

THE DURATION OF IMMUNITY TO PULPY KIDNEY DISEASE OF SHEEP

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Jansen (1960) showed that sheep were immune to pulpy kidney disease so long as the circulating epsilon antitoxin titre was 0·15 unit/ml serum or more. In 1966 he also found that a maximal response was obtained by injecting two doses, each containing 90 Lf, of alum-precipitated trypsinactivated toxoid (APT).

In the present investigation the duration of immunity following the primary, secondary and subsequent stimuli has been investigated.

MATERIALS AND METHODS

The APT was prepared by growing *Clostridium welchii* Type D in meat particle broth, toxoiding the culture filtrate and adding potassium alum to a final concentration of 1·5 per cent (Jansen, 1966). Experimental sheep were obtained from farmers who could guarantee that they had never been vaccinated against pulpy kidney disease. They were further tested for the presence of circulating epsilon antitoxin by injecting a mixture of 0·5 ml of the serum from each sheep and 3 MLD toxin intravenously into mice. Only sheep whose serum contained no antibody were used.

For determining the antitoxin content of the serum of vaccinated sheep, toxin neutralization tests were done in mice (Jansen, 1966). The quantity of antigen was expressed in Lf (one Lf equivalent to 1 IU of antitoxin).

RESULTS

In the first experiment, designed to establish the immunity resulting from a primary and a secondary stimulus, a group of 20 fully susceptible sheep was given two doses of APT containing 135 Lf per dose at a three weeks interval. The larger dose, in Lf, of toxoid was chosen to obtain information on the longest possible duration of protection. Three weeks after the second dose of vaccine the sheep were bled and their serum antibody titres determined. The results are recorded in Table 1.

From Table 1 it can be seen that by the ninth week after the secondary stimulus five sheep had serum antitoxin titres below the protective level, and by the twelfth week all but three would have been susceptible to pulpy kidney disease. They were consequently given a third injection twelve weeks after the second one. At one and at three weeks after the third injection and subsequently at three-weekly intervals they were bled and their circulating antibody titres determined. By the twelfth week after the third injection one sheep was susceptible, by the eighteenth week four, and by the twenty-first week, five.

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TABLE 1.—*The duration of immunity after the injection of vaccine containing 135 Lf antigen per dose*

Sheep No.	Weeks after second injection						Weeks after third injection															
	3		6		9		12		3		6		9		12		15		18		21	
1.....	4.00	5.00	2.00	0.80	10.00	3.30	2.00	0.67	0.50	0.44	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
2.....	2.00	0.57	0.20	0	20.00	10.00	6.70	3.30	2.00	2.00	6.70	2.00	3.30	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
3.....	1.00	0.33	0.10	0	20.00	6.70	2.20	1.00	0.80	2.00	2.20	2.00	1.00	0.80	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
4.....	2.00	0.80	0.20	0	50.00	20.00	10.00	5.00	2.00	10.00	5.00	5.00	2.90	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
5.....	10.00	2.50	1.00	0.57	50.00	20.00	10.00	5.00	2.00	5.00	2.00	5.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
6.....	1.30	0.57	0.22	0	1.30	1.00	0.50	0.27	0.25	1.00	0.50	0.27	0.27	0.25	0.27	0.25	0.27	0.25	0.27	0.25	0.27	0.25
7.....	20.00	2.00	0.57	0	25.00	20.00	10.00	5.00	2.00	10.00	5.00	5.00	2.90	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
8.....	2.20	1.00	0.30	0	10.00	3.30	1.00	0.40	0.27	1.00	0.40	1.00	0.40	0.27	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.20
9.....	5.00	0.50	0.20	0	20.00	6.70	2.90	2.00	1.30	2.90	2.00	2.90	2.00	1.30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.57
10.....	1.30	0.57	0.20	0	20.00	5.00	2.50	1.30	1.00	2.50	1.30	2.50	1.30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80
11.....	0.44	0.20	0.07	0	6.70	3.30	0.57	0.27	0.10	0.57	0.27	0.57	0.27	0.10	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0
12.....	1.30	0.30	0.10	0	6.70	3.30	1.00	0.33	0.20	6.70	3.30	1.00	0.33	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10
13.....	1.30	0.33	0.20	0	10.00	3.30	0.57	0.27	0.20	10.00	3.30	0.57	0.27	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10
14.....	1.00	0.29	0.10	0	4.00	2.00	0.40	0.27	0.20	4.00	2.00	0.40	0.27	0.20	0.27	0.20	0.27	0.20	0.27	0.20	0.27	0.24
15.....	2.00	0.29	0.10	0	10.00	3.30	1.00	0.33	0.20	10.00	3.30	1.00	0.33	0.20	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0
16.....	2.20	1.30	1.30	0.57	2.90	2.00	1.00	0.67	0.50	2.90	2.00	1.00	0.67	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.33
17.....	2.00	0.57	0.20	0	10.00	4.00	2.00	1.00	0.67	10.00	4.00	2.00	1.00	0.67	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.33
18.....	3.30	1.30	0.44	0	10.00	3.30	2.90	1.30	1.00	10.00	3.30	2.90	1.30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.40
19.....	3.30	2.00	0.67	0	33.00	6.70	2.50	1.30	1.00	33.00	6.70	2.50	1.30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80
20.....	2.00	0.30	0.20	0	25.00	6.70	1.00	0.57	0.40	25.00	6.70	1.00	0.57	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.33

Figures in columns represent antibody titre in units/ml serum
 The interval between the 1st and 2nd injections was 3 weeks
 The interval between the 2nd and 3rd injections was 12 weeks

TABLE 2.—*The duration of immunity after four injections of 135 Lf toxoid. The first two injections at three-weekly and the others at ten-weekly intervals*

Sheep No.	Weeks									
	1	2	3	6	9	12	15	18	21	1
1.....	1.00	0.80	0.80	0.57	0.33	0.24	0.20	0.10	0—	20.00
2.....	20.00	20.00	20.00	10.00	10.00	10.00	3.30	3.30	3.30	100.00
3.....	5.00	2.50	2.90	1.30	0.57	0.57	0.33	0.24	0.20	10.00
4.....	20.00	20.00	20.00	4.00	2.90	2.50	1.30	1.00	0.80	50.00
5.....	1.30	1.30	1.30	0.67	0.57	0.57	0.33	0.24	0.20	20.00
6.....	33.00	33.00	25.00	10.00	10.00	6.70	2.90	2.00	1.30	100.00
7.....	20.00	20.00	20.00	10.00	6.70	5.00	2.90	2.00	1.30	100.00
8.....	5.00	3.30	3.30	2.00	1.30	1.00	0.67	0.67	0.67	25.00
9.....	6.70	6.70	6.70	4.00	3.30	3.30	2.00	2.00	2.00	20.00
10.....	4.00	2.50	2.00	0.57	0.33	0.27	0.20	0.10	0—	25.00

Figures in columns represent ϵ antitoxin titres in units/ml serum

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These rather unexpected results were followed up in a group of ten sheep. Each was given a primary and a secondary stimulus of 135 Lf at a three weeks interval. Ten weeks after the second injection a third was given and ten weeks later a fourth. From one week after the fourth injection the sheep were bled at weekly intervals and the antibody titres of their sera determined. The results are recorded in Table 2.

The results given in Table 2 show that by the eighteenth week after the fourth injection the serum titre of two sheep was below the protective level, and that by the twenty-first week antitoxin was not detectable in their sera. The fall in antitoxin noticed in the previous experiment after the third injection also occurred in this experiment after the fourth injection, although after a somewhat longer period.

This experiment was repeated using booster doses of 25 Lf. Similar results were obtained and the titres after the third and fourth injections did not differ significantly from those obtained with the 135 Lf doses.

From results in Table 2 it will also be noticed that, when the sheep were given a booster injection of 135 Lf antigen twelve months after the primary stimulus, the titres promptly rose to a high level.

In the second experiment the duration of immunity after a booster injection given one year after the establishment of a basic immunity was investigated. Three different groups of sheep were treated as follows:

Group I

A basic immunity was established in this group by a primary and a secondary injection of 135 Lf antigen at three weeks interval. The sheep were left for a year and then given a booster injection of 135 Lf. They were bled one week after the booster injection and subsequently at three-weekly intervals. The results are summarized in Table 3.

TABLE 3.—*The e antitoxin titres of the serum of sheep which received two doses of 135 Lf antigen at 3 weeks interval and a booster dose of 135 Lf one year later*

Sheep No.	Weeks after booster				
	1	12	24	42	52
1.....	100·00	20·00	6·70	5·00	5·00
2.....	100·00	10·00	5·00	5·00	5·00
3.....	50·00	2·90	2·00	4·00	4·00
4.....	33·00	10·00	5·00	4·00	3·30
5.....	50·00	20·00	6·70	2·90	2·90
6.....	50·00	5·00	2·00	1·30	1·30
7.....	20·00	2·90	2·50	2·00	2·00
8.....	10·00	2·00	1·00	1·30	1·30
9.....	20·00	10·00	4·00	2·00	2·00
10.....	25·00	2·00	1·30	0·67	0·67
11.....	25·00	6·70	4·00	1·00	1·00
12.....	20·00	4·00	2·90	0·67	0·67
13.....	20·00	2·90	2·00	0·67	0·57
14.....	100·00	4·00	2·90	0·67	0·67

Figures in columns represent antibody titres in units/ml serum

From the results in Table 3 it can be seen that the titres increased to a very high level after the booster dose, declined fairly steeply during the first twelve weeks and thereafter gradually to the 52nd week. At the last sampling, i.e. after one year, all animals were still protected.

Group 2

To find if a booster dose of only 25 Lf antigen given after the establishment of a sound basic immunity would have as lasting an effect as one of 135 Lf, a group of ten sheep were basically immunized in the same way as those of Group 1. One year after the first injection each received a booster of 25 Lf. The results are given in Table 4. (Three sheep died of an intercurrent infection.)

TABLE 4.—*The antibody titres in sheep receiving two doses of 135 Lf antigen at 3 weeks interval and a booster dose of 25 Lf one year later*

Sheep No.	Weeks after booster				
	1	9	21	33	52
1.....	20·00	5·00	2·90	2·90	2·00
2.....	33·00	10·00	4·00	3·30	2·20
3.....	33·00	10·00	4·00	3·30	2·90
4.....	2·50	0·80	0·27	0·27	0·20
5.....	5·00	2·00	0·67	0·67	0·50
6.....	250·00	50·00	25·00	20·00	20·00
7.....	20·00	10·00	6·70	5·00	4·00

Figures in columns represent antibody titres in units/ml serum

The sheep of this group were as well protected after one year as those of Group 1.

Group 3

The sheep of this group received 25 Lf for their primary, secondary and booster injections. One sheep died during the course of the experiment. The results are given in Table 5.

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TABLE 5.—The antibody titres in sheep receiving two doses of 25 Lf antigen at 3 weeks interval and 25 Lf one year later

Sheep No.	Weeks after booster				
	1	9	21	33	52
1.....	100.00	33.00	10.00	6.70	5.00
2.....	20.00	6.70	4.00	3.30	2.50
3.....	100.00	33.00	20.00	20.00	6.70
4.....	250.00	25.00	4.00	3.30	2.50
5.....	0.57	0.40	0.27	0.20	0.10
6.....	20.00	2.90	2.00	1.30	1.30
7.....	25.00	33.00	20.00	20.00	20.00
8.....	50.00	10.00	3.30	3.30	2.20
9.....	2.90	1.30	0.44	0.44	0.25

Figures in columns represent antibody titres in units/ml serum

All, but sheep No. 5, were fully protected after one year. When these results are compared with those obtained in Group I, no statistically significant difference can be detected either by the U-test of Wilcoxon or by the t-test at the 5 per cent level ($Z_u = -0.6612$; $t = 0.5213$).

From Fig. 1, where the log of the geometric mean value of the titres obtained for each group at each sampling time is plotted against time, it can be seen that there is no difference in the response of the three groups.

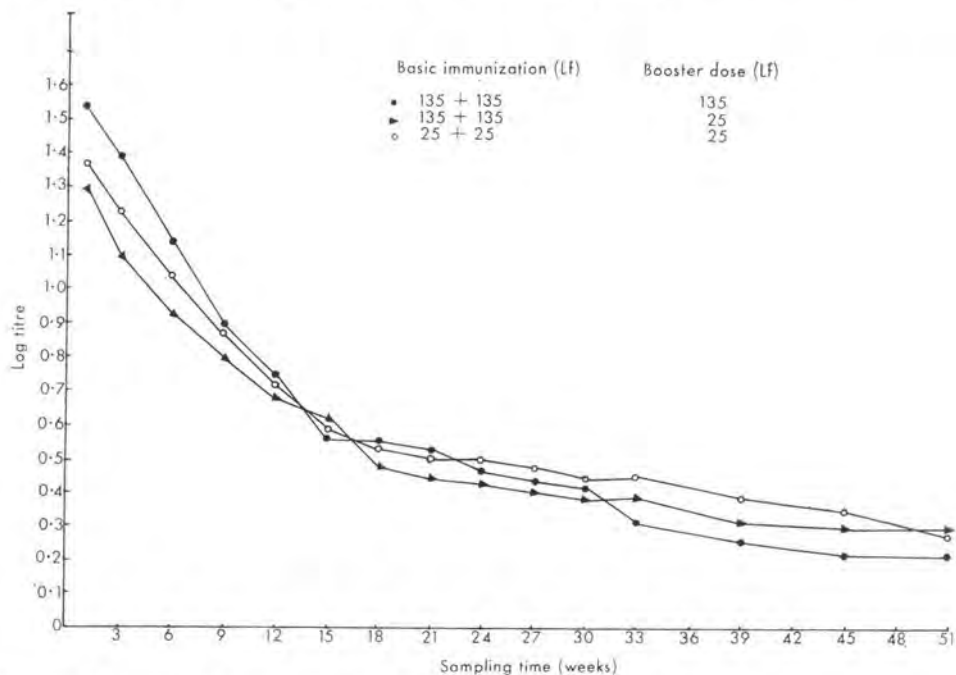


FIG. 1.—Response of sheep with varying basic immunity to various booster stimuli

A reasonable conclusion from these results is that, when a sound basic immunity is established, e.g. with two doses of 135 Lf, the booster dose given one year later may be large (135 Lf) or small (25 Lf) for the same degree of protection over one year after the booster.

To investigate the response to a second booster dose of antigen a group of sheep received a primary and a secondary stimulus of 135 Lf at an interval of three weeks. One year after the primary injection and again one year later they received an injection of 135 Lf. They were bled one week after the last injection and every six weeks thereafter and the titre of their sera was determined at every bleeding. Table 6 records the results.

TABLE 6.—*The serum antibody level in sheep after two booster doses of 135 Lf*

Sheep No.	Weeks after second booster					
	1	21	52	94	130	160
1.....	250·00	100·00	50·00	25·00	20·00	20·00
2.....	100·00	20·00	20·00	10·00	4·00	4·00
3.....	100·00	20·00	20·00	2·00	1·30	1·00
4.....	50·00	20·00	20·00	10·00	6·70	5·00
5.....	100·00	20·00	10·00	6·70	5·00	3·30
6.....	250·00	25·00	25·00	6·70	4·00	2·90
7.....	100·00	6·70	4·00	10·00	6·70	4·00
8.....	50·00	5·00	5·00	2·00	1·00	1·00
9.....	50·00	20·00	10·00	10·00	5·00	4·00
10.....	100·00	10·00	10·00	2·00	2·00	1·00
11.....	50·00	4·00	2·50	2·20	1·30	1·30
12.....	50·00	3·30	2·50	2·00	1·30	1·30
13.....	100·00	4·00	2·90	2·00	1·30	0·67
14.....	33·00	2·20	2·00	20·00	10·00	6·70

Figures in columns represent antibody titres in units/ml serum

From these results it can be seen that the serum antibody titre is very high in every sheep one week after the second booster dose, declines slowly and at 160 weeks is still higher than the minimum protective level.

An obvious feature of the results obtained thus far is the decline in antibody level where some sheep become fully susceptible twelve weeks after the secondary stimulus, some 15 weeks after the first booster stimulus and some 18 weeks after the second booster (the boosters being given within twelve months of the primary stimulus). The finding of Jansen (1962) that the immune response to an injection of epsilon toxoid is prolonged when the toxoid is emulsified in Freund's complete adjuvant (Freund, Thomson, Hough, Sommers & Pisani, 1948) seemed to offer a possible solution. Three groups of fully susceptible sheep were given 5, 25 and 50 Lf respectively of toxoid in Freund's adjuvant. They received but one dose consisting of the required number of Lf in 0·5 ml saline emulsified in 2 ml adjuvant.

For the first five weeks after the injection the sheep were bled at weekly intervals and thereafter at four-weekly intervals. Table 7 summarizes the results.

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TABLE 7.—*The antitoxin titres in the sera of sheep that received toxoid in Freund's adjuvant*

Dose in Lf	Sheep No.	Weeks after injection					
		2	4	30	50	82	106
5.....	1	2.90	5.00	4.00	4.00	2.00	2.00
	2	2.00	50.00	2.00	2.00	1.30	1.00
	3	10.00	100.00	2.00	20.00	6.70	6.70
	4	0.10	2.00	1.00	1.00	0.67	0.67
	5	1.30	50.00	10.00	10.00	4.00	2.50
	6	0	10.00	0.67	0.57	0.33	0.25
	7	0	1.00	2.00	2.00	0.67	0.33
	8	6.70	100.00	6.70	6.70	4.00	4.00
	9	0	0.80	0.44	0.44	0.44	0.29
25.....	1	20.00	100.00	2.00	2.00	2.00	0.80
	2	0.50	50.00	20.00	10.00	5.00	2.90
	3	2.00	20.00	1.00	1.00	0.44	0.29
	4	1.30	25.00	5.00	5.00	2.20	2.00
	5	1.30	20.00	2.50	2.00	1.30	0.80
	6	0	5.00	2.90	2.20	1.30	1.30
	7	10.00	250.00	6.70	6.70	6.70	5.00
	8	33.00	50.00	10.00	5.00	4.00	2.90
	9	0.10	3.30	3.30	2.00	2.00	1.00
	10	0	1.00	2.50	2.00	0.67	0.57
50.....	1	5.00	10.00	20.00	10.00	10.00	5.00
	2	6.70	250.00	6.70	6.70	3.30	2.90
	3	1.00	50.00	50.00	50.00	20.00	20.00
	4	5.00	33.00	10.00	10.00	6.70	5.00
	5	2.50	100.00	50.00	50.00	33.00	20.00
	6	6.70	25.00	2.00	2.00	2.00	2.00
	7	5.00	100.00	7.50	5.00	4.00	3.30
	8	5.00	100.00	25.00	20.00	10.00	6.70
	9	0.07	2.90	10.00	6.70	2.90	2.00

Figures in columns represent antitoxin titres in units/ml serum

Although some of the sheep had no detectable circulating antitoxin two weeks after the injection, most had high levels at four weeks. After two years all the sheep were fully protected.

To determine if a similar result could be obtained with Freund's incomplete adjuvant, the experiment was repeated using 0.5 ml toxoid (suitably diluted) in 2 ml of a mixture of Arlcel A and Bayol F in the proportion as they occur in Freund's adjuvant, but without the killed mycobacteria. The results are given in Table 8.

TABLE 8.—*Antitoxin titres of the sera of sheep that received toxoid in Freund's incomplete adjuvant*

Dose in Lf	Sheep No.	Weeks after injection					
		2	4	30	50	82	106
5.....	1	0	0	0·67	0·67	0·57	0·57
	2	0	0·27	1·00	0·57	0·25	0·22
	3	0·10	2·90	10·00	4·00	2·00	1·30
	4	0	0	2·00	2·00	1·30	1·30
	5	0	0·36	0·67	0·50	0·29	0·22
	6	0	0·57	2·20	1·30	0·67	0·50
	7	0	0	2·00	2·00	2·00	1·30
	8	0·57	20·00	10·00	10·00	6·70	5·00
25.....	1	0·67	3·30	5·00	5·00	5·00	5·00
	2	0·50	1·00	0·67	0·44	0·25	0·20
	3	0·07	1·00	2·50	2·50	2·00	1·00
	4	2·50	10·00	10·00	6·70	2·90	2·50
	5	1·00	2·20	1·30	0·80	0·36	0·30
	6	2·50	20·00	20·00	20·00	20·00	10·00
	7	6·70	100·00	33·00	33·00	20·00	10·00
	8	1·00	2·00	1·30	1·30	1·30	1·00
50.....	1	5·00	100·00	10·00	10·00	6·70	5·00
	2	0	2·00	3·30	3·30	2·20	2·20
	3	0·20	3·30	5·00	5·00	2·50	2·00
	4	0·67	20·00	33·00	20·00	5·00	4·00
	5	0·57	20·00	5·00	3·30	2·00	1·30
	6	1·30	1·30	0·50	0·40	0·36	0·36
	7	2·00	2·20	20·00	20·00	6·70	4·00
	8	0·25	20·00	10·00	10·00	3·30	3·30
	9	2·20	20·00	25·00	25·00	10·00	10·00
	10	2·20	5·00	2·90	2·90	3·30	3·30

Figures in columns represent antitoxin titres in units/ml serum

The serum titres were lower than those obtained in the previous experiment, but in spite of this all the sheep were immune two years later. Half the number of sheep receiving 5 Lf took more than four weeks to become immune, but those of the other two groups were immune at four weeks.

DISCUSSION

These experiments show that the serum antitoxin titres of some sheep were below the protective level nine weeks after the secondary stimulus. This happened even when the quantity of antigen contained in the primary and secondary injections was large (135 Lf). When a third injection was given twelve weeks after the second all but one of 20 sheep were protected for a further period of 15 weeks. If a fourth injection was given ten weeks after the third, all the sheep were protected for a period of 15 weeks after the last injection.

These results, no doubt, account for the complaints by farmers of losses from pulpy kidney disease in spite of having vaccinated their sheep three months previously.

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When sheep have a sound basic immunity (such as that produced by primary, and secondary stimuli of 135 Lf in APT), the immunity following a booster dose given twelve months after the primary stimulus will remain at a protective level for twelve months. The quantity of antigen used in the booster injection is of secondary importance because 25 Lf gives results comparable with those obtained with 135 Lf.

However, when sheep are given primary and secondary stimuli of 135 Lf and, one and two years afterwards, booster injections of 135 Lf, they will remain fully immune for at least three years subsequent to the last injection. Whether the same applies when the primary and secondary stimuli are 135 Lf and both booster doses are 25 Lf is still under test, but there is little reason to believe that it will be different. This finding is significant to both farmers and to veterinarians investigating outbreaks of disease among sheep. Farmers have the assurance that sheep vaccinated for three consecutive years are immune for virtually the rest of their lives, and need expend no further money and effort in re-vaccinating them and veterinarians can be certain that sheep immunized in this way will not die of enterotoxaemia.

The rather sharp decline in titre occurring after the secondary stimulus and any booster doses given within one year of the primary injection, offer difficulties in badly infected areas. A farmer is not always sure of the degree of protection of his sheep without going to the trouble of having the antibody titre of a representative sample of his flock determined. Repeated injections for the sake of playing safe are expensive and laborious. It has been proved, however, that the difficulty can be effectively overcome by using toxoid in Freund's adjuvant. The complete adjuvant contains killed mycobacteria, the large-scale production of which is costly and time-consuming. But the finding that toxoid emulsified in Freund's adjuvant without mycobacteria can stimulate a protective level of immunity lasting for at least two years, offers a feasible solution.

A dose of vaccine containing 50 Lf antigen in Freund's or other adjuvant need be used only once in two years while APT may have to be injected four times to give the same sort of protection. This fact has an important bearing on the cost of protecting sheep.

SUMMARY

It was shown that the immunity induced by a primary and secondary dose of antigen varying from 25 to 135 Lf APT, protected sheep for only about nine weeks. Any booster dose given before a year after the primary injection resulted in protective titres lasting for periods up to about 20 weeks.

When a sound basic immunity is established, e.g. with two doses of 135 Lf, sheep are protected to the same extent by a large (135 Lf) or small (25 Lf) booster dose given one year after the primary injection. The protection thus effected lasts for at least one year.

When sheep with a sound basic immunity receive a booster of 135 Lf after one year and a second booster of 135 Lf after a further year, they are protected against pulpy kidney disease for at least three years following the last injection.

A single injection of epsilon toxoid in Freund's complete or incomplete adjuvant protects sheep for at least two years.

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