

our batches of vaccines have been slightly stronger and produced a higher degree of immunity. This type of vaccine is used in areas where the horses do not seem to possess such a marked susceptibility to attenuated organisms and particularly where the animals had been inoculated previously. As mentioned during the discussion on "Duration of Immunity," (see page 497) there appears to be good reason for believing the immunity conferred by spore vaccine to last for more than 12 months; at any rate, animals inoculated about a year previously, do not show well marked reactions after subsequent vaccination.

In practice at least two strengths of vaccine are made use of, and they are distributed as indicated above, namely (a) the weaker one to parts of the country where anthrax had not been prevalent for any length of time and where equines are known to be very susceptible, and (b) the stronger one, to well known infected areas, where annual vaccination is generally practised as a routine measure. In these latter areas the ordinary cattle and sheep vaccine has been employed without any untoward results, the equines there having sufficient immunity left over from previous inoculations to make them more resistant than normal. Experience in the field has shown that donkeys can be immunized with greater safety than mules and that mules react less severely to vaccination than horses.

By working on the lines indicated above, namely, (a) special selection of a suitable vaccine strain, (b) growing vaccine cultures for shorter periods, sufficient to ensure good sporulation, (c) careful tests on guinea-pigs, rabbits, goats and sheep, and (d) issue of different strengths of vaccine to different parts of the country, we have achieved great success in the vaccination of equines against anthrax.

Where four years ago we were repeatedly called upon to investigate deaths and dangerous swellings resulting from vaccination, during the last twelve months it has been necessary to enquire into only one such complaint. In this case vaccination was resorted to during an active outbreak of anthrax in equines, at a time when probably 50 per cent. of the animals had already become infected. The owners expected the vaccination to show beneficial results immediately, but this could, of course, not be expected. Our experience is that in such cases the disease will not stop completely for about 10 to 14 days after vaccination; animals already infected will die of the disease, while during the first five or six days after vaccination very little or no immunity will be present and further animals may contract the disease. When anthrax breaks out in a troop of horses and horse flies (*Hippobosca*) become infected, it is very difficult to check the outbreak, simply because of the rapid manner in which the infection is spread. In areas where this fly is prevalent, inoculation during the late winter or early spring is strongly recommended; for by this means the disease can be controlled successfully even on the worst infected farm.

Experiments were also conducted to find a preparation which could be used to kill *Hippobosca* on horses as the fly remains on the skin of animals sufficiently long to be reached by a fluid spray. A large number of preparations was tried and the most successful one was found to be a proprietary article, containing tar products. Where dipping tanks are available *Hippobosca* can be gradually exterminated by regular dipping of all stock which serve as a host.

In conclusion we may be permitted to draw attention to the relatively enormous scale on which vaccination of equines is carried out; in Table 14, previously given, it will be seen that during the twelve months ended March, 1925, 128,700 doses had been used in this species alone.

(4) *Swine*.—Our experience with the vaccination of pigs has not been extensive. The same vaccine as used for equines has been issued for inoculation of pigs, with entirely satisfactory results. Apparently this species is not very susceptible to attenuated organisms and can, therefore, be immunized with comparative safety.

(5) *Ostriches*.—Here again the same remarks apply. Protective inoculation of this species is not practised to any extent, but one farmer in the Eastern Cape Province does so regularly. Not having had much experience with vaccination of ostriches we prefer to be on the safe side by issuing vaccines of the milder type, namely that prepared for horses. The farmer above referred to has been using the ordinary cattle and sheep vaccine for a number of years, and he reports good results. It would appear therefore as if the ostrich were not particularly susceptible and may be treated with the stronger vaccines.

(6) *Goats*.—Elsewhere in this report it has been shown very clearly that the goat is extraordinarily susceptible not only to virulent anthrax but also to attenuated organisms. It has been shown that as many as 30 per cent. of goats may succumb to injection with ordinary vaccine intended for use in cattle and sheep, and, further, that some goats appear to be even more susceptible than the average guinea-pig.

Another remarkable phenomenon is that it is not always the larger doses of vaccine which are responsible for deaths in goats. At first we were inclined to think that this could be explained by great variations in individual susceptibility, but the same thing has occurred with such regularity and in so many cases (over 30 per cent.) that one begins to doubt whether individual susceptibility affords sufficient explanation.

The following experiment will illustrate the point at issue:—

*Experiment No. 20.—September, 1924.*

Goats Nos.	Dose of Vaccine.	Result.	Dose of Virulent Material.	Result.
9656.....	10 c.c.	Negative.	500 M.L.D.	Survived.
9901.....	1 "	"	"	"
9924.....	1 c.c.	"	"	"
9668.....	.02 "	"	"	"
9923.....	.01 "	"	"	"
9880.....	.005 "	+ Anthrax.	"	"

+ Equals died.

In this case the goat which received the smallest quantity of vaccine died from the effects, whereas those receiving bigger doses, up to 2,000 times the amount, responded merely by a temperature

reaction. This animal may be said to have had a remarkably high susceptibility to anthrax, but, as already stated, over 30 per cent. of deaths after vaccine tests have occurred in goats receiving the smaller amounts of vaccine, namely, .005, .01 and .02 c.c. One does not wish to theorise too much, but it may be that the following explanation meets the case:—When a big dose of vaccine is introduced, the body defences are at once made aware of the potential danger, and consequently its protective forces are immediately mobilised and the invasion is checked. On the other hand, if a small dose is administered, there may not be an immediate response on the part of the body tissues, with the result that the spores have time to germinate, multiply and develop protective capsules. By the time the defences of the body are brought into action the invader has become thoroughly entrenched and can now not only maintain itself but assume the offensive and succeed in killing the individual.

It is true that the same phenomenon has never been observed in sheep, but this species is remarkably resistant against infection by attenuated anthrax organisms.

Whatever the explanation may be, this factor is of the utmost importance as far as vaccination of goats is concerned, because it shows that dilution of the vaccine alone will not be sufficient to ensure its safety.

The following experiment is inserted here to show what happens when a strong vaccine is injected into goats:—

*Experiment No. 21.—April, 1923.*

Goats Nos.	Dose of Vaccine.	Result.	Dose of Virulent Material.	Result.
1.....	10 c.c.	+ Anthrax.		
2.....	1 "	"		
3.....	.1 c.c.	"		
4.....	.02 "	Survived.	1,000 M.L.D.	Survived.
5.....	.01 "	"	"	+ Anthrax.
6.....	.005 "	+ Anthrax.		
7.....	.001 "	Survived.	1,000 M.L.D.	Survived.

+ Equals died.

In this case four out of the seven goats died from the effects of the vaccine which was a particularly strong one. Most of the deaths occurred, as one would have expected, in the animals receiving the larger doses, but goat No. 6 was again an exception. In the immunity tests one out of the three goats died from anthrax, thus showing that even when animals have passed successfully through a strong vaccine reaction, they do not always possess a strong immunity against the disease.

This brings us to another difficult point in connection with the vaccination of goats, namely, the uncertain manner in which some of them respond to vaccination, in so far as the development of immunity is concerned.

We have on record many cases where goats receiving the larger doses of vaccine did not develop immunity, whereas those receiving

smaller quantities were protected against virulent anthrax. It would seem, therefore, as if the individual again played a role in this case, that, in other words, some individuals do not lend themselves to being immunized against anthrax. Apart from the case referred to in the previous experiment, a further example may be given to illustrate this point, as follows:—

*Experiment No. 22.—March, 1924.*

Goats Nos.	Dose of Vaccine.	Result.	Dose of Virulent Material.	Result.
3384.....	10 c.c.	Survived.	500 M.L.D.	Survived.
3385.....	1 „	„	„	„
3404.....	·1 c.c.	„	„	+ Anthrax.
3388.....	·02 „	„	„	Survived.
3389.....	·01 „	„	„	„
3304.....	·005 „	„	„	„

+ Equals died.

In this case one goat did not survive the immunity test, in spite of the fact that it had received a relatively large dose of vaccine. All other animals, including one which had received twenty times less vaccine, had developed sufficient immunity to withstand the injection of 500 M.L.D. of virus. It seems reasonable to conclude that this individual goat did not lend itself to immunization.

Numerous experiments have been made to find a suitable method of vaccinating goats against anthrax. In some cases fairly weak vaccines intended for use in equines have been employed, after laboratory tests in goats had given satisfactory results. When such a vaccine was issued for use in practice, it was found to produce a mortality of anything up to 10 per cent., and this in spite of the fact that no goats were killed by it at the laboratory. We soon realised, however, that under laboratory conditions where only six to twelve goats were employed in a test, a 10 per cent. mortality would not always be brought to light. In practice one has to deal with goats, varying greatly in susceptibility, some not in too good condition, others either in advanced pregnancy or nursing young kids. The result is that when a vaccine, which appeared to be quite safe when tested under laboratory conditions, is issued for use in practice, entirely different results are obtained.

A double method of vaccination has been tried, and, although the first vaccine was very weak, it still produced sufficiently severe reactions to make some individuals so weak that they could not stand the second injection. A parallel experiment carried out with single and double vaccine under field conditions, showed the single method to be slightly superior to the double method of vaccination, and this in spite of the fact that the single vaccine used was exactly the same as the second of double.

Having now established the fact that quite a large percentage of goats possessed extraordinarily marked susceptibility to attenuated (vaccine) anthrax organisms, a susceptibility which in some cases is

greater than that of the average guinea-pig, we began to realise that a safe vaccine for goats could be obtained only by employing a specially mild attenuated strain. The observation that dilution of the ordinary vaccine did not make it any safer for goats supported this view. With this information before us, we can come to no other conclusion than that the vaccine strain employed for the manufacture of goat vaccine must be so *mild that it does not kill guinea-pigs*, even in relatively large doses. Along these lines we have proceeded, and the results in practice have been extremely satisfactory. The following experiments show the results of the animal tests carried out in connection with the goat vaccines:—

*Experiment No. 23.*

Animal Nos.	Dose of Vaccine.	Result.	Dose of Virulent Material.	Result.
Sheep 6093.....	20 c.c.	Survived.	1,000 M.L.D.	Survived.
"   6060.....	20 "	"	"	"
"   6053.....	1 "	"	"	"
"   6020.....	1 "	"	"	+ Anthrax.
"   6089.....	.1 c.c.	"	"	Survived.
"   6023.....	.1 "	"	"	"
"   6090.....	.02 "	"	"	"
"   6054.....	.02 "	"	"	"
"   6091.....	.01 "	"	"	+ Anthrax.
"   6092.....	.01 "	"	"	"
"   6094.....	.005 "	"	"	Survived.
"   6029.....	.005 "	"	"	"
Goat 6697.....	10 "	"	500 M.L.D.	"
"   6704.....	1 "	"	"	"
"   6707.....	.1 "	"	"	+ Anthrax.
"   6677.....	.02 "	"	"	"
"   6676.....	.01 "	"	"	"
"   6688.....	.005 "	"	"	Survived.

+ Equals died.

It will be seen that a moderate degree of immunity was conferred on sheep, but that the goats were not protected too well.

The test on guinea-pigs gave the following results:—

- 1 guinea-pig received subcutaneously .1 c.c. glycerinised emulsion.
- 1 guinea-pig received subcutaneously .01 c.c. glycerinised emulsion.
- 1 guinea-pig received subcutaneously .02 c.c. glycerinised emulsion.

All survived, showing that the vaccine strain was a particularly mild one. For use in the field this vaccine was issued in a dilution of 1 in 50, the dose for a goat being  $\frac{1}{2}$  c.c.

Over 50,000 doses have so far been used, and up to now no serious mortality resulting from vaccination has been brought to our notice. An unexpected result is that apparently the immunity conferred by this rather weak vaccine has been sufficient to protect goats against natural infection; at any rate, no authentic reports concerning breakdowns in immunity have been received. This is certainly remarkable,

especially in view of the fact that the vaccine is nearly two years old, and has undoubtedly become considerably weaker. Concerning this latter remark it may be stated that the indications are that attenuated spores appear to be affected adversely by suspension in 60 per cent. glycerine-saline for long periods. Some spores seem to be destroyed and further attenuation of the remaining ones appears to take place. Before a definite statement can be made on this very interesting point, we shall have to await the results of more extensive observations.

A further example of what is considered a suitable goat vaccine, is furnished by the following experiment:—

*Experiment No. 24.—January, 1925.*

Animal Nos.	Dose of Vaccine.	Result.	Dose of Virulent Spores.	Result.
Sheep 9370.....	20 c.c.	Survived.	500 M.L.D.	Survived.
„ 8958.....	20 „	„	„	„
„ 9130.....	1 „	„	„	„
„ 8914.....	1 „	„	„	„
„ 9486.....	.1 c.c.	„	250 M.L.D.	+ Anthrax.
„ 8954.....	.1 „	„	„	Survived.
„ 9442.....	.02 „	„	„	+ Anthrax.
„ 9507.....	.02 „	„	„	„
„ 9545.....	.01 „	„	100 M.L.D.	„
„ 9459.....	.01 „	„	„	Survived.
„ 9436.....	.005 „	„	„	+ Anthrax.
„ 9106.....	.005 „	„	„	Survived.
Goat 9892.....	10 „	„	500 M.L.D.	„
„ 9881.....	1 „	„	„	„
„ 9956.....	.1 „	„	250 M.L.D.	„
„ 9921.....	.02 „	„	„	+ Anthrax.
„ 9905.....	.01 „	„	100 M.L.D.	Survived.
„ 9912.....	.005 „	„	Not tested.	„

+ Equals died.

Two tests on guinea-pigs were carried out as follows:—

- (1) 1 guinea-pig received .1 c.c. spore emulsion, died of anthrax.
- (1) 1 guinea-pig received .01 c.c. spore emulsion, survived.

This test was made at the time when the spores were first suspended in glycerine and when the results would be influenced by the vegetative forms that were still present.

(2) The second test was made a few months later when it was thought that all vegetative forms would be killed.

- 1 guinea-pig received .1 c.c. glycerinised emulsion—survived.
- 1 guinea-pig received .1 c.c. glycerinised emulsion—survived.
- 1 guinea-pig received .01 c.c. glycerinised emulsion—survived.
- 1 guinea-pig received .01 c.c. glycerinised emulsion—survived.
- 1 guinea-pig received .001 c.c. glycerinised emulsion—survived.

From this it became quite clear that the vaccine was a particularly weak one. The results obtained from the test in sheep and goats confirmed this, showing that the immunity conferred on the experimental animals was not very strong, but that, on the other hand, the vaccine was perfectly safe. One sheep, receiving a dose of .01 c.c. vaccine, and the goat injected with the same quantity of vaccine

both survived the immunity test against 100 M.L.D. for sheep. It was therefore decided to issue the vaccine in this dilution, especially in view of the fact that field experience appears to show that this degree of immunity is sufficient to protect goats against natural infection.

We fully realise that this standard is very low, but experience has taught us that it is safer to err on the low side, especially when a vaccine is required purely for preventive purposes. In cases of this kind it would be a fatal mistake to issue a vaccine that might cause the loss of even one or two per cent. animals, not to mention ten per cent. or more, as could easily be the case.

In cases where an active outbreak exists in goats the advisability of employing stronger vaccines might be considered, and, in any case, a stronger vaccine should always be kept on hand for the purpose of reinoculation, in the event of breakdowns in immunity being reported. Needless to say, we admit that on the experimental evidence available, vaccination of goats does not appear to be on an entirely sound basis, but experiments and observations still in progress will decide any further modifications that may be necessary. The indications are that vaccination by the method of scarification of the skin will prove to be much safer and that with this method goats can be done with comparative safety, even when relatively strong vaccines are employed.

In concluding this chapter on vaccination against anthrax, it is considered to be highly desirable to refer to one very important aspect, namely, the possibility of anthrax being established on a farm by carcasses of animals that have died from the effects of the vaccine. This point has been raised by farmers and others in connection with the preventive inoculation of stock on what are believed to be "clean" farms. Provided properly attenuated strains are employed in the manufacture of the vaccine, we cannot believe that there is the slightest risk of the farm becoming infected with virulent anthrax. We have often recovered the organisms from goats that have died from the effects of vaccination, and have always found these organisms to *retain their attenuated character*.

Large doses have been injected repeatedly into sheep and rabbits, but we have never found the organisms to have assumed a more virulent character.

Animals may pick up attenuated spores on a farm, but it is extremely doubtful whether such infection would ever succeed in setting up a fatal disease.

## H.—SUMMARY AND CONCLUSIONS.

All the observations and facts referred to in this article have been discussed at great length under various headings in the different chapters, so that a further detailed discussion at this stage is not called for.

It is, however, necessary to summarize the position briefly and to emphasize the more important conclusions arrived at.

(1) *Early History of Anthrax*.—No mention was made of anthrax in the very early history of this country, probably because it was not very prevalent, rarely recognised, and other more serious stock diseases claimed the attention of the stock owner. It was not until towards the end of the 19th century that serious outbreaks of the

disease were recorded and since then anthrax has become increasingly prevalent.

(2) *Distribution of Anthrax*.—Although anthrax is a notifiable disease under the Stock Diseases Act, no reliable data on its distribution are available. Many stock owners do not always recognise the disease and this is especially the case in small stock (sheep and goats). Others fear quarantine measures and in order to avoid these neglect to report outbreaks, preferring to apply methods of control in their own way and in their own time; since free vaccine can be obtained by direct application to the laboratory, the stock owner finds no difficulty in controlling an outbreak of the disease. Owing to the extensive farms, scattered population, including the ignorant and careless native, it is of course impossible to enforce veterinary police measures in all instances.

In spite of these difficulties we have endeavoured to give the reader a fair idea of the distribution of anthrax in this country. The period selected was the year ended June, 1924, but it must be clearly understood that the same figures can naturally not be accepted for any other period; since then outbreaks of the disease have been suppressed in many of the districts mentioned, while new outbreaks in other districts have occurred. Generally speaking, anthrax has been placed under much better control since 1924 and owing to preventive inoculation applied on a large scale outbreaks have become much fewer.

(3) *Factors determining the Maintenance and Spread of Anthrax*.—These have been discussed very fully, and will not be referred to again in any detail. By better farming methods and closer veterinary supervision some of these factors are gradually being eliminated.

For the complete eradication of anthrax from a farm it is necessary to have some knowledge of the mode of life of the causal organism under natural field conditions. Information on this point is entirely lacking, and, owing to the difficulties in the way of obtaining definite experimental evidence, very little progress has been made.

We have already started some experiments that may throw light on the question, but it is clear that definite results can be expected only after long and patient research.

(4) *Seasonal Prevalence of Anthrax*.—By analysing the figures at our disposal we have been able to show quite clearly that anthrax is more prevalent during the summer months, but that cases of the disease occur all the year round. This seasonal prevalence is attributed to summer rains, resulting in flooding, exposing of buried anthrax spores, growth of new vegetation, etc.

(5) *Anthrax in Industry*.—Industries in connection with animal products have not developed to any great extent in the Union, so that anthrax in human beings is rarely derived from this source. Cases of anthrax that are observed in man can usually be traced back to careless handling of infected carcasses.

Under the Stock Diseases regulations skinning or dissecting of carcasses of animals suspected to have died of anthrax is strictly prohibited, but such cases do occur, just as in other parts of the world. For this reason a certain amount of risk in connection with the handling of hides and skins must be accepted. This danger is



probably not greater than in other parts of the world where anthrax is prevalent.

The danger attached to handling baled wool derived from this country is, however, so small as to be almost negligible; it is a well-known fact that those parts of South Africa where sheep farming is carried out on a large scale are comparatively free from anthrax. Not only that, but even if rare cases of the disease did occur and the dead animals were skinned, the skins would be sold with the wool attached to them. It is therefore unlikely that anthrax infection would gain access to wool contained in a wool pack.

(6) *Animals affected under Natural Conditions.*—Regarding the relative frequency of anthrax in the different domestic animals, no accurate statistics are available, but we have no hesitation in placing cattle first and thereafter sheep and goats. In certain parts of the country the disease is most prevalent in equines. Pigs suffer less frequently, while cases in the dog are practically unknown. Wild ruminants are very susceptible, and heavy losses have been observed in springbok and hartebeest.

(7) *Susceptibility under Experimental Conditions.*—Experimentally the goat is the most susceptible of our domestic animals, both to virulent and attenuated strains. Apart from this exceptional "species" susceptibility we still have to record a most remarkable "individual" susceptibility, which may be present in 30 per cent. of cases.

The merino sheep is also very susceptible to virulent anthrax strains, but shows a strong resistance to attenuated strains; this makes it an easy animal to immunise against the disease.

Neither equines nor cattle are easy to kill by the administration of virulent bacilli or spores; a fatal infection of the disease can be set up by subcutaneous injection of relatively large quantities. On the other hand, equines sometimes show a marked susceptibility to attenuated strains, so much so that 1 c.c. of vaccine has been known to produce fatal results, while alarming swellings were quite commonly observed.

(8) *The Methods of Natural Infection.*—These are not known with certainty. Herbivora are commonly believed to contract the disease by ingestion of infected material. Experimentally it requires a great deal of virulent anthrax spores before one can succeed in producing the disease by administration per os, and even then infection does not always occur.

In the case of both cattle and equines the subcutaneous route of infection is not always certain and in any case the disease can be set up this way only by administration of relatively enormous quantities of virulent material. Both sheep and goats can be infected very easily by injecting virulent material under the skin, even when minute quantities are used. Besredka and other workers have suggested that the skin is the only sensitive organ in so far as anthrax infection is concerned, but we have not been able to confirm this. As a matter of fact, we have found the outer layers of the skin less sensitive than other routes of infection.

(9) *Symptomatology.*—Regarding the symptomatology of anthrax we are not able to bring forward much information additional to what has already been recorded in the literature; swellings in horses

are extremely common and have a certain amount of diagnostic value. From our experimental cases information has been obtained in connection with the incubation period and duration of the disease.

(10) *Diagnosis*.—No difficulty is experienced in the diagnosis of anthrax, always provided blood smears are obtained from the carcase while still in a fairly fresh condition. In many cases a definite diagnosis can still be made when the blood is teeming with putrefactive organs. In such cases reliance has to be placed on one's knowledge of the morphology of the organism and its staining characters. Giemsa solution has been used for the purpose in this Institute for many years and for bringing out the special staining reaction of *B. anthracis* is still found to be superior to any other method of staining.

In cattle, sheep and goats, fatal cases are always of a typically septicaemic character, while even in horses the bacilli appear in the blood stream near the time of death. In natural cases of anthrax in the horse, lasting several days, we have been able to demonstrate bacilli in the blood some hours before death. In goats dying from attenuated strains, a septicaemic condition is often present, but in equines dying under similar conditions it is often very difficult to demonstrate any organisms even when cultural methods are employed. The diagnosis of anthrax in dried material, such as pieces of hide, bones, etc., can be accomplished successfully by cultural methods and small animal inoculations.

(11) *Control*.—For the control of anthrax all the necessary legislative powers under the Stock Diseases Act are in existence and it only remains to enforce the regulations that have been drawn up. This is being done with great success in badly infected areas, but, owing to the scattered population, vastness of the country, etc., veterinary police measures cannot be applied and supervised everywhere.

Owing to the sporadic nature in which the disease very often appears cases sometimes go unrecognised and are not reported to the authorities. By means of lectures, popular pamphlets, etc., the farming population is being enlightened on the dangers of the disease and this propaganda work has already borne very good fruit. Farmers in many parts of the country are extremely afraid of the disease, and call in veterinary assistance when the slightest suspicion occurs. Ever since the beginning of 1924 when the issue of free vaccine was decided upon, vaccination has become progressively more popular, so much so that it is now often being applied when there is only a suspicion of the disease in the neighbourhood.

For the proper control of anthrax the closest co-operation of stock owners is essential and this is now being secured to a very large extent.

We can safely say that in spite of its increased prevalence up to 1924 anthrax is now under better control by the State than it has ever been in this country.

(12) *Protective Inoculation*.—One of the chief weapons against anthrax is protective inoculation with a reliable vaccine. By such a vaccine we understand one that (a) is safe for use, (b) produces a sound immunity, (c) has good keeping properties, and (d) is cheap. Up to a few years ago a vaccine answering this description was not

available in South Africa. Pasteur vaccines were imported or vaccines prepared according to Pasteurian methods were employed, but they all failed mainly because of the lack of good keeping properties.

Moreover, *one vaccine* was considered safe for use in all domestic animals and this has now been found to be erroneous. There is such a remarkable difference in susceptibility of the various species of domestic animals to attenuated anthrax strains that each species requires separate treatment.

Since 1920 the so-called spore vaccines have been prepared at this Institute; full details concerning its preparation from selected strains are given elsewhere in this article. We claim that this vaccine possesses all the properties which we have laid down for a *reliable vaccine*, especially as far as cattle, sheep and equines are concerned.

(13) *Duration of Immunity.*—On an infected farm anthrax can be suppressed entirely by rendering all susceptible stock immune to the disease. Unfortunately the nature of the immunity which is developed against anthrax is not a life long one and hence to keep the stock insusceptible to the disease reinoculation has to be practised at definite intervals. This brings us to the question of duration of immunity in anthrax, on which reliable data are not so easily obtained. To obtain such information one would have to keep vaccinated animals away from natural infection for long periods and then submit them to immunity tests. Owing to the expense involved we have been able to carry out only a few tests on these lines and the results indicate that the immunity begins to decline after about six months and is present only to a slight degree after an interval of nine to twelve months. This confirms observations in the field, namely that vaccinated animals exposed on an infected farm are likely to contract anthrax after an interval of about twelve months. That some immunity is still present after this interval is proved by the fact that reinoculation of equines is hardly ever followed by severe reactions, even if a particularly strong vaccine is employed.

In one or two cases we have observed immunity to be present after a much longer interval, but for practical purposes annual inoculation on infected farms must be adhered to.

(14) *Use of Sero-vaccines.*—It has been claimed that sero-vaccines give much better results when dealing with active outbreaks of anthrax, but this has not been our experience. In such cases the use of serum for the production of passive immunity is indicated, but apparently the so-called sero-vaccines which have come under our observation have not been sufficiently active to produce the desired effects.

We have succeeded in producing a very potent serum, but for use on a large scale its cost is prohibitive.

According to some workers the development of immunity depends on the path through which vaccine is introduced into the body, some methods resulting in immunity being developed much earlier than others. We have found very little difference after introducing the vaccine by the different routes described, well marked immunity in all cases being present only after eight to ten days. This is in agreement with experience obtained in practice, active outbreaks of anthrax only ceasing ten to fourteen days after vaccination.

(15) *Use of Anthrax and Blackquarter Vaccines simultaneously.*—An observation worth drawing attention to is that cattle can be immunised successfully against both anthrax and blackquarter by injecting anthrax vaccine and blackquarter aggressin (culture filtrate) simultaneously; the latter is harmless, producing hardly any disturbance in the animal and hence does not tend to aggravate the reaction resulting from vaccination against anthrax. At the same time it has been found that the animal body will respond by manufacturing immune substances against both diseases. This is of great practical importance in countries where cattle farming is carried out under ranching conditions and where inoculation has to be performed under great difficulties.

(16) *Results of Spore Vaccination in the Practice.*—The practical application of our method of inoculation against anthrax has been attended by great success; whereas a few years ago we were repeatedly called upon to enquire into undesirable sequelae following on vaccination and breakdowns in immunity in vaccinated animals, not a single complaint has reached us during the past twelve months. When it is remembered that well over two million animals, including equines and goats, were vaccinated during the past year, the success achieved will be more readily appreciated.

It has to be emphasized that, provided properly attenuated strains are used, and the vaccine is prepared and tested under strict supervision, no difficulty is experienced in obtaining a reliable vaccine for cattle and sheep, but that a vaccine which is safe for these species cannot be used with impunity for the inoculation of either equines or goats.

For equines specially selected vaccine strains have to be employed and the vaccine itself has to be a good deal milder than that used for cattle and sheep. This is particularly necessary when the equines have to be vaccinated for the *first* time.

In the case of goats still greater care has to be taken, only weak strains being employed for vaccine production. Such strains have to be practically a-virulent for guinea-pigs.

(17) *Is the Complete Eradication of Anthrax possible?*—Lastly, the question of complete eradication of anthrax from a farm has to be referred to. If all sources of infection were known they could be eliminated by disinfection, fencing, etc., but under South African conditions it hardly ever occurs that the source of infection can be traced, and for all practical purposes the whole farm has to be considered as infected. Further cases of the disease are prevented by annual inoculation and in the event of straying animals dying on the farm their carcasses are carefully disposed of by burning or burial. Provided annual inoculation is carried out regularly, no further animals contract the disease and further infection of the farm is avoided. In the meantime the original infection must be dying out, but we are unfortunately not in the position to say how long this dying out process will take and consequently how soon the farm will be "clean," always provided no fresh infection is introduced. We do not even know whether the anthrax organisms can persist only in the *spore forms* or whether the spores can vegetate under certain favourable conditions and in this way increase in numbers. As previously mentioned, all this requires elucidation, but in the meantime we may accept that the anthrax bacillus is a strict obligatory

parasite, only thriving and multiplying in the animal host and persisting outside the animal body in the spore form. If we accept this, then we can discuss the complete eradication of the disease from any particular area, because it is quite certain that the organism even in the spore form, will not live for ever. As a matter of fact, in the outer world it may be exposed to so many adverse conditions that it will not last very long. We have fairly definite proof that exposure to unfavourable conditions has a marked influence on anthrax spores; for instance, when kept for long periods in 60 per cent. glycerine it has been found that some spores will die out while others have become *weakened or attenuated in character*. This is well known in the case of the vegetative forms but it is remarkable that the same phenomenon can occur in organisms while in the spore form.

Further observations in this connection are in progress and it is hoped to throw more light on this question at a later stage.

For the purpose of eradicating the disease all we can do is to avoid all further infection by the careful disposal of infected carcasses and the application of annual inoculation over a number of years.

While the State supplies a reliable vaccine free of charge there is no reason whatever why stock owners in the Union should not succeed in eradicating this dangerous disease from their farms.

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## APPENDIX.

DIRECTIONS FOR USING ANTHRAX SINGLE SPORE VACCINE  
IN CATTLE AND SHEEP.

NOTE.—The vaccine is to be *well shaken before use*.

DOSE.—For cattle, 1 c.c.; for sheep,  $\frac{1}{2}$  c.c.

NOTE.—For horses, mules, donkeys, and goats special vaccines are supplied.

1. The vaccine should be used as soon as possible after it is received, and on no account should it be used later than the date specified on the label.

2. The vaccine is supplied in bottles with a white label printed in green ink, containing sufficient material for the vaccination of 10, 25, 50, or 100 head of cattle, and 20, 50, 100, or 200 sheep.

3. Instruments necessary for the operation: A hypodermic syringe and, if possible, at least five hypodermic needles to fit the nozzle of the syringe. The syringe must be graduated, with a revolving wheel on the piston-rod to act as a stop and to regulate the size of the dose to be injected.

When obtainable, a syringe of a capacity of 1 c.c. or 2 c.c., graduated in half-parts of a cubic centimetre, is convenient for use; syringes of 5 c.c. or 10 c.c. capacity can also be employed.

4. Sucking animals between the ages of one and three months should receive half the dose of a full-grown animal. The dose for young animals over three months is the same as for full-grown animals.

5. *Sterilization*.—Complete sterilization of the instruments used and of anything that the vaccine may come in contact with before the injection is made, is of the utmost importance.

Sterilization is effected by boiling the instruments for fifteen minutes in clean water; the syringe must be first unscrewed and taken to pieces, and the needles should be cleaned by passing through them one of the small pieces of wire provided for this purpose.

It is important also, before using the vaccine, to see that the syringe works well; this should be tested by sucking up into the syringe, by means of the piston, some of the boiled water and ejecting it once or twice. If, after sucking up the water, the barrel of the syringe is not completely filled, the screw at the base of the piston needs tightening, but if the piston works too tightly the screw may be slightly loosened.

6. *Operation*.—Cattle can be vaccinated behind the shoulder or on the neck, while sheep can be injected under the skin on the inside of the thigh. Working animals should be vaccinated in a place where the harness will not chafe.

Clip the hair from a patch of skin about the size of the hand and brush this part thoroughly with hard bristles. Take the syringe with needle fitted on to its nozzle, *shake well the bottle of vaccine*, and see that the vaccine in the bottle is thoroughly mixed; remove the cork from the bottle, and suck up the vaccine into the syringe. Instead of uncorking the bottle, and thus exposing the contents to the danger of contamination, it is preferable to remove some of the sealing-wax, then to disinfect the surface of the cork, and to push the needle through the cork. The vaccine can then be sucked directly into the syringe if the bottle be inclined or inverted. Withdraw the syringe and set the wheel on the piston-rod to the figure indicating the size of the dose. Insert the point of a fresh needle through the skin, and push the needle right under the skin (not into the flesh), then press in the piston of the syringe until it is stopped by the wheel on the piston-rod. One dose will then have been injected. Withdraw the needle and syringe together, pressing at the same time firmly with the finger on the puncture of the skin.

This completes the operation, and only one inoculation is necessary in using the single spore vaccine.

If any doubt exists that an animal has not received the correct dose, the operation may be repeated immediately.

Each needle should be resterilized after being used for the inoculation of one animal. It is advisable to have as many needles as possible. Make sure that each needle is boiled for at least ten minutes before being used. To ensure this, keep a vessel with water continually boiling. Place the needles in a row. Take your needle from one end of the row and, after use, put it at the opposite end.

If the same needle be used for the vaccination of more than one animal, there is a danger that the first animal may have been infected with anthrax, even though it looked perfectly healthy, and that the germs may be carried to the animals treated subsequently. The use of the same needle may result in the development of swellings as a result of other infections.

7. Female animals in advanced pregnancy (heavy in young) should not be vaccinated, as they may abort as a result of the inoculation. If, however, the farm is badly infected, it may be advisable to take this risk and inoculate these animals. In this way it may be possible to save the mothers, even though the young be lost.

8. Any vaccine which is not used immediately after a bottle has been opened must be destroyed.

9. Working animals should be allowed to rest for about a week following inoculation, and may then again be used unless they show a marked reaction to the inoculation and appear visibly ill. In the latter case they must be treated as sick animals and not put to work until fully recovered from the reaction.

The more rest given to animals before and after inoculation, the less likely are they to show any ill effects.

10. No danger is anticipated from the use of milk of vaccinated animals, save in such exceptional cases which may occur where the animals show a very marked reaction to the inoculation with signs of being visibly ill, and a marked diminution in the yield of milk; and in these cases the milk should not be used until the animal has completely recovered from the reaction.

11. It is also not anticipated that any ill results will ensue from the consumption by human beings of the meat of animals that have been vaccinated, provided that the animals have shown no marked symptoms of illness following vaccination, and that no signs of the disease are apparent on post-mortem inspection.

12. The vaccine will not cure animals that have already contracted the disease, and immunity may not be conferred until fifteen days after vaccination. This means that deaths due to naturally acquired infection may continue to occur up to about a month after the injection, as the incubation period of the disease varies from one to fourteen days.

In practice, however, cases of this kind will be exceedingly rare. In most cases immunity will be present before the fifteenth day. Nevertheless, inoculations should always be carried out as a preventive measure and not after an outbreak of the disease.

13. In the majority of cases, vaccination is expected to protect animals against the natural contraction of the disease for about twelve months, and it is recommended that stock be inoculated again at the end of that time. In some cases, however, the protection given does not last for such a long period, and reinoculation may have to be performed at an earlier date. To such cases the following section on the nature of the immunity particularly refers.

#### NATURE OF IMMUNITY.

14. With regard to the immunity produced by the vaccine, it has been pointed out that, whilst the vaccine gives excellent general results, it is not to be regarded as an infallible agent in conferring protection against the disease, and it is a recognized possibility that in certain cases animals, subsequently to having been inoculated, may naturally contract the disease and sometime die as a result.

These breakdowns in immunity may depend on one or more of several circumstances, the more apparent of which are—

- (a) that as the dose of vaccine is small, certain animals may not receive the full dose of material, this depending on the care with which the inoculation is performed;
- (b) that in some localities there apparently exists an excessively heavy infection of the veld or a local strain of anthrax of a higher degree of virulence than usual;
- (c) that certain animals may resist ordinary attempts at immunization;
- (d) that certain animals are individually exceptionally susceptible to the disease.

Where breakdowns in immunity occur, it is requested that full particulars be communicated to the Director immediately, so that, if necessary, a fresh issue of vaccine can be made without further delay.

15. *Warning.*—Owing to the danger of carrying infection from diseased to healthy animals by means of the inoculating needle, any animals showing signs of sickness should not be vaccinated.

16. *Note.*—In herds or flocks when infection is already present, a measure which is recommended, in addition to the vaccination, is the movement of the stock from the portion of the farm where the infection has been acquired to another portion of the same farm believed to be clean or less infected. This movement, to have full value, should be carried out immediately following inoculation, and subsequent movement of the stock back again to the infected portion, if desired, is not recommended until after a period of about four weeks from the time of completion of vaccination.

17.—*Request.*—It is particularly requested that blood-smears of all animals that die after the inoculation be sent to the Division of Veterinary Education and Research, P.O. Box 593, Pretoria, so that the cause of death may be determined. The name and number of the farm, the postal address of sender, as well as the batch number of vaccine, should be quoted.

18. Every care is taken in the preparation of anthrax vaccine, and each batch is tested before issue, but no guarantee is given regarding its safety or efficacy.