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NOTE

Mycoplasma Agalactiae V – Comparison of three different contagious agalactia vaccine (*)

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After Bridré and Donatien (1), in 1925, stated that recovered animals are immune to and resist intra-articular inoculation, many investigators started to make a vaccine for control of disease in sheep and goats. However, Carré (2), in 1921, and Bridré and Donatien (3), in 1925, had encountered great difficulty in eliciting active immunity to prevent the development of disease in animals speciallys in lacting ewes.

Different kinds of vaccine have been prepared and presented by different investigators. Zavagli (4), in 1951, claimed excellent results by using a live vaccine prepared from infected tissues, and modified by the addition of aluminium hydroxide as a localizing adjuvant. This vaccine is usually preceded by a formaline-killed vaccine made from a mixture of infected milk and a broth culture of the mixed contaminating bacterial usually found in the milk of agalactia cases.

Lopez and Lopez (5), in 1952, had also prepared a vaccine by mixture of serum peptone veal infusion broth culture and of total chick embryo cultures killed with formalin.

Shamir (6), in 1954, attenuated the organism by serial chick embryo passaegs and reported that vaccine prepared from it immunized goats successfully.

Bory and Entessar (7), in 1955, prepared an inactivated saponinized vaccine with good results on immunizing goats.

Blanco Loizolier (8), in 1959, prepared an avianized vaccine for sheep and goats.

Popoviçi (9), in 1962, had used an inactivated and live vaccine for control the disease in Roumania.

Ivanov (10), in 1962, using a live vaccine prepared from Ag1 Strain of M. agalactiae with more satisfactory results than adsorbed vaccine.

Ozsoy (11), in 1961, compared three different contagious agalactia vaccines. The nature of these vaccines were as follows :

1) The culture of M. agalactiae and Ringer solution were mixed and

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inactivated with formalin and adsorbed by Aluminium hydroxide gel.

2) The culture of M. agalactiae was mixed with glycerine and phosphate buffer solution and partially inactivated with 2/10.000 of formaline, adsorbed by Aluminium hydroxide gel.

3) Zavagli's tissue vaccine.

Ozsoy showed that, the partially inactivated vaccine afforded complete protection; and Zavagli's vaccine gave 50-75% protection.

In this communication we are going to describe the results which we have obtained by using three different vaccines. The comparison has been made by serological test and also by challenge. The vaccines which we have used were as follows :

1) Egg propagated inactivated vaccine.
2) Inactivated and adsorbed vaccine.
3) Inactivated and saponinized vaccine.

MATERIAL AND METHODS

1) Egg propagated inactivated vaccine: Embryonated eggs 6-7 days old are injected into the yolk sac with 0.2 ml of 24 hours culture of M. agalactiae. After 3-4 days re-incubation at 37°C. all the dead embryos are harvested for vaccine production. The embryos plus corio-allantoid membrane and yolk, representing a high concentration of Mycoplasma, are harvested aseptically. These materials are ground up twice in the Atomix blender for five minutes. 60 ml of a 0.25% formolized physiological saline for each embryo is added and then blended for an additional 5 minutes. This suspension, after being filtered through sterile gauze and checked for sterility by culturing on different microbiological media, is ready to be used as vaccine.

2) Inactivated and adsorbed vaccine: 36 hours culture of M. agalactiae, in Difco PPLO Broth w/o CV supplemented by 20% horse serum and 1% of Difco Yeast Extract, is conjugated with 1.5 g.% of aluminium hydroxide solution and kept for 24 hours in +4°C. and then inactivated by addition of 0.25% formalin. The emulsion after being checked for sterility is ready for using as a vaccine.

3) Inactivated and saponinized vaccine: This vaccine is prepared by the method which described previously (12).

RESULTS

60 sheep and 30 goats were divided in 3 groups, each group inoculated with 2 ml of different vaccines. From 5 days after inoculation the serum of sheep and goats were tested for detection of antibodies corresponding to the M. agalactiae antigen. The method of serological test which we used for this purpose was Antiglobuline Test which has been described previously (13).

The titer of serum of vaccinated animals is tabulated in table No 1, for egg propagated inactivated vaccine, No 2, for inactivated and adsorbed vaccine, and No 3, for inactivated and saponinized vaccine.

Two months after vaccination all sheep and goats were inoculated with 10MID of a virulent culture of M. agalactiae.

DISCUSSION

Table No 1,2 and 3 indicates that the egg propagated vaccine is not very valuable, because 75% of sheep and 100% of goats serologically were negative after 40 days post-vaccination. All vaccinated animals were infected by challenge dose after two months.

The adsorbed vaccine is of good value for goats, because 20% of them were negative after 40 days, while 45% of vaccinated sheep were negative in that time. On challenging also, the per centage of resistant goats was 12.5% more than sheep, because 25% of vaccinated sheep and only 2% of vaccinated goats were infected after two months. This shows that, this vaccine has protected the goats better than sheep.

The saponinized vaccine on the contarary has a good protection effect for sheep and goats, because all vaccinated sheep and goats showed the titer between 10-20 after 40 days. In challenge, all vaccinated animals (Sheep and Goats) were resistant to the challenge dose after two months.

According to the results which we have mentioned, the saponinized vaccine has a good protective effect and also is very easy to produce, and economically is cheaper than other vaccines and besides in minimum time a good batch of vaccine can be prepared.

SUMMARY

The egg propagated, the adsorbed and saponinized vaccine against contagious agalactia were compared. In serological and challenge we find that saponinized vaccine is better than other two vaccines for vaccination in sheep and goats. The saponinized vaccine is also very easy to produce and economically is cheaper than other vaccines.

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Table No 1 = Evaluation of Serum Titers after Vaccination

(Egg Vaccine)

Days Post - Vaccination	Serum Titers (Number of Animals)
5	1/10 (5S & 7G) - 1/20 (15S & 3G)
10	1/40 (15S & 9G) - 1/80 (5S & 1G)
15	1/80 (12S & 9G) - 1/160 (8S & 1G)
20	1/80 (1S & 5G) - 1/160 (19S & 5G)
25	1/20 (12S & 10G) - 1/40 (8S)
30	1/10 (12S & 10G) - 1/20 (8S)
35	U (12S & 10G) - 1/10 (8S)
40	N (16S & 10G) - U (4S)
45	N (20S & 10G)
50	N (20S & 10G)
55	N (20S & 10G)

S = Sheep G = Goat U = Undiluted N = Negative

Table No 2 - Evaluation of Serum Titers after Vaccination

(Adsorbed Vaccine)

Days Post - Vaccination	Serum Titers (Number of Animals)
5	1/10 (5S & 1G) - 1/20 (14S & 9G) - 1/40 (1S)
10	1/20 (1S) - 1/40 (11S & 1G) - 1/80 (8S & 9G)
15	1/40(1S) - 1/80(10S & 1G) - 1/160(8S & 9G) -1/320(1S)
20	1/80(2S) - 1/160(9S & 2G) - 1/320(8S & 8G) - 1/640(1S)
25	1/10(1S) - 1/20(7S & 2G) - 1/40(6S & 1G) - 1/80(6S & 7G)
30	1/10 (8S & 2G) - 1/20 (6S & 1G) - 1/40 (6S & 7G)
35	U (8S & 2G) - 1/10 (6S & 1G) - 1/20 (6S & 7G)
40	N (8S & 2G) - U (6S) - 1/10 (6S & 8G)
45	N (14S & 2G) - U (2S & 4G) - 1/10 (4S & 4G)
50	N (16S & 6G) - U (4S & 4G)
55	N (20S & 10G)

S - Sheep G - Goat U - Undiluted N - Negative

Table No 3 = Evaluation of Serum Titers after Vaccination

(Saponinized Vaccine)

Days Post - Vaccination	Serum Titers (Number of Animals)
5	1/10 (6S & 1G) - 1/20 (14S & 9G)
10	1/20 (1S) - 1/40 (7S & 5G) - 1/80 (12S & 5G)
15	1/80 (8S & 4G) - 1/160 (12S & 4G) - 1/320 (2G)
20	1/160 (1G) - 1/320 (9S & 5G) - 1/640 (11S & 4G)
25	1/40 (1G) - 1/80 (9S & 5G) - 1/160 (11S & 4G)
30	1/20 (1G) - 1/40 (9S & 5G) - 1/80 (11S & 4G)
35	1/10 (1G) - 1/20 (9S & 5G) - 1/40 (11S & 4G)
40	1/10 (9S & 6G) - 1/20 (11S & 4G)
45	U (4S & 2G) - 1/10 (16S & 8G)
50	N (4S & 2G) - U (5S & 6G) - 1/10 (11S & 2G)
55	N (9S & 8G) - U (11S & 2G)

S = Sheep G = Goat U = Undiluted N = Negative