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دراسة عن المناعة المكتسبة في الصغار من الأمهات المحصنة ضد مرض التفحم العضلي

اقبال فراج ، فهمي عوض *، عبدالسلام زكي ، محمد عبيد **
رقية عثمان

١ ـ أثبتت الدراسة عن المناعة المكتسبة في الصغار من الأمهات المحصنة ضد مرض التفحم العضلي وجود صلة كبيرة بين وجود الأجسام المناعية عند الأمهات في وقت الولادة وبين صغارها ، وكلما ارتفعت كمية هذه الأجسام في سرسوب الأم زادت كمية الأجسام المناعية في الصغار • وقد وجد أن نسبة الأجسام المناعية في السرسوب أعلى من المصل في الدم • كما أثبتت الدراسة أن متوسط اختفاء الأجسام المناعية من الحيوانات الصغيرة سواء في الحوالي أو العجول الصغيرة كان بعد شهرين من الولادة •

٢ ـ ثبت بواسطة التحليل الكهربائي أنه لا يمكن التفريق بين السرسوب المأخوذ من أغنام محصنة بالتفحم العضلي أو غير المحصنة ، وكذلك لايمكن التفريق بهذه الطريقة بين الأمصال المأخوذة من حملان من أمهات محصنة بمرض التفحم العضلي وأخرى من حملان مولودة من أمهات غير محصنة •

* كلية الطب البيطري ـ جامعة القاهرة •
** كلية الطب البيطري ـ جامعة بنها •

Vet. Serum and Vaccine Res. Institute,
Head of anaerobic Dept. Prof. Dr. Doreyea Sharaf.

STUDIES ON THE MATERNAL TRANSMISSION OF CLOSTRIDIUM CHAUVOEI ANTIBODIES FROM VACCINATED PREGNANT ANIMALS

(With 4 Tables & 6 Figs.)

By

IKBAL FARRAG; F.L AWAD* A.Z. HUSSEIN; M.H. EBEID** and ROUKAYA OSSMAN

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SUMMARY

Studies on the maternal transmission of antibodies from vaccinated pregnant ewes and buffaloes showed a remarkable degree of correlation between the agglutination levels found in the dams sera at the time of parturition and the antibody titre of the offspring. The titre of colostral whey was mostly higher than that of the dams serum. The average agglutination titre dropped at 2 months to 3.3 ul of serum in lambs and to 4.6 ul in calves.

Electrophoretic analysis of colostral whey of ewes showed an increase in the lacta-albumin, the B_2 lacta-globulins and the B_1 lacta-globulins in colostral whey positive to the agglutination test using Cl.chauvoei antigen compared with the negative samples. This increase was statistically insignificant concerning the lacta-albumin and the B_2 lacta-globulins. The increase of B_1 lacta-globulins was statistically significant.

Electrophoretic analysis of sera from lambs suckling immune dams and of lambs from non-immunized dams from birth to 4 months old were studied. There was no statistically significant difference between the percentage of albumin, alpha, B_1 B_2 and gamma globulins in the sera of lambs from immunized dams when compared with those of non-immunized dams. There was only a statistically significant increase in the B_2 globulins of lambs from immunized dams at two weeks old.

INTRODUCTION

Blackleg may affect newly born lambs through the ambalical cord. In bovines there is a difference in age suceptibility between buffaloes and cattle. An analysis of 763 cases of blackleg in bovines showed that young buffalo calves of a few days old are susceptible to the disease but cattle are usually afected over 6 months old (FARRAG, 1975).

Effective vaccine is available, but to secure the full protection of newly born animals, reliance must be placed on a vaccinated mothers ability to transfer antibodies via the clostrum.

* Faculty of Vet. Med., Cairo University.
** Faculty of Vet. Med., Banha University.

FARRAG, et al. (1983) stated that the best time for the booster dose of clostridial vaccines is 2-3 weeks before parturition particularly for Cl.chauvoei. The importance of clostrum in protecting the young ruminant against neonatal disease is well established and the serum globulin levels of neonatal lambs have been recorded by McCARTHY and McDOUGALL (1953), HALLIDAY (1966, 1968, 1970 and 1971). REID (1972) reported that at birth small amounts of immune globulin were found in the lambs sera. VARELA-DIAZ and SOULSBY (1972) found that only trace amounts of immunoglobulins occur in antigenically unstimulated foetal lambs and the neonatal animals has the ability to synthesize IgG soon after birth and also acquries it through the clostrum.

The experiment described in this paper was design to study the maternal transmission of Cl.chauvoei agglutinins to lambs and calves. Also to study the difference in serum immunoglobulins fractions between lambs born to ewes immunized with blackleg vaccine and others of non immune ewes by polyacrylamid gel electrophoresis.

MATERIAL and METHODS

Vaccine:

Blackleg oil adjuvant vaccine was used for immunization of the ewes. The preparation of the vaccine was described by FARRAG (1975).

Immunization of experimental animals:

Nine pregnant ewes were obtained from Tahrir Province. Seven buffaloes in late pregnancy were obtained from dairy herd of Dokki farm. Before vaccination sera were collected from all the animals and tested for the presence of Cl.chauvoei agglutinins. Two sheep and one buffalo sera were free from antibodies against Cl.chauvoei and were kept as unvaccinated control.

The 7 ewes and 6 buffaloes possitive to the agglutination test were vaccinated intramuscularly with 2 and 3 ml respectively oil adjuvant blackleg vaccine 2 weeks before parturition. Blood samples were collected from the lambs and calves immediately after parturition and before suckling and then 12 hours, 3 days, 7 days, 14 days and one month after parturition then monthly until the lamb reached the age of 4 months. Serum separated from blood were stored at - 20 C° until used.

Plate agglutination test:

The test was applied as sescribed by CLAUS and MAHEAK (1972).

Collection of colostral whey:

Colostrum was collected immediately after parturition in 20 ml amounts in one ounce McCartney bottles, and centrifuged at 5000 r.p.m. for 30 minutes. Ater centrifugation, the lipid portion was removed form the surface and the fat free colostrum was treated with reninto remove casein (PIERCE, 1955). The samples were then incubated at 37°C for one hour, and after clotting placed in the refrigirator overnight. The whey was then removed by a pasteur pipette, clarified by centrifugation at 3000 r.p.m. for 15 minutes and stored at -20°C until required.

Technique of electrophoretic analysis:

The technique employed was that described by DAVIS (1964) in which the serum and colostrum were fractionated in columns of polyacrylamide gels. Reading of the gels ws carried out by scaning through Extintion recorder ERI 10 Carl Zeiss. The relative mobility value (Rm) was determined according to PEACOCK, et al. (1965) and GLICK (1968).

RESULTS

Passive responce of calves and lambs to immune mothers:

The sera and colostrum of the buffaloes and ewes as well as lambs and calves sera were tested by plate agglutination test against Cl.chauvoei antigen. The results are shown in Table 1,2 and Fig. 1,2.

Tables 1,2 Fig. 1,2 show that the agglutination titre of the colostral whey was higher than that of the serum in 6 ewes and all the buffaloes. There was no detectable agglutinins in the sera of the lambs or calves before suckling their dams. Agglutination titre rose rapidly in all animals 12 hours after suckling colostrum and then declined gradually until it completely disappeared between 2-6 months according to the level of antibodies in individual animals. The average group titre of lambs, and calves dropped to a low level at 2 months.

Electrophoretic studies on colostral whey of ewe containing Cl.chauvoei agglutinins:

The results obtained are illustrated in Table 3, Fig. 3 and 4. Table 3, Fig. 3 and 4 show that there was an increase in the lactalbumin, the B_2 lacta-globulins and the B_1 lacta-globulins in colostral whey positive to the agglutination test using Cl.chauvoei antigen when compared with the negative samples. However, this increase was statistically insignificant concerning the lacta-albumin and the B_2 lacta-globulins. The increase of B_1 lacta-globulins at P 0.05, T = 2.85.

Electrophoretic studies on sera of lambs sucling immune dams:

Sera of 7 lambs from ewes which had been vaccinated during pregnancy with blackleg vaccines, and sera of 2 lambs of non-vaccinated ewes were subjected to electrophoretic analysis on polyacrylamid gels.

Results obtained are illustrated in Table 4 and Fig. 5 and 6. Table 4 shows that there was no statistical significance between the different serum globulins in sera of lambs of immunized dams and those of non immunized are except a significant increase in B globulins in lambs of 2 weeks old from immunized dams.

DISCUSSION

In the present study it was considered necessary to investigate maternal immunity in animals vaccinated against Cl.chauvoei, as it is a very importanat factor in the protection of young animals and determining the optimum time for vaccination. Results obtained indicated that the agglutination titre of the colostral whey was higher than that of the dames sera both in buffaloes and ewes. No evidence of agglutinins was found in calves or lambs before their first feed of colostrum. Maximum titres were found in their sera 12 hours after colostrum feeding. Agglutination titre of the calves and lambs exceeded that of their dams sera. There was a remarkable degree of correlation between the agglutinin levels found in the dams colostrum and the antidody titre attained by their offspring. The agglutination titre of lambs and calves gradually declined. The protective level obtained from the mothers in the young animals lasted for two months only. However individual animals with high titre retained a good level of antibodies up to three months which dropped gradually until traces only were found at 6 months. Our results proved that the offspring of vaccinated dams were born with no detectable antibodies against blackleg in their sera and that they obtained the antibodies from the colostrum the amounts of antibodies found in their sera seems to correspond to their level in the colostrum, and the duration of immunity in young animals depended on the level of antibodies in their sera. In conclusion our results indicated that pregnant animals should be vaccinated with oil adjuvant vaccine two weeks before parturition to ensure a high level of colostral antibodies capable of protecting the young for at least two months at which time it should receive the first dose of oil adjuvant vaccine to be repeated at the age of six months then yearly.

The electropherograms of colostral whey revealed 9-10 protein bands namely; prealbumin, albumin, alphalactaglobulin ($_1$, $_2$ and $_3$) $beta_1$ lactaglobulin, $beta_2$ lactaglobulin (B_{2a} B_{2b} and B_{2c}) and 2 gamma immunoglobulins ($_1$ and $_2$) according to their electrophoretic mobilities when compared to serum proteins. Results indicated that there was an increase in the lacta-albumin, the B_2 lacta-globulins and the B_1 lacta-globulins in colostral whey, from vaccinated ewes. However, this increase was statistically non-significant concerning the lacta-albumin and the B_2 lacta globulins. The increase of the B_1 lacta-globulins was statistically significant.

The electropherograms of lambs sera revealed 7-12 protein bands in lambs from immune or non-immune ewes. No significant difference in the electrophoretic mobilities of the major serum proteins was found between the electrophoretic components of lambs and adult sera. In some of the lambs sera the transferrin (B_1) was divided into two fractions (B_{1a} and B_{1b}). Results obtained showed that there was no statistically significant difference between the percentage of albumin, alpha-globulins transferrin (B_1, B_2 globulins) and gamma globulins in sera of lambs from immunized dams and those from non-immunized dams except statistically significant increase in the B_2 globulins of lambs 2 weeks old from immunized mothers.

Comparing the percentages of albumin, alpha, B_1 and B_2 fractions in the sera of lambs immediately after birth with those in sera 12 hours after colostrium feeding showed a decrease in those fractions in both lambs from immune and non immune dams; but there was statistically significant increase in the gamma globulins only. The presence of a low percentage of gamma globulins before colostral feeding in our experiments disagrees with SMITH and HOLM (1948), CHARLWOOD and THOMSON (1948) who reported that newly born lambs are completly a gamma globinaemic. However, our findings are supported by those of McCARTHY and McDOUGAL (1949), SILVERSTEIN, et al. (1963), VARELA-DIAZ and SOULSBY (1972).

Absorption of immune globulins in lambs after suckling colostrum resulted in a rapid increase in serum gamma globulins particularly $_1$ fraction, reaching a peak at 12 hours then declining gradually till the lambs were two weeks old, after which the gamma globulins exihbited slight flactuation until the animals were four months old. The flactuation in the percentage of the gamma globulins has been explained by BRAMBELL (1970) who claimed that the young ruminant after taking the colostrum and aquiring concentration of maternal immune globulins by the second day enters upon a period when the decline in the passively acquired globulins overlaps the rise in autogenous gamma globulins and tends to obscure it. Consequently, a point is reached some time after birth when the gamma globulin concentration is at a minimum and the loss of pasively aquired gamma globulins was equalled by the production of autogenous gamma globulins. The rate of disappearance of one is compilicated by the rate of appearance of the other.

In conclusion our results indicated that electrophoretic studies did not reveal any significant difference in the sera of lambs from immunized and non immunized dams, or even in the colostrum of immunized and non immunized ewe. This shows that electrophoretic analysis of sera although useful for followign the behaviour of different sera fractions from birth to weaning, it can not be used specifically for detecting antibodies against blackleg disease.

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Table 1

Agglutination titre (µL) in the colostrum and in the sera of ewes at parturition and in the sera of their lambs.

Ewe No.	Lamb No.	agglutination titre of ewes		agglutination titre (µ L) in the sera of lambs										
		sera	colos-rum	presu-ckling	12 hours	3 days	7 days	2 weeks	one month	2 months	3 months	4 months	5 months	6 months
6417	179	0.2	0.02	00	0.05	0.05	0.2	0.2	0.2	0.5	5	00	00	00
14145	149	0.5	0.004	00	0.04	0.05	0.2	0.4	0.5	0.5	5	00	00	00
14437	112	0.2	0.005	00	0.02	0.04	0.4	0.5	0.5	0.5	0.5	5.0	00	00
8035	180	0.04	0.05	00	0.2	0.2	0.2	0.4	0.5	1.0	10.0	00	00	00
8998	265	0.04	0.005	00	0.02	0.02	0.02	0.04	0.2	0.5	20.0	Dead	-	-
10175	190	0.0004	0.0004	00	0.0005	0.0005	0.001	0.002	0.004	0.02	0.5	5.0	5.0	20.
13486	103	0.2	0.02	00	0.2	0.4	0.5	2.0	2.0	20.0	00	00	00	00
Average		0.16	0.01	00	0.07	0.11	0.25	0.5	0.55	3.3	00	00	00	00
11281"C"	167	00	00	00	00	00	00	00	00	00	00	00	00	00
12075"C"	155	00	00	00	00	00	00	00	00	00	00	00	00	00

00 = No detectable antibodies .

Table 2

Agglutination titre (µ L) in the colostrum and the sera of buffaloes at time of parturition and the sera of their calves.

buffalo No.	calf No.	agglutination titre of buffaloes		agglutination titre (µ L) in the sera of calves										
		sera	colostrum	pre-sucling	12 hours	3 days	7 days	14 days	one month	2 months	3 months	4 months	5 months	6 months
701325	67	2	0.5	00	1.0	2.0	5.0	5.0	10.0	dead	-	-	-	-
3304	45	0.4	0.05	00	0.1	0.1	0.2	0.2	0.2	0.5	5.0	00	00	00
3330	46	0.05	0.05	00	0.4	0.4	0.4	0.4	0.5	0.5	1.0	2.0	4.0	5.0
229	27	0.5	0.4	00	0.5	0.5	1.0	2.0	5.0	20.0	20.0	00	00	00
18501	48	0.1	0.05	00	0.5	0.5	0.5	0.5	0.5	2.0	5.0	5.0	5.0	20.0
2[illegible]3	49	0.1	0.005	00	0.01	0.01	0.02	0.02	0.04	0.1	2.0	5.0	5.0	40.0
Average		0.52	0.17	00	0.41	0.58	1.18	1.35	2.7	4.62	6.6	00	00	00
89302"C"68		00	00	00	00	00	00	00	00	00	00	00	00	00

00 = No detectable antibodies

Table (3)

Electrophortic analysis of colostral whey

Ewe No.	percentage of				
	lactalbumin	alpha lacta-globulin	B_1 lacta-globulin	B_2 lacta-globulin	gamma globulins
14145	23.91	3.1	10.5	1.6	58.95
6417	22.86	7.9	6.03	3.7	58.63
8998	18.64	7.4	8.5	1.6	63.23
8035	20.17	7.2	8.7	5.5	57.29
14437	21.58	6.9	7.8	3.2	59.60
13486	16.29	4.9	7.7	3.2	66.91
10175	19.88	8.25	8.7	2.6	59.81
Mean	20.47	6.53	8.27	3.06	60.6
S.E	1 ±	0.43 ±	0.74 ±	0.5 ±	1.46 ±
12075"C"	16.15	5.4	4.8	1.8	70.31
11281"C"	21.89	7.2	3.7	3.2	64.48
Mean "C"	19.02	6.3	4.25	2.5	67.39
S.E.	2.8 ±	1 ±	0.55 ±	0.7 ±	3 ±
T.value	0.48	0.23	2.85	0.56	2.05

"C" = Neg. control

Table 4

Average percentage of different serum fractions in sera of lambs from birth to 4 months of age

Protein fraction	Percentage of different fraction at								
	birth	12 h	3 days	7 days	2weeks	1month	2month	3month	4month
Albumin									
Mean	25.02	17.83	19.43	20.0	25.31	29.06	28.58	28.53	23.36
S.E	1.73	0.65	0.92	1.25	1.5	2.0	1.13	1.06	0.81
Mean"C"	30.49	18.39	17.57	18.7	22.24	29.99	26.89	23.69	25.33
S.E "C"	7.36	2.5	0.14	1.22	3.9	2.24	3.75	5.39	4.0
T.Value	0.55	0.21	1.80	0.75	0.72	0.31	0.42	0.88	0.11
Alpha globulin									
Mean	24.4	14.1	12.9	10.02	11;8	6.9	9.2	13.3	11.66
S.E	1.41	0.6	0.6	0.74	0.88	1.09	0.43	0.74	1.0
Mean"C"	26.05	10.9	13.5	14.1	7.2	5.5	9.00	13.50	10.65
S.E "C"	5.2	2.0	4.13	1.0	2.0	1.22	0.02	2.0	2.34
T.Value	0.33	1.6	0.14	1.43	1.74	0.62	0.2	0.08	0.41
Transferrin (B_1)									
Mean	15.6	10.2	11.9	17.8	18.3	17.3	16.2	12.8	18.43
S.E	0.57	1.25	0.65	2.03	0.92	4.0	4.9	3.2	1.3
Mean"C"	16.3	10.5	13.6	14.2	23.9	22.1	19.75	17.4	18.5
S.E "C"	2.1	1.22	1.0	1.0	1.0	2.4	3.08	4.31	2.9
T.Value	0.31	0.18	1.2	1.3	1.69	1.02	0.61	0;85	0.02
B_2 globulin									
Mean	8.06	4.8	4.7	4.8	6.08	5.4	5.2	7.05	5.9
S.E	0.4	0.4	0.92	0.65	0.56	5.4	0.75	1.0	1.2
Mean"C"	10.3	5.1	6.05	8.2	4.3	4.8	9.35	10.65	3.75
S.E "C"	3.2	3.2	8.7	1.8	0.56	3.75	0.7	2.0	0.22
T.value	0.70	0.15	0.15	1.7	3.9	0.09	4.15	1.61	1.8
Gamma globulin									
Mean	15.8	54.66	50.2	48.8	39.07	39.5	39.08	38.1	40.49
S.E	0.7	1.69	3.16	2.4	1.4	1.7	1.7	1.7	1.2
Mean"C"	16.31	54.57	53.5	42.5	41.5	35.4	36.7	36.15	41.47
S.E"C"	1.8	4.2	5.06	7.4	2.5	1.4	4.9	5.1	4.3
T.Value	0.25	0.02	0.61	0.81	0.86	1.83	0.46	0.36	0.2

"C" = Control lambs of unvaccinated ewes .

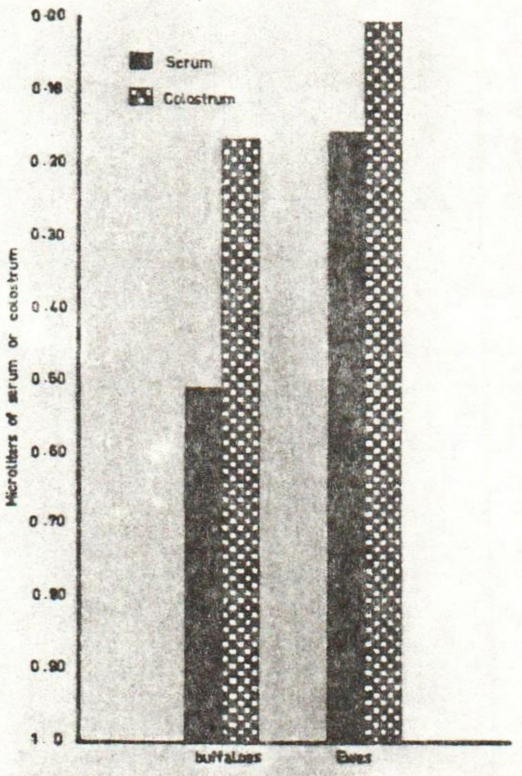


Fig.1: Average agglutination titre in the sera and colostrum of buffaloes and ewes at parturition

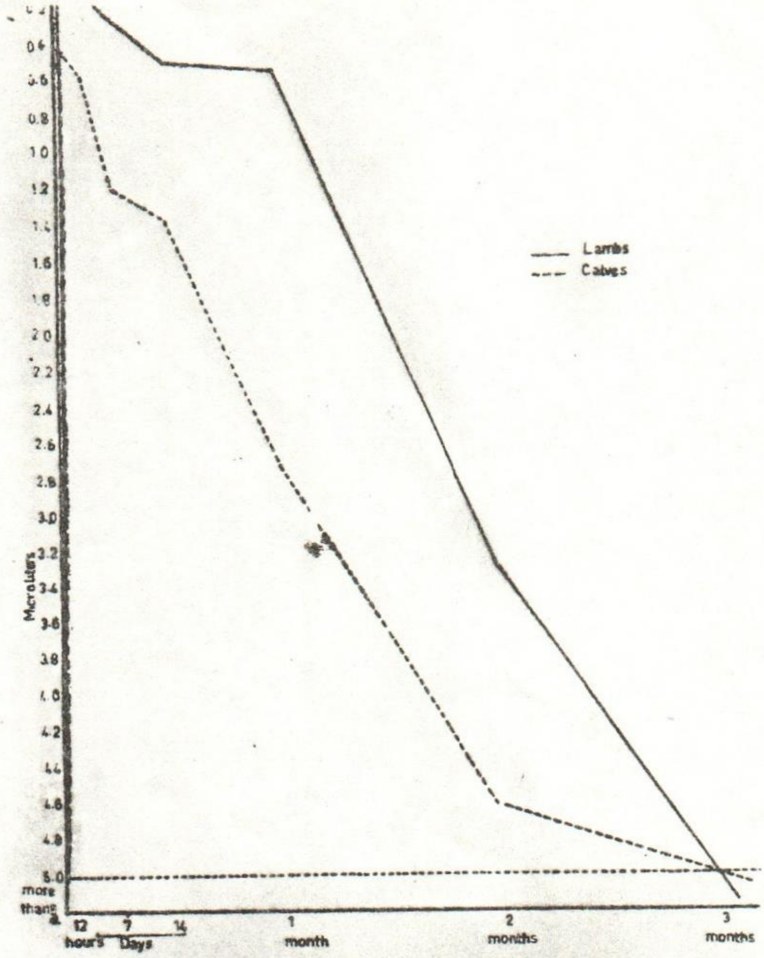


Fig.2: Average curves of agglutinin disappearance in lambs and calves

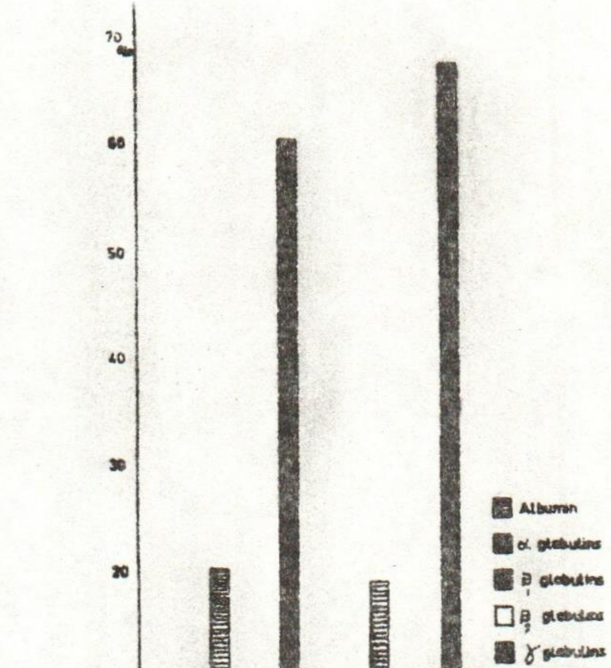


Fig.3: Percentage of different fractions in colostral whey of sheep

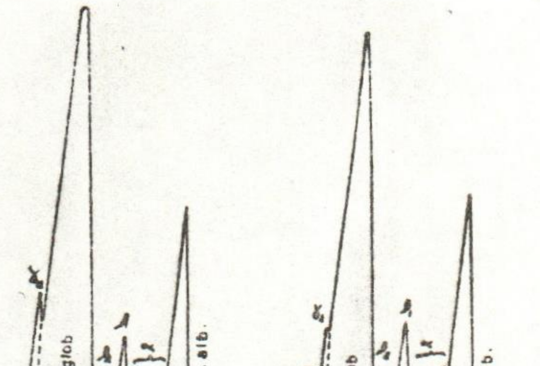


FIG. 4: ELECTROPHORETIC PATTERN OF COLOSTRUM SAMPLES FROM EWES VACCINATED AND NON VACCINATED WITH BLACKLEG VACCINE

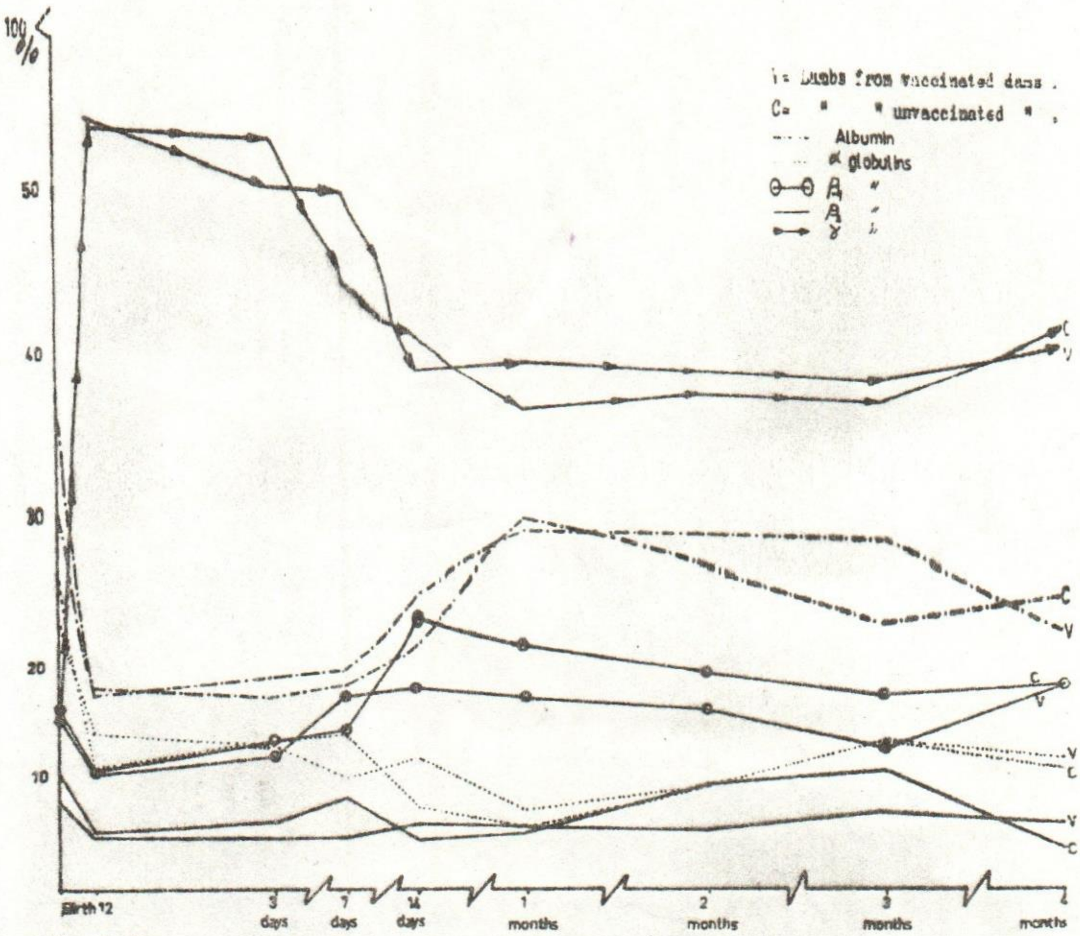
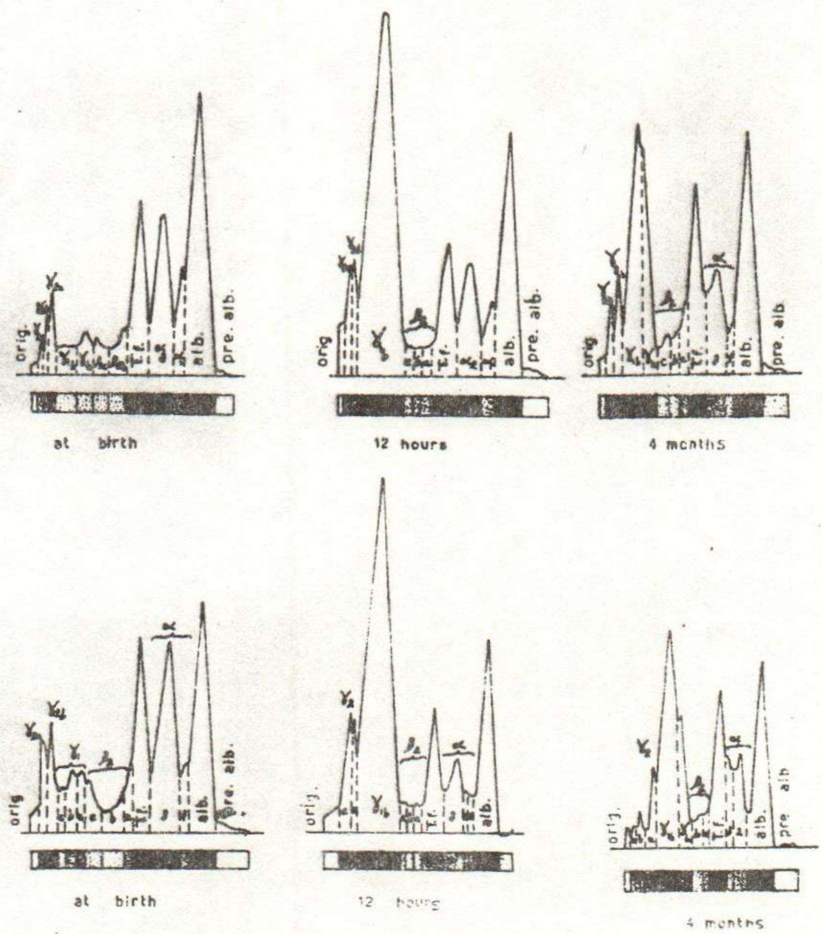


Fig. 5: Percentage of different protein fractions in lambs sera from birth to 4 months .

Fig. 6: LAMB FROM VACCINATED DAM



LAMB FROM NON VACCINATED DAM