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QUANTITATIVE STUDIES OF IMMUNOGLOBULINS IN VACCINATED COWS WITH A K99 VACCINE

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SUMMARY

Pregnant cows were injected I / M two times at 6 - 8 weeks before the expected time of parturition and again 3 to 4 weeks later, with a formalized killed whole cell bacterin containing enterotoxigenic E. coli K99. Immunoglobulin concentration levels were measured in plasma of vaccinated cows prevaccination, post first dose of vaccine and at time of parturition, using single radial immunodiffusion test and monospecific antibodies against each class and subclass. The means of immunoglobulin levels were increased to 38.875, 3.190, 0.594, 14.2, 15.308 mg / ml compared to 22.35, 2.394, 0.258, 10.750, 8.773 for plasma IgG, IgM, IgA, IgG1, IgG2 of non vaccinated control cows respectively.

INTRODUCTION

An animal's capacity to resist infection involves the interaction of cellular and humeral factors of which specific antibodies are subdivided in cattle into IgG1, IgG2, IgM, IgA (Butler and Maxwell 1972). There is also evidence for the existence of a forth class of immunoglobulin (IgE) present in very low concentrations in plasma (Hammer et al. 1971; Wells and Eyre, 1972). Mansa, 1965; Kulkarni, 1971; Nansen, 1972 reported that IgG2 in the Danish red breed in Denmark was selectively deficiency. Calves which develop colisepticemia have little or no circulating (colostral) immunoglobulins. Their particular problem is a lack of IgM (Selman 1981). Enterotoxigenic Echerichia coli (ETEC) is the major cause of diarrhea and death in neonatal calves (Moon et al. 1976; Myers, 1975). Previous studies have indicated that the organisms have at least 2 known virulence factors (Moon, 1978; Saeed et al. 1983), colonization factors that mediate adhesion to the epithelium, thus allowing rapid proliferation of ETEC in the small intestine and enterotoxins, either heat stable or heat labile that cause diarrhea and dehydration. The K99 pilus antigen is one of the major adherence factors found on ETCE isolated from neonatal calves (Guinee et al. 1976; Gaastra and Graaf, 1982).

Immunization of pregnant cows with purified pilus antigen conferred pilus specific protection to their neonates against experimental challenge exposure (Acres et al. 1979 & Nagy 1980). Myers 1975, reported that a crude K99 extract effectively produce antibody response in cows. The objctives of this study are first to determine the levels of protective immunity induced by formalin - killed bacterin containing K99 by estimation of immunoglobulin classes and subclasses concentration levels in plasma of pregnant cows, second to evaluate the immunoglobulin profile in the sera of vaccinated and nonvaccinated cows at parturition time.

MATERIAL AND METHODS

Forty pregnant cows belonged to two farms were divided to four groups, each two groups from every farm were vaccinated I / M on back of the neck with NOB1 VAC K99 (Inter Vet. Holland), formalized antigen formalized killed vaccine contain K99. A dose of 2 ml was given at 6 - 8 weeks before the expected time of parturition and 2 ml again at 3 to 4 weeks later for every cow. Heparinized blood samples were taken from Jugular vein before vaccination, before inoculation of second dose, and at time of parturition. The plasma collected by centrifugation at 2000 rpm / 10 minutes. All samples were kept frozen at - 20 until tested.

Serum immunoglobulin concentration levels were measured by single radial immunodiffusion test (SRD).

SRD test was adopted as method of Mancini et al. 1965 using monospecific antibodies against IgG, IgG1, IgG2, IgM, IgA. Precipitin ring diameters were measured after one day for IgG or two days for IgM using RID reader. Calibration curves drawn for the standers corresponded closely to those described by Mach and Pahud 1971.

RESULTS

IgG1 and IgG2 gave a linear relationship between ring size and log of the antigen concentration over a wide range wherease IgM calibration curve was not linear for small ring diameters (below 4 mm). Furthermore, three concentric rings of precipitation form with some undiluted plasma samples but two of these rings are weak and disappear after dilution of tested samples to 1 / 5.

Immunoglobulin concentration levels in farm 1 was shown in Table (1). Which shows that IgG levels Immunoglobulin concentration in plasma of non vaccinated cows in both farm1 and farm2. As shown in this table concentration of Immunoglobulin IgM concentration levels were 3.016 and 2.013 in plasma of vaccinated and nonvaccinated cows of farm 1 as shown in Table (1) while they were 3.363 and 2.775 mg / ml in vaccinated and nonvaccinated cows in farm 2 as shown in table (2). IgA concentration in farm 1 measured 0.348 and 0.970 mg / ml in plasma of nonvaccinated and vaccinated cows of farm 1 respectively. It was 0.218 and 0.167 mg / ml in plasma of vaccinated and nonvaccinated cows in farm 2 respectively. Concentration of subclass IgG1 in plasma of vaccinated cows in farm 1 was 14.45 mg / ml and

Table (1): Plasma immunoglobulin levels (mg / ml) in pregnant cows vaccinated with formalized killed K99 in farm 1.

Immunoglobulin class. Subclasses	Time of plasma collection		
	Post one dose	Post two doses at Parturition	Prevaccination
IgG			
Group 1	32.400	18.0	14.2
Group 2	39.0	24.0	23.80
Mean	35.7	21.0	19.0
IgM			
Group 1	3.257	2.425	1.8
Group 2	2.757	2.55	2.225
Mean	3.016	2.488	2.013
IgA			
Group 1	0.98	0.8	0.58
Group 2	0.96	0.94	0.116
Mean	0.97	0.87	0.348
IgG1			
Group 1	15.0	13.5	13.3
Group 2	13.9	12.6	13.3
Mean	14.45	13.05	13.3
IgG2			
Group 1	10.0	8.12	6.84
Group 2	19.120	10.0	8.12
Mean	9.56	9.06	7.48

Table (2): Plasma immunoglobulin levels (mg / ml) in pregnant cows vaccinated with K99 formalized killed vaccine in farm 2.

Immunoglobulin class. subclasses	Time of plasma collection		
	Post one dose	Post two doses at Parturition	Prevaccination
IgG			
Group 1	41.5	32.4	19.8
Group 2	42.6	42.6	31.6
Mean	42.05	37.5	25.7
IgM			
Group 1	3.875	3.0	2.85
Group 2	2.85	2.55	2.55
Mean	3.363	2.775	2.775
IgA			
Group 1	0.26	0.226	0.186
Group 2	0.176	0.166	0.148
Mean	0.218	0.196	0.167
IgG1			
Group 1	12.2	13.2	7.80
Group 2	15.8	14.2	8.24
Mean	13.95	13.70	8.20
IgG2			
Group 1	9.01	9.01	8.53
Group 2	11.6	10.8	11.60
Mean	10.305	9.905	10.06

13.300 mg / ml in plasma of nonvaccinated cow. Immunoglobulin concentration levels for plasma in farm 1 measured at time of parturition were shown in table 1. It measured 21 mg/ml for IgG, 2.488 for IgM, IgA 0.870, IgG1 13.05 and 9.06 mg/ml. for IgG2. Immunoglobulin concentration levels in plasma of vaccinated cows received two doses of vaccine and measured at time of parturition in farm 2 were 37.50, 2.775, 0.196, 13.70 and 9.905 mg / ml for IgG, IgM. IgA, IgG1, IgG2 respectively.

Table (3) summarized the effect of formalized K99 vaccine in plasma of cows received one dose and those received two doses but measured at time of parturition, compared toimmunoglobulin concentration in plasma of non vaccinated cows in both farm1 and farm2. As shown in this table concentration of immunoglobulin IgG increased 16.525 while this amount was declined in plasma collected at time of parturition. IgM immunoglobulin concentration increased to level equal to 0.796 mg / ml in cows inoculated with one dose of formalized k99 Vaccine in both farms while this amount gave decrease amount equal to 0.558 mg / ml for IgM concentration level in cows vaccinated with two doses of the same vaccine and plasma was collected at time of parturition. The concentration of IgA gave an increasing level equal to 0.336 mg / ml in plasma of vaccinated cows in both farms received one dose of K99 vaccine while this amount declined to 0.051 mg / ml when measured at parturition in cows received two doses of the same vaccine.

Table (3 :Plasma immunoglobulin levels (mg / ml) in pregnant cows vaccinated with K99 formalized killed vaccine in farm 2.

Immunoglobulin class. Ssubclasses	Time of plasma collection		
	Post one dose	Post two doses at Parturition	Prevaccination
IgG			
Group 1	35.7	21.0	19.0
Group 2	42.05	37.5	25.70
Mean	38.875	29.25	22.35
IgM			
Group 1	3.016	2.488	2.013
Group 2	3.363	2.775	0.775
Mean	3.190	2.632	2.394
IgA			
Group 1	0.970	0.870	0.348
Group 2	0.218	0.196	0.187
Mean	0.594	0.533	0.258
IgG1			
Group 1	14.45	13.05	13.3
Group 2	13.95	13.70	0.20
Mean	14.2	13.375	10.75
IgG2			
Group 1	9.56	9.06	7.48
Group 2	10.305	9.905	10.065
Mean	15.308	9.482	8.773

IgG1 recorded a rise equal to 3.450 mg / ml in cows received one dose of K99 vaccine in both farms, this amount gave 0.825 mg / ml less in plasma of cows received two doses of the same vaccine and measured at parturition time. IgG2 recorded an increasing amount equal to 6.535 mg / ml in plasma of vaccinated cows received one dose of K99 vaccine in both farms, this amount equal to 5.826 mg/ml in plasma of vaccinated cows recieved two doses of the same vaccine and measured at parturition time. showed decrease equal to 5.826 mg / ml in plasma of vaccinated cows received two doses of the same vaccine and measured at parturition time.

DISCUSSION

Immunization of pregnant cows with killed K99 can be used to increase the level of both plasma and lacteal immunoglobulins. Immunoglobulin concentration means were increased 16.7 gm / ml; 1.003, 0.622, 1.14, 1.58 for IgG, IgM, IgA, IgG1, IgG2 in farm 1 respectively. Immunoglobulin concentration level means in plasma of vaccinated cows in farm 2 recorded increasing values equal to 16.35, 0.588, 0.051, 5.75, 0.24 mg / ml for IgG, IgM, IgA, IgG1, IgG2 respectively. Variation in immunoglobulin concentration levels in plasma of cows in farm 1 and farm 2 might be due to the difference immune status of individual cow as a result of antigenic stimulation of other agent in their environments as reported by (Panhale and Christie 1969 & Curtain 1971), Table (3) indicated the means of immunoglobulin concentration levels in both farm 1 and farm 2. IgG concentration was increased to 16.525 mg / ml in vaccinated cows in

both farms compared to the concentration in plasma controls in both farms. Elevation of IgG could be due to stimulation of K99 antigen. IgG class has the ability to cross placenta and provides a major line of defence against collibacillosis for the first weeks of neonatal life which may be further reinforced by the transfer colostral IgG across the gut mucosa in neonates. IgG diffuses more rapidly than the other immunoglobulins into the extravascular body space and neutralize K99 entertoxines besides binding to the microorganisms to enhance their phagocytosis. IgM concentration levels were increased 0.796 mg / ml in plasma of cows in both farms after injection of K99 vaccinal antigen. IgM antabodies are extremely efficient agglutinating and cytolytic agents for colibacillosis and activate complement.

IgA immunoglobulin concentration levels were increased in plasma of vaccinated cows in both farms (0.336 mg / ml) compared to those measured in nonvaccinated control cows. IgA antibodies have activity against enterotoxins and K99 bacteria (iso - agglutinins). Immunoglobulin IgG1 subciass concentration levels were increased in plasma of vaccinated cows of farm 1 and farm 2 (3.450 mg / ml). It is the major secretory immunoglobulin in the mammary gland as a result of selective transferred to blood stream. It is able to bind complement to produce lysis of E. coli.

In this study evaluation of immune status of pregnant cows vaccinated with formalized killed K99 antigens indicated by increasing levels of immunoglobulin classes and subclasses in their plasma. **Acres et al. (1979)** used challenge test to determine the protection rate in calves from vaccinated dams with K99. **Nagy (1980)** evaluated his vaccin against crude K99 extract in pregnant cows using challenge test in their neonates and determined colostral anti K99 titers.

Immunoglobulin concentration of classes and subclasses were decreased in plasma of caccinated cows measured at parturition time in both farms 1, 2 indicated transfer of immunoglobulin into the mammary gland.

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