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PASSIVE IMMUNIZATION OF LAMBS THROUGH COLOSTRUM OF EWES VACCINATED BY DIFFERENT E. COLI K99 VACCINES

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SUMMARY

A total of 80 Barki lambs were fed on colostrum of 3 groups vaccinated dams by E.coli K99 vaccines & one group was left non vaccinated as control. A total of 80 colostrum samples were collected at parturition time and blood samples were collected at different intervals, from the newborn lambs (born to vaccinated and non - vaccinated ewes). The indirect method of ELISA technique and agglutination test were preformed for the detection of K99 antibodies in sera and colostrum samples.

Vaccination of pregnant ewes., during the late period of pregnancy, resulted in an increase of the K99 antibody levels in the colostrum samples when compared with that of non - vaccinated dams. The mean antibody reading of lamb serum samples showed a gradual increase during the first week of life. It reached the maximum level in samples of the 3rd to the 7th day of age. There was a decrease in the antibody titers later on, but a detectable level of antibody was still recorded up to 12th week after parturition.

Lambs nursing ewes vaccinated with aluminum hydroxide formalin killed vaccine showed a significant abrupt rise in their K99 antibody levels, followed by those vaccinated with scourguard vaccine and then lambs nursing ewes vaccinated with irradiated vaccine.

Kay words : lambs, ewes, colostrum, E.coli K99, immunity, vaccination protective rate .

INTRODUCTION

Sheep and goats are considered to be very important animals among livestock especially in arid, semiarid and new reclaimed lands in Egypt . Diarrhoea is of common occurrence in young animals, and it is considered to be one of the main causes of great losses in sheep and goats production (Reid, 1976). Colibacillosis is the most important cause of morbidity and mortality in young lambs (Ansari et al. 1978., Blanco et al., 1991 Munoz et al., 1996 Ibrahim et al., 1997). Earlier Studies found that most enterotoxigenic E. coli strains isolated from pre-weaning lambs had K99 pili, antigenic structures which would facilitate colonization of the gut lining (Moon et al., 1978).

The enterotoxins produed by these strains caused secretion of body fluids and electrolytes in the gut resulted in severe dirrhoea whieh leads to dehydration (Wary and Thomlinson, 1972)

Passive maternal immunity through vaccination of the pregnant ewes was considered as an important means for lamb protection against E.coli K99 infection (Moon, 1979). For effective passive immunization, new born lambs should immediately consume adequate amounts of colostrum containing high levels of antilbodies (Penhale et al., 1973).

The present wark was carried out to evaluate the immune response of pregnant ewes to a local prepared and imported E.coli K99 vaccrines with regard to the level of E.coli K99 antibody titers in celostral of the vaccinated ewes, and in sera of newly born lambs fed such colostrum. Determinate the maternal immunity in challenged lambs nursing from vaccinated ewes and challenging them with virulent E.coli K9 strain.

MATEIAL AND METHODS

1- Animals

A total number of 80 pregnant Barki ewes were, equally divided into 4 groups. Three groups were vaccinated with two doses (2 weeks in between) of three different K99 vaccines (group I was vaccinated with scourguard vaccine, group II with aluminium hydroxide formalin killed vaccine and, group III with irradiated vaccine) while group IV was kept as non - vacciated control group. A total of 40 lambs, delivered from both non-vaccinated and vaccinated ewes, were challenged with E.Coli K99. All lambs received the colostrum from their dams soon after birth and were reared similarly but separately.

II Vaccines

a. Scourguard3 (K). an imported vaccine. It is a liquid commereial trivelent killed adjuvent vaccine, from Smith kline, Beechman U.S.A
b. Aluminum hydroxide formalin killed vaccine, localy prepared vaccine. Formalin 37% was, used for the preparetion of formalin inactivated bactrin.
c. Irradiated Vaccine , by using gamma irradiation which leads to detoxification of the endotoxin.

III- Samples:

a- Colostrum samples:

From 60 vaccinated as well as 20 non- vaccinated ewes, colostrum samples were collected within 6 hours after parturition to determine the antibody levels against the different K99 E. coli vaccines used. Whey was separated according to the method reported by kohler et al. (1968) then preserved frozen at -20°C till the time of examination .

b- Blood samples:

Blood samples were collected from 80 newborn lambs delivered from vaccinated and non-vaccinated dams at the 1st day . 1st week, 2nd week, 4th week, 8th week and 12th week after birth. Serum was separated and stored at-20°C until used.

The indirect ELISA and agglutination test were preformed to estimate the antibody titers against E.coli K99 antigen in sera of new born lambs and colostrum of vaccinated and non- vaccinated dams according to Farid et al., (1997).

The Elisa antibody titers were estimated by measuring the optical density of serum dilutions. While the agglutination antibody titers was measured as the reciprocal of the highest serum dilution showing postive agglutination.

The Protective capacity of colostrum of ewes vaccinated with different E.coli K99 vaccines was determined by challenging lambs with virulent *E.coli* K99 strain after ingestion of the colostrum. Ten lambs from each group were used for the protective capacity rate. The statistical analysis of the obtanined results was done as described by Cochran and Cox (1960).

RESULTS

The mean colostral K99 antibody titers of ewes vaccinated with different E.coli K99vaccines using ELISA were shown in Table (1). From this table it is clear that the optical density values of anti k99 antigen of colostrum samples were highest in gruoup II, followed by groups I,III and IV. the differences between the vaccinated and non vaccinated animals were highly significant.

Meanwhile Table (2) showed the mean colostral K99 titers of ewes vaccinated with different *E. coli* vaccines using agglutination test in which these titers demonstrated comparable result to that of the ELISA.

From Tables (1) and (2) it is clear that there is a significant increase in the mean colostral K99 antibidy titers in the vaccinated groups (No. I, II, III) rather than the non-vaccinated control group (No. IV) . On the other hand, there are non-significant differences ($P > 0.01$) in the mean colostral K99 antibody titers in the vaccinated groups by both techniques.

Tables (4) and (5) showed the mean K99antibody titers in serum of lambs delivered from both non-vaccinated and vaccinated ewes with different E. coli K99 vaccines and received colostrum of their dams using EIISA and agglutination test.

The tables showed that lambs of group II delivered from ewes vaccinated with aluminum hydroxide formalin killed vaccine showed the highest antibody levels.

T-test of the obtained data revealed that there were very highly significant ($P < 0.001$) and highly significant ($P<0.01$) increase in K99 antibody titers in serum of lambs delivered form vaccinated ewes groups I, II, III from the first day to the 4th week after birth in comparison with the control non- vaccinated group IV.

Comparable results as shown in table (5) where lambs of group II delivered from the vaccinated ewes with aluminum hydroxide formalin killed vaccine exhibited a significant ($P < 0.01$) rise in their antibody levels at different intervals, followed by the mean titers of lambs delivered from those in group I, and finally, the mean antibody titers of lambs delivered from group No. III. All lambs showed the peak of antibodies within the first week, then the antibody levels began to decline gradually.

The protective capacity of colostrum of ewes vaccinated with different E.Coli K99 vaccines was presented in Table (3). It is clear from this table that the protection percentage rate were 100% , 100% ,80% among the 1st ,2nd and 3rd groups respectively. while the poor protection rate was 30% in lambs nursing form non vaccinated ewes and its mortality rate was 70% .

DISCUSSION

Colibacillosis or *E.coli* infection is one of the main causes of diarrhoea in sheep (Wolk et al., 1992). This infection results in high mortality in the young lambs. One of the most successful approaches to counteract the problem of colibacillosis in sheep is strengthening the host defense mechanism to be able to withstand the constant attacks of enterotoxigenic *E.coli.* This might be partially reached through artificial means of vaccination against colibacillosis.

The primary source of lamb protection is the colostrum that mediates passive maternal immunity. It was proved that the vaccination programme of ewes before parturition was successful in increasing the specific K99 antibody level in both colostrum of vaccinated ewes and sera of lambs reared on such high antibody colostrum level (Cameron and Fuls, 1970, Nagy, 1980, Snodgrass et al, 1982 and Altmann and Mukkur, 1983).

The colostrum collected within 6 hours post parturition from previously vaccinated ewes as well as from non- vaccinated ewes were subjected to both ELISA and agglutination test for the detection of colostrum K99 antibody titers against the different E.coli tested vaccines.

In the present study, it was clear from Tables (1), (2) that vaccination of pregnant ewes during the late period of pregnancy with 3 types of vaccines, resulted in an increase in K99 antibody levels in colostrum samples when compared with that of non-Vaccinated ewes as determined by ELISA and agglutination test. These findings agreed with those of Ellens et al., (1979), and Valente et al., (1984) .

In order to prevent colibacillosis, it was necessary to ensure that the newly born lambs receivd adequate amounts of colostrum early in life as colostrum must be fed during the absorptive phase at birth because it is richest in terms of immunoglobulins and specific antibodies . (Dobbaloar et al., 1987)

The mean readings of lambs serum samples showed gradual increase during the 1st day of life. It reached the maximum level in samples of the 1st week of their age in group II, then in group I and lastly in group III. There was a decrease in the reading later on, but detectable level was still recorded up to the end of the experiment on the 12th week after parturition. These findings agreed with those of Cameron and Fuls (1970)& Gergory et al., (1983).

The high protective rate in lambs delivered from ewes of group II was indicative due to the highest titers of immunoglobulins while in control group the newborn lambs which exposed to oral challenge inoculation of E.coli K99 strain were developed diarrhea and sever dehydration and capable of causing deaths that is might be due to lock of colostoal antibody .

Generally, lambs nursing from ewes vaccinated with aluminum hydroxide formalin killed vaccine showed a significant abrupt rise in their antibody levels , followed by those vaccinated with scourguard vaccine and then lambs nursing from ewes vaccinated with irradiated vaccine .

So to protect the offspring from colibacillesis a proper vaccinaton of pregnant ewes before lambing was highly recommended.

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Table (1): Mean colostral K99 Antibody Titers of Ewes Vaccinated with Different E.coli k99+Vaccines Using ELISA.

Group No.	No. of Ewes	Mean K99 Antibody Titers
I	20	11308.0+3911.96***
II	20	2012050.67+553.56***
III	20	206676.67+128.44**
IV	20	213.4+ 11.30

Table (2): Mean Colostral K99 Antibody Titers Of Ewes Vaccinated with Different E. coli k99+ Vaccines Using Agglutination Test.

Group No.	No. of Ewes	Mean K99 Antibody Titers
I	20	595.0+149.0***
II	20	630.0+153.0***
III	20	425.0+59.0**
IV	20	14.0+ 5. 0

Table (3): Procction rate of lambs Delivered from ewes vaccinated with E. coli K99 vaccines after challenging with virulent E.coli K99 strain.

Group No.	No. of lamds	No. of Dead	No. of Survived	Mortality rate	Protection rate
I	10	0	10	0%	100
II	10	0	10	0%	100
III	10	2	8	20%	80
IV	10	7	3	70%	30

Mean + standard error

*** Significant at < 0.001

** Significant at < 0.01

Group I Ewes vaccinated with Scourguard vaccine

Group II Ewes vaccinated with Alum. Hydro. Form. killed vaccine.

Group III Ewes vaccinated with Irrdiated vaccin

Group IV Non Vaccinated ewes.

Table (4): Immune response of lambs to passive immunization by E.coli using ELISA.

Group No.	No. of lambs	Mean K99 Antibody Titers					
		1st day	1st day	2nd day	4thday	8th day	12th day
I	10	2000.35 ± 40.1***	3310.50 ± 199.10**	2555.0 ± 250.90**	1335.0 ± 88.93***	714.0 ± 41.33*	215.90 ± 9.31*
II	10	2317.83 ± 92.73***	4113.71 ± 34.63***	3219.11 ± 85.38***	1560.99 ± 81.55***	898.30 ± 31.10**	277.0 ± 9.110**
III	10	191.0 ± 39.93***	2310.73 ± 211.98**	1945.37 ± 191.77**	1094.0 ± 130.11**	559.94 ± 45.9*	201.19 ± 31.94*
IV	10	141.30 ± 5.92	166.49 ± 155.91	173.80 ± 9.39	130.10 ± 7.83	110.0 ± 5.1	104.80 ± 2.11

Table (5): Immune response of lambs to passive immunization by E.coli using Agglutination Test

Group No.	No. of lambs	Mean K99 Antibody Titers					
		1st day	1st day	2nd day	4th day	8th day	12th day
I	10	173.0 ± 34.0***	305.0 ± 83.0**	182.0 40.0**	105.0 ± 23.0***	59.0 ± 17.2*	10.0 ± 2.4*
II	10	179.0 ± 32.0***	399.0 ± 80.0***	265.0 ± 53.0***	138.0 ± 52.0***	71.0 ± 31.0**	15.0 ± 3.0**
III	10	156.0 ± 45.0***	263.0 ± 80.1**	161.0 ± 30.0**	76.0 ± 26.2**	45.0 ± 13.0*	10.0 ± 5.0*
IV	10	5.80 ± 1.3	6.30 ± 1.50	5.0 ± 1.1	4.8 ± 1.3	2.88 ± 0.5	2.3 ± 0.5

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