of parasites and organisms as those which are responsible for the acariases, fungus diseases like ringworm, and diseases such as tuberculosis, Johne's disease, contagious abortion, foot rot and mastitis, in addition to foot-and-mouth disease. In this country, at any rate, the low incidence of anthrax enables us to ignore this disease as one requiring special attention when considering the disinfection of means of transport.

The results which have been obtained in Great Britain in the disinfection of foot-and-mouth disease infected places over a long period seem to justify the recommendation that disinfectants of the coal tar type essentially bactericidal agents are eminently suitable for use against the virus of foot-and-mouth disease and therefore can be recommended for general use in the disinfection of vehicles, railway trucks, ships, lairs and railway stations.

They are easily obtainable in most countries and comparatively cheap. They have, however, one serious disadvantage in that they taint meat and other foodstuffs.

Paper No. 30.

MEMORANDUM ON HORSE-SICKNESS IMMUNIZATION.

By S. H. Whitworth, B.V.Sc., D.V.M., M.R.C.V.S., Veterinary Research Officer, Department of Agriculture, Kenya.

The disease African horse-sickness has been extensively investigated by Sir Arnold Theiler in South Africa, and his researches on the disease have been published in the various reports of the Director of Veterinary Education and Research for the Union of South Africa.

As the result of Sir Arnold Theiler's investigations it has been proved possible to protect horses and mules against fatal horse-sickness infection in the majority of cases by (1) paying particular attention to the stabling and prevention of bites of blood-sucking insects, and (2) by means of artificial immunization.

It is suspected that horse-sickness is insect-borne but, as yet, the particular insect concerned has not been definitely determined. As the result of observations made at Onderstepoort over a number of years during the horse-sickness season, some species of culicinae is regarded with suspicion. Accordingly, if horses or mules be stabled in mosquito proof stables, they are efficiently proteced against infection. If such accommodation be not possible, it has also been proved that satisfactory results can be obtained by smoking the stable out and maintaining the smoke during the night. Where horses are to be used at night, deterrent skin dressing, e.g. paraffin, may be applied with good results. Such treatment has obvious disadvantages. Experiments carried out to determine the value of short interval dipping in specially prepared insect repellant fluids indicated the method to have some value, although not entirely satisfactory.

The movement of horses from horse-sickness infected areas to higher altitudes where the disease is relatively rare or absent, can be resorted to with advantage, but is not always possible. Moreover,

horse-sickness does occur even at the higher altitudes; in Kenya Colony outbreaks of the infection have occurred at altitudes of 10,000 feet.

The above methods, therefore, in the control of horse-sickness can have only limited application, and are of special value in valuable horses or where a small number of animals come under consideration.

Artificial immunization of equines against horse-sickness has received a great deal of attention, and as a result a method has been introduced by Sir Arnold Theiler, and which, taken over a large number of animals, gives satisfactory results. The method consists in the simultaneous inoculation of a specially selected horse-sickness virus and hyperimmune serum obtained from a hyperimmune animal, followed 72 hours later by a similar inoculation. This inoculation actually produces a horse-sickness reaction which in some cases is scarcely noticeable, in others definite and severe, and in a few cases is followed by mortality. The experience in South Africa is that in horses so treated a mortality of 5 per cent. may occur, and in mules a mortality of 1—2 per cent. from horse-sickness produced by the inoculation. Occasional mortality results directly from the inoculation itself. Both the virus and the serum are injected intravenously, and since the amount of serum injected, viz.; 400 c.c. is large, death may occur apparently from shock, especially if the serum be injected too quickly. Since there are many strains of horse-sickness virus, horses immune to one or more strains may not be immune to some other particular strain. Indeed, horses may react more than once to one particular strain. In the practice, however, the mortality amongst artificially immunized horses on exposure to infection is not great, and may be considered to be 5—10 per cent. in the case of horses and 2—3 per cent. in the case of mules. Breakdowns in immunity on exposure with recovery also occur. Although this method of immunization has proved fairly satisfactory, it must be considered to have certain disadvantages and to present certain difficulties in its application. These disadvantages may be indicated as follows:—

(1) The inoculation of horse-sickness virus may give rise to a horse-sickness reaction, and so maintain horse-sickness infection.

(2) The horse-sickness reaction may prove fatal, or may be so severe as to affect the constitution of the inoculated animal. Horses which have reacted severely may not be able subsequently to withstand hard work.

(3) Certain diseases other than horse-sickness may be transmitted in the virus itself. These may be pernicious anaemia, biliary fever, and trypanosomiasis. Special precautions can be taken to avoid transmitting these latter infections by using blood virus which has been kept sufficiently long for the parasites of biliary fever and trypanosomiasis to die. In the case of pernicious anaemia the blood may also be kept sufficiently long for this virus to die out, and since this may take a year, the blood can then be inoculated to horses known not to transmit pernicious anaemia. Old horse-sickness virus sometimes does not produce horse-sickness on inoculation to susceptible equines.

(4) Cases of liver atrophy with fatal issue sometimes follow horse-sickness immunization. The relation of this condition to horse-sickness immunization is not known, but has been observed to follow the process both in South Africa and Kenya Colony.

(5) The technique of the inoculation itself requires considerable skill to be applied with safety.

(6) The care necessary in the production of serum with regard to its sterility and the exclusion of sera haemolytic for the blood of other equines entails much work, while the expense of serum production and the maintenance of virus for inoculation is considerable. In view of the satisfactory results which have been recorded in artificial immunization against diseases such as distemper and rinderpest by the use of treated tissue extracts, e.g. spleen, experiments were commenced at the Veterinary Research Laboratory early in 1929, and are still in progress, to determine whether a similar prophylactic vaccine could be prepared from treated tissues, e.g. spleen, of horses reacting to horse-sickness. Such a method, if successful, might obviate some of the disadvantages of the virus-serum method indicated above, and it was with this object in view that the work was undertaken. The cost of equines for experimental work in Kenya Colony prevents any work on a large scale which would give conclusive results, and also does not allow of much work being done with various modifications in the preparation of inoculation material, or observations on the value of various material, e.g. spleen, liver, gland, kidney, etc.

The tissue selected was spleen obtained from equines slaughtered when showing high temperature reactions to horse-sickness virus inoculation. The spleen, collected as aseptically as possible after slaughter of the reacting animal, was passed through a Latapie pulper, the mass weighed, and to each gram of spleen pulp was added 4 c.c. of sterile saline. To the total bulk was added either Toluol or Formalin in certain proportions (see below), and the mixture well shaken in a flask and incubated at 37 deg. C. for a period varying from four to eight days, and then stored in the ice-chest. The mixture was well shaken daily while stored in the incubator.

The details of the inoculations are given in the following experiments:—

(A) Treatment of Spleen with Toluol.

(B) Treatment of Spleen with Toluol and then Formalin.

(C) Treatment of Spleen with Formalin.

(A) Treatment of Spleen with Toluol.

Origin of Spleen.—Horse 1253 inoculated 2/3/29 with horse-sickness virus *ex* Onderstepoort. Horse-sickness reaction commenced 5/3/29, and the horse was shot in extremis on 7/3/29. The material was prepared as described above, and Toluol added in the proportion

of 3 parts of Toluol to 100 of the mixture (i.e. 3% Toluol, placed in the incubator for 8 days, and then stored in the ice-chest.

Inoculations.

(1) *Horse* 1254 inoculated 14/3/29 subcutaneously with 20 c.cs. of the above treated tissue extract(i.e. material 7 days old).

Result.—Horse-sickness temperature reactions commenced 17/3/29 and continued until 24/3/29, when dikkop symptoms appeared. This horse eventually recovered although the reaction was very severe.

Immunity Tests were carried out on 2/4/29, 12/4/29, and 20/4/29 by inoculation of various known virulent viruses, but no reactions resulted.

(2) *Horse* 1256 inoculated 25/3/29 subcutaneously with 20.c.cs. of the above treated tissue extract (i.e. material 18 days old).

Result.—Horse-sickness temperature reaction commenced 28/3/29 and the horse died of typical acute horse-sickness on 31/4/29.

(3) *Mule* 875 inoculated 2/5/29 subcutaneously with 20 c.cs. of the above treated tissue extract (i.e. material 56 days old).

Result.—Horse-sickness temperature reaction commenced 6/5/29 and the mule was shot on 11/5/29 (spleen collected for preparation of more spleen material. See below).

Conclusion.

Horse-sickness spleen material prepared as above and treated with Toluol in the proportion of 3 parts of Toluol to 100 parts of the mixture was still virulent up to a period of 56 days (see Mule 875), and produced horse-sickness in all three susceptible equines inoculated.

(B) Treatment of Spleen with Toluol and then with Formalin.

In another experiment (see below) it was found that spleen treated with Formalin in the proportion of one part of Formalin to 100 parts of spleen mixture (i.e. 1%) was rendered avirulent. The material prepared under (A) (3% Toluol) was therefore subsequently treated with commercial formalin—one part of formalin to 100 parts of the mixture. This new preparation was then incubated for 4 days and then stored in the ice-chest.

Inoculations.

Horse 1271 inoculated 15/5/29, 27/5/29, and 4/6/29 subcutaneously on each occasion with 30 c.cs. of the above treated tissue extract.

Result.—No reactions.

Immunity Test.—Inoculated 11/6/29 10 c.cs. intravenously and 10 c.cs. subcutaneously with H.S. virus 1253 of 6/3/29 and H.S. virus 1256 of 30/3/29.

Result.—No reactions.

2. *Mule* 822 inoculated 20/5/29, 30/5/29, and 10/6/29 subcutaneously on each occasion with 30 c.cs. of above extract.

Result.—No reactions.

Material Inoculated.	Method of Treatment of Tissue.	Lab. No. of Equine in Expt.	Age of Material at Time of Inoculation.	Date of Inoculation.	Dose.	Result.	Immunity Test.			Remarks.
							Date.	Method.	Result.	
Horse-sickness virus *ex* Onderstepoort..	Nil.....	H. 1253...	—	2/3/29	5 c.c. Intravenously.	Reacted...	—	—	—	Slaughtered in extremis for collection of spleen.
Blood *ex* H. 1253...	Nil.....	M. 450....	—	19/3/29	10 c.c. Intravenously.	,, ...	—	—	—	Died of H.S. Control on virulence of H. 1235 virus.
Spleen *ex* H. 1253...	3% Toluol.....	H. 1254...	7 days.....	14/3/29	20 c.c. Subcutaneously	,, ...	2/4/29 12/4/29 20/4/29	Virus intravenously..... ,, ,, ,, ,,	No reaction	Recovered, but severe reaction.
,, ,,	,, ...	H. 1256...	18 days.....	25/3/29	,, ,,	,, ...	—	—	—	Died of H.S.
	,, ...	M. 875....	56 days.....	2/5/29	,, ,,	,, ...	—	—	—	Slaughtered in extremis for collection of spleen.
	3% Toluol plus 1% Formalin	M. 822....	74 days Toluol, 20 days Formalin	20/5/29	30 c.c. Subcutaneously	No reaction	17/6/29	Virus intrav. and subcutaneously	No reaction	—
	,, ,, ,,	M. 822....	84 days Toluol, 30 days Formalin	30/5/29 10/6/29	,, ,, ,, ,,	,, ,,	— —	— —	— —	— —
	,, ,, ,,	H. 1271...	69 days Toluol, 15 days Formalin	15/5/29	,, ,,	,,	11/6/29	Virus intrav. and subcutaneously	No reaction	—
,,	,, ,, ,,	,,	81 days Tolyol, 27 days Formalin	27/5/29	,, ,,	,,	—	—	—	—
,, ,,	,, ,, ,,	H. 1276...	79 days Toluol, 25 days Formalin	25/5/29	,, ,,	,,	—	Not yet done.	—	—
Horse-sickness virus *ex* Onderstepoort..	Nil.....	H. 1259...	—	5/3/29	5 c.c. Intravenously..	Reacted...	—	—	—	Slaughtered in extremis for collection of spleen.
Spleen *ex* H. 1259	Nil.....	M. 1203...	—	16/3/29	10 c.c. Subcutaneously	,, ...	2/4/29 12/4/29 20/4/29	Virus intravenously..... ,, ,, ... ,, ,, ...	No reaction ,, ,,	Recovered. (Control on virus H 1259.) —
,, ,,	1% Formalin.....	H. 1265...	9 days.....	23/3/29	20 c.c. Subcutaneously	No reaction	20/4/29	,, ,, ...	,,	—
,, ,,	,, ...	,,	19 days.....	2/4/29	30 c.c. Subcutaneously	,,	2/5/29	,, ,, ...	,,	—
,, ,,	,, ...	,,	29 days.....	12/4/29	,, ,,	,,	—	—	—	—
,, ,,	,, ...	H. 1272...	35 days.....	18/4/29	,, ,,	,,	15/5/29	Virus intrav. and subcutaneously	No reaction	—
,, ,,	,, ...	,,	43 days.....	26/4/29	40 c.c. Subcutaneously	,,	—	—	—	—
,, ,,	,, ...	,,	51 days.....	4/5/29	30 c.c. Subcutaneously	,,	—	—	—	—
,, ,,	,, ...	H. 1274...	68 days.....	21/5/29	20 c.c. Subcutaneously	,,	—	Not yet done.	—	—
,, ,,	,, ...	H. 1275...	68 days.....	21/5/29	,, ,,	,,	—	Not yet done.	—	—
Spleen *ex* M 875	1—100 Formalin.....	H. 1277...	11 days.....	22/5/29	30 c.c. Subcutaneously	No reaction	—	Not yet done.		
,, ,,	1—100 ,, ...	,,	20 days.....	31/5/29	,, ,,	,,	—	—	—	—
,, ,,	1—400 ,, ...	H. 1273...	11 days.....	22/5/29	,, ,,	,,	22/6/29	Virus intrav. and subcutaneously	—	—
,, ,,	1—800 ,, ...	,,	20 days.....	31/5/29	,, ,,	,,	—	—	? Temp. reaction	—
,, ,,	1—1000 ,, ...	,,	28 days.....	8/6/29	,, ,,	,,				
,, ,,	1—1000 ,, ...	H. 1278...	19 days.....	30/5/29	,, ,,	,,	—	Not yet done.	—	—

Immunity Test.—Inoculated 17/6/29 10 c.cs. intravenously H.S. virus 875 of 10/5/29; 10 c.cs. intravenously H.S. virus 1256 of 30/5/29; 10 c.cs. subcutaneously H.S. virus 1253 of 6/3/29, and 1241 of 1/3/29.

Result.—No reactions.

3. *Horse* 1276 inoculated 25/5/29, 4/6/29, and 13/6/29 subcutaneously on each occasion with 30 c.cs. of the above extract.

Result.—No reactions.

Immunity Test.—Not yet done.

Conclusions.

1. Horse-sickness spleen material which proved still virulent after treatment with 3% Toluol was rendered avirulent on addition of 1% Formalin in so far as no horse-sickness temperature reactions and no symptoms of horse-sickness were produced on inoculation of the material to three equines.

2. Two of these equines when tested on their immunity by inoculation of horse-sickness virus gave no reaction.

(C) Treatment of Spleens with Formalin.

Two spleens, Nos. 1 and 2, have been used in these experiments.

Origin of Spleen No. 1.

Horse 1259 inoculated 5/3/29 with horse-sickness virus *ex* Onderstepoort. Horse-sickness reaction commenced 9/3/29, and the horse was shot in extremis on 14/3/29. The material was prepared as described above and commercial Formalin added in the proportion of 1 part of Formalin to 100 parts of the mixture (i.e. 1% Formalin). This was then incubated at 37 deg. for 5 days and then stored in the ice-chest.

Inoculations.

1. *Horse* 1265 inoculated subcutaneously 23/3/29 with 20 c.cs.; 2/4/29 with 30 c.cs.; 12/4/29 with 30 c.cs. of the above extract.

Result.—No reactions resulted from any of the three inoculations.

Immunity Tests.—20/4/29 inoculated with 10 c.cs. horse-sickness virus.

Result.—No reaction.

2/5/29 inoculated with 10 c.cs. subcutaneously and 10 c.cs. intravenously horse-sickness viruses.

Result.—No reaction.

2. *Horse* 1272 inoculated subcutaneously 18/4/29 with 30 c.cs. 26/4/29 with 40 c.cs. and 4/5/29 with 30 c.cs. of the above extract.

Result.—No reactions resulted from any of the three inoculations.

Immunity Test.—15/4/29 inoculated intravenously and subcutaneously with horse-sickness virus.

Result.—No reaction.

3. *Horse* 1274 inoculated subcutaneously 21/5/29 with 20 c.cs. of the above extract.

Result.—No reaction.

Immunity Test.—Not yet done.

4. Horse 1275 inoculated subcutaneously 21/5/29 with 20 c.cs. of the above extract.

Result.—No reaction.

Immunity Test.—Not yet done.

Conclusions.

Spleen material prepared as above and treated with Formalin in the proportion of 1 part of Formalin to 100 parts of the mixture did not produce horse-sickness reactions on inoculation to 4 horses. Two horses when tested on their immunity did not show horse-sickness reactions.

It should be noted that immunity tests were made with the same strain of virus as that contained in the spleen material used.

Blood has been collected from the horses at various periods after the inoculations, and it is intended to determine by inoculation of susceptible horses whether horse-sickness virus was present in the blood at any period after the inoculation. Owing to the few numbers of horses available for experiment this has not yet been done.

A Mule (No. 1203) inoculated subcutaneously with *untreated* spleen material *ex* Equine 1259 from which the spleen extract was prepared, gave a temperature reaction to horse-sickness and recovered.

(C) Treatment of Spleen No. 2 with Formalin.

Origin of Spleen No. 2.—Mule 875 (see above) inoculated 2/5/29 subcutaneosuly with 20 c.cs. Toluol treated spleen *ex* horse 1253.

Horse-sickness reaction commenced 11/5/29 and the mule was shot on 13/5/29. The temperature reaction was marked but prolonged, and clinical symptoms were not severe. The mule would probably have recovered. The spleen was passed through the mincer, weighed, and divided into 5 equal lots. To each lot was added saline as described above, and then formalin to each lot to make the following dilutions of formalin in the mixture:—

1 in 100, 1 in 200, 1 in 400, 1 in 800, 1 in 1,000.

Each lot was put in the incubator at 37 deg. for 8 days, and the following inoculations made:—

Horse 1273 inoculated 22/5/29 subcutaneously with 30 c.cs. 1 in 400 Formalized spleen; inoculated 31/5/29 subcutaneously with 30 c.cs. 1 in 800 Formalized spleen, inoculated 8/6/29 subcutaneously with 30 c.cs. 1 in 1,000 Formalized spleen.

Result.—No reactions.

Immunity Test.—22/6/29 inoculated intravenously 5 c.cs. H.S. virus 1256 of 30/3/29; inoculated intravenously 5 c.cs. H.S. 875 of 10/5/29; inoculated intravenously 5 c.cs. H.S. virus 450 of 25/3/29; inoculated subcutaneously 5 c.cs. H.S. virus 1253 of 6/3/29; inoculated subcutaneously 5 c.cs. H. S. virus 1241 of 1/3/29; inoculated subcutaneously 5 c.cs. H.S. virus 1239 of 30/4/29.

Result.—? Temperature reaction.

Note.—Blood collected from Horse 1273 during the ? temperature reaction will be inoculated to a susceptible equine when the opportunity occurs.

2. *Horse* 1277 inoculated 22/5/29 subcutaneously with 30 c.cs. 1 in 100 Formalized spleen; inoculated 30/5/29 subcutaneously with 30 c.cs. 1 in 100 Formalized spleen; inoculated 7/6/29 subcutaneously with 30 c.cs. 1 in 400 Formalized spleen; inoculated 17/6/29 subcutaneously with 30 c.cs. 1 in 1,000 Formalized spleen.

Result.—No reactions.

Immunity Test.—Not yet done.

3. *Horse* 1278 inoculated 30/5/29 subcutaneously with 30 c.cs. 1 in 1,000 Formalized spleen; inoculated 13/6/29 subcutaneously with 30 c.cs. 1 in 800 Formalized spleen; inoculated 22/6/20 subcutaneously with 30 c.c.s. 1 in 800 Formalized spleen.

Result.—No reactions.

Immunity Test.—Not yet done.

Conclusions.

Spleen material prepared as above and treated with Formalin in the proportions of 1 part of Formalin to 100 parts of the mixture; to 400 parts; to 800 parts; and to 1,000 parts of the mixture did not produce horse-sickness temperature reactions or symptoms in the three horses on experiment.

One horse (No. 1273) showed a ? temperature reaction on immunity test, and blood from this horse will be inoculated to a susceptible equine to confirm or otherwise a horse-sickness reaction. It should be noted that the spleen material used in this experiment was obtained from a mule which showed a prolonged temperature reaction to horse-sickness and which did not show severe symptoms of the disease, and may have recovered.

It is intended to repeat the experiment with spleen taken from a horse at the height of a reaction and to confirm or otherwise the action of formalin in such dilutions as 1 in 400, 1 in 800, and 1 in 1,000 on horse-sickness virus in spleen.

Summary.

The experiments described give indications of the actions of Formalin and Toluol in various strengths on horse-sickness virus in spleens taken from equines reacting to the disease. The number of animals under observation is insufficient to enable one to draw any definite conclusions as to the value of the method for artificial immunization against horse-sickness, but the indications are that such a method may be of some value.

The cost of such experiments in Kenya prohibits work on any large scale. Modifications in technique may prove advantageous, and the use of tissue other than spleen might be attempted. The virus used for immunity test is known to produce fatal reaction on inoculation to susceptible equines, but here again, on account of the cost of equines for experiment, actual control on the virulence of each bottle of virus used in immunity test is not possible in this Colony.

A table giving the summary of inoculations and results is attached (see page 255).