Antibody Titers of Dairy Heifers following Vaccination with a Staphylococcal Toxoid[1]

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INTRODUCTION

Bovine mastitis, a very common infectious malady of the mammary gland of the cow, causes heavy economic losses to the dairyman. These economic losses include decrease in milk yield, production of poor-quality milk unfit for human consumption, destruction of the affected quarters of the udder which may render a cow totally unproductive, and expenditures on feed, labor, medicines, and professional veterinary care used on sick or unproductive cows. In Puerto Rico, the average loss per cow has been estimated at $28 annually, but it is probably much higher than this.

The most prevalent infectious agents in mastitis are the streptococci and staphylococci. Pomales-Lebrón *et al.* (*2*)[3] demonstrated that 87 percent of the mastitis cases he studied were caused by *Streptococcus agalactiae*, and only 2.1 percent were of staphylococcal origin. Rivera-Anaya and Berrocal (*3*) reported an incidence of 54.25 percent for streptococcal as against 27.37 percent for staphylococcal mastitis, in a study extending from 1950 to 1958.

Treatment of staphylococcal mastitis with antibiotics has not been very successful and has persuaded investigators to work on immuno-therapeutic measures. Among these, a toxoid made from the Slanetz strain No. 7 of staphylococcus has reached the commercial market. The widespread use of this toxoid in Puerto Rico has been empirically recommended. There is no previous evaluation of the use of staphylococcal toxoids in Puerto Rico, and this preliminary work was done from August 1961 to September 1962, to study certain prophylactic properties of this Slanetz Staphylococcal Toxoid.

PROCEDURE

Forty-three pregnant heifers were selected at random at two commercial dairies near Caguas and at the Lajas Substation. Fifteen heifers were selected at random from one of the commercial herds, 12 from the other, and 16 from the Substation's herd. Four of the animals selected—two in each

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[3] Italic numbers in parentheses refer to Literature Cited, p. 98.

of the two commercial dairy herds—were left as controls. These were injected with sterile saline solution in the same dose-schedule as the vaccinates.

At the start of the observations, blood samples were drawn from the 43 heifers to establish the prevaccination antibody level in their sera. The first dose of 5 ml. of staphylococcal toxoid was injected intramuscularly at that time. Four weeks later, a booster dose of 5 ml. of the toxoid was similarly injected into each heifer, and individual blood samples were again drawn.

Thereafter, blood samples and composite milk samples for antibody-level determinations, as well as quarter milk samples for bacteriological observations, were obtained bimonthly from each heifer. The first milk samples were collected from each heifer on the closest scheduled visit following her parturition. Antibody levels were determined by the observation of macroscopic agglutination in twofold serial dilutions of the blood sera and milk wheys against the staphylococcal antigen diluted 1:20 with isotonic saline solution.

The blood samples were left at room temperature for 2 hours and then stored overnight in the refrigerator to allow the clot to retract. The clear serum was poured off into another tube and centrifuged at 2,000 r.p.m. for 20 minutes; the supernatant liquid was poured off into a clean tube. Serial twofold dilutions of the sera were made by mixing 1 ml. of serum with 1 ml. of isotonic salt solution. One milliliter of the diluted antigen was added to 1 ml. of the serially diluted serum for obtaining final dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc. The highest dilution having a 2+ reading was taken as the end-point to determine the antibody level.

Composite milk samples were treated with acetic acid and calcium chloride, and incubated at 77° C. for 1 hour. The curd was separated from the whey by filtration through a rapid-filtration filter paper, and the filtrate centrifuged at 2,000 r.p.m. for 20 minutes. Serial dilutions of the whey were made following the same procedure as used with the serum, the highest dilution with a 2+ reading was taken as the end-point to determine the antibody titer.

RESULTS AND OBSERVATIONS

During the 13 months of observations, 161 quarter milk samples were examined bacteriologically, and the antibody titers of sera from 161 composite milk samples and from 204 blood samples were determined. Seven of the heifers from the two commercial herds had to be eliminated because of postpartum paralyses and accidental deaths; besides this, one of the control animals was eliminated because of a fistula of the udder from streptococcal mastitis.

TABLE 1.—*Staphylococcal antibody response in blood sera of 43 heifers vaccinated with Slanetz No. 7 Strain Toxoid, on 3 farms in Puerto Rico, 1961–62*

Farm and animal No.	Titers (1:X)							
	Pre-vaccine	Booster	Postvaccination				Pre-booster	Annual booster
	1	2	3	4	5	6	7	8
A								
1	16	64	64	64	128	16	4	32
2	0	2	128	128	32	8	16	64
3	0	8	64	128	128	16	8	32
4	0	8	64	32	64	8	8	64
5	0	4	64	32	32	8	16	64
6	0	32	32	32	32	8	8	32
7	0	8	64	64	64	8	4	64
8	8	8	16	64	32	8	4	64
9	64	16	128	128	32	16	4	128
10	2	32	32	64	32	8	0	64
11	0	16	128	128	16	8	0	32
12	16	16	64	32	32	8	4	32
13	4	8	32	32	16	4	2	32
14	4	16	32	64	16	16	0	16
15	32	32	32	128	32	8	4	32
16	0	2	32	32	32	8	2	32
Average	9	17	61	72	45	9	5	49
B								
17	2	32	64	32	16	16	0	16
18	2	128	64	64	16	16	4	32
19	0	8	4[1]					
20	0	32	32	64	16	16	4	16
21	2	32[2]						
22	0	8	32	32	32	16	4	16
23	2	8	8	16	8	4	2	4
24	2	8	64	64	16	16	4	16
25	2	32	32	64	8	8	4	16
26	0	16	64	64	32	8	4	16
27	2	32	32	64	16	8	4	32
28	2	64	32	64	16	8	8	8
Average	1	33	39	53	18	11	4	24

TABLE 1.—*Continued*

Farm and animal No.	Titers (1:X)							
	Pre-vaccine	Booster	Postvaccination				Pre-booster	Annual booster
	1	2	3	4	5	6	7	8
C								
29	2	128		32	8	8[3]		
30	4		16[4]					
31	8	64	64	32	8	4	4	
32	2	128	64[5]					
33	0	8	32	32	32	16	4	32
34	0	8	8	8	8	16	4	32
35	0	64	32	16	16	8	2	32
36	0	128	32	32	16	8	0	32
37	0	128	128	32	32	16	0	32
38	0	128	64	64	16	8	2	64
39	2	128	64	64	32	8	2	64
40	2	256	64	64	32[3]			
41	64	256	32[3]					
42	0	2	2	2[6]				
43	2	2	8	4	0[7]			
Average	6	102	44	32	18	9	2	41
Overall Average	6	50	49	54	30	10	4	40

[1] Taken off the milking line.
[2] Dead on calving.
[3] Dried up.
[4] Accidental death after calving.
[5] Eliminated from herd.
[6] Sold, fistulated udder.
[7] Dead.

BLOOD-SERUM TITERS

The blood-serum antibody levels of the three groups of heifers appear in table 1. On farm A, the prevaccination titer of 16 heifers ranged from 1:0 to 1:64, with an average of 1:9. The postvaccination titer at the time of the booster shot ranged from 1:2 to 1:64 with an average of 1:17. The subsequent postvaccination average levels were 1:61, 1:72, 1:45, and 1:9, and for the prebooster 1:5. The annual booster was given. Four weeks later the average blood-serum titer rose to 1:49.

The 12 heifers on farm B had an average prevaccination blood-serum titer level of 1:1, with subsequent postvaccination average levels of 1:33,

1:39, 1:53, 1:18, and 1:11. The level at the annual prebooster time was 1:4, and 4 weeks later it was 1:24.

The average prevaccination blood-serum titer level for the 15 heifers on farm C was 1:6. The subsequent postvaccination average levels were 1:102,

TABLE 2.—*Staphylococcal antibody response in milk whey of 37 heifers vaccinated with Slanetz No. 7 Strain Toxoid, on 3 farms in Puerto Rico, 1961–62*

Farm and animal No.	Titers (1:X)							
	Pre-vaccine	Booster	Postvaccination				Pre-booster	Annual booster
	1	2	3	4	5	6	7	8
A								
1				16	32	0	2	8
2		256	512[1]					
3					16	0	0	4
4		512	128	16[1]				
6							8	64
7				256	32	0	0[1]	
8						4	2	128
9						4	4	16
10			64	256	16	0	2[1]	
11				32	16	4	4	128
12						16	8	16
13				8	32	8	4	8
14					8	4	8	4
15		512[2]						64
16		512	512	32[1]				
Average		448	304	88	22	4	4	44
B								
17			256	128		8	0	4
18			16	128	4	0	2[1]	
20			256	32		4	8	4
22			256	32	4	2	2[1]	
23			16	16		2	0	2
24			64	16	4	2	0	2
25		16	16	16	4	0[3]		0
26				16	256	4	0	2
27			256	128	128	0	2	2
28			64	16	32	2	2	4
Average		16	133	53	62	3	2	3

TABLE 2.—*Continued*

Farm and animal No.	Titers (1:X)							
	Pre-vaccine	Booster	Postvaccination				Pre-booster	Annual booster
	1	2	3	4	5	6	7	8
C								
29		4[3]			64	4	0[1]	
31				16	64	4	0[1]	
33				32	8	4	0	4
34				16	8	4	2	2
35			128	64	64	4[3]		8
36			4	4[3]		8	4	2
37		8	128	32	32[3]		0	8
38		8	256	32	32	4	2	32
39		0	256	32	32[3]		2	4
40			128	32	32[1]			
42				0	2[4]			
43				16[5]				
Average		5	150	25	34	5	1	9
Overall Average		201	175	51	38	4	3	21

[1] Dried up.
[2] Aborted, no milk.
[3] Not taken.
[4] Sold, fistulated udder.
[5] Dead.

1:44, 1:32, 1:18, and 1:9. The annual prebooster level was 1:2. The postbooster reading went up to 1:41.

MILK-WHEY TITERS

Table 2 shows the milk-whey titer levels observed at intervals corresponding with those mentioned for blood sera of the 43 heifers. The milk-whey titer level averaged 1:448 for the 4 heifers on farm A which had calved at the time of the booster injection. A general decreasing trend on the titer was observed thereafter down to 1:4 prebooster, *i.e.* before the annual booster sample. The level went up to 1:44 on the postannual booster observation. An average of eight milking heifers from farm A was included in these observations.

On farm B the postvaccination milk-whey antibody level was 1:16 for the single heifer which had calved at the time of the second or booster visit.

Subsequently, there was a peak of 1:133 on the third postvaccination observation and a steady decrease to a low of 1:2 prebooster immediately prior to the annual booster shot. The postannual-booster level was only 1:3. These observations are for an average of nine heifers.

An average postvaccination milk-whey titer level of 1:5 was observed on the four heifers from farm C which had calved by the time of the booster-shot visit. A peak reading of 1:150 was reached on the third postvaccinal samples. A decreasing level occurred thereafter with a low of 1:1 on the sixth postvaccinal sampling at the time of the annual-booster injection.

TABLE 3.—*Bacteriological results from milk samples of 38 cows vaccinated with Slanetz No. 7 Strain Staphylococcal Toxoid in Puerto Rico, 1961–62*

Sampling period No.	Period after vaccination	Streptococcal infections		Staphylococcal infections	
		Animals	Quarters	Animals	Quarters
	months	*Number*	*Number*	*Number*	*Number*
1	0	0	0	0	0
2	1[1]	4	7	1	1
3	3	3	8	0	0
4	5	5	9	0	0
5	7	12[2]	16[3]	4[2]	6[3]
6	9	0	0	0	0
7	11[1]	14[2]	28[4]	7[2]	9[4]
8	12	10[2]	13[5]	6[2]	7[5]

[1] Booster shots given after samples were taken.
[2] Includes animals having both infections.
[3] Includes 2 quarters having both infections.
[4] Includes 3 quarters having both infections.
[5] Includes 1 quarter having both infections.

The titer went to 1:9 a month after that injection. These observations included an average of 8 of the 12 heifers from farm C.

BACTERIOLOGICAL RESULTS

Quarter milk samples were studied bacteriologically. Table 3 shows the results of these observations.

Streptococci started appearing in the milk samples at the second sampling period, and increased steadily in numbers until the seventh sampling period, when 28 quarters were shedding streptococci.

One quarter was shedding staphylococci at the second sampling period, but 6 quarters of 4 heifers were shedding staphylococci at the fifth sampling, while these organisms were seen in 9 quarters of 7 heifers, and 7 quarters of 6 heifers at the seventh and eighth sampling periods, respectively.

These increases in number of staphylococci-positive milk samples appear to be in indirect relationship to the antibody titers. The increase in numbers of staphylococci-postive milk samples appears to correspond with a decrease in the antibody levels.

Figure 1 charts the range of the blood-sera antibody levels for the three herds, and the resulting average. Farm C heifers had a sharp increase after the vaccination dose but, contrary to expectations, had a noticeable decrease following the booster dose. Heifers on farms A and B had a rapid

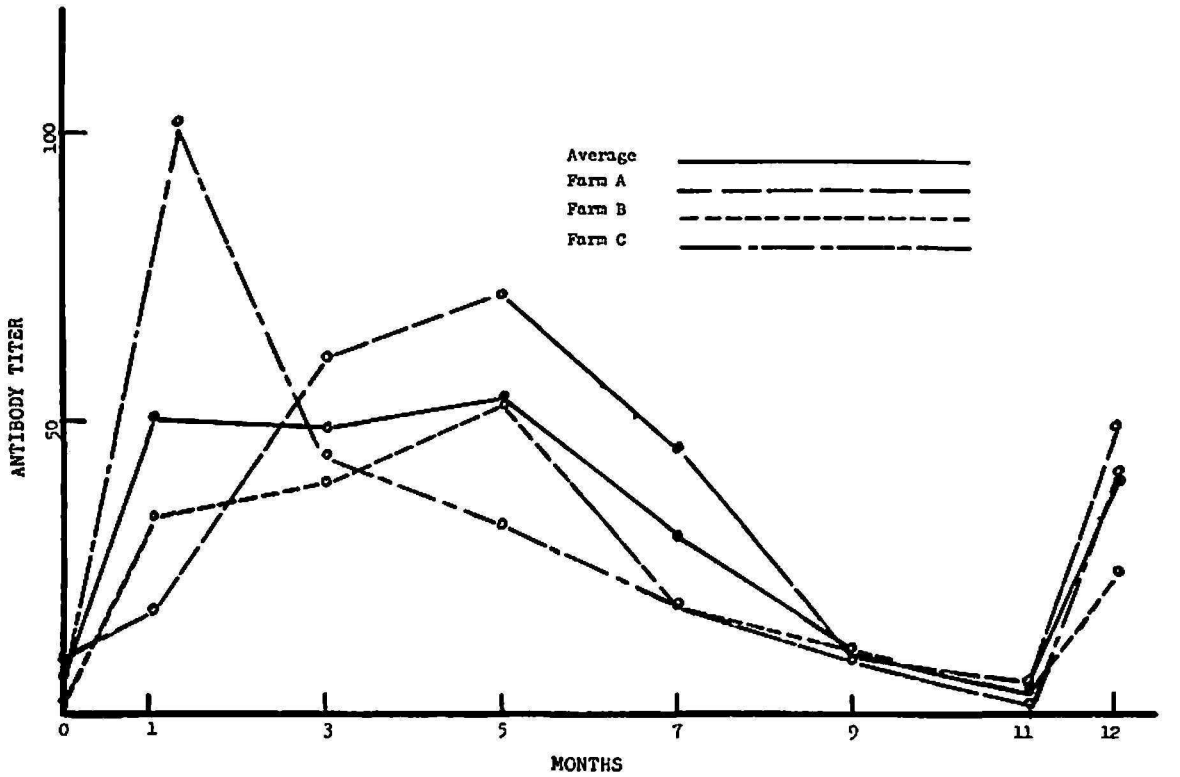

FIG. 1.—Staphylococcal antibody titer in blood sera of 43 heifers vaccinated with Slanetz No. 7 Toxoid on 3 farms in Puerto Rico, 1961–62.

increase in level after the vaccination dose and a slower increase for the two subsequent samples after the booster dose. After the third postvaccinal samples, or the second postbooster samples equivalent to a 5-month period, the average level for all three herds decreased steadily down to a low of around 1:5 on the 9th and 11th months, postvaccination, equivalent to the 8th and 10th month postbooster. One month after the annual booster, the levels had risen to an average of 1:40.

The range of milk-whey antibody titer levels is shown on figure 2. The postvaccination sample, taken at the time of the booster injection was very high (1:449) for the farm A heifers, and dropped to 1:304 after the booster dose.

Farms B and C animals had a low postvaccinal milk-whey titer that

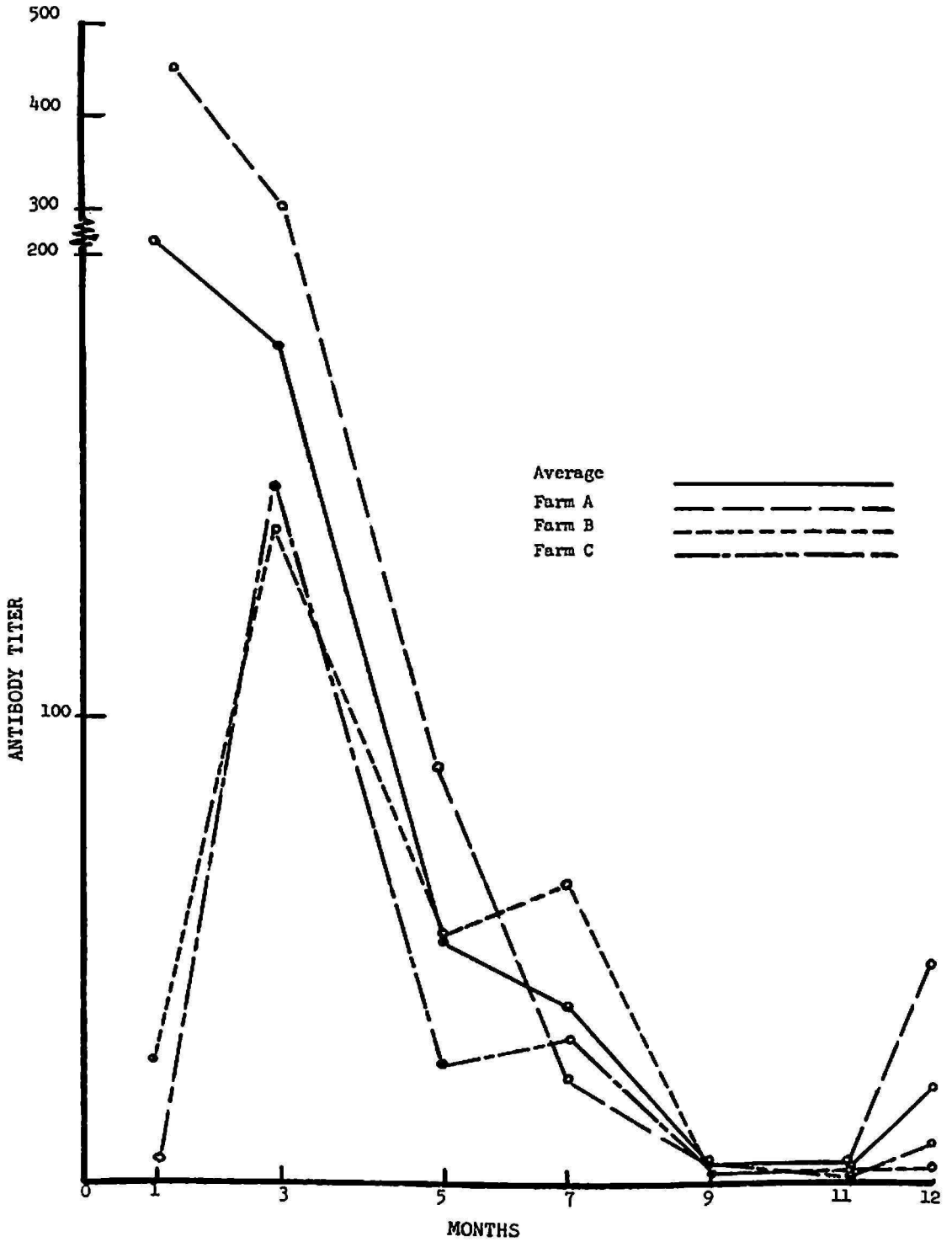

FIG. 2.—Staphylococcal antibody titer in milk whey for 43 heifers vaccinated with Slanetz No. 7 Toxoid on 3 farms in Puerto Rico, 1961–62.

went up sharply after the booster dose. Thereafter, the milk-whey titer level for all heifers from the herds went steadily and sharply down, (*3*) until a low of around 1:3 was reached 6 to 8 months after the booster dose. A terminal observation 1 month after the annual booster injection showed the level starting to increase.

DISCUSSION

The rapidity of decrease of antibody titers, both in the blood-sera and the milk-wheys of the 43 heifers from the 3 herds included in these observations, indicate that whatever protection these animals might have developed from the staphylococcal toxoid vaccination was of low intensity and short duration.

These observations show that the value of vaccination as a measure for the control of staphylococcal mastitis is not as yet clearly defined and needs further study under more controlled conditions.

In this respect, Slanetz *et al.* (*4*), in an article published while this paper was being prepared for print, pointed out that

> It is evident that further controlled studies are needed to more definitely establish the value of vaccines for the prevention and control of staphylococcic bovine mastitis. More information is needed on the various toxic factors related to the virulence of different strains of *Staph. aureus*, on the antigenic structure of these organisms, and on the various problems related to the development of specific antigenic preparations and their administration to dairy cattle.

Likewise, Blobel and Berman (*1*) concluded that

> Considerably better-controlled studies of the factors which play a determinant role in the ability of staphylococci to become established in the udder and to cause disease appear to be necessary. Until these substances have been properly identified, particularly with respect to their immunologic significance, attempts at large-scale vaccination as a means to control staphylococcic mastitis are likely to remain empirical and probably fruitless.

SUMMARY

Five milliliters of Slanetz No. 7 Staphylococcal Toxoid were injected intramuscularly into 43 pregnant heifers in 3 herds; a booster dose was given 1 month later. The antibody level in both the blood sera and the milk wheys of these heifers was determined through a 13-month period.

The average blood-serum titer reached a 1:50 level 1 month after vaccination, and remained around that level until the fifth postvaccinal month. Thereafter, a continuous and rapid decrease in titer was observed until the 11th postvaccinal month. An increase in titer was observed after the annual booster dose was given.

Although the staphylococcal antibody titers in milk wheys from two herds showed a peak in the third month, the levels went steeply down at the 5th-month observation. The general average trend was a steady and steep decrease in milk-whey antibody titer through the 11-month postvaccinal observation period, with a very feeble response after the annual booster dose.

RESUMEN

Cuarenta y tres novillas preñadas se injectaron intramuscularmente con una dosis de 5 ml. de toxoide estafilocóccico Slanetz núm. 7, seguida de una inoculación reforzadora un mes más tarde. Se determinaron los niveles de anticuerpos estafilocóccicos en el suero sanguíneo y en el suero lácteo de estos animales durante un período de observación de 12 meses.

La concentración promedio de anticuerpos en el suero sanguíneo alcanzó un nivel de 1:50 al mes de la vacunación, nivel que se mantuvo hasta el quinto mes. Subsiguientemente, se observó un rápido y continuo descenso de la concentración hasta el undécimo mes, notándose un ligero aumento después de la dosis reforzadora anual.

Aún cuando las concentraciones de los anticuerpos estafilocóccicos de los sueros lácteos de dos de los hatos alcanzaron su punto máximo durante el tercer mes, el nivel bajó precipitadamente al quinto mes. En general, la tendencia fue una de reducción continua y rápida en las concentraciones de los anticuerpos en el suero lácteo durante los once meses de observación, notándose una reacción muy leve después de la revacunación reforzadora anual.

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