

## The use of Anthrax Vaccines Prepared from Avirulent (Uncapsulated) Variants of *Bacillus anthracis*.

By MAX STERNE, Section of Bacteriology, Onderstepoort.

A TECHNIQUE has been described (Sterne 1937a, 1937b) for consistently obtaining avirulent, immunogenic variants from virulent anthrax strains. The results of field and laboratory tests with vaccines prepared from such variants will be described in this paper.

### EXPERIMENTS.

*To Find Out How Long Suspensions of Avirulent Variants Retained their Immunizing Power.*

Avirulent variants 22A<sub>2</sub>, 33B<sub>2</sub>, 34A<sub>2</sub>, and 34F<sub>2</sub> were grown on nutrient agar until fully sporulated. The spores were suspended in 50 per cent. glycerine-saline. Their concentration was approximately 300,000 per c.c., which is, roughly, that in vaccine prepared here ordinarily. Sheep were inoculated, subcutaneously, with 1.0 c.c. Table I gives the results of these laboratory tests.

TABLE I.

Avirulent Variant	Isolated from Virulent Strain on	Vaccine prepared from Avirulent Variant on	Sheep Immunized with Vaccine on	RESULT OF TEST WITH VIRULENT SPORES ON	
				Immunized Sheep.	Normal Controls.
22A <sub>2</sub> .....	5.2.36	13.7.36	31.7.36	6/6	0/4
			22.9.36	6/6	0/4
33B <sub>2</sub> .....	27.7.36	13.8.36	14.8.36	5/6	0/4
			7.8.37	5/6	0/4
34A <sub>2</sub> .....	7.8.36	13.8.36	14.8.36	5/6	0/4
			7.8.37	6/6	0/4
34F <sub>2</sub> .....	10.8.36	10.6.38	14.6.38	6/8	0/2
			12.6.39	6/6	0/2

5/6 = 5 sheep out of 6 lived.

The vaccine prepared from strain 22A<sub>2</sub> was potent after nearly two months, and that prepared from strains 33B<sub>2</sub>, 34A<sub>2</sub>, and 34F<sub>2</sub> after a year. It should also be noted that the vaccine from 34F<sub>2</sub> was prepared two years after the isolation of this variant.

TABLE II.

*Duration of Immunity Produced in Guinea-pigs by an Avirulent Variant.*

Guinea Pigs.	Immunized with 34F <sub>2</sub> on	Tested with 100 M.L.D. Vaccine Strain on	RESULT OF TEST ON	
			Immunized Guinea Pigs.	Normal Controls.
3.....	20. 3.39	5. 6.39	3/3	0/4
5.....	25.11.38	13.12.38	5/5	0/12
3.....	25.11.38	19.12.38	3/3	0/6
3.....	25.11.38	5. 6.39	3/3	0/4

The results showed that guinea-pigs were solidly immune six months after vaccination.

*Immunity Tests with Vaccines Prepared from Avirulent Variants 22A<sub>2</sub> and 34F<sub>2</sub>.*

All batches of avirulent vaccine, except the first, were prepared from strain 34F<sub>2</sub>. This was isolated from a virulent strain on 10.8.36.

*Preparation of vaccine.*—The strain was grown on buffered nutrient agar, pH 7.4, at 37° C. Sporulation was always complete after three days. The spores were washed off with saline and this suspension added to twice its weight of glycerine. The growth should not be washed off later than the third day, because it then adheres tenaciously to the medium. The stock glycerine-saline suspension was diluted 1:50 to 1:25 with 50 per cent. glycerine-saline for issue to the field. The dose for cattle, 1.0 c.c., contained 600,000 to 1,200,000 spores per c.c. After the middle of 1938, avirulent vaccine issued to the field was suspended in 0.5 per cent. saponin in 50 per cent. glycerine-saline. This improved the immunizing power of the vaccine. [Sterne, Robinson and Nicol (1939)]. No saponin was added for the laboratory tests. Table III summarizes the results of the laboratory titrations on sheep. Guinea-pigs could always be immunized solidly against 1 to 500 M.L.D. of a Pasteur II vaccine. These titrations are omitted for the sake of brevity.

Thus, under laboratory conditions, batches prepared from variant 34F<sub>2</sub> consistently elicited a sound immunity in sheep.

TABLE III.

Strain.	Batch No.	No. of Doses Prepared.	Prepared on	Sheep Immunized on	Result of Test with Virulent Spores on	
					Immunized Sheep.	Normal Controls.
22A <sub>2</sub> .....	4	80,000	13. 7. 36	31. 7. 36	6/6	0/4
34F <sub>2</sub> .....	14	300,000	11. 3. 37	—	—	—
34F <sub>2</sub> .....	19	300,000	18. 5. 37	18. 5. 37	10/10	0/2
34F <sub>2</sub> .....	21	250,000	7. 6. 37	8. 6. 37	7/8	0/2
34F <sub>2</sub> .....	24	500,000	6. 9. 37	7. 9. 37	10/10	0/2
34F <sub>2</sub> .....	25	500,000	20. 9. 37	21. 9. 37	10/10	0/2
34F <sub>2</sub> .....	39	300,000	10. 6. 38	14. 6. 38	10/12	0/2
34F <sub>2</sub> .....	50	500,000	21. 2. 39	23. 2. 39	10/12	0/2
34F <sub>2</sub> .....	51	700,000	4. 4. 39	4. 4. 39	10/10	0/2
34F <sub>2</sub> .....	52	900,000	21. 4. 39	24. 4. 39	9/20	0/2
34F <sub>2</sub> .....	53	700,000	9. 5. 39	9. 5. 39	8/10	1/2*
34F <sub>2</sub> .....	54	700,000	22. 5. 39	22. 5. 39	7/10	0/2
TOTALS.....		5,730,000	—	—	97/108	1/24

\* During the three years covered in Table III, 63 further normal sheep were used as controls for other batches of ordinary vaccine. All died. The one control that lived (Batch 53) showed no reaction at all, and also no reaction to a second inoculation of a large virulent test dose. It is possible that a sheep immunized previously was used here as a control.

#### FIELD TESTS WITH VACCINES PREPARED FROM AVIRULENT VARIANTS.

These were started in 1936, and the amount of vaccine issued was increased as experience was gained of its use. To date, June 1939, about 2,700,000 doses have been used.

*Batch 4.*—In 1936, 1,350 doses were issued for cattle. Reactions were inconsiderable, and a severe outbreak of anthrax was stopped.

*Batch 14.*—In May and June 1937, about 300,000 doses were used for cattle. There was an absence of severe reactions, even in highly-bred stock. Four animals were reported to have died, but it is doubtful whether the vaccine was responsible for the deaths.

*Batch 19.*—Only a few doses used.

*Batches 21, 24, 25.*—These were issued (1,250,000 doses) up to April 1938. By this time the avirulent vaccines had shown themselves safer than the ordinary vaccine and were being used, as a routine, for dairy cattle and highly-bred stock.

*Batches 39, 50, 51.*—Issued (1,100,000 doses) suspended in saponin, from April 1938 to date (June 1939). No complaints have been received.

It is difficult to produce statistically sound evidence of a vaccine's efficacy in the field. The avirulent vaccine has been tested on a large scale and its innocuousness established. The only complaints (4) of

deficiency in immunizing power concerned Batch 14. Complaints ceased when the concentration of spores was increased. The avirulent vaccine was used largely in those areas from which most of the complaints about the ordinary vaccine had come; that is in districts with a large proportion of dairy farmers. The complaints ceased when the new vaccine was introduced.

A questionnaire was sent to the farmers of one of these districts. Replies were received from 216 farmers who owned 49,000 cattle. The replies were classified as follows:—

1. *No or negligible reactions.*—200 farmers owning 46,100 cattle.
2. *Mild reactions.*—8 farmers owning 1,600 cattle. Transient drop in milk yield; 14 head reported lame.
3. *Severe reactions.*—8 farmers owning 1,300 cattle. More serious drop in milk yield; 12 animals were badly swollen; one animal died.

Questionnaires are of dubious value. Replies are usually obtained from those that are very pleased and those highly displeased. The deleterious results were certainly not all due to the vaccine, although possibly due to the inoculation procedure. There were no complaints of lack of immunity.

*Comparative Field Test of Avirulent Spore Vaccine (without Saponin) and Ordinary Saponin Spore Vaccine.*

Every year, in May and June, 1,700,000 cattle, the entire cattle population of the Transkei Territories, are inoculated against anthrax. In 1938 all the cattle (271,500) in one area of the Transkei were inoculated with avirulent vaccine. The cattle (1,288,030) of the rest of the Transkei were inoculated with ordinary saponin spore vaccine. Table IV gives the result of these and, for comparison, the previous year's inoculations.

TABLE IV.

*Comparison of Avirulent Vaccine with Ordinary Saponin Spore Vaccine in Transkeian Territories.*

Area Inoculated.	Period May-June.	No. of Cattle Inoculated.	Type of Vaccine Used.	Deaths from Anthrax in Year following Inoculation.	Percentage Deaths from Anthrax.
Butterworth.....	1937	273,730	Spore vaccine.....	17	0.0062
	1938	271,500	Avirulent vaccine...	5	0.0018
Rest of Transkei..	1937	1,276,240	Spore vaccine.....	60	0.0047
	1938	1,288,030	Saponin vaccine....	25	0.0019

Thus the avirulent vaccine was as effective as the ordinary saponin spore vaccine. A few years ago thousands of cattle died annually from anthrax in the Transkei. The reduction in mortality shown in Table IV is the continuation of a trend that commenced in 1928, when systematic immunization was started in the Transkei. A detailed discussion of this anti-anthrax campaign will be given in another publication.

*Use of Avirulent Vaccine on Animals other than Bovines.*

*Sheep.*—Has been used on a considerable scale; results entirely satisfactory.

*Horses.*—Used on a large scale in 1939; results entirely satisfactory.

*Camels.*—A few were inoculated and these showed no reactions.

*Goats.*—Under laboratory (stabled) conditions, very large doses were harmless. Under field conditions, far smaller doses provoked swellings. Of about 400 goats done in the field four died. Avirulent vaccines are not, therefore, being issued, as a routine, for goats. None of the goats that died showed anthrax septicaemia.

ACKNOWLEDGEMENT.

I am indebted to Mr. W. G. Barnard, Government Veterinary Officer, for preparing and sending out questionnaires, and for supervizing inoculations in his area.

SUMMARY AND CONCLUSIONS.

(1) During the last three years (1936-1939) several batches of anthrax vaccine were made from avirulent (uncapsulated) variants. These batches, as judged by laboratory tests on guinea-pigs and sheep, were uniformly good.

(2) The avirulent strains sporulated rapidly, completely and regularly.

(3) Vaccine prepared from avirulent variants retained its immunizing power for at least a year.

(4) Field tests were carried out on 2½ million cattle and several thousand horses and sheep. The vaccine was safer and produced slighter reactions than ordinary saponin spore vaccine. Highly-bred animals tolerated inoculation very well; horses and sheep could be safely inoculated with cattle vaccine.

(5) Preliminary field tests on goats indicated that these might be more affected by the uncapsulated strains than other domestic animals.

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