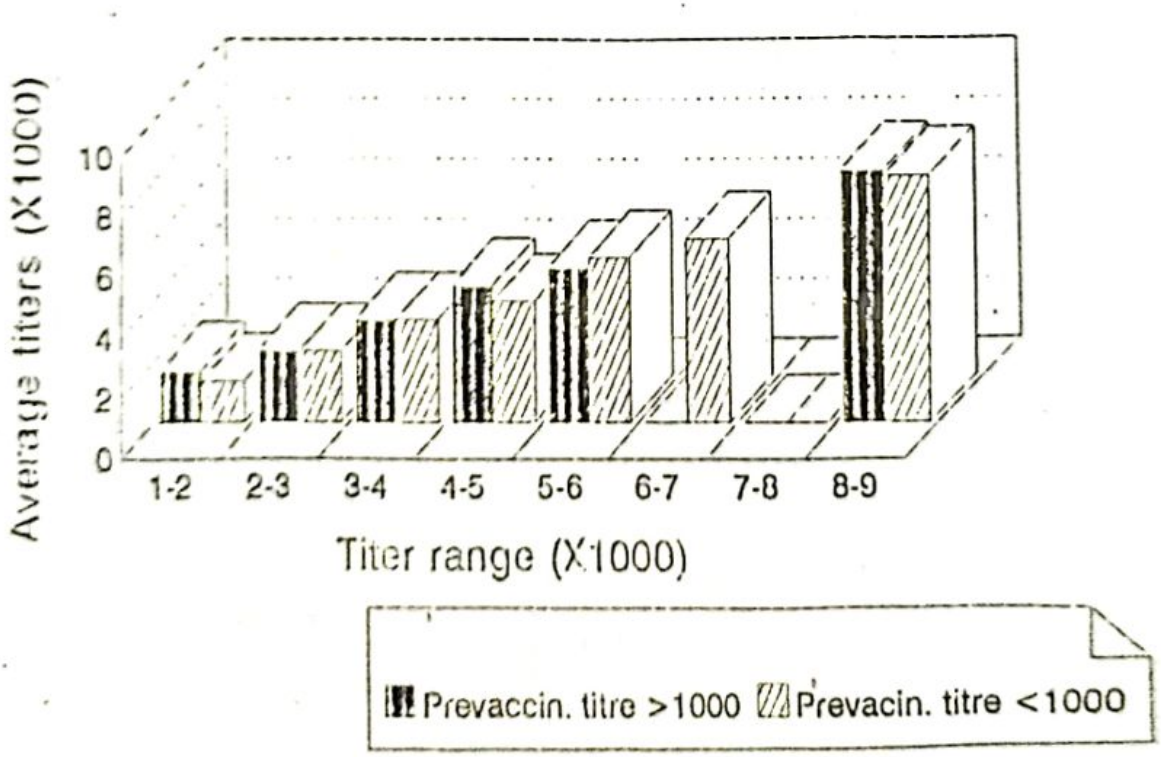

Table (3): Levels and distribution of antibodies to Bovine Rota Virus in sera, colostrum of vaccinated, non vaccinated dams and their calves as measured by Indirect ELISA.

Time of sampling	Average ELISA titers in vaccinated dams	Average ELISA titers in non vaccinated dams
Dams 14 days PB (serum)	9303	1143
At parturition (colostrum)	13254	2491
Calves sera 0 day	-ve	-ve
Calves sera 24 hrs (PCF)	8515	1042
Calves sera 1 week (PCF)	7643	970
Calves sera 2 weeks (PCF)	5303	596
Calves sera 3 weeks (PCF)	4532	260

PB = Post Booster
PCF = Post Colostrum Feeding

Comparative distribution of antibodies to Bovine Rota virus in sera and colostrum of cattles & their calves

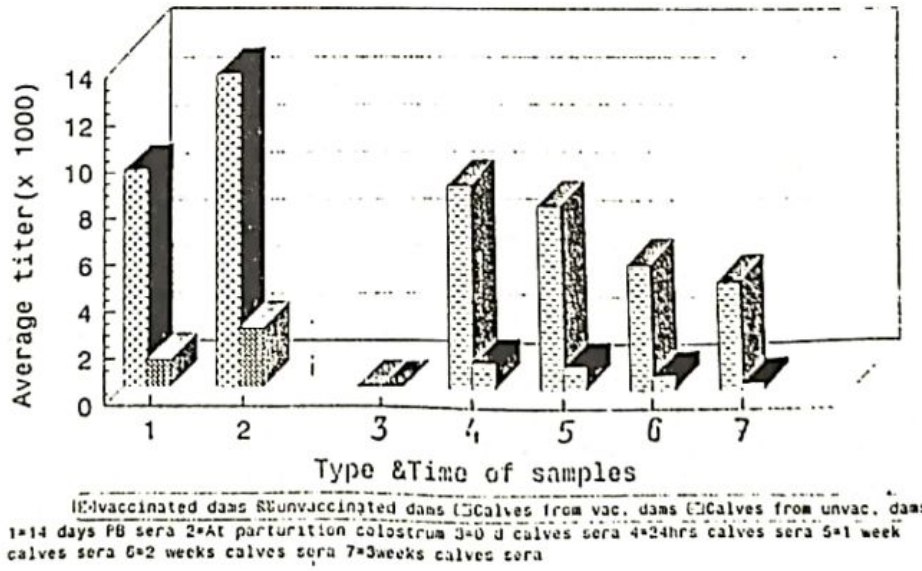

Table 3 document the significant rise in antibody titer (13254) estimated in the colostrum of vaccinated dams obstained directly just after parturition if compared with that given by colostrum obtained from non-vaccinated dams (2491).

Sera of calves which suckled from their vaccinated mothers showed a sharp increase in antibody titer from zero to 8515 24 hrs post colostrum feeding. This high level corresponds to the low titer (1042) given by sra of calves delivered from non-vaccinated dams. Table 3 also shows the gradual decrease of this antibody level to reach a titer of 7643, 5303 and 4532 at 1,2 and 3 weeks post colostrum feeding.

DISCUSSION

Rotavirus infection in bovine neonates constitutes in the last few years together with coronavirus and E. coli infection a serious economic problem threatening animal production in Egypt, The prvalence and incidence of rotavirus infection among calves in critical specially in the first two weeks after birth (1,3). Therefore passive immunization of these animals has been found possible through suckling or giving the colostrum of vaccinated dams to these calves just within the first 24 hrs after delivery.

The experimental field study which has been done in this work describes the successful application of trivalent killed adjuvated vaccine which included rota-, corona viruses and E. coli K99 bacterin. The vaccination approach considered the importance of vaccinating pregnant cattle by two doses: the first one was 8-10 weeks and the second booster dose was 2-4 weeks before parturition to ensure the availability of high protecting antibody titers against rotavirus in the sera and colostrum of these animals.

We have tried in this experiment to estimate the level and distribution of rotavirus specific antibodies in serum samples collected from vaccinated and non-vaccinated cattle and their neonates, as well as in colostrum collected from these animals just after deliver. Most of the animals used in this experiment showed a preexisting level of rotavirus specific antibodies as clearly shown in table 1. This table categurized our animals with respect to their natural antibodies into two groups: those animals with antibody titers > 1000 and those with titers < 1000. The immune response of these two groups showed a pronounced differences i the titer ranges of these vaccinated animals specially in samples tested 14 days post boosting. This effect could ot be observed among vaccinated animals 14 days post vaccination as clearly illustrated in fig. 1. In addition, table 2 translated the significance of these natural antibodies in the immune response after boosting which has been expressed in lower antibody titers (in group 1) if compared with the higher response given by group 2.

The high antibody titers given by vaccinated animals (average titer 9303) might be attributed to the enhancing effet of the adjuvant, thus increasing the antigenic input of the given trivalent vaccine. This enhancing adjuvant effect lies in full agreement iwht the findings reported by (2), who emphasized the importance of adjuvant in increasing the neutralizing antibody titer against rotavirus in the sera and colostrum of vacinated heifers and cows.

The importance of colostrum in providing newborn calves with the protecting neutralizing antibodies has been emphasized by sveral investigators (5), (4), (9) and (6). For this reason and based on the necessity of lactogenic immunity within the gut of calves, we have tried in this communication to estimate the level of rotavirus antibodies in colostrum of vaccinated and non-vaccinated dams and the sera of their neonates. At parturition, the average antibody titer was 13254 in colostrum of vaccinated animals, while it was 2491 in colostrum of non-vaccinated group.

The sharp elevation of specific rotavirus antibodies in clves sera 24 hrs after suckling the colostrum (Fig. 2) indicates the fast absorption capability of the gut lumen of these calves, a signal which might lead to a good protection of these calves against natural infection with rotavirus. A suggestion which stand in full support with the findings reported by (5), (7) and (6) . This capability of colostrum uptake by calves

delivered by vaccinated dams in a good explanation to the much lower antibody titers given by sera of calves delivered by non-vaccinated animals (Table 3). The protective threshold of these titers in both vaccinated and non-vaccinated cattle and their calves should be elucidated by testing the colostrum and sera of these animals with serum neutralization test (SNT).

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