charge of Government experimental stock farms, etc., to practise what we preach. The public is always watching, and will adopt our methods and recommendations when these have been proved to "deliver the goods."

Gentlemen, let we African Veterinarians acquaint ourselves with local conditions and then preach what we feel can be practised.

Paper No. 13

CONTAGIOUS BOVINE PLEURO-PNEUMONIA.—CULTURE VACCINES.

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The following is a short summary of the work carried out in the Sudan during the last three years.

Introduction.

Earlier knowledge, on which the work has been based, can be briefly set out as follows:—

1. Injections of natural lymph induce a strong immunity, but are too dangerous. By reducing the dose and introducing into selected sites mortality can be reduced, but risk has never by these means been eliminated.

2. The virus has been cultivated in various media, but most of them are rather troublesome to prepare.

3. Cultures of the virus have been used with varying success as vaccines. It has been recorded that cultures of the first and second generation are approximately as dangerous to inject as the natural lymph, but cultures of several generations are safe.

4. Regarding the best age (in generations of transplants—or length of time in artificial culture) at which to use cultures for vaccination, no very definite evidence has been recorded. From some records, however, it has been argued that to be an efficient vaccine a culture should be of such an age that it provokes a mild reaction when injected—if there is no "reaction," there is no subsequent immunity. Such a principle of vaccination entails a second vaccination of the non-reactors, which in a country such as the Sudan is in most cases impracticable, if not impossible.

Without attempting to discuss the literature in any way, it seems that evidence is either lacking or unsatisfactory on many points; those that have received attention in the Sudan are as follows:—

1. The possibility of growing the virus in very simple culture media.
2. The determination, if possible, of the exact length of time a virus must be maintained in artificial culture medium for it to become harmless when injected into cattle.
3. Assessment of the antigenic value of cultures of different ages expressed in terms of the time they have been maintained in artificial media since original isolation.

4. The duration of the immunity produced by culture vaccines.
5. Routine methods of preparation.
6. Vaccinations in the field.
7. Summary.

To deal with these points on as individual lines as possible, the results have been as follows:—

1. Simple Culture Media.

The virus grows very well in plain serum broth. Various sera have been used and all are useful; the two best seem to be horse and camel serum, but as a routine horse serum is preferred, because horse-blood clots more firmly and it is easier to obtain large quantities of clear serum. There is no advantage in using fresh serum; in Khartoum a practice is made of carrying out bleedings on a large scale and storing the serum until required in sterile bottles.

The only variation from the stereotyped "plain broth" of bacteriologists is that more peptone is incorporated; the pleuro-pneumonia virus is a poor grower in weak media, but responds to increased nourishment. In practice it has been found that there is no great advantage in using more than 2 to 3 per cent. of peptone.

Regarding the "reaction" of the medium, the only references in the literature indicate the popularity of a reaction of P.H. 8.0. This is not an easy reaction to maintain, particularly in media stored for a short time, as these tend to become neutral. Several estimations of the reaction both of pleuritic fluid and "tumour lymph" have shown that the reaction is about P.H. 7.3–7.4 (the usual reaction of body fluids). Cultural tests have shown that the virus will grow in media whose reactions vary from P.H. 6.2 to P.H. 8.8, and that at about midway between these reactions the best growths are obtained. At present the medium in Khartoum is adjusted to P.H. 7.8 before final sterilization, and many tests of the finished medium have shown that the final reaction is approximately P.H. 7.2 to 7.4.

Estimation of luxuriance of growth on the basis of opacity in broth culture is accurate. The virus grows well on the surface of serum agar, and opacity in broth cultures has been found to run parallel with the number of colonies on agar plates.

2. Loss of Virulence in Artificial Culture.

It has been claimed that after a time in artificial culture the virus arrives at a stage of "fixed attenuation." *A priori*, this claim is difficult to admit, unless by "fixed attenuation" one means "established non-virulence." With a view to co-ordinating studies on this point with others designed to ascertain whether there is any difference in the value of young and old cultures as vaccines, it was thought necessary to determine the shortest time required in artificial medium for the virus to become harmless when injected subcutaneously into cattle. Small-scale experiments have shown that up to the fourth week (weekly transplants in serum broth) cultures are dangerous; fifth week cultures have caused swellings of about 7–8 cm. radius around the site of injection; sixth week cultures have been followed by no reaction either local or thermal; while seventh week cultures have been issued on a large scale to officers in the field, and no more harm has resulted than in the case of cultures of forty–fifty or more generations.

3. Antigenic Value of Cultures of Different Ages.

This is the most important point to be discussed, since on one's conclusions depends the exact nature of cultures to be used as vaccines in the field. The general objective of studies under this head has been to correlate the relative losses of "virulence" and "antigenic power" on the part of the pleuro-pneumonia virus in artificial culture, on the assumption that it does not reach a stage of fixed attenuation. The particular objective has been to establish a sector in the range of attenuation over which the culture virus is (*a*) harmless and (*b*) useful as a single dose vaccine. A subsidiary practical point to receive attention has been the necessity or otherwise for "reaction" on the part of vaccinated cattle.

1. *Method of Testing.*—Immunity of vaccinated cattle has always been tested by injecting one cubic centimetre of "tumour lymph" subcutaneously behind the shoulder, and in order to assess results the following records of controls and virus passage cattle should be consulted. Of 84 such cattle that have come under my own observation, the results of injecting virus have been:—

(*a*)	Progressive local swelling, etc.:	
	Died or destroyed *in extremis*	67
	Destroyed early	7
(*b*)	Arthritic form:	
	Died	2
	Severe reaction, recovered	1
(*c*)	Mild fever, small swelling, recovered	3
(*d*)	Immune (no reaction of any kind)	3

All the cattle considered in the above statement were young adult bulls with the exception of three sucking calves. It was observed incidentally that the calves all succumbed to the commonest form of the infection and the three arthritic cases were in adults. The immune cattle all came up for post-mortem examination at later dates and were found to be quite normal as regards pleuro-pneumonia infection. In regard to testing immunity, it thus seems that to use as few as two cattle in any one test, with two controls to any one virus injection, will with considerable certainty eliminate errors of assessment owing to the employment of immune animals.

2. *Actual Tests.*—No information could be gleaned from available literature as to the length of time required after vaccination for a solid immunity to develop; cultures of various ages had been tested at various intervals—usually several weeks—but fortunately the generation of the culture was in most cases recorded. Certainly no attempt had been made to correlate the data in order to assess the antigenic value of cultures in terms of the speed with which they produced immunity. Since, however, a new culture medium was being used, it was decided provisionally to leave earlier experiments out of consideration. In order to get some rough idea of the time required a preliminary experiment was carried out, using a culture of moderate age—actually of the 47th (weekly) generation. Tested by the standard method, no immunity was observed at the end of two weeks, at four and six weeks there were definite signs of developing immunity, and at nine weeks immunity was solid. Following on this, another experiment was performed, using (*a*) very young, dangerous

cultures, (*b*) slightly older and proved safe cultures, and (*c*) relatively old cultures. Actually cultures of the 2nd, 17th, and 115th generation were used. The results were:—

(*a*) Very young cultures of the 2nd generation killed half the cattle in the process of vaccination, but produced a solid immunity in less than three weeks.

(*b*) Fairly young cultures of the 17th generation produced a fairly solid immunity in three weeks and a solid immunity in less than six weeks.

(*c*) Old cultures of the 115th generation seemed to produce a low immunity which showed no signs of strengthening with advancing time—at any rate, not up to the termination of the experiment.

The results of these two experiments showed that the culture vaccine should consist of the youngest safe cultures; safety is assured after about six weekly transplants and immunity is produced within about two months by cultures of 40 odd generations. Actually in the Sudan the vaccines now issued are from ten to twenty-six weekly generations in age; the latter number is chosen for the sake of simplicity, i.e. six months.

3. "Reactions."—The hypothesis, in favour of which experimental records have by some workers been interpreted as proof, that in order to produce a solid immunity a vaccine must first induce some form of visible "reaction" in a vaccinated beast, cannot be supported by one's personal observations. In no case has any form of reaction—local, thermal, or general—been observed in any beast injected behind the shoulder with cultures of six or more generations, whereas subsequent tests with living virus have proved the existence of solid immunity; probably because sheer mechanical injury has always been avoided by the use of fine-calibre hypodermic needles.

4. Duration of Immunity.

No very deliberate experiments have been carried out to determine the duration of the immunity conferred by vaccination. Two cattle that had been vaccinated one year and seven months earlier were found to be immune to subcutaneous injection of tumour lymph, while controls succumbed. The culture used in vaccination was about 50 generations of age (in Martin's broth).

5. Routine Methods of Preparation and Issue.

The routine method in the Sudan is to maintain cultures of the virus in serum peptone broth, transplanted weekly. Every transplantation is controlled by surface cultures on serum agar. Before issue of the tenth and older generations any new strain is tested for safety at the sixth generation.

Vaccine is issued as a four days' growth in broth medium in 2-ounce bottles with rubber stoppers, the seed material being controlled by surface cultures and the final product judged by its physical appearance. The dose employed is 2 cubic centimetres; this dose was originally used in the Sudan, and there seems to be no reason for changing it—it is of convenient size and everybody is used to it.

All bottles issued are dated, and standing instructions are to use as fresh as possible and in any case within a fortnight. Possibly the vaccine remains useful, even under field conditions, for more than a

fortnight (experiments are now in progress to determine this point), but it is considered that meanwhile the best policy is to use as soon after issue as possible.

In order to compensate for possible contamination in the process of stoppering—the only manipulation that cannot be " controlled "—5 per cent. of bottles are sent above the number requisitioned, and at the time of use any bottles that are obviously contaminated are discarded. This method is considered better than further incubation after stoppering; contamination occurs in very few bottles, and it is preferred to issue vaccine without too lengthy a sojourn in incubators.

6. Vaccinations in the Field.

Very extensive vaccination has not yet been carried out in the Sudan, issues having only been made during the last three years. The general results have been good in the sense that both veterinary officers in the field and owners are satisfied that the vaccine prevents pleuro-pneumonia for some indefinite period.

The only difficulty encountered is the occasional development of large swellings at the site of vaccination, responsibility for which is not willingly accepted either by the vaccinating officer or by the laboratory. Reference to records of such complaints does not show that more swellings have occurred with younger cultures; in fact, the most serious complaint yet received was after the injection of cultures of 59th generation.

Fortunately for the field officer, these swellings are rarely fatal, although many of them burst and leave large wounds (not in itself a symptom of local pleuro-pneumonia infection), and on behalf of the laboratory it is nearly always possible to quote the issue of identical vaccine to another station without adverse report.

In consideration of the general policy of vaccination, the accidents are so few as to be of no account, but, since one accident is more trouble to explain away than a thousand successful vaccinations are to carry out, it seems that an attempt should be made to explain and prevent them.

In the first place, the swellings, as far as can be gathered from reports, are not typical " pleuro-pneumonia " tumours; they commonly burst and suppurate, indicating the presence of pyogenic bacteria. The presence of such organisms is assumed, although no material has yet been received in the laboratory for examination. The main point at issue is to determine the source of these organisms. Every precaution is taken in the laboratory to issue pure vaccine, including the provision of a few additional bottles to replace possibly contaminated ones, and it seems unlikely that contamination enters at this source.

The hypothesis is therefore brought forward that the most serious factor in causing trouble is the difficulty of skin sterilization. That skin is difficult to sterilize in any circumstances is well known, while in the process of vaccinating a herd of semi-wild cattle in a dusty country it must be even more difficult. The vaccine consists of a relatively sparse growth of pleuro-pneumonia virus in a rich culture medium without any preservative, and this would provide ideal conditions for the multiplication of extraneous bacteria introduced with it. It is recognized that most of the bacteria in sheer " dirt " are not very pathogenic, but an occasional few probably are; it is these random few that give rise to the occasional swellings that are observed.

7. Summary.

1. The virus of contagious bovine pleuro-pneumonia can be cultivated in serum peptone broth, and in this medium gives a richer culture than in "Martin's broth." It also grows very well on solid media made by adding agar to the above. It is recommended that 2 per cent. of peptone be used instead of the 1 per cent. employed in the commoner bacteriological media.

2. In the above liquid medium the virus becomes harmless after about six weekly subcultures. At this stage no visible reaction of any kind is noticed in test cattle.

3. Cultures of few generations in subculture produce immunity more rapidly than those of many generations.

4. No "reaction" is necessary in a vaccinated beast in order to ensure immunization. A single injection of a small volume of culture of the correct degree of attenuation is adequate.

5. Few observations have been made on the duration of immunity, but two cattle were found to be immune after one year and seven months.

6. In the field, vaccinations carried out on a basis of the foregoing principles have been very satisfactory. The only apparent objection is the occasional development of large swellings, and these seem not to be referable to the nature of the vaccine, but to the difficulty of skin sterilization under field conditions.

Paper No. 14.

PLEURO-PNEUMONIA CONTAGIOSA BOVUM.

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(Bovine Pleuro-pneumonia: Lung-sickness of cattle, British; Lungenseuce der Rinder, German; Peripneumonie contagieuse, French; Polmonera-polmonite essudativa, Italian.)

Bovine pleuro-pneumonia is a contagious disease naturally confined to bovines; it is caused by a filterable virus which produces an exudative fibrinous pneumonia and pleuritis, and specific histological lesions.

GEOGRAPHICAL DISTRIBUTION.

It is said that bovine pleuro-pneumonia occurred from time immemorial in Central Europe. It remained localized in a portion of Germany, Switzerland, France, and Italy, up till the end of the 17th century, and subsequently extended in various directions. About the middle of the 19th century it had invaded the countries of Western Europe, and as the result of the exportation of infected cattle was introduced into other parts of the world.