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Immunological Studies on Bluetongue in Sheep.*

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Introduction.

Bluetongue has been recognized as a specific disease of sheep since the commencement of the sheep breeding industry in the early days of the colonization of the Cape Colony. Theiler (1905) reported that bluetongue was caused by an ultravisible virus and he, together with Spreull, Hutcheon and Robertson, during the early years of the present century described many of the characteristics of the disease. Theiler (1906) mentioned that Spreull had introduced a method of immunizing sheep based on the use of hyper-immune serum and virus, and that he had conducted some experiments on the lines indicated. During the course of this work, which necessitated passage of the virus through numbers of sheep, he noticed that the virus had lost its ability to set up a fatal infection. He then described a new method of immunization based upon vaccination with a virus attenuated by serial passage through sheep. Laboratory experiments on 897 sheep gave satisfactory results so that after an additional preliminary field trial during the season 1906-07 on 5,875 sheep the vaccine was released for general use. This somewhat remarkable phenomenon of attenuation of a virus by passage through the host, which it is intended ultimately to protect, has been the subject of comment on many occasions, and was confirmed by du Toit (1929). In the present article the contention is submitted that the virus is not amenable to attenuation by what may be regarded almost as the classical procedure, and an explanation for the misconception is tendered.

Undoubtedly the introduction of Theiler's method of immunization and the persistence with it, with only minor modifications (du Toit, 1929), until the present day has been a boon to the sheep breeding industry, in fact it is no exaggeration to state that without it, profitable sheep breeding would have been impossible in many areas where it has thrived. However, the decreased margin of profit resulting from keen competition in the wool industry, the greatly increased value of individual animals and flocks of stud sheep, and finally the increased attention being paid to the breeding of hyper-susceptible English mutton breeds for the production of high quality fat lambs, have served to focus attention upon two cardinal defects, viz. that it is not safe and that the resultant immunity is inadequate. These defects are mentioned by du Toit (1929) and prompted his investigation into the nature and duration of immunity. In the introduction to that report the complaint that the vaccine produced unduly severe reactions is dismissed with the statement that " whenever such complaints could be investigated

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it was found that other causes and not the bluetongue vaccine were responsible for the severe reactions or the mortality in sheep ", e.g. natural infection with virulent virus prior to immunization or intercurrent heavy worm infestation. It will be shown that at least in many instances this explanation is not acceptable, and that more adequate explanations based on direct experimental evidence may be advanced. As regards the failure to produce an adequate immunity, du Toit (1929) drew the tentative conclusion that this was due at least in part to the short duration of solid protection produced by the vaccine. Belief in this transient active immunity led to the fairly general practice in some parts of the country of the immunization of sheep twice during a season, i.e. in October and again in January immediately before the incidence of natural bluetongue reaches its peak. This practice does not appear to have achieved the expected results. It will be shown that solid protection has been found to persist for 12 months, the limit of the experiment, and that a probable explanation of the previous conception is probably due to failure to recognize the existence of a plurality of virus strains.

To illustrate the validity of persistent and frequent complaints that the vaccine produces unduly severe reactions and that insufficient protection is conferred upon the treated sheep particulars of two out of several detailed investigations are given:—

(1) Towards the end of January 1944 a report was received about a serious outbreak of bluetongue on several farms in the vicinity of Nelspoort in the Karoo. The incidence of bluetongue in this district is a rare occurrence, in fact it is from this area that some 2,000 sheep are purchased annually to serve as susceptibles for the production of Theiler's bluetongue vaccine* and for research purposes at Onderstepoort. The position was reported to be serious and assistance to control the outbreak was urgently solicited so arrangements were made to carry out a full investigation in company with the Government Veterinary Officer of De Aar (Mr. S. W. de Villiers), whose knowledge of local conditions would be invaluable. A number of farms were visited and several flocks of Merino sheep varying in number from two to five thousand were inspected. It was the general consensus of opinion of the farmers that no comparable outbreak of bluetongue had previously been known. The previous year a few cases had been diagnosed, approximately 10 to 25, in the different flocks. One farmer stated that he had lost 9 per cent. of his flock during the outbreak in 1944. In view of the low incidence of the disease in the past, routine annual immunization had never been practised so that the vast majority of the sheep could be regarded as fully susceptible. The care and treatment of the sick sheep was a severe burden to the owners so that, in desperation one farmer had decided to immunize his entire flock of 4,000 Merino sheep. When 1,400 of these sheep, which had been treated nine days previously were inspected, approximately 500 (35 per cent.) were showing clinical symptoms of bluetongue indistinguishable in severity from those affected with the naturally contracted disease. The comparatively low incidence of natural cases of bluetongue in the remainder of the flock justified the conclusion that the severe reactions in these fully susceptible

* The method of preparation has been described by Du Toit (1929).

sheep had been produced by the vaccine and no contributory factor other than the weather conditions (see Neitz and Riemerschmid, 1944) could be found to account for it.

From one of the naturally contracted cases on one of the farms a strain of virus was isolated subsequently, which is referred to in the text as the " Nelspoort " strain.

(2) In January 1939 a visit was paid to the farm Mimosa Park in the East London district to study the results of a field experiment which had been planned to throw some light on a previous complaint from the owner of the farm. The history of this farm was that for three years prior to 1937 the Onderstepoort vaccine prepared from the " Veglia " strain had been used with satisfactory results. In October 1937 all the sheep (Merinos) approximately 3,000 in number had been vaccinated. During the ensuing bluetongue season a large but undetermined number contracted bluetongue and approximately 300 (±10 per cent.) died. In addition the marked loss of condition, protracted convalescence of a large number of recovered sheep had had a very serious effect upon the following wool clip. In 1938 arrangements had been made for the immunization of all the lambs on the farm under veterinary supervision and on the 27th of October 848 lambs, approximately five months old, were injected by the Government Veterinary Officer of East London, (Mr. A. Matthews). Mild reactions followed in these lambs and no mortality or loss of condition was observed. Six weeks later the owner reported that cases of bluetongue were occurring. When the flock was examined on the 18th January 1939 it was found that approximately 30 per cent. of the flock had contracted bluetongue and that 55 sheep (6·5 per cent.) had died. On the whole the flock was in a miserable condition and it was apparent that there had been a serious set-back in the normal development. No cases of bluetongue had occurred in the main flock, which had been immunized several times and in addition had been exposed to natural infection during several seasons. As the general system of animal husbandry was beyond reproach and from a veterinary point of view strictly in accordance with our own instruction, it was obvious that immunity produced by the vaccine was insufficient to protect against the natural virulent infection.

From one of the affected sheep a strain of virus was isolated subsequently, which is referred to in the text as the " Mimosa Park " strain.

The experience at Nelspoort indicates that the use of the vaccine in a fully susceptible herd under field conditions is dangerous and the Mimosa Park incident that the immunity conferred may be inadequate. It was a full appreciation of these defects which led to reinvestigation of the entire problem of bluetongue by a small team of workers and the results of one phase of that investigation form the basis of this report.

Experimental Observations.

(1) *The Isolation of the Strains.*

Over a period of forty years a number of strains of blue tongue virus have been isolated from different sources, and as these are constantly referred to in the text it is necessary to detail their origin.

(i) *The "Theiler" Strain.*—This is the original strain isolated by Theiler, who used it for research work upon which he based his classical description of the disease. The origin is somewhat obscure, but apparently it was obtained from a natural case of the disease in the Cape Province at the beginning of this century. It is the strain which was used for the routine production of vaccine from 1905 to 1929, when it was replaced by the "Veglia" strain. In 1942 the Government Veterinary Officer, Vryheid, Natal, found a bottle of vaccine which had been misplaced in the office for 25 years from the information obtained from the batch number and the record of the date of preparation. It was forwarded to Onderstepoort and on test it was found to produce mild febrile reactions in sheep kept in the stable. A supply of virus was collected and the strain was brought into general use again in 1942.

(ii) *The "Veglia" Strain.*—In February 1927 a lamb (No. 16016) developed bluetongue at Onderstepoort as a result of natural infection (du Toit, 1929). The lamb died, but before death infective blood was collected. This strain was used to confirm Theiler's work on the attenuation of the virus by serial passage through sheep and from 1929 to 1942 it was used for the production of vaccine.

(iii) *The "Bekker" Strain.*—In March 1933 a strain of virus was isolated from a cow suffering from the so-called pseudo-foot and mouth disease in the Standerton district of the Transvaal highveld. It was brought to Onderstepoort where its identity to bluetongue was established and has been described by Bekker, de Kock and Quinlan (1934).

(iv) *The "Camp" Strain.*—In March 1937, somewhat earlier than usual, a number of sheep were brought to Onderstepoort for the routine production of vaccine. Before being drafted into experiment they were confined in a small camp and kept under observation. After an interval of 19 days after arrival eight sheep contracted bluetongue and of these 4 died. From one of the fatal cases (No. 48736) the "Camp" strain of virus was isolated. It will be noticed that this and the "Veglia" strain were isolated in precisely the same locality but at an interval of ten years.

(v) *The "Mimosa Park" Strain.*—The virus was isolated on the farm Mimosa Park in the East London district from a sheep reacting to a natural infection after previous immunization against the "Veglia" strain. Full details have been given above.

(vi) *The "Byenespoort" Strain.*—During the course of investigations into an enzootic of redwater in cattle due to *Babesia bovis* (Babes, 1888) infection during March 1941 on the farm Byenespoort in the Pretoria district fresh blood was subinoculated from a sick heifer into a sheep. A febrile reaction was produced and the virus was subsequently identified as bluetongue.

(vii) *The "University Farm" Strain.*—During February 1942 several cases of bluetongue were diagnosed in a small flock of sheep on the experimental farm run by the Agricultural

Research Institute in conjunction with the Faculty of Agriculture, University of Pretoria. These sheep were on open grazing, had been immunized the previous November with Onderstepoort vaccine ("Veglia" strain). Through the courtesy of Prof. M. W. Henning, who reported the breakdown in immunity, blood was collected from reacting animals and the strain was isolated.

(viii) *The "Nelspoort" Strain.*—Full details of the isolation of this strain in January 1944 from a susceptible sheep in the Beaufort West district of the Cape Province have been given above.

(ix) *The "C.43" Strain.*—In February 1943 Mr. R. du Toit produced a case of bluetongue by injecting into a susceptible sheep an emulsion of a species of *Culicoides* trapped at Onderstepoort. I am indebted to him for a supply of this strain of virus originating from an insect vector. This was the third strain isolated at Onderstepoort over a period of 16 years.

(x) *The "Cyprus" Strain.*—In February 1944 Mr. R. J. Roe, Senior Veterinary Officer of Cyprus, forwarded to Onderstepoort various blood samples obtained from sheep which were suffering from an acute febrile disease accompanied by heavy mortality. Bluetongue was suspected, and this was supported by the detailed report on the clinical symptoms and post mortem lesions. The diagnosis was confirmed by sub-inoculation, immunity and cross-immunity experiments and the strain has been retained for comparative purposes.

The origin of these ten strains of bluetongue virus may, therefore, be summarized as:—

(*a*) From susceptible sheep:—"Theiler", "Veglia", "Camp", "Nelspoort" and "Cyprus" strains.

(*b*) From sheep immune to the "Veglia" strain:—"Mimosa Park" and "University Farm" strains.

(*c*) From cattle:—"Bekker" and "Byenespoort" strains.

(*d*) From an insect vector (*Culicoides* spp.):—"C.43" strain.

The nomenclature is purely arbitrary, being based on common usage in the laboratory. It will be possible to introduce some standard nomenclature only when an adequate classification has been worked out.

(2) *Methods Employed for these Studies.*

The experimental sheep were obtained from large consignments of Merino forwarded to Onderstepoort from time to time. These sheep were purchased from areas in the Karroo, where bluetongue does not normally occur and a long experience has shown that there is no reason to doubt their full susceptibility. While in experiment the sheep were maintained in loose boxes in a stable, fed on adequate maintenance ration prescribed by the Section of Nutrition of the Institute, from the feeds available at the time were allowed fresh water *ad lib.* and were not permitted outside even for exercise. Under these conditions they do remarkably well, but it will be noted that they were continuously in the shade, and at no time were they exposed to the sun or any adverse weather conditions. These conditions, therefore, were entirely artificial. Temperatures were taken

twice daily at approximately 7.30 a.m. and 4 p.m. and these temperatures were always checked whenever deemed necessary. All animals were examined every morning and a careful record kept of any clinical reactions. From time to time blood smears stained with Giemsa were examined to exclude the incidence of some protozoal infections e.g. *Babesia ovis*, Babes, 1892, *Anaplasma ovis*, Lestoquard, 1924, *Eperythrozoon ovis*, Neitz, Alexander and du Toit, 1934, and *Rickettsia ovina*, Lestoquard and Donatien, 1936, as a complicating factor. This is a precaution which has not always received adequate attention in the past and its importance in *in vivo* immunity tests of this nature cannot be too strongly emphasized.

Immunity tests with homologous virus strains were carried out from three to six weeks after the immunizing infection, and in the vast majority of cases on the 28th day. Care was taken that for any immunity test a sheep did not receive an injection of its own blood collected during a previous reaction. Sheep were submitted to a cross-immunity test three to six weeks after an homologous immunity test had been applied, i.e. to avoid any possibility of an error. The immunity to any one strain of virus was established and confirmed before the immunity to another strain was tested.

Adequate supplies of virus were maintained by collecting blood from reacting susceptible sheep at what was judged to be the height of the febrile reaction. With aseptic precautions the blood was tapped from the jugular vein into bottles containing 1/10 of the final volume of anticoagulant and preservative fluid consisting of 500 parts of glycerine, 5 parts potassium oxalate, 5 parts of phenol in 500 parts of distilled water. This virus was stored at about 10° C until required and except in serial passage experiments was only used after it had been stored for a minimum period of two weeks to ensure that any protozoan contaminants had become inactive. In passage experiments fresh infective blood was used for subinoculation.

It was appreciated that, in spite of the most rigorous precautions, there was some risk of mixing up virus strains or alternatively of using for test purposes a strain which had been modified in some way by passage or by storage. To obviate this a stock supply of virus (R.A. Alexander, personal communication) was prepared from spleens of selected sheep destroyed at the height of the reaction and dried *in vacuo* over anhydrous calcium sulphate at a pressure of less than 0·001 mm. of mercury after rapid preliminary prefreezing in an alcohol-carbon dioxide snow mixture at −70° C. This dried powder sealed in ampoules in an atmosphere of dry air if properly prepared retains infectivity for several years. From time to time sheep were infected with an emulsion prepared from this desiccated spleen and a fresh supply of virus collected in the usual way.

The dose of infective blood for all tests was 1 or 2 c.c. of blood given subcutaneously unless specifically stated to the contrary.

(3) *The Interpretation of the Nature of the Reactions.*

In work of this nature, where death or survival is not the chief criterion of the existence of immunity, it is exceedingly difficult to devise any fixed standard by which to compare differences in the degree of immunity. The reaction produced by each strain of virus in a number of susceptible sheep was judged according to the severity and duration of the febrile reaction, and the severity of the pathognomonic clinical symptoms e.g. buccal and nasal hyperaemia, stomatitis, swelling of the lips, coronitis, loss

in condition, and appetite. Any deviation from this picture was estimated in the case of each individual sheep and designated in the conventional manner by crosses e.g. xxxx = severe febrile reactions with pronounced symptoms, x = mild febrile reaction with no other clinical symptoms and ? = an indefinite or doubtful reaction. The estimate of the severity of a reaction was further complicated by the fact that in many cases the degree of the temperature reaction was not correlated in any way with the severity of the clinical symptoms e.g. a slight fever never rising above 105° F. may be accompanied by the most severe nasal and buccal lesions, inappetence, extensive pododermatitis with subsequent exungulation, general weakness, torticollis and emaciation, or alternatively a fever with a temperature persisting at 108° F for some days might be the only symptom. In spite of this, however, it is confidently believed that although errors of judgement may have occurred in isolated cases there has been no gross error in the general interpretation of the severity of the reaction.

A. *The Plurality of Virus Strains.*

The general object of the investigation was to determine the reciprocal cross-immunity between each of the ten virus strains. It is obvious that in order to obtain results which are statistically significant in *in vivo* work of this nature, it would be necessary to draft excessively large numbers of animals into experiment. Consequently the investigation was spread over a number of years and full use was made of animals in a wide variety of experiments providing the full history was accurately known and the conditions enumerated above were fulfilled. This is the explanation for the gaps in the tables of results and indicates that it will be many years before this investigation can possibly be completed. The results of the cross-immunity tests on merino sheep which have been completed to date are presented in tabular form in Table 1.

1. The first broad review of this table establishes a point of the utmost significance, viz. that there exists a plurality of antigenically different strains of virus in that the immunity to one strain may be broken down after reinfection by another strain.

2. Each strain of virus produces a solid immunity against the same strain as shown by the fact that in no single instance did the homologous immunity test produce any detectable reaction.

3. Between all the strains investigated there is a variable degree of common or basic immunity. This is shown numerically by the animals which failed to react to a heterologous immunity test. From a clinical point of view it was just as apparent. Even in the case of the sheep immune to the "Veglia" strain, which were tested with the "Camp" strain, where 71 out of 79 sheep tested reacted, the reactions were modified and only in rare instances was the severity of the clinical lesions equal to those seen in a fully susceptible animal. There was no increase in the period of incubation and frequently an equally high maximum temperature might be developed during the fever, but usually the course of the disease was shortened as shown by a sudden rise in temperature and after a few days a sudden return to normal by crisis. In addition to the decreased severity of the buccal and feet lesions the period of convalescence was considerably decreased. It must be stated however that in several instances the appearance of two distinct bands of hyperaemia as a result of two successive bouts of pododermatitis were noted.

TABLE 1.

The *in vivo* cross-immunity Tests between the various Strains of Bluetongue Virus.

Sheep Tested with Virus Strains.	Sheep Immunized Against Virus Strains.									
Strains.	"Theiler."	"Veglia."	"Bekker."	"Camp."	"Mimosa Park"	"Byenespoort."	"University Farm."	"C.43."	"Nelspoort"	"Cyprus."
"Theiler".....	0+0+25	0+0+15	0+0+12	0+0+0	1+0+6	0+0+0	2+0+6	0+0+0	0+0+2	4+0+4
"Veglia".....	2+0+23	0+0+40	0+0+20	12+2+20	1+0+25	0+0+0	0+0+0	0+0+0	0+0+0	0+0+0
"Bekker".....	1+0+24	24+6+134	0+0+90	33+11+76	17+1+22	0+0+4	0+0+1	0+0+0	0+0+8	5+0+4
"Camp".....	9+0+16	71+3+5	5+1+19	0+0+90	13+1+25	1+0+2	1+1+3	0+0+0	0+0+0	0+0+0
"Mimosa Park".	13+1+11	47+7+30	14+7+8	27+10+20	0+0+100	1+0+1	0+0+0	0+0+0	3+0+3	0+0+5
"Byenespoort"..	4+5+16	15+1+9	14+6+30	5+1+10	3+1+8	0+0+5	1+1+7	0+0+1	0+0+0	0+0+0
"University Farm".	18+1+6	11+1+3	8+2+8	5+0+0	2+0+0	1+0+2	0+0+30	0+0+0	0+0+0	0+0+0
"C. 43".....	9+4+13	1+0+0	5+3+2	0+0+1	3+1+5	0+0+2	14+0+6	0+0+1	1+0+1	1+0+11
"Nelspoort".....	3+0+22	0+0+0	6+2+4	0+0+0	13+1+3	0+0+0	3+0+2	0+0+0	0+0+20	0+0+1
"Cyprus".....	19+0+6	0+0+0	12+3+11	0+0+0	0+0+20	0+0+0	9+1+4	1+0+1	3+0+1	0+0+50

NOTE.—1+2+3 signifies—6 sheep tested :— 1 reacted (= no or only partial immunity).
2 doubtful reactors (= almost complete immunity).
3 did not react (= complete immunity).

4. The "Theiler", "Veglia" and "Bekker" strains show almost complete reciprocal cross-immunity. The only breakdowns in immunity were observed in 24 out of 164 "Veglia" immunes and one out of 25 "Theiler" immunes when tested with the "Bekker" strain. This observation suggests the possibility of placing these three strains together in one group, but a definite antigenic identity is negatived when the differences in reactions to heterologous strains is examined, e.g. the "Camp" virus was able to break down the immunity of only 5 out of 25 (=20 per cent.) sheep immune to "Bekker" virus, but caused reactions in 71 out of 79 (=90 per cent.) sheep immunized against the "Veglia" strain and 9 out of 25 (=36 per cent.) immunized against the "Theiler" strain. It would seem therefore that there is a variation within very wide limits in the antigenic structure of strains even though the common or basic resemblance may be pronounced.

5. From the data available it is not possible to group the strains on the basis of identity of antigenic structure.

Before discussing the significance of the above results it is of interest to examine in greater detail the results of those cross-immunity tests in which the number of animals tested was sufficiently large (that portion of Table I enclosed by the dotted line), i.e. sheep immune to the "Theiler", "Veglia", "Bekker", "Camp" and "Mimosa Park" tested with these strains and with the "Byenespoort" strain in addition. The results are set out diagrammatically in Chart I.

In the chart the length of each column covered by diagonal hatching represents the percentage of complete protection, horizontal hatching represents little or no immunity and the dotted area partial or incomplete immunity. The final block is a composite representation of the immunity produced by four of the strains ("Veglia", "Bekker", "Camp" and "Mimosa Park") against five of the heterologous strains. The number of cross-immunity tests on sheep immune to the "Camp" and "Mimosa Park" strains respectively with the "Theiler" strain is statistically inadequate.

Again it is immediately apparent that each strain protects completely against itself and that there is a variable degree of protection against the heterologous strains. Further it becomes quite clear that the selection of the "Theiler" and "Veglia" strains for routine mass immunization purposes was unfortunate, because these strains produce by far the least polyvalent immunity (c/f Table 1), whilst any one of the others, with a slight advantage in favour of the "Bekker" or "Mimosa Park" strains, would have been preferable. If there is no antagonistic action between all the components represented in a mixture of two or more strains to be used for immunization purposes, then it is readily apparent that a mixture of say the "Bekker" and "Mimosa Park" virus would produce a polyvalent immunity far superior to anything, one could hope to obtain with a single strain.

No opportunity presented itself of testing the immunity produced by the simultaneous injection of two virus strains, since this aspect of the problem became apparent only later in the course of the investigation. However, a number of sheep, which had reacted to one strain of virus, and which had subsequently received an immunity test by injection with a heterologous strain, were retained for a test with a third strain. Full details of these experiments are given in Appendix I, but the results are summarized in tabular form in Table 2.

CHART I.

Cross-Immunity Tests between Homologous and Heterologous Virus Strains.

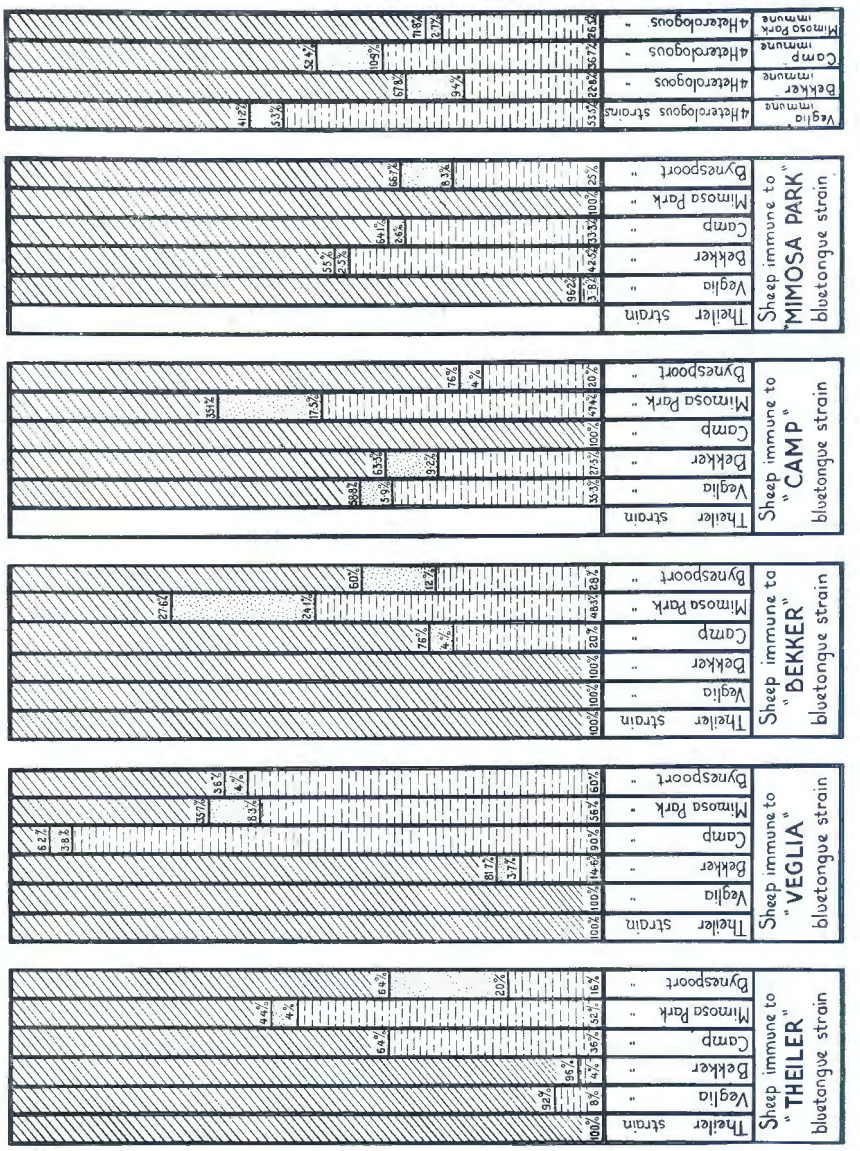

TABLE 2.

Immunity Produced by Two Strains of Virus against a Third Strain.

Experiment.	Number of Sheep.	Virus.	Reaction.	Interval in days.	Virus.	Reaction.	Interval in days.	Virus.	Reaction.
1.....	17	"Mimosa Park"..	All reacted..	28–40	"Bekker".....	8 Reacted..... 9 No reaction..	47–67	"Camp"..	3 Reacted. 14 No reaction.
2.....	15	"Mimosa Park"..	All reacted..	26–73	"Camp".....	7 Reaeted..... 8 No reaction.	38–93	"Bekker".	5 Reacted. 10 No reaction.
3.....	17	"Camp".....	All reacted..	18–63	"Mimosa Park"..	7 Reacted..... 10 No reaction.	38–76	"Bekker".	5 Reacted. 12 No reaction.
4.....	7	"Camp".....	All reacted..	21–23	"Bekker".....	3 Reacted..... 4 No reaction.	47–60	"Mimosa Park"....	3 Reacted. 4 No reaction.

Results.—Although the number (56) of sheep used in this series of experiments is small, the results, particularly if read in conjunction with the cross-immunity tests shown in Table 1, are of great importance.

(1) From experiments No. 2 and 3, it appears that the sum total of the immunity produced by two strains of virus is the same no matter in which order they are injected, e.g. of 15 sheep immunized first against "Mimosa Park" and then against "Camp", 5 reacted to "Bekker" and 10 were solidly immune, whereas in 17 sheep when "Camp" was injected first and "Mimosa Park" subsequently 5 reacted and 12 were immune. This slight difference is quite insignificant and consequently the group of 32 sheep may be regarded as being immune to "Camp" and "Mimosa Park" when tested with the "Bekker" strain.

(2) A combination of two strains of virus produces the same immunity against a third strain as that which would be produced by the best immunizing strain alone, e.g.:—

(*a*) Sheep immune to "Mimosa Park" tested with "Camp": 13 out of 39 reacted =33 per cent. (c/f Table 1).

Sheep immune to "Bekker" tested with "Camp": 5 out of 25 reacted =20 per cent. (c/f Table 1).

Sheep immune to "Mimosa Park" and "Bekker" tested with "Camp": 3 out of 17 reacted =18 per cent., i.e. the immunity of the combination was that produced by the better antigenic strain "Bekker".

(*b*) Sheep immune to "Mimosa Park" tested with "Bekker": 17 out of 40 reacted 42·5 per cent. (c/f Table 1).

Sheep immune to "Camp" tested with "Bekker": 33 out of 120 reacted 28 per cent. (c/f Table 1).

Sheep immune to "Mimosa Park" and "Camp" tested with "Bekker": 10 out of 32 reacted =31 per cent., i.e. the immunity of the combination was approximately that produced by the better antigenic strain "Camp", since the difference between 28 and 31 per cent. is hardly significant, but the difference between 31 and 42·5 per cent. is significant.

(*c*) Sheep immune to "Camp" tested with "Mimosa Park": 27 out of 57 reacted =47 per cent. (c/f Table 1).

Sheep immune to "Bekker" tested with "Mimosa Park": 14 out of 29 reacted 48 per cent. (c/f Table 1).

Sheep immune to "Camp" and "Bekker" tested with "Mimosa Park": 3 out of 7 reacted =43 per cent.

The number of sheep in this group is particularly small, but nevertheless the close agreement in the figures is worthy of note.

(3) The immunity against one strain of virus does not interfere with the antigenic action of another strain. In other words a partially immune sheep will respond to the stimulus of the specific antigenic components of a heterologous strain of virus in spite of the presence of the basal immunity common to all strains, or of additional common antigens.

(4) There is a marked variation either in the susceptibility of individual sheep to bluetongue or in the ability of individual sheep to develop immunity. Of the 56 sheep in the series of experiments all reacted to the first injection of virus; 23 reacted to the second injection but not to the third; 11 did not react to the second injection, but did react to the third and 5 reacted to all three injections.

Comment.—From a clinical point of view it was found that the degree of common or basal immunity was not increased by giving a sheep immune to one strain of virus an injection of a heterologous strain. This is illustrated by the fact that when a sheep reacted to a third strain of virus, the period of incubation was not lengthened; the decrease in severity of the lesions and the reduced course of the febrile reaction was not further enhanced. This may be interpreted as showing that repeated injections of a homologous strain of virus cannot increase the degree of immunity to that strain or to other strains of different antigenic structure, and that repeated injections of heterologous strains will only result in the production of immunity against the specifically new antigenic components introduced with each injection. In other words polyvalent immunity can only be produced by the injection of a number of antigenically different strains of virus and cannot result from the repeated injection of the same strain.

Discussion.—The establishment of the existence of a plurality of antigenically different virus strains is of prime importance in obtaining a clear picture of the requirements for any campaign of mass immunization in the field, and in offering an adequate explanation for the reported failures of the present vaccine. This aspect will be discussed fully after the experiments on the duration of immunity have been detailed.

Two criticisms may be levelled against the present series of experiments:—

(1) They are conducted under laboratory and not under field conditions. Apart from the difficulty of organising similar controlled investigations in the field it is desirable to have uniform conditions for a series of experiments which had to be prolonged over a long period of time so that the only variable factor in each case was the strain of virus used. It is believed that attention to this factor is of greater importance than an exaggeration of minor differences which may have escaped attention.

(2) The observations are not complete. Only one portion of the cross-immunity tests has been completed, but the results are so striking that an early report is necessary in the interest of the immediate problem. Greater speed in the completion of a more comprehensive invesigation will only be possible upon the elaboration of an *in vitro* test to be applied to a suitable small laboratory animal available in large numbers. Up to the present no such test has been worked out, but it must be borne in mind that the final criterion must always remain the direct *in vivo* resistance test of immunized sheep. In this instance the *in vivo* tests have been presented first in the hope that they will stimulate the development of some simple laboratory test.

B. *The Duration of Immunity.*

Du Toit (1929) investigated the nature and duration of immunity to bluetongue in sheep. He concluded that " generally speaking there was a

gradual, but slow decrease in immunity from the third to the twelfth month after vaccination, but even at the latter stage the immunity was strong enough to prevent losses ". Apart from the general interest of this rapid loss of immunity to a virus the phenomenon is of practical importance because it is the justification of the fairly widespread practice of immunizing sheep twice each year, in October at the begining of summer and again in January immediately prior to the highest incidence of the disease. This practice which has been followed by many breeders in the bad bluetongue areas has not been uniformly effective as a control measure. It was therefore decided to reinvestigate the problem in the light of the recently acquired conception of a plurality of strains.

Method.—A total of 111 sheep was selected from those which had shown typical febrile reactions to the " Veglia " strain of virus during the course of the annual test of the routine vaccine to be issued. From September to the end of December, when the risk of natural infection is considered negligible the sheep were confined in a small camp. From January onwards they were housed in a stable. When they were required for immunity test they were transferred to another stable where they were maintained under the same conditions as all the other experimental sheep. The " Bekker " strain of virus was used for the immunity tests because:—

(i) It could be relied upon to produce a well-defined febrile reaction with marked clinical lesions and a fairly high mortality.

(ii) It is antigenically closely related to, but not identical with the " Veglia " strain so that if anything a decrease or immunity would be accentuated. In any case the percentage of reactors would be compared with the statistically significant series shown in Table 1, i.e. 24 reactors out of 164.

Groups of sheep, 9 to 12 in number, were given their immunity tests at monthly intervals from 2 to 12 months after the immunity injection. The dose of infective preserved blood was 1·0 c.c. subcutaneously in about 75 per cent. and 5·0 c.c. in approximately 25 per cent. in an attempt to obtain some information on the effect of a larger dose; one or two fully susceptible controls were included in each group, the one receiving 1·0 c.c. and the other 5·0 c.c.

The results are given in Table 3.

Results.—Of the 111 sheep tested 98 (=88 per cent.) were solidly immune. This percentage is somewhat better than the 82 per cent. shown in Table 1, but the difference is insignificant. The 13 sheep which did react showed only mild febrile reactions without any clinical lesions of bluetongue. The reactions were incomparably milder than those shown by the 20 controls of which all showed marked febrile reactions with severe clinical lesions of bluetongue, and 14 died.

Of the 79 immune sheep which received 1·0 c.c. of virulent blood 10 (12·6 per cent.) reacted and of the 32 which received 5·0 c.c. 3 (9·4 per cent.) reacted. It is apparent, therefore, that within the limits of the challenging dose of virus there was no significant difference in the percentage of reactors, nor could any difference be detected clinically. Among the controls of those given 1·0 c.c. 8 died, and 3 recovered, while those given 5·0 c.c. 6 died and 3 recovered.

TABLE 3.

Duration of Immunity.

Sheep Immune to Strain "Veglia" Tested with Strain "Bekker".

No. of Sheep.	Interval in months.	Dose.	Reaction.	Controls.
12	2	*9–1·0 cc.	8 No reaction..... 1 reaction x, day 8–9.....	1·0 cc. Rxxxx and died.
		3–5·0 cc.	3 no reaction.....	
12	3	9–1·0 cc.	8 no reaction..... 1 reaction x, day 10–17.....	1·0 cc. Rxxxx and died.
		3–5·0 cc.	3 no reaction.....	5·0 cc. Rxxxx and died.
11	4	8–1·0 cc. 3–5·0 cc.	8 no reaction..... 2 no reaction..... 1 reaction x, day 11–16.....	1·0 cc. Rxxxx and recovered.
10	5	8–1·0 cc.	7 no reaction..... 1 reaction x, day 8–14.....	1·0 cc. Rxxxx and died.
		2–5·0 cc.	1 no reaction..... 1 reaction ? day 12–18.....	5·0 cc. Rxxxx and died.
9	6	6–1·0 cc.	4 no reaction..... 2 reaction x, day 9–15 and 8–24 respectively.	1·0 cc. Rxxxx recovered.
		3–5·0 cc.	3 no reaction.....	5·0 cc. Rxxxx recovered.
9	7	6–1·0 cc. 3–5·0 cc.	6 no reaction..... 3 no reaction.....	1·0 cc. Rxxxx and died. 5·0 cc. Rxxxx and died.
9	8	6–1·0 cc. 3–5·0 cc.	6 no reaction..... 3 no reaction.....	1·0 cc. Rxxxx and died. 5·0 cc. Rxxxx and died.
9	9	6–1·0 cc. 3–5·0 cc.	6 no reaction..... 3 no reaction.....	1·0 cc. Rxxxx and died. 5·0 cc. Rxxxx recovered.
9	10	6–1·0 cc.	4 no reaction..... 2 reaction x, day 8–13, and 10–16 respectively.	1·0 cc. Rxxxx recovered.
		3–5·0 cc.	2 no reaction..... 1 reaction x, day 7–12.....	5·0 cc. Rxxxx recovered.
9	11	6–1·0 cc.	5 no reaction..... 1 reaction ?, day 7–9.....	1·0 cc. Rxxxx and died.
		3–5·0 cc.	3 no reaction.....	5·0 cc. Rxxxx and died.
9	11	6–1·0 cc.	5 no reaction..... 1 reaction ? day 7–9.....	1·0 cc. Rxxxx and died.
		3–5·0 cc.	3 no reaction.....	5·0 cc. Rxxxx and died.
12	12	9–1·0 cc.	7 no reaction..... 2 reaction x, day 7–9.....	1·0 cc. Rxxxx and died.
		3–5·0 cc.	3 no reaction.....	5·0 cc. Rxxxx and died.
Total 111..	2–12		98 no reaction = 88·3 percent 13 reacted..... = 11·7 percent.	14 reacted and died. 6 reacted and recovered.

Rxxxx signifies:—Severe febrile reaction with pronounced clinical symptoms.
*9–1·0 cc. signifies that nine sheep received an injection of 1 cc.
3–5·0 cc. signifies that three sheep received an injection of 5 cc.

In the immunity test there was no significant difference in the percentage reactors in any of the groups of sheep so that there was no correlation whatever between the time factor and the degree of immunity. In fact, it is worthy of note that of 27 animals in the 7, 8 and 9 months interval groups there was not a single reactor.

Conclusion.—It is concluded that over a period of 12 months there is no detectable decrease in the immunity of sheep to the more virulent, but antigenically closely allied strain of virus. Within the limits of the experiment a five-fold increase in the test dose of virus had no effect upon the ultimate reaction.

Discussion.—This result is completely at variance with that reported by du Toit (1929) and it is not possible to offer any explanation for the difference. It should be noted, however, that in that work a virulent strain of virus (No. 16357) was used for rather more than half the immunity tests, and as no mention is made of the origin of that strain, there is very good reason to believe that an antigenically different strain had been selected. The conclusion which was drawn, namely that there was a gradual loss of immunity, should therefore be viewed in the light that at that time nothing was known about the existence of heterologous strains. This, however, does not explain the apparent decrease in immunity where the homologous virus was used.

C. *The Transmitted Immunity.*

Alexander and Mason (1941) investigated the transmissions of immunity from dam to offspring in the case of horsesickness and discussed the significance of the results in relation to the problem of immunization against that disease. They showed that the whole question of the protection of foals was a matter that required the attention of the breeder and not the immunologist, due to the fact that mares are bred to foal from August to December, and consequently the foals, which develop a strong but transient passive immunity enter the natural horsesickness season at a time when this immunity is inadequate to protect against natural infection, but is sufficiently potent to neutralize the attenuated virus in the present vaccine (Alexander, 1935). With regard to bluetongue Dixon (1909) concluded from observations in the field that suckling lambs possess a marked degree of resistance to this disease. No record whatever was found in the literature of any investigations on bluetongue in sheep similar to those of Alexander and Mason (1941). From an analogy with other diseases reviewed by Schneider and Szathemary (1939 and 1940), it is clear that transmitted immunity is a factor which cannot be taken into account, particularly in view of the fairly general practice of lambing down in autumn or winter approximately six months before the incidence of natural bluetongue.

Method.—The general plan of the investigation was to mate approximately equal numbers of bluetongue susceptible ewes as well as ewes immune to the "Veglia" and "Bekker" strains of virus, to test the susceptibility of each group of lambs to the "Bekker" strain at various ages, and then to ascertain the nature of the immunity to the "Bekker" strain approximately nine months later. After lambing the sheep and lambs were to be maintained in a stable away from exposure to natural infection. The test dose of virus in every case was 2·0 c.c. subcutaneously.

The results are given in tabular form in Table 4.

TABLE 4.

The Susceptibility of and the Immunity present in Lambs from Susceptible and Immune Ewes.

Ewes.	Lamb No.	Age in days.	Reaction (1)	Interval in days.	Reaction (2).
SUSCEPTIBLE.	66528	4	R xxx	—	—
	66415	16	R xx	285	N.R.
	66375	66	R xx	285	N.R.
	66372	68	R xxx	285	N.R.
	66353	79	R xxxx	—	—
	66350	80	R xx	—	—
	66351	80	R xx	—	—
	66352	80	R xxx	285	N.R.
	66345	88	R xx	—	—
	66324	109	R x	285	N.R.
	66322	109	R xxx	285	N.R.
IMMUNE.	66529	4	N.R.	281	N.R.
	66433	8	N.R.	285	R xxx
	66320	68	R xx	272	N.R.
	66338	93	R xx	285	N.R.
	66331	100	R xxx	285	N.R.
	66328	101	R xx	—	—
	66329	101	R xx	—	—
	66327	103	R xx	285	N.R.
	66318	103	R xxx	—	—

NOTE: (1) Bekker virus sheep No. 66206—2 controls R xxxx.
(2) Bekker virus sheep No. 68709—4 controls 3 R xxx.
1 R xxxx and died.

R x signifies:—Mild febrile reaction;
R xx ,, Typical febrile reaction;
R xxx ,, Typical febrile reaction and mild clinical symptoms;
R xxxx ,, Typical febrile reaction and pronounced clinical symptoms;
N.R. ,, No reaction.

Result.—Unfortunately the conditions under which it was necessary to maintain and rear the lambs were hardly suitable to the purpose and the mortality from causes not associated with bluetongue, particularly coccidiosis was high, so that only a limited number survived to the end of the experiment.

(*a*) *Observations on Lambs from Susceptible Ewes.*—All reacted to bluetongue after the injection of the "Bekker" virus. The course of the disease as shown by the febrile curves and the severity of the lesions were much less severe than those shown by the controls. After an interval of 285 days they were still solidly immune.

(*b*) *Observations on Lambs from Immune Ewes.*—Two lambs aged 4 to 8 days respectively failed to show any reaction after the injection of the "Bekker" virus. After an interval of 281 days the younger lamb was immune, and after 285 days the older lamb showed a reaction comparable in severity to that of all the other lambs which did react. The balance of the lambs from 68 to 103 days old at the time of immunization reacted mildly and all the survivors were still solidly immune 285 days later.

Conclusion.—(1) Lambs from susceptible ewes of any age between 4 and 109 days are susceptible to bluetongue, but there appears to be a somewhat variable resistance of youth which causes them to react less severely than adult sheep.

(2) At some age between 4 and 68 days lambs from immune ewes lose their passive transmitted immunity and attain a susceptibility equal to that of lambs from susceptible ewes.

(3) During the phase of passive protection non-reacting lambs may or may not develop a durable active immunity.

(4) Immunity in lambs vaccinated under the age of 109 days persists for at least 285 days.

Discussion.—It was most unfortunate that the mortality in the lambs prevented any observations being made on lambs from immune ewes between the 8 and 68 day period. From a practical point of view, however, it is of little importance because as a general rule lambs are never vaccinated before the age of 2 months. Such lambs may be immunized with impunity. From a purely scientific point of view it is realized that during that period when the passive immunity is waning *in vivo* tests unless carried out on very large numbers of lambs will probably show conflicting results depending upon the degree of immunity of the ewes and the antibody content of the colostrum. Consequently a final solution of the problem must again await the development of an *in vitro* test, which may be applied on an accurate quantitative basis.

The duration of immunity (285 days) is of interest in view of the previously recorded experiments on the duration of immunity in adult sheep.

D. *Attenuation by Serial Passage through Sheep.*

(1) *The "Bekker" Strain.*—This strain of virus was passaged serially through fifteen generations of sheep. Full details of the reactions produced in each individual sheep will be found in Appendix 2, while a concise summary is given in Table 5.

Result.—From the above it will be seen that the percentage mortality dropped progressively with each successive five generations of sheep, the respective percentage being 72, 60 and 14. There was a well-marked variation in the severity of the reactions seen in individual sheep, but when the experiment was discontinued at the fifteenth passage, the clear impression had been gained that there had been no progressive general decrease in the severity of the clinical symptoms produced. For the first successive five generations 18 sheep were used and for the last five successive generations only 7 animals. The wide variation in the susceptibility of individual sheep and the fact that less than half the number were used, should also be considered as a possible reason for the smaller mortality rate in the latter group.

Remarks and Conclusions.—At the end of 1939 after the outbreak of war it was found necessary to curtail some of the experimental work in progress at the time. Unfortunately this and the following experiments on the attenuation of bluetongue virus by passage were amongst those that had to be discontinued, so that no definite conclusions can be drawn from the limited series of passages.

TABLE 5.

Passage of the "Bekker" Strain through Sheep.

Generation.	Number of Sheep.	Reactions.	Deaths.	Percentage Mortality.
1.....	2	xxx, xxx.....	2	
2.....	3	xxxx, xxx, xxx.....	3	
3.....	5	xxxx, xxxx, xxx, xxx, xxx	3	72 per cent.
4.....	5	xxxx, xxxx, xxx, xxx, xxx	3	
5.....	3	xxxx, xxxx, xxx.....	2	
6.....	2	xxx, xxx.....	0	
7.....	3	xxxx, xxxx, xxxx.....	2	
8.....	3	xxxx, xxxx, xx.....	2	60 per cent.
9.....	1	xxxx.....	1	
10.....	1	xxxx.....	1	
11.....	1	xx.....	0	
12.....	1	xxx.....	Killed.	
13.....	2	xxxx.....	1	14 per cent.
14.....	1	xx.....	0	
15.....	2	xxxx, xxx.....	0	

NOTE.—The x's are an estimate of the severity of the reaction as judged from the febrile reaction and the clinical lesions. An anomaly was that an animal might show a comparatively mild febrile reaction but would die and *vice versa*.

If the progressive decrease in mortality can be regarded as an index of the attenuation of the virus then the reported experience of Theiler (1906) and du Toit (1929) was being confirmed. However, the exceedingly severe reactions produced in the higher generations indicate that passage through a very large number of generations of sheep would be required to attenuate the virus to a degree which would permit its use for routine vaccine purposes. A tentative conclusion will be deferred until the results can be discussed in the light of the other passage experiments detailed below.

(2) *The "Camp" Strain.*—This strain was passaged through 95 generations of sheep. Details of the reactions in all the sheep are shown in Appendix 3. For the sake of brevity the observations in Table 6 are shown separately for the first five generations and then each subsequent five generations combined.

Results.—No deaths occurred amongst the sheep for the first 43 generations, although severe reactions accompanied by pronounced clinical lesions were observed in generations 1, 2, 3, 29 and 30. The remainder of the sheep up to this stage had shown febrile reactions of varying severity, but the clinical lesions were mild. Both sheep used in generation 44 showed very severe reactions and one died. From generation 45 onwards there was a wide variation in the nature of the reactions. The majority showed a febrile reaction only, but of the ten sheep in generations 69, 70, 78, 79 and 83 all reacted severely and five died. An additional sheep in generation 88 reacted severely, but no further deaths occurred. Two sheep, one in generation 13 and one in generation 92 failed to react. Both these sheep were tested subsequently with the "Bekker" strain and were found to be solidly immune.

TABLE 6.

Passage of "Camp" Strain through Sheep.

Generation.	Number of Sheep.	Reactions.										Deaths	Percentage. mortality.
1	1	xxxx	—	—	—	—	—	—	—	—	—	0	0
2	2	xxxx	xxxx	—	—	—	—	—	—	—	—	0	0
3	2	xx	xxxx	—	—	—	—	—	—	—	—	0	0
4	2	xx	xx	—	—	—	—	—	—	—	—	0	0
5	2	xx	xx	—	—	—	—	—	—	—	—	0	0
6–10	10	xx	xx	xx	xx	xxx	xx	xx	xx	xx	xx	0	0
11–15	10	xxx	xxx	xx	xx	—	xx	xx	xx	xx	xx	0	0
16–20	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
21–25	10	xx	xx	xxx	xx	xx	xx	xx	xx	xx	xx	0	0
26–30	10	xx	xx	xx	xx	xx	xx	xxxx	xxxx	xxxx	xxxx	0	0
31–35	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
36–40	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
41–45	10	xx	xx	xx	xx	xx	xx	xx	xxxx	x	xx	1	10
46–50	10	xx	xx	xxxx	xxxx	xxxx	xxxx	xx	xx	xx	xxx	0	0
51–55	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xxxx	0	0
56–60	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
61–65	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
66–70	10	xx	xx	xx	xx	xx	xx	xx	xxxx	xx	xxxx	2	20
71–75	10	xx	xx	xxxx	xxxx	xx	xxx	xxx	xxxx	xx	xx	0	0
76–80	10	xx	xxx	xx	xx	xx	xxxx	xx	xxxx	xx	xx	2	20
81–85	10	xxxx	xxxx	xxxx	xxxx	xxx	xxx	xx	xx	xx	xx	1	10
86–90	10	xx	xxx	xx	xxx	xxx	xxxx	xx	xx	xx	xx	0	0
91–95	10	xxx	xxx	—	xx	xx	xx	xx	xx	—	—	0	0

Conclusion.—From this experiment it was concluded that:—

(i) The susceptibility of individual sheep varies within very wide limits.

(ii) After 95 passages through sheep the "Camp" strain of virus had probably not been attenuated at all, or alternatively if some attenuation had occurred it was not sufficiently constant to justify the use of the strain for vaccine purposes.

(3) *The "Mimosa Park" Strain.*—The results of the passage of this strain are given in detail in Appendix 4, but are summarized in Table 7.

TABLE 7.

Passage of the "Mimosa Park" Strain through Sheep.

Generation.	Number of Sheep.	Reaction.										Deaths	Percentage mortality.
1	1	xx	xx	—	—	—	—	—	—	—	—	0	0
2	2	xx	xxxx	—	—	—	—	—	—	—	—	0	0
3	2	xx	xxx	—	—	—	—	—	—	—	—	0	0
4	2	xx	xx	—	—	—	—	—	—	—	—	0	0
5	2	xxx	xxx	—	—	—	—	—	—	—	—	0	0
6	10	xx	xxx	xx	xx	xx	xx	xx	xx	xx	xxx	0	0
11–15	10	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	0	0
16–20	10	xx	xx	xx	xx	xx	xxxx	xx	xx	xx	xx	1	10
21–25	9	xx	xx	xx	xx	xx	xxxx	xx	—	xx	—	1	10
26–28	5	xx	—	xx	—	xxxx	xxx	xx	—	—	—	1	20

Results.—In the earlier passages the number of sheep which showed any evidence of disease other than a variable febrile reaction was small. From the 8th to the 17th generation only 1 out of 22 sheep showed mild clinical lesions. In each of generations 18 and 23 one of the two subinoculated sheep died and in generation 28 one sheep was killed *in extremis* for post mortem examination.

Remarks and Conclusions.—As in the case of the passage of " Bekker " virus there was no alternative but to discontinue this experiment just when it had reached a most interesting stage at the 28th passage. The origin of the strain justified the belief that a virulent strain of virus had been isolated, but this was not confirmed by the behaviour under laboratory conditions. The reactions at all times were mild, and if anything appeared to increase in severity with passage. If mortality is taken as an index of virulence then there was a slight enhancement rather than a decrease.

It is concluded that after 28 passages there was no attenuation of the " Mimosa Park " strain of virus.

(4) *The " Byenespoort ", " University Farm ", " C.43 " and " Nelspoort " Strains.*—In connection with other work these strains were passaged for 8, 7, 8 and 4 generations respectively. Each strain is characterized by the production of clinical symptoms of varying severity, but the percentage mortality in the limited number of sheep was comparatively low. No good purpose would be served by discussing the details of the limited number of passages, since no definite conclusion as regards attenuation is possible. To serve as an index of the virulence of the strains, records of the reactions in individual sheep are given in Appendix 5, 6, 7 and 8 respectively.

(5) *The " Cyprus " Strain.*—This strain was maintained by serial passage through 8 generations at Cyprus and for a further 3 generations in sheep at Onderstepoort. From the very severe reactions observed in the sheep at Cyprus and from those noticed locally one was fully justified in concluding that no attenuation whatsoever had been achieved by passaging the strain through 11 generations. The records of the reactions made in the experimental sheep at Onderstepoort are given in Appendix 9.

Discussion.—It is essential to review the results of these experiments on the passage of the virus strains through sheep in the light of the reports by Theiler (1906) and du Toit (1929). From the protocols of Theiler it is seen that in the first five generations 6 out of 28 sheep (21·4 per cent.) died and in the subsequent five generations 4 out of 71 (5·6 per cent.). From the 10th to the 23rd generation, the limit of the record, no further deaths occurred and symptoms were limited to those of a mild febrile reaction sometimes associated with mild mouth lesions. In du Toit's work only three sheep died, two in the third and one in the 11th generation. The passage was continued until the 80th generation with the production of mild febrile reactions accompanied by very mild clinical symptoms in a limited number of animals. On these results, particularly since the " Veglia " strain was isolated from a fatal case of the disease, it was reasonable to conclude that attenuation had been achieved and that these attenuated strains were safe for general vaccination purpose in the field. This conclusion was supported by the fact that good results were obtained in the preliminary field trials.

In the present series of experiments passage of the " Camp " strain is comparable with the earlier work except that there was no mortality until the 44th passage and further deaths together with severe reactions occurred in still later passages. Prior to the 44th passage it might have been concluded that the virus had become attenuated, but persistence with subinoculations showed that such a conclusion would have been incorrect. The experience with the " Mimosa Park " strain was similar but less conclusive.

It has been shown (Neitz and Riemerschmid, 1944) that solar radiation is a factor which has a marked adverse effect upon the course of bluetongue and that under precise experimental conditions the avirulent " Theiler " strain in exposure tests at the laboratory may produce lesions practically as severe as those produced by virulent strains in the field. From the available evidence therefore, it is considered that the original conception of attenuation by passage through sheep must be modified. The present investigations and those on the transmission of bluetongue by the intermediate host (*Culicoides* spp.) by du Toit (1944), suggest that there was no more than an adaptation of the virus to a mammalian host, and that the apparent attenuation was due entirely to the conditions under which the work was carried out. The consideration of the clinical manifestations under stable conditions produced by the " Theiler " and " Veglia " strains, justifies the conclusion that they are merely strains of low virulence, that the " Camp " and " Mimosa Park " strains are of slightly greater virulence, while the others, for example the " Bekker " strain is one of varying degree of greater virulence.

The conception of adaptation of a virus to a new host is no new one. For instance Burnet and Bull (1943) describe in detail distinctive properties of their original and derivative forms of influenza virus obtained by serial amniotic passage in the developing chick embryo and emphasize that the derivative form arises very rapidly from the original form under particular experimental conditions. This rapid adaptation to a new host is a phenomenon which should not be confused with a slow transformation of a pantropic strain of virus to one with essentially neurotropic characters, e.g. horsesickness (Alexander, 1935) and yellow fever (Theiler, 1930).

In the case of bluetongue du Toit (1944) demonstrated that *Culicoides* spp. are the vectors of bluetongue. This observation shows that ruminants are not the only hosts of the virus. The serial passage of several strains of vira (" Theiler ", " Veglia ", " Bekker ", " Camp ", and " Mimosa Park ") after transmission *via* the insect vector to sheep, showed that there is an adaptation to the environment of the new host. The chief change on adaptation is then the development of a virus, which produces a febrile reaction with or without the concurrent production of specific lesions of variable severity, and the virulence of any given strain of virus after adaptation remains practically constant under the same environmental conditions. This means that there was no true progressive attenuation of any of the virus strains investigated, but merely an adaptation to a particular set of conditions and that this adaptation reached a constant level after approximately ten subinoculations. This conception of adaptation rather than attenuation by serial passage is supported by:—

1. The failure to attenuate the " Bekker ", " Mimosa Park " and " Camp " strains.

2. The report by de Kock, du Toit and Neitz (1937) of the failure to attenuate their " Tzaneen " strain of virus by passage through fifteen generations of sheep immune to the " Veglia " strain of virus and through nine generations of susceptible sheep. At one stage of their investigations the non-attenuation of the virus was regarded as a point which did not permit of its classification as a strain of bluetongue.

3. The complaint by Mr. R. J. Roe (personal communication) of failure to attenuate the " Cyprus " strain by serial passage.

It is believed, therefore, that just as there is a plurality of antigenically different strains of bluetongue virus, so there is a plurality of strains of different virulence on adaptation to sheep represented by the " Theiler " strain as the least virulent and the " Cyprus " strain as the most virulent.

E. *The Relative Susceptibility to Bluetongue of the Merino and English Mutton Breeds of Sheep.*

On the 16th October, 1940, 54 Dorset Horn rams imported from Australia for the purpose of breeding prime fat lambs were delivered to the quarantine station at Cape Town. On the 22nd October they were injected with bluetongue vaccine batch 267 (" Veglia " strain) as a routine immunization measure prior to their distribution. Some time later the veterinary officer in charge (Mr. C. H. Flight) reported that reactions commenced after the normal incubation period of seven days; the reactions were exceedingly severe being accompanied by severe lesions of the nose, mouth and feet. The period of convalescence was so prolonged that it was at least two months before the animals could be released and even at that time the condition of the majority was so poor that they would be of no use for immediate service.

As a result of this report six Dorset Horn sheep were brought into the experimental stable at Onderstepoort and were vaccinated with material from three different batches of vaccine (" Veglia " strain). An equal number of Merinos acted as controls. There was no difference in the severity of the febrile reactions between the two groups, but the difference in the other clinical symptoms was most pronounced. Whereas the Merinos were never off their food and at most showed only slight reddening of the buccal mucosa, the Dorset Horns showed complete inappetence for almost a week. There were severe lesions of the nostrils and mouth. The pododermatitis was so severe that the animals were unable to stand for several days. There were no deaths, but this was ascribed to the conditions under which the animals were maintained, throughout the experiment.

Remarks and Conclusions.—It is concluded that the Dorset Horn as a breed is far more susceptible to bluetongue than the Merino. It is not possible to express any opinion as to whether this hyper-susceptibility is shared by other English mutton breeds simply because no animals of other breeds were available for experimental purposes. Attention has already been directed to the variation in susceptibility of different individuals of the Merino breed. Since hyper-susceptibility of another breed has been shown experimentally it is quite possible that still further varieties will be found amongst other breeds.

General Discussion.

In the introductory remarks to this report it was stated that the work to be recorded formed one portion of a general re-investigation of the whole problem of bluetongue and its control in South Africa, necessitated by the fact that complaints regarding the two major defects of the present method of immunization had been substantiated. The defects referred to are:—

1. That the vaccine did not produce an adequate immunity;
2. that reactions to the vaccine may be so severe as to cause considerable direct and indirect losses.

It is believed that direct experimental evidence has been brought forward to show that these complaints based on observations in the field are justified and that adequate explanations may be offered.

The inadequate immunity is due not to short duration but to the existence of a plurality of antigenically different virus strains. Between these virus strains there is a variable degree of common or basic immunity, but this is insufficient to protect against infection with heterologous strains. Repeated injections of a homologous strain of virus has no detectable effect upon the common immunity and did not widen the polyvalent range of the immunity. It is apparent therefore that a system of immunization based on the use of a single strain of virus can only be effective in an area where the same strain predominates in nature.

The danger attached to the use of the vaccine is due to an erroneous belief in the phenomenon of attenuation by serial passage through sheep, and not due to the presence of intercurrent infections. It is believed that after the isolation of a virus from a naturally contracted case of disease, there is no more than a rapid adaptation to a new host with possible changes which could not be detected by the technique used, but with no progressive attenuation by serial passage. The two strains of virus used for extensive immunization in the field were strains whose chief characteristic was low virulence. This low virulence was accentuated by the laboratory conditions under which the observations were made and being more apparent than real was capable of producing excessively severe reactions under adverse conditions of which one is solar radiation of high intensity.

Mention has been made of the fact that in spite of the defects of the present immunization the vaccine has been used with considerable success in the past. It must be remembered that its use has been confined principally to the enzootic areas, where annual immunization and frequent bi-annual injection was practised. Once a general flock immunity had been established it was easily maintained since the duration of the artificially induced immunity, whether it was re-inforced by natural infection or not, was sufficient to afford protection against the repeated injections of the vaccine. The passive transmitted immunity of the lambs from immune ewes, together with the natural resistance of all lambs, was sufficient to protect the young stock. Hence in the vast majority of cases it was safe to use the vaccine, but under present day conditions of the sheep industry the extension of the method to fully susceptible flocks, or to breeds of hyper-susceptible sheep cannot be advocated. It is difficult to offer any completely feasible explanation for the reports that an adequate immunity was produced in the past whereas it has been shown to be deficient in recent years. Possibly a more complete understanding of the diseases of sheep in South Africa has shown that bluetongue is directly responsible for more losses than was previously

believed to be the case and in consequence the value of the vaccine was exaggerated. Alternatively an annual flock mortality which might have been considered quite satisfactory twenty years ago, would probably be the cause of serious complaint to-day. But due consideration should be paid to the opinion that prolonged passage of strains of virus through sheep *via* the insect vector may have resulted in the differentiation of antigenic variants, which have now become so widespread as to be of considerable importance.

The limitations of the technique of *in vivo* tests, which was adopted in the present study are fully appreciated since it is difficult to assess degrees of immunity on any quantitative basis. It must be pointed out again, however, that the value of any *in vitro* test applied by any laboratory procedure can be assessed only after the consideration of the comparative results in sheep. Consequently this record of experiments by a laborious method on some 1,500 sheep over a number of years must be of value to other investigators in the same field. A wide extension of the scope of the investigations is essential, because it is quite apparent that an extension of the present method of vaccination cannot solve the problem of immunization against bluetongue. A universally successful vaccine must have as its basis a full appreciation of the plurality of virus strains, a plurality which comprises antigenic structure as well as degrees of virulence and the reaction must either be controlled or some method of true attenuation evolved.

Summary.

1. Complaints that the present method of immunization against bluetongue is not safe and that it produces an inadequate immunity have been justified.

2. A plurality of antigenically different virus strains has been established.

3. There appears to be an antigenic component common to all strains investigated and in addition an unknown number of different specific components.

4. There is a wide variation in the virulence of different strains.

5. The virus is not attenuated by serial passage through sheep.

6. The significance of these findings is discussed.

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Appendix I (*a*).

(See Table 1 in Text).

The Cross-Immunity Tests on Sheep Immune to the "Camp" with the "Mimosa Park" and the "Bekker" Bluetongue Strains.

Number of Sheep immune to the "Camp" strain.	Interval in days between the immunization and the first immunity test.	Tested with strain.	Result.	Interval in days between the immunization and the second immunity test.	Tested with strain.	Result.
1	18	"Mimosa Park"	No reaction.....	38	"Bekker"	No reaction.
1	18	"Mimosa Park"	No reaction.....	38	"Bekker"	Reacted and recovered.
1	26	"Mimosa Park"	No reaction.....	39	"Bekker"	No reaction.
1	26	"Mimosa Park"	No reaction.....	39	"Bekker"	Reacted and recovered.
2	26	"Mimosa Park"	Reacted and recovered	47	"Bekker"	No reaction.
1	28	"Mimosa Park"	Reacted and recovered	75	"Bekker"	No reaction.
2	32	"Mimosa Park"	Reacted and recovered	75	"Bekker"	Reacted and recovered.
1	35	"Mimosa Park"	Reacted and recovered	56	"Bekker"	No reaction.
1	35	"Mimosa Park"	No reaction.....	56	"Bekker"	No reaction.
1	36	"Mimosa Park"	No reaction.....	60	"Bekker"	Reacted and recovered.
2	36	"Mimosa Park"	Reacted and recovered	60	"Bekker"	No reaction.
2	50	"Mimosa Park"	Reacted and recovered	69	"Bekker"	No reaction.
1	63	"Mimosa Park"	No reaction.....	76	"Bekker"	No reaction.
Total 17	18-63	"Mimosa Park"	7 no reaction..... 10 reacted and recovered	38–76	"Bekker"	12 no reaction. 5 reacted and recovered
2	21	"Bekker"	No reaction.....	47	"Mimosa Park"	Reacted and recovered.
1	28	"Bekker"	No reaction.....	40	"Mimosa Park"	Reacted and recovered.
1	30	"Bekker"	Reacted and recovered	50	"Mimosa Park"	No reaction.
1	32	"Bekker"	No reaction.....	53	"Mimosa Park"	No reaction.
2	33	"Bekker"	Reacted and recovered	60	"Mimosa Park"	No reaction.
Total 7	21–33	"Bekker"	4 no reaction..... 3 reacted and recovered	47–60	"Mimosa Park"	4 no reaction; 3 reacted and recovered.

APPENDIX I (*b*).

(See Table 2 in Text).

The Cross-Immunity Tests on Sheep Immune to the "Mimosa Park" with the "Bekker" and the "Camp" Bluetongue Strains.

Number of Sheep immune to the "Mimosa Park" strain.	Interval in days between the immunization and the first immunity test.	Tested with strain.	Result.	Interval in days between the immunization and the second immunity test.	Tested with strain.	Result.
1	28	"Bekker"	Reacted and recovered	47	"Camp"	No reaction.
1	28	"Bekker"	No reaction.....	47	"Camp"	Reacted and recovered.
1	30	"Bekker"	Reacted and recovered	57	"Camp"	No reaction.
1	30	"Bekker"	No reaction.....	57	"Camp"	No reaction.
1	32	"Bekker"	No reaction.....	50	"Camp"	No reaction.
1	32	"Bekker"	No reaction.....	50	"Camp"	Reacted and recovered.
2	32	"Bekker"	No reaction.....	55	"Camp"	No reaction
2	32	"Bekker"	Reacted and recovered	50	"Camp"	No reaction.
1	34	"Bekker"	Reacted and recovered	53	"Camp"	No reaction.
1	34	"Bekker"	No reaction	53	"Camp"	No reaction.
1	34	"Bekker"	No reaction.....	60	"Camp"	No reaction.
1	35	"Bekker"	Reacted and recovered	59	"Camp"	No reaction.
1	35	"Bekker"	No reaction.....	59	"Camp"	No reaction.
1	40	"Bekker"	Reacted and recovered	67	"Camp"	No reaction.
1	40	"Bekker"	Reacted and recovered	67	"Camp"	Reacted and recovered.
TOTAL 17	28–40	"Bekker"	9 no reaction;..... 8 reacted.....	47–67	"Camp"	14 no reaction; 3 reacted and recovered
2	26	"Camp"	No reaction.....	39	"Bekker"	No reaction.
1	26	"Camp"	No reaction.....	47	"Bekker"	No reaction.
1	26	"Camp"	No reaction.....	47	"Bekker"	Reacted and recovered.
1	28	"Camp"	No reaction.....	44	"Bekker"	Reacted and recovered.
2	30	"Camp"	Reacted and recovered	50	"Bekker"	No reaction.
1	34	"Camp"	No reaction.....	55	"Bekker"	No reaction.
2	42	"Camp"	Reacted and recovered	62	"Bekker"	No reaction.
1	44	"Camp"	Reacted and recovered	44	"Bekker"	No reaction.
1	49	"Camp"	No reaction.....	69	"Bekker"	No reaction.
1	50	"Camp"	No reaction.....	70	"Bekker"	Reacted and recovered.
1	50	"Camp"	Reacted and recovered	78	"Bekker"	Reacted and recovered.
1	73	"Camp"	Reacted and recovered	93	"Bekker"	Reacted and recovered.
TOTAL 15	26–73	"Camp"	8 no reaction;..... 7 reacted and recovered	39–93	"Bekker"	10 no reaction; 5 reacted and recovered.

Appendix II.

(See Table 5 in Text).

Passage of the "Bekker" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	S. 18	Cow								
2.....	37454	S. 18	5·0 cc.	3	6	105·0	XXX	—	N.F.	Died from bluetongue.
	37679	S. 18	5·0 cc.	7	12	107·0	XXX	X	N.F.	Died from bluetongue.
3.....	40633	37454	5·0 cc.	5	11	106·0	XXX	X	N.F.	Died from bluetongue.
	37691	37454	1·0 cc.	6	6	106·0	XXX	—	N.F.	Died from bluetongue.
	37810	37454	1·0 cc.	6	3	106·0	XXX	—	N.F.	Died from bluetongue.
4.....	40965	40633	5·0 cc.	5	12	107·0	XX	—	N.F.	Died from bluetongue.
	40980	40633	5·0 cc.	4	7	107·6	XXX	X	N.F.	Died from bluetongue.
	41044	40633	5·0 cc.	6	14	107·0	XXX	X	N.F.	Recovered.
	40717	40633	1·0 cc.	6	6	106·2	XXX	—	N.F.	Died from bluetongue.
	40945	40633	1·0 cc.	5	11	108·0	X	X	N.F.	Recovered.
5.....	46071	40980	5·0 cc.	4	10	106·8	XXX	—	N.F.	Died from bluetongue.
	41570	40980	5·0 cc.	4	9	107·8	XXX	XXX	N.F.	Died from bluetongue.
	41531	40980	5·0 cc.	5	12	107·6	X	—	F.	Recovered.
	41517	40980	1·0 cc.	4	12	107·7	XXX	—	N.F.	Died from bluetongue.
	41577	40980	1·0 cc.	5	9	107·0	XX	—	N.F.	Recovered.
6.....	48815	46071	5·0 cc.	6	3	107·0	XXX	—	N.F.	Died from bluetongue.
	47019	46071	2·0 cc.	4	4	106·0	XXX	X	N.F.	Died from bluetongue.
	46068	46071	1·0 cc.	5	12	108·2	XXX	XX	N.F.	Recovered.
7.....	49968	48815	10·0 cc.	5	7	107·4	X	X	N.F.	Recovered.
	50164	48815	10·0 cc.	5	7	107·6	X	—	N.F.	Recovered.
8.....	49941	49968	5·0 cc.	5	10	107·0	XXX	XXX	N.F.	Died from bluetongue.
	49900	49968	5·0 cc.	6	4	108·0	XXX	XXX	N.F.	Died from bluetongue.
	50169	49968	5·0 cc.	6	10	107·4	X	XXX	N.F.	Recovered.

APPENDIX II.—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
9.....	52726	49900	5·0 cc.	4	10	107·2	XXX	XX	N.F.	Died from bluetongue.
	53742	49900	5·0 cc.	7	8	105·0	XXX	XX	N.F.	Died from bluetongue.
	52696	49900	5·0 cc.	4	7	106·6	—	—	N.F.	Recovered.
10.....	55160	53742	2·0 cc.	4	9	107·2	XXX	XX	N.F.	Died from bluetongue.
11.....	54412	55160	5·0 cc.	3	6	106·8	X	X	N.F.	Died from bluetongue.
12.....	55890	54412	2·0 cc.	5	9	105·2	X	—	N.F.	Recovered.
13.....	55875	55890	10·0 cc.	4	—	107·0	—	—	—	Killed on 2nd day of reaction.
14.....	56550	55875	5·0 cc.	6	10	106·6	XX	XX	N.F.	Recovered.
	58797	55875	5·0 cc.	6	10	106·6	XX	XX	N.F.	Recovered.
15.....	59636	58797	2·0 cc.	6	8	106·0	X	—	N.F.	Recovered.

NOTE :—Mouth lesions x = Hyperaemia of the buccal mucosa.
xx = Hyperaemia and oedema of the lips.
xxx = Hyperaemia, oedema, and ulcers on the buccal and nasal mucosa.

Foot lesions x = Light form of pododermatitis.
xx = Severe form of pododermatitis.
xxx = Very severe form of pododermatitis.

Appetite F = Feeding.
N.F. = Not feeding.

Appendix III.

(See Table 6 in Text).

Passage of the "Camp" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	48927	Natural infection	—	—	—		XXX	XX	N.F.	Recovered.
2.....	48268	48927	5·0 cc.	7	14	107·4	XXX	XX	N.F.	Recovered.
	48275	48927	5·0 cc.	7	12	107·4	XXX	XX	N.F.	Recovered.
3.....	48731	48268	5·0 cc.	5	3	105·0	—	—	N.F.	Recovered.
	48816	48268	5·0 cc.	7	14	107·0	XX	XX	N.F.	Recovered.
4.....	48019	48816	1·0 cc.	4	11	106·8	X	—	N.F.	Recovered.
	47972	48816	1·0 cc.	5	9	106·0	X	—	N.F.	Recovered.
5.....	47951	47972	1·0 cc.	6	8	106·6	—	—	N.F.	Recovered.
	47999	47972	1·0 cc.	5	4	106·0	—	—	N.F.	Recovered.
6.....	47918	47951	1·0 cc.	6	7	107·0	—	—	F.	Recovered.
	47973	47951	1·0 cc.	5	21	106·0	—	—	N.F.	Recovered.
7.....	47904	47918	5·0 cc.	3	10	107·0	X	—	F.	Recovered.
	48058	47973	5·0 cc.	3	7	107·0	—	—	F.	Recovered.
8.....	47793	48058	5·0 cc.	3	17	107·0	XX	—	N.F.	Recovered.
	47963	48058	5·0 cc.	6	16	107·8	X	—	N.F.	Recovered.
9.....	48012	47793	1·0 cc.	8	8	106·4	—	—	F.	Recovered.
	48048	47793	1·0 cc.	7	3	106·4	—	—	F.	Recovered.
10.....	47988	48012	1·0 cc.	5	6	107·0	—	—	F.	Recovered.
	48056	48048	1·0 cc.	3	3	106·0	—	—	F.	Recovered.
11.....	47987	47988	1·0 cc.	2	10	106·8	XX	—	N.F.	Recovered.
	47954	47988	1·0 cc.	4	9	108·0	XX	—	N.F.	Recovered.
12.....	48032	47954	1·0 cc.	6	7	106·4	—	—	F.	Recovered.
	47934	47954	1·0 cc.	3	10	107·0	—	—	F.	Recovered.
13.....	48052	47934	1·0 cc.	—	—	103·0	—	—	F.	No reaction.
	48050	47934	1·0 cc.	5	10	107·0	—	—	F.	Recovered.
14.....	47959	48050	1·0 cc.	6	7	106·4	—	—	F.	Recovered.
	48087	48050	1·0 cc.	3	9	107·0	—	—	F.	Recovered.

APPENDIX III—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
15.....	47961	48087	1·0 cc.	6	7	106·8	X	—	N.F.	Recovered.
	48013	48087	1·0 cc.	6	6	106·4	X	—	N.F.	Recovered.
16.....	47983	48013	1·0 cc.	4	8	107·0	—	—	F.	Recovered.
	48097	48013	1·0 cc.	4	8	106·0	—	—	F.	Recovered.
17.....	48021	47983	1·0 cc.	4	7	106·4	—	—	F.	Recovered.
	48054	47983	1·0 cc.	3	7	106·6	—	—	F.	Recovered.
18.....	47923	48054	1·0 cc.	8	9	108·0	—	—	F.	Recovered.
	48082	48021	1·0 cc.	4	7	108·0	—	—	F.	Recovered.
19.....	48087	47923	1·0 cc.	5	6	107·4	—	—	F.	Recovered.
	47936	48082	1·0 cc.	5	6	107·0	—	—	F.	Recovered.
20.....	48010	47936	1·0 cc.	3	10	107·0	—	—	F.	Recovered.
	48069	47936	1·0 cc.	3	10	106·4	X	—	N.F.	Recovered.
21.....	48014	48069	1·0 cc.	5	7	106·2	—	—	F.	Recovered.
	49883	48069	1·0 cc.	7	4	106·2	—	—	F.	Recovered.
22.....	50190	48014	1·0 cc.	6	12	107·0	X	X	N.F.	Recovered.
	50009	48014	1·0 cc.	7	3	105·0	—	—	F.	Recovered.
23.....	49908	50190	1·0 cc.	6	10	107·0	X	—	N.F.	Recovered.
	50176	50190	1·0 cc.	6	4	107·0	X	—	N.F.	Recovered.
24.....	49413	49908	1·0 cc.	6	7	108·0	—	—	F.	Recovered.
	49899	49908	1·0 cc.	4	6	106·0	—	—	F.	Recovered.
25.....	50051	49413	1·0 cc.	5	9	107·0	—	—	F.	Recovered.
	49947	49413	1·0 cc.	7	7	107·0	—	—	F.	Recovered.
26.....	50110	49947	1·0 cc.	6	7	107·2	—	—	N.F.	Recovered.
	49880	49947	1·0 cc.	6	7	106·8	—	—	F.	Recovered.
27.....	49992	49880	1·0 cc.	5	8	107·6	—	—	F.	Recovered.
	50191	49880	1·0 cc.	6	4	108·0	—	—	F.	Recovered.
28.....	47912	50191	1·0 cc.	6	8	107·0	—	—	F.	Recovered.
	49977	50191	1·0 cc.	6	8	107·0	—	—	F.	Recovered.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
29.....	49253	47912	1·0 cc.	4	17	107·0	XXX	XX	N.F.	Recovered.
	50247	47912	1·0 cc.	5	17	106·0	X	XX	N.F.	Recovered.
30.....	49888	49253	1·0 cc.	3	18	107·2	XXX	XX	N.F.	Recovered.
	49934	49253	1·0 cc.	3	11	107·0	XX	XX	N.F.	Recovered.
31.....	49931	49888	1·0 cc.	6	7	106·0	—	—	F.	Recovered.
	50182	49888	1·0 cc.	5	6	108·0	—	—	F.	Recovered.
32.....	50165	49931	1·0 cc.	3	13	107·2	—	—	N.F.	Recovered.
	49885	49931	1·0 cc.	7	14	106·2	—	—	N.F.	Recovered.
33.....	49978	50165	1·0 cc.	7	5	105·6	—	—	F.	Recovered.
	49933	50165	1·0 cc.	6	6	106·0	—	—	F.	Recovered.
34.....	50091	49978	1·0 cc.	6	7	107·0	—	—	N.F.	Recovered.
	49972	49978	1·0 cc.	6	5	106·4	—	—	F.	Recovered.
35.....	50069	50091	1·0 cc.	6	4	107·0	—	—	F.	Recovered.
	50195	50091	1·0 cc.	6	4	107·0	—	—	F.	Recovered.
36.....	49894	50069	1·0 cc.	5	9	107·6	—	—	F.	Recovered.
	49937	50069	1·0 cc.	7	9	106·2	—	—	F.	Recovered.
37.....	50099	49894	1·0 cc.	6	6	106·0	—	—	N.F.	Recovered.
	50153	49894	1·0 cc.	6	7	106·8	—	—	N.F.	Recovered.
38.....	50171	50153	1·0 cc.	6	7	106·6	—	—	F.	Recovered.
	50265	50153	1·0 cc.	6	4	107·0	—	—	F.	Recovered.
39.....	50161	50265	1·0 cc.	7	7	106·4	—	—	F.	Recovered.
	50245	50265	1·0 cc.	6	5	107·0	—	—	F.	Recovered.
40.....	49961	50245	1·0 cc.	6	4	107·4	—	—	F.	Recovered.
	50255	50245	1·0 cc.	4	10	107·4	—	—	F.	Recovered.
41.....	49996	50295	1·0 cc.	6	9	107·4	—	—	F.	Recovered.
	50052	50295	1·0 cc.	6	7	105·8	—	—	F.	Recovered.
42.....	49887	49996	1·0 cc.	4	6	107·6	—	—	N.F.	Recovered.
	50251	49996	1·0 cc.	5	5	108·0	—	—	N.F.	Recovered.

APPENDIX III—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
43.....	49940	49887	1·0 cc.	3	9	107·0	—	—	F.	Recovered.
	50079	49887	1·0 cc.	8	4	106·0	—	—	F.	Recovered.
44.....	50200	49940	5·0 cc.	6	5	107·0	—	—	F.	Recovered.
	49951	49940	5·0 cc.	3	14	107·0	XXX	X	N.F.	Died from bluetongue.
45.....	50055	50200	1·0 cc.	5	3	106·8	X	—	F.	Recovered.
	50126	49951	1·0 cc.	4	3	104·8	—	—	F.	Recovered.
46.....	49990	50055	5·0 cc.	5	4	106·6	—	—	F.	Recovered.
	50011	50126	5·0 cc.	6	5	107·4	—	—	F.	Recovered.
47.....	50063	49990	5·0 cc.	6	4	106·0	XXX	XX	N.F.	Recovered.
	49986	50011	5·0 cc.	6	15	106·2	XXX	XX	N.F.	Recovered.
48.....	49889	49986	5·0 cc.	6	10	106·4	XXX	X	N.F.	Recovered.
	49881	49986	5·0 cc.	6	14	105·8	XXX	—	N.F.	Recovered.
49.....	49925	49889	5·0 cc.	6	5	106·2	X	—	F.	Recovered.
	50098	49881	5·0 cc.	6	8	107·6	X	—	F.	Recovered.
50.....	51979	49925	5·0 cc.	7	2	105·0	—	—	F.	Recovered.
	52826	50098	5·0 cc.	6	7	106·0	XXX	—	F.	Recovered.
51.....	50082	51979	5·0 cc.	5	8	107·0	—	—	F.	Recovered.
	52843	52826	5·0 cc.	7	7	105·2	—	—	F.	Recovered.
52.....	52921	52843	5·0 cc.	6	7	107·0	—	—	F.	Recovered.
	52897	52843	5·0 cc.	6	3	107·0	—	—	F.	Recovered.
53.....	52867	52921	5·0 cc.	6	7	106·0	—	—	F.	Recovered.
	52825	52921	5·0 cc.	7	6	105·4	—	—	F.	Recovered.
54.....	52878	52867	5·0 cc.	4	5	106·0	—	—	F.	Recovered.
	52705	52867	5·0 cc.	5	9	106·4	—	—	F.	Recovered.
55.....	52780	52705	5·0 cc.	6	3	107·4	—	—	F.	Recovered.
	52950	52705	5·0 cc.	5	4	107·0	XXX	—	N.F.	Recovered.
56.....	52677	52780	5·0 cc.	3	8	106·0	—	—	F.	Recovered.
	52799	52780	5·0 cc.	6	7	106·2	—	—	F.	Recovered.

Appendix III—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
57.....	52839	52799	5·0 cc.	5	4	104·8	—	—	F.	Recovered.
	52707	52799	5·0 cc.	6	3	105·8	—	—	F.	Recovered.
58.....	52855	52707	5·0 cc.	4	10	106·0	—	—	F.	Recovered.
	52871	52707	5·0 cc.	4	7	107·0	—	—	F.	Recovered.
59.....	52853	52855	5·0 cc.	5	7	105·8	—	—	F.	Recovered.
	52829	52871	5·0 cc.	6	7	106·8	—	—	F.	Recovered.
60.....	52881	52829	5·0 cc.	9	3	105·6	—	—	F.	Recovered.
	52849	52853	5·0 cc.	6	9	105·4	—	—	F.	Recovered.
61.....	52690	52881	5·0 cc.	6	7	106·0	—	—	F.	Recovered.
	52772	52849	5·0 cc.	6	4	106·4	—	—	F.	Recovered.
62.....	52947	52690	5·0 cc.	3	9	107·4	—	—	F.	Recovered.
	52700	52772	5·0 cc.	3	9	107·4	—	—	F.	Recovered.
63.....	52859	52947	5·0 cc.	5	7	106·0	—	—	F.	Recovered.
	52857	52700	5·0 cc.	5	7	107·2	—	—	F.	Recovered.
64.....	52930	52859	5·0 cc.	4	6	107·0	—	—	F.	Recovered.
	52907	52857	5·0 cc.	4	7	106·8	—	—	F.	Recovered.
65.....	52702	52930	5·0 cc.	5	6	107·4	—	—	F.	Recovered.
	52666	52907	5·0 cc.	7	3	106·6	—	—	F.	Recovered.
66.....	52934	52702	5·0 cc.	5	4	107·2	—	—	F.	Recovered.
	52706	52666	5·0 cc.	6	16	107·0	—	—	F.	Recovered.
67.....	52719	52934	5·0 cc.	4	6	107·0	—	—	F.	Recovered.
	52790	52706	5·0 cc.	4	1	106·4	—	—	F.	Recovered.
68.....	52763	52719	5·0 cc.	5	4	105·0	—	—	N.F.	Recovered.
	52918	52719	5·0 cc.	6	3	105·0	—	—	F.	Recovered.
69.....	52785	52763	5·0 cc.	6	3	106·4	—	—	F.	Recoveerd.
	52864	52918	5·0 cc.	6	9	107·2	—	—	N.F.	Died from bluetongue.
70.....	52731	52864	5·0 cc.	7	6	107·0	—	—	F.	Recovered.
	52885	52785	5·0 cc.	6	12	107·0	XXX	XX	N.F.	Died from bluetingue.

APPENDIX III—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
71.....	52810	52731	5·0 cc.	4	7	106·4	—	—	F.	Recovered.
	52798	52885	5·0 cc.	4	7	105·6	—	—	F.	Recovered.
72.....	52922	52810	5·0 cc.	5	10	107·0	XXX	XX	N.F.	Recovered.
	52691	52798	5·0 cc.	5	10	107·0	XXX	XX	N.F.	Recovered.
73.....	51964	52922	5·0 cc.	4	9	105·2	—	—	N.F.	Recovered.
	52910	52691	5·0 cc.	5	5	106·4	X	—	N.F.	Recovered.
74.....	52801	51694	5·0 cc.	6	5	107·0	X	X	N.F.	Recovered.
	52805	52910	5·0 cc.	4	17	108·0	XX	XXX	N.F.	Recovered.
75.....	52806	52801	5·0 cc.	5	5	107·0	—	—	F.	Recovered.
	53741	52805	5·0 cc.	5	8	107·0	—	—	F.	Recovered.
76.....	53666	52806	5·0 cc.	5	6	107·8	XX	—	F.	Recovered.
	53684	53741	5·0 cc.	7	6	106·0	—	—	F.	Recovered.
77.....	53612	53666	5·0 cc.	7	7	105·4	—	—	F.	Recovered.
	53675	53681	5·0 cc.	—	—	103·0	—	—	F.	No reaction.
78.....	53708	53612	5·0 cc.	7	11	107·0	XXX	XX	N.F.	Died from bluetongue.
	53717	53612	5·0 cc.	10	7	107·0	—	—	N.F.	Recovered.
79.....	53746	53708	5·0 cc.	5	6	107·4	XXX	XX	N.F.	Died from bluetongue.
	53712	53717	5·0 cc.	5	9	106·4	—	—	N.F.	Recovered.
80.....	53707	53712	5·0 cc.	5	7	107·0	—	—	F.	Recovered.
	53601	53746	5·0 cc.	6	4	107·2	—	—	F.	Recovered.
81.....	53656	53707	5·0 cc.	6	21	106·0	XXX	XXX	N.F.	Recovered.
	53625	53601	5·0 cc.	5	21	107·4	XXX	XX	N.F.	Recovered.
82.....	53693	53656	5·0 cc.	6	10	108·0	XX	X	N.F.	Recovered.
	53667	53625	5·0 cc.	6	8	107·0	XX	X	N.F.	Recovered.
83.....	53718	53693	5·0 cc.	6	7	107·0	X	—	N.F.	Recovered.
	53632	53667	5·0 cc.	5	7	106·2	X	—	N.F.	Died from bluetongue.
84.....	53688	53632	5·0 cc.	4	8	105·0	—	—	F.	Recovered.
	52816	53718	5·0 cc.	7	9	106·4	—	—	F.	Recovered.

APPENDIX III.—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
85.....	54253	53688	5·0 cc.	5	11	107·0	—	—	N.F.	Recovered.
	54381	52816	5·0 cc.	6	5	106·0	—	—	N.F.	Recovered.
86.....	54268	54381	5·0 cc.	4	20	107·0	X	—	N.F.	Recovered.
	54294	54253	5·0 cc.	4	14	107·4	—	—	N.F.	Died.
87.....	55028	54294	5·0 cc.	6	9	106·2	—	—	N.F.	Recovered.
	55284	54268	5·0 cc.	5	9	106·0	X	—	N.F.	Recovered.
88.....	55218	55028	5·0 cc.	4	9	109·0	X	—	N.F.	Recovered.
	55345	55284	5·0 cc.	6	13	106·8	XXX	X	N.F.	Recovered.
89.....	55793	55345	5·0 cc.	4	8	106·4	—	—	F.	Recovered.
	55828	55218	5·0 cc.	5	8	106·6	—	—	F.	Recovered.
90.....	55747	55828	5·0 cc.	8	7	106·4	—	—	F.	Recovered.
	55816	55793	5·0 cc.	10	2	107·0	—	—	F.	Recovered.
91.....	55887	55747	5·0 cc.	9	6	107·0	X	—	F.	Recovered.
	55885	55816	5·0 cc.	6	7	106·0	X	—	F.	Recovered.
92.....	55842	55887	5·0 cc.	—	—	103·0	—	—	F.	No reaction.
	55914	55885	5·0 cc.	7	9	106·4	—	—	F.	Recovered.
93.....	55927	55914	5·0 cc.	6	7	106·0	—	—	F.	Recovered.
	55936	55914	5·0 cc.	5	8	106·8	—	—	F.	Recovered.
94.....	55902	55936	5·0 cc.	5	7	106·4	—	—	F.	Recovered.
	55946	55927	5·0 cc.	3	7	105·4	—	—	F.	Recovered.
95.....	55919	55902	5·0 cc.	6	4	106·4	—	—	F.	Recovered.
	55877	55946	5·0 cc.	5	—	107·4	—	—	F.	Killed.
96.....	59021	55919	5·0 cc.	6	4	107·0	XX	—	F.	Recovered.

Appendix IV.

(See Table 7 in Text).

Passage of "Mimosa Park" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	52884	Mimosa Park Strain	10·0 cc. i.v.	8	5	104·8	—	—	N.F.	Recovered.
	52741		10·0 cc. i.v.	7	4	106·8	—	—	N.F.	Recovered.
2.....	52808	52741	5·0 cc.	12	7	106·6	—	—	F.	Recovered.
	52927	52741	5·0 cc.	8	12	107·6	XX	X	N.F.	Recovered.
3.....	53679	52808	5·0 cc.	6	5	106·6	—	—	N.F.	Recovered.
	53652	52808	5·0 cc.	6	9	106·0	—	—	N.F.	Recovered.
4.....	52831	53679	5·0 cc.	6	2	105·4	—	—	F.	Recovered.
	51960	53652	5·0 cc.	6	4	107·4	—	—	F.	Recovered.
5.....	53690	52831	5·0 cc.	7	7	107·8	X	—	N.F.	Recovered.
	53694	51960	5·0 cc.	5	5	107·2	X	—	N.F.	Recovered.
6.....	53701	53690	5·0 cc.	7	12	105·8	X	—	F.	Recovered.
	53647	53694	5·0 cc.	6	4	107·0	—	—	F.	Recovered.
7.....	53647	53647	5·0 cc.	7	6	106·0	—	—	F.	Recovered.
	53642	53701	5·0 cc.	6	3	107·0	—	—	N.F.	Recovered.
8.....	53719	53642	5·0 cc.	18	1	107·0	—	—	F.	Doubtful reaction.
	53639	53678	5·0 cc.	4	13	106·6	—	—	F.	Recovered.
9.....	53671	53639	5·0 cc.	6	7	106·8	—	—	F.	Recovered.
	53622	53639	5·0 cc.	4	2	106·0	—	—	F.	Recovered.
10.....	53721	53671	5·0 cc.	4	5	107·0	X	—	N.F.	Recovered.
	53698	53622	5·0 cc.	6	3	106·6	—	—	N.F.	Recovered.
11.....	53730	53698	5·0 cc.	7	5	106·0	—	—	F.	Recovered.
	53604	53721	5·0 cc.	7	10	107·0	—	—	F.	Recovered.
12.....	53624	53604	5·0 cc.	7	6	107·2	—	—	F.	Recovered.
	53664	53730	5·0 cc.	4	8	108·0	—	—	F.	Recovered.
13.....	54351	53624	5·0 cc.	6	6	106·4	—	—	F.	Recovered.
	54365	53664	5·0 cc.	7	7	106·0	—	—	F.	Recovered.
14.....	53655	54351	5·0 cc.	6	5	107·0	—	—	F.	Recovered.
	54561	54365	5·0 cc.	6	7	106·0	—	—	F.	Recovered.

APPENDIX IV—(*continued*).

Generation.	Number of Sheep.	Injected from	Dose of blood subcu-taneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temper-ature.	Mouth lesions.	Foot lesions.	Appetite.	
15.....	55273	{ 53655	5·0 cc.	9	7	107·0	—	—	F.	Recovered.
	55323	{ 54561	5·0 cc.	4	9	107·0	—	—	F.	Recovered.
16.....	55258	{ 55273	5·0 cc.	5	8	107·0	—	—	N.F.	Recovered.
	55650	{ 55323	5·0 cc.	5	17	106·4	—	—	N.F.	Recovered.
17.....	55868	{ 55650	5·0 cc.	—	—	103·0	—	—	F.	No reaction.
	55850	{ 55258	5·0 cc.	8	7	106·4	—	—	N.F.	Recovered.
18.....	55924	55850	5·0 cc.	10	5	105·0	—	—	F.	Recovered.
	55918	55850	5·0 cc.	6	11	106·8	XX	XX	N.F.	Died from bluetongue.
19.....	55879	{ 55924	5·0 cc.	6	7	106·4	—	—	F.	Recovered.
	55655	{ 55918	5·0 cc.	4	7	107·8	—	—	N.F.	Recovered.
20.....	55920	55655	5·0 cc.	—	—	104·0	—	—	N.F.	Died from peritonitis.
	55925	55879	5·0 cc.	6	7	106·8	—	—	N.F.	Recovered.
21.....	55832	55925	5·0 cc.	7	5	106·0	—	—	F.	Recovered.
	55907	55925	5·0 cc.	7	6	106·0	—	—	F.	Recovered.
22.....	55820	{ 55907	5·0 cc.	4	10	107·0	—	—	N.F.	Recovered.
	55921	{ 55832	5·0 cc.	4	7	108·0	—	—	N.F.	Recovered.
23.....	55922	{ 55921	5·0 cc.	5	3	107·6	XX	—	N.F.	Died from bluetongue.
	55900	{ 55820	5·0 cc.	4	10	107·0	—	—	N.F.	Recovered.
24.....	59677	59922	5·0 cc.	7	12	105·6	X	—	F.	Recovered.
25.....	59147	59677	5·0 cc.	4	—	106·6	—	—	F.	Killed for collection of specimens.
26.....	59545	59147	5·0 cc.	5	16	108·0	X	—	N.F.	Recovered.
27.....	56218	59545	2·0 cc.	6	8	107·0	X	—	N.F.	Killed for collection of specimens.
28.....	59521	56218	1·0 cc.	5	18	105·8	XXX	XXX	N.F.	Killed in extremis.
	59566	56218	1·0 cc.	8	7	107·0	X	—	N.F.	Recovered.
	59562	56218	1·0 cc.	6	7	106·2	X	—	N.F.	Recovered.

Appendix V.

Passage of the "Byenespoort" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	59108	Calf 8633	10·0 cc. *	3	7	106·8	X	—	N.F.	Recovered.
	59361	Calf 8633	10·0 cc. *	3	5	105·4	X	—	N.F.	Recovered.
2.....	59332	59108	10·0 cc. *	3	6	106·8	XX	—	F.	Recovered.
	59388	59108	10·0 cc. *	2	6	105·8	X	—	F.	Recovered.
3.....	59483	{ 59332 59388	10·0 cc. *	3	8	105·8	X	—	F.	Recovered.
	60228	59388	10·0 cc. *	7	3	107·6	XX	—	F.	Recovered.
	60247	59388	10·0 cc. *	4	8	106·8	XX	—	F.	Recovered.
	62064	59332	10·0 cc. *	6	2	108·0	X	—	F.	Killed on 2nd day of reaction.
	62048	59332	10·0 cc. *	6	4	107·2	XX	—	N.F.	Died from bluetongue.
4.....	61405	62064	10·0 cc. *	6	10	107·0	XX	X	N.F.	Killed in extremis.
	60502	62048	2·0 cc.	4	7	107·0	XX	—	N.F.	Recovered.
5.....	64173	61405	1·0 cc.	7	7	107·0	XX	—	F.	Recovered.
6.....	60462	64173	2·0 cc.	5	8	106·4	X	—	F.	Recovered.
7.....	68844	60462	5·0 cc.	10	5	107·2	XX	—	N.F.	Recovered.
8.....	72074	68844	2·0 cc.	11	6	106·0	XX	—	N.F.	Recovered.

* = Blood injected intravenously.

Appendix VI.

Passage of the "University Farm" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	62060	Sheep at University Farm.	2·0 cc.	5	—	107·0	X	—	—	Killed for collection of specimens.
2.....	60513	62060	2·0 cc.	4	4	107·6	XX	X	N.F.	Died from bluetongue.
3.....	62031	60513	2·0 cc.	7	9	107·0	XX	X	N.F.	Died from bluetongue.
	62302	60513	2·0 cc.	3	14	107·0	XX	X	N.F.	Recovered.
	62239	60513	2·0 cc.	3	14	107·0	XX	X	N.F.	Recovered.
	64506	60513	2·0 cc.	3	—	106·8	X	—	—	Killed for collection of specimens.
4.....	64482	64506	2·0 cc.	3	11	107·2	XX	X	N.F.	Killed for collection of specimens.
	62121	64506	2·0 cc.	3	12	107·4	XX	—	N.F.	Recovered.
5.....	68680	64482	2·0 cc.	4	7	107·2	XX	X	N.F.	Recovered.
6.....	69155	68680	2·0 cc.	6	7	107·0	XX	—	N.F.	Recovered.
7.....	71992	69155	2·0 cc.	5	12	106·4	XX	X	N.F.	Recovered.

Appendix VII.

The Passage of the "C 43" Bluetongue Strain.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	66230	*Culicoides* sp.	10·0 cc.* emulsion.	3	12	106·0	XXX	XX	N.F.	Died from bluetongue.
2.....	66520	66230	5·0 cc.*	2	7	105·4	XXX	X	N.F.	Killed in extremis.
3.....	68713 68718	66520 66520	5·0 cc.* 10·0 cc.*	6 4	6 5	105·2 106·8	X XXX	— XXX	F. N.F.	Recovered. Killed in extremis.
4.....	*Culicoides* sp.	Fed on 68713								
5.....	68604	*Culicoides* sp. fed on 68713	—	6	9	107·2	XXX	XX	N.F.	Recovered.
6.....	69026	68604	2·0 cc.	6	10	107·6	XX	X	N.F.	Recovered.
7.....	69079	69026	2·0 cc.	6	10	108·0	XX	—	N.F.	Recovered.
8.....	71966	69079	2·0 cc.	5	11	107·4	XXX	XXX	N.F.	Killed in extremis.

* = Blood injected intravenously.

Appendix VIII.

Passage of the "Nelspoort" Strain through Sheep.

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
1.....	69006	Sheep at Nelspoort	5·0 cc.	6	8	105·6	X	X	N.F.	Recovered.
	69056		5·0 cc.	3	7	107·0	X	—	N.F.	Recovered.
2.....	69080	69056	10·0 cc. *	4	9	105·6	XX	X	N.F.	Died from bluetongue.
3.....	69133	69080	2·0 cc.	7	2	107·0	XX	—	N.F.	Killed for collection of specimens.
	69213	69080	5·0 cc. *	4	12	106·0	XX	XXX	N.F.	Recovered.
	69157	69080	2·0 cc.	4	5	107·2	X	—	F.	Recovered.
4.....	71951	69213	2·0 cc.	4	13	107·0	XXX	XXX	N.F.	Died from bluetongue.

* = Blood injected intravenously.

Appendix IX.

Passage of the "Cyprus" Strain through Sheep (Generations 1-8 maintained in Cyprus).

Generation.	Number of Sheep.	Injected from	Dose of blood subcutaneously.	Incubation period in days.	Duration of disease in days.	Clinical Symptoms.				Result.
						Highest temperature.	Mouth lesions.	Foot lesions.	Appetite.	
9.....	69218	Sample B	5·0 cc. *	8	17	106·8	XX	XX	N.F.	Recovered.
	69220	Cyprus.	5·0 cc. *	6	17	106·6	XX	XXX	N.F.	Killed in extremis.
10.....	70119	69220	2·0 cc. *	4	24	107·4	XXX	XXX	N.F.	Killed in extremis.
11.....	71971	70119	2·0 cc.	3	7	106·4	XXX	XXX	N.F.	Died from bluetongue.
	71943	70119	2·0 cc.	6	8	105·8	XX	XX	N.F.	Killed for collection of specimens.
	71959	70119	5·0 cc.	5	10	105·8	XX	XXX	N.F.	Died from bluetongue.
	72217	70119	5·0 cc.	6	7	105·2	XX	XXX	N.F.	Killed in extremis.

* = Blood injected intravenously.