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# Some Remarks on Black Quarter Vaccines.

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For some years past very little has been published in South Africa on blackquarter vaccines. A number of advertising leaflets have been issued by commercial firms supplying vaccines. Such leaflets are not always reliable. Some experimental evidence on the efficacy of the vaccines which are being used at the present time will, therefore, be of interest.

These vaccines are of two types, the cultural aggressins and the precipitated bacterins.

Cultural aggressins are clear, germ-free filtrates of cultures of Clostridium chauvoei. This type of vaccine was issued by the Onderstepoort Laboratory from 1922 to 1934.

Bacterins are vaccines which contain, in addition to the antigenic substances found in the filtrates, the dead blackquarter bacteria. At present such bacterins are treated with alum or aluminium hydroxide. A heavy deposit is thereby produced, which contains the antigenic substances. It is assumed that these are continuously being liberated in the animal body in small quantities over a considerable period of time. In this way the effect of numerous vaccinations with very small doses is obtained, resulting in a much stronger immunity.

## REACTIONS PRODUCED BY THE VACCINE.

Cultural aggressins usually cause a very mild reaction, seldom amounting to more than a slight rise of temperature and a moderate lameness. The precipitated bacterins produce a more marked reaction. This is to be expected, when one considers that they contain a large quantity of solid material, with an irritating substance in it, such as alum or aluminium hydroxide. In addition to a rise of temperature and at times a fairly severe lameness, one may notice a swelling at the site of inoculation. It appears shortly after vaccination as a subcutaneous oedema in the form of a flat swelling, and in the course of two to four weeks becomes hard and fibrous and often takes the shape of a nodule which may persist for many months. As a rule, however, such indurations do not seem to worry the animals. The following may serve as an illustration.

All cattle of a susceptille age on our experimental farm Kaalplaats were inoculated with Onderstepoort alum-precipitated blackquarter bacterin. The stockman reported that there were no reactions. Nearly 200 animals were then examined by the writer with the following results: Of 92 calves which had been vaccinated for the first time, 5 showed very small hard nodules. Of 100 animals vaccinated for the second time 6 had developed similar lesions. In 6 Jersey cows this type of vaccine produced similar swellings which were, however, distinctly painful. The preparations used were two aluminium hydroxide-adsorbed bacterins, one imported and the other one locally prepared, the third one being an alum-precipitated vaccine of an Onderstepoort routine batch. Each was given to two animals subcutaneously in doses of 5 c.c. No difference was noticed between the reactions caused by the three vaccines. On the other hand, experiments on 16 sheep showed that aluminium hydroxide bacterin produced a noticeable milder reaction than did alum-precipitated vaccine prepared at the same time from the same culture.

In the following tests all vaccines were injected subcutaneously and the virulent test cultures intramuscularly.

### MAXIMUM PROTECTION AFFORDED.

Cultural Aggressins.

Repeated experiments with Onderstepoort filtrates has shown that good batches of blackquarter filtrates should protect against 20 minimal lethal doses (m.l.d.) of culture. Recently an imported cultural aggressin was tested. Eight sheep were injected, each with 5 c.c. of vaccine, and tested 3 weeks later against varying doses of virulent culture (two sheep with each dose) with the following results:—

Table 1. (Experiment S. 6778.)

Dose of culture	0.2 c.c.	0.6 c.c.	2 c.c.	5 c.c.	10 c.c.
Results	LL	. ++	LL	+L	++
Controls	0.05 c.c.+		0·1 c.c.++		

<sup>+</sup> Indicates that the animal died of blackquarter.
L Indicates that the animal survived the experiment.

The m.l.d. would be 0.05 c.c. and the test doses would therefore correspond to 4, 12, 40, 100 and 200 m.l.d.

The susceptibility of Merino sheep to blackquarter varies considerably, and so does the ability to produce antibodies. Therefore, apparently conflicting results are sometimes obtained and no conclusion can be drawn unless a larger number of sheep is used. In the above experiment it may be assumed that the 2 sheep receiving 0.6 c.c. of culture showed a subnormal antibody development, and the protection given by the vaccine probably corresponded to about 40 m.l.d. (2 c.c. of culture).

Alum Bacterins.

An important alum-adsorbed bacterin was tested at the same time and in the same way. No sheep were lost; it protected against at least 200 m.l.d. (Exp. S. 6779).

Locally prepared vaccines of the same type were tested with similar results. The dose of vaccine was 5 c.c.

TABLE 2.

Dose of c	ulture	2 c.c.	5 c.c.	10 c.c.			
Results:	Batch 25 (Exp. S6988)	LL	LL	LL			
	Controls	0·1 e.e. ++	0·2 cc ++				
	The m.l.d. would be about 0.1 c.c. of culture and the inoculated sheep surviv 100 m.l.d.						
-							
	Batch 49 (Exp. S7138)			LL			
	Batch 49 (Exp. S7138)	-		LL			

#### DURATION OF IMMUNITY.

It is generally accepted that the immunity from vaccination in blackquarter decreases in the course of time. It is therefore recommended that re-vaccination be carried out during the susceptible period of life at intervals of 9-12 months. But certain commercial firms claim that the vaccine sold by them protects for life. The following experiments were carried out to obtain information on this point:—

Five sheep were vaccinated each with 5 c.c. of an imported cultural aggressin (Exp. S 6771) and another 5 with the same dose of an imported alum-precipitated bacterin (Exp. S.6772). All were tested 6 months later, each sheep receiving 0.2 c.c. of culture. All the aggressin sheep died; all the bacterin sheep survived. Controls: 0.1 c.c. +L, 0.2 c.c. ++.

The aggressin sheep were no longer protected against a dose of what was probably 1-2 m.l.d.

As a result of this test, showing the superiority of the bacterin, the experiments with cultural aggressin were discontinued. All further work was done with either imported or locally prepared precipitated bacterins.

Twelve sheep were injected each with 5 c.c. of imported alum adsorbed bacterin and tested at various intervals:

Table 3. (Experiment S. 6772.)

Number of Sheep Tested.	Time after Vaccination.	Dose of Culture.	Results.	Controls.	Protection against About.
4	6 months	1 e.e.	LLL+	0·1 c.c.++, 0·2 c,c.++	10 m.l.d.
4	8½ months	0.5 e.c.	L+++	0.05 c.c.++, 0.1 c.c.+L	Less than 10 m.l.d.
4	10 months	0 · 2 e.c.	++++	0.025 e.e.LL, 0.05c.e.++	Less than 4 m.l.d.

A similar experiment was made with Onderstepoort alum-precipitated bacterin (Batch 25):—

Table 4. (Experiment S. 6989.)

Number of Sheep Tested.	Time after Vaccination.	Dose of Culture.	Results.	Controls.	Protection against About.
4	4 months	1 e.e.	LLLL	0·1 c.c. ++, 0·5 c.c. ++	10 m.l.d.
4	10 months	0·2 c.c.	LL++	0.05 e.e. ++, 0.1 e.e. ++	4 m.ld.: unsatisfactory.
4	12 months	0.05 c.c.	LLLL	0.025 c.c. LL, 0.05 c.c.++	1 m.l.d.

N.B.—It will be remembered that this batch of vaccine was shown to protect sheep against at least 100 m.l.d., when tested a short time after vaccination.

These experiments show conclusively that the immunity in sheep decreases during the course of a year. In both cases there was a protection against ten m.l.d. six months after immunization. But ten months after vaccination the immunity against four m.l.d. was unsatisfactory. In the second experiment, however, there was still a definite protection against one m.l.d. twelve months after vaccination:

One would expect the immunity in cattle to decrease in the same way, but not enough experiments have been carried out to allow of a definite conclusion being reached.

#### KEEPING PROPERTIES.

It is customary to stipulate the date up to which the blackquarter vaccine is expected to be fully satisfactory. We favour the use of fresh vaccine and discourage farmers from storing it over a long period. Therefore a rather early date of expiration is given. Commercial firms find it more convenient to allow longer periods for the use of these products. A few experiments were made to obtain more accurate information on the deterioration of blackquarter vaccine after various periods of time.

Four sheep were vaccinated with an imported alum-adsorbed bacterin two weeks before its expiring date. It had been stored in the dark at room temperature for nearly a year. The dose given was 3 c.c. as recommended in the accompanying instructions. After 24 days the sheep were tested against 1 c.c. of culture.

### TABLE 5.

# (Experiment S. 7233.)

Results: L L L+. Controls: 0.05 c.c.++, 0.1 c.c. LL.

This protection against what was probably 20 m.l.d. is considered satisfactory.

Some Onderstepoort vaccine, batch 25, which had also been stored at room temperature, protected from strong light was tested as follows:—

Six months after preparation 10 sheep were injected; 5 received 2 c.c. and the other 5 got 5 c.c. of vaccine.

Table 6. (Experiment S. 6970.)

Dose of culture	1 e.c.	2 c.c.	5 c.c.
Sheep vaccinated with 2 c.c	L	LL	++
Sheep vaccinated with 5 c.c	L	LL	+ L
Controls	0·1 c.c. ++,	0.5 c.c. ++	

The protection against 20 m.l.d. was still satisfactory.

Six months later the same vaccine was re-tested in the same way: -

Table 7. (Experiment S. 6970.)

Dose of test culture	l e.c.	2 c.c.	5 c.c.
Sheep vaccinated with 2 c.c	++	+ L	++
Sheep vaccinated with 5 c.c	LL	LL	+ L
Controls	0·1 c.c. + L	0.2 e.e. + L	

This shows that the immunizing power, at least of the smaller dose, had decreased very considerably. Two other batches (48 and 50) were re-tested when a year old. (Exp. S. 7337.) A dose of 2 c.c. of vaccine protected all 8 sheep (4 for each batch) against 1 c.c. of culture; controls 0.1 c.c. + +, 0.2 c.c. + +. There was complete protection against 10 m.l.d. Higher doses were not tried.

#### CONCLUSION.

The experiments detailed here tend to the following conclusions:-

- (1) Slight persistent local reactions may sometimes be expected to follow inoculation with blackquarter vaccines precipitated by alum or aluminium hydroxide.
- (2) Blackquarter vaccines containing alum afford a very strong protection against the disease.
- (3) The immunity from the best vaccines at present in use becomes gradually reduced after six to twelve months.
- (4) There is probably some diminution in the efficiency of all types of blackquarter vaccine after 6-12 months, but a fairly good protection was still afforded by one-year-old vaccine.