

## Avirulent Anthrax Vaccine.

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A FEW years ago a report was published on field tests with anthrax vaccines made from avirulent unencapsulated strains [Sterne (1939)]. The vaccine was subsequently brought into regular use, and at present more than six million doses are used annually. Altogether, more than 30 million doses have been used.

The results have equalled expectations and far surpass those obtained with spore vaccines of the Pasteur type. In the last three years, for example, during which more than fifteen million animals have been inoculated, there have been only twenty complaints to the laboratory.

Four (4) were outbreaks of *Cl. chauvoei* infection following vaccination.

Four (4) were of deaths following inoculation. In all, about 14 animals died. The association of inoculation and death was fortuitous.

Ten (10) complaints were of abscesses, swellings, or severe reactions following inoculation.

Two (2) complaints were of insufficient immunity. In one case a farmer lost two animals two months after immunization. The description of the post mortem made it fairly clear that the deaths were not due to anthrax. Another farmer lost an animal two months after immunization. This was definitely anthrax.

It is not suggested that every misfortune is reported. Complaints are, however, a small fraction of the number we used to get.

### PREPARATION OF THE VACCINE.

Apart from slight modifications, the method of obtaining avirulent variants is the same as that described in earlier papers [Sterne (1937a, 1937b)]. The strains are preserved by drying in the frozen state. Stock cultures are prepared from the ampoules of dried spores and kept on agar slants under sterile liquid paraffin.

A batch of vaccine is prepared by sowing a broth suspension of 24-hour agar cultures on to Woodhead flasks of Gladstone and Fildes' (1940) casein hydrolysate agar. We simply mix the hydrolysate, the tryptic digest of casein, and the yeast brew with water and agar. Nothing else is added. This medium is very simple to prepare, very cheap and very satisfactory. Sporulation is usually complete after 48 hours and the batch is harvested on the third day by washing off with physiological saline. Twice the amount by weight of glycerine is then added. This stock suspension is adjusted to contain  $6 \times 10^8$  spores per cubic centimetre. The immunizing power is tested on guinea-pigs as follows:—

AVIRULENT ANTHRAX VACCINE.

Six (6) guinea-pigs receive 0.01 c.c., and six (6) guinea-pigs 0.001 c.c. of the stock suspension subcutaneously on the abdomen. Three weeks later, these guinea-pigs, together with six controls receive 0.1 c.c. of a glycerine-saline spore suspension of a Pasteur II strain subcutaneously in the hind leg. This dose of the Pasteur strain contains about 100 guinea-pig lethal doses. It is important that the test should be given well away from the site of the immunizing dose to avoid non-specific effects. If the guinea-pigs immunized with the 0.01 c.c. dose of vaccine resist the test dose, the vaccine is passed for a field test. The field test is merely a precaution. Several hundred animals are injected with a dose of the vaccine four times the strength of that finally issued. So far no batch has failed in this test.

Below is a typical laboratory test. It is an average test; neither very good nor very bad. Duplicate titrations of the same batch agree reasonably well.

BATCH 15.

No. of Guinea Pig.	Amount of Concentrated Vaccine.	Date.	Result.	Amount of Test Dose (100 M.I.D.)	Date.	Result.
1.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
2.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
3.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
4.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
4.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
5.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
6.....	0.01 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
7.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
8.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
9.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
10.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	L
11.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	†2*
12.....	0.001 c.c.	22/11/43	L	0.1 c.c.	11/12/43	†2
13.....	Nil	—	—	0.1 c.c.	11/12/43	†2
14.....	Nil	—	—	0.1 c.c.	11/12/43	†2
15.....	Nil	—	—	0.1 c.c.	11/12/43	†2
16.....	Nil	—	—	0.1 c.c.	11/21/43	†2
17.....	Nil	—	—	0.1 c.c.	11/12/43	†2
18.....	Nil	—	—	0.1 c.c.	11/12/43	†3

L = Lived.

\* † 2 = dead by second day.

Before issue, the vaccine is diluted  $\frac{1}{50}$  with 50 per cent. glycerine-saline and  $\frac{1}{8}$  per cent. Evan's saponin or  $\frac{1}{4}$  per cent. Merck's saponin. The strengths of different brands of saponin can be compared by injecting serial dilutions intra-dermally into guinea-pigs. The dose of vaccine used is one cubic centimetre, containing about 10 million spores.

*Response of different animals to the vaccine.*

The same vaccine is now used for all animals. Cattle and sheep react very mildly. Horses react more vigorously; but no farmer has yet complained about the reactions. Goats, in general, show little reaction; but now and then may show large swellings, and deaths have been reported. Work

done on guinea-pigs indicates that the oedema-producing property of our strains is not entirely a function of the immunizing power, and that the medium used is an important factor. The casein medium has proved the least provocative in this respect.

Guinea-pigs develop a good immunity within a few days, and field results indicate that the response of cattle is equally fast. In horses, on the other hand, a sound immunity takes a month to six weeks to develop.

#### SUMMARY AND CONCLUSIONS.

More than 30 million doses of anthrax vaccine made from avirulent unencapsulated variants have been used in South Africa. The same vaccine is used for all domestic animals, and the results far surpass those obtained with the Pasteur type of spore vaccine. The strains sporulate very readily, and their immunizing power is easily tested in guinea-pigs. It has been found possible to dispense with large-animal immunity tests. Thus these strains lend themselves very well to laboratory manipulations.

#### REFERENCES.

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