Paper No. 32.

MEMORANDUM ON RESEARCH ON EAST AFRICAN SWINE FEVER IMMUNIZATION IN KENYA.

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THE diagnosis of East African swine fever in Kenya (late British East Africa Protectorate) dates from June, 1910; up till December, 1915, fifteen naturally occurring outbreaks were reported in the Colony involving 1,366 pigs of which 98.9 per cent. succumbed to the disease.

Nineteen outbreaks are recorded in the Annual Reports of the Chief Veterinary Officer during the period 1915-1927. Montgomery was the first to study this disease and to attempt immunization and from 1910 to 1917 carried out research work hereon at the Veterinary Pathological Laboratory, Kabete, Kenya.

He established that the virus is filterable and found that the English and European anti-serum prepared against the swine fever of those countries did not protect against the East African virus; pigs immune to the English type succumbed when infected with the East African virus and the serum of a pig recovered from the latter disease did not protect against the English virus.

Serum prepared locally from the single available domestic pig which survived injection with the East African virus and from wild pig respectively, was found valueless even when employed in very large doses.

He attempted to obtain immune pigs by other methods, viz.:—

(*a*) Mixing " in vitro " the virus, with the sera of naturally refractory animals such as the horse, mule, donkey, sheep, and goat respectively.

(*b*) Attenuation of the virus by heat.

The former was found of no assistance, and although by the latter method one of a number of pigs recovered and was proved to be immune, heating was found unsatisfactory inasmuch as the virus when heated below the thermal death point either produced, in some animals, a lengthened incubation period and reaction and the animals died of secondary infection, or in others no reaction and no immunity was conferred.

Montgomery was thus confronted with the difficulty of obtaining immune and hyperimmune pigs.

For some time past the writer has endeavoured to immunize and hyperimmunize domestic pigs against the East African virus by various methods. The experiments carried out and results obtained are recorded in the Annual Reports of the Chief Veterinary Research Officer for the following years, viz., 1921, 1922, 1924, 1925, 1926, 1927 and which are embodied in the Annual Report of the Agricultural Department, Kenya, for those years. The experiments and results obtained during 1929 to date are also included in this memorandum.

Briefly it was found that—

(1) the serum of wart hogs which had been inoculated with the East African virus and the serum of wart hogs inoculated with comparatively large doses of East African virus, possessed no protective properties;

(2) the prescribed and increased doses of American anti-swine fever (hog cholera) serum did not protect;

(3) pigs which had recovered from simultaneous inoculation of the American swine fever (hog cholera) serum were not protected against the East African virus;

(4) treatment of virulent blood with Lugol's solution for varying periods and at varying concentrations, either rendered the virus inert in which case no reaction occurred and no immunity was conferred, or the virus was attenuated and when inoculated produced, in some pigs, a reaction after a lengthened incubation period, ending in the death of the animal or, in others, either a slight or no reaction and no immunity. Variations in susceptibility of individual pigs was found to be one of the difficulties in the standardising of an attenuated virus;

(5) treatment of virulent blood with trypanblue solution and sodium potass. bismuth tartrate was of no assistance. The virulency was not affected by these;

(6) passage of virulent blood through naturally refractory animals, e.g., the horse, cattle, rabbit, and guinea pig, was unsatisfactory. Blood collected from these after they hand been inoculated with virulent blood did not transmit the disease to susceptible domestic pigs and no immunity was conferred.

So far attempts to hyperimmunize domestic pigs had failed; the few which reacted and recovered, when subsequently inoculated with a comparatively large dose of virulent blood with a view to hyper-immunization, died of swine fever and immunization by other methods was resorted to, viz.:—

(1) *Immunization with an attenuated virus.*
(2) *Prophylactic vaccination with tissue extract.*

IMMUNIZATION WITH AN ATTENUATED VIRUS.

The most promising results were obtained with virus ex domestic pig No. 1,125; the history of this animal is as follows, viz.:—

Pig No. 1,125 was injected subcutaneously on 11/5/25 with 2 c.c. blood in O.C.G., equal parts, of domestic pig No. 1,035.

Result.—A temperature reaction commenced on 14/5/25 which continued until 18/5/25, maximum 104.4° Fahr., animal was visibly sick during the reaction. (Blood was collected from 1,125 in equal parts of O.C.G. on 18/5/25 and stored in the ice-chest at a temperature of approximately 48° Fahr.).

NOTE.—Pig No. 1,035 was injected on 21/2/25 with virus, collected at the time of an outbreak of the disease in the Naivasha area, and died on 28/2/25 of swine fever.

Pig 1,125 was tested on its immunity on the following dates with the following results:—

28/5/25 with 1 c.c. blood of pig 1,035 (for history of virus see above).

Result.—No temperature reaction or clinical symptoms.

15/7/25 with 10 c.c. blood ex pig 1,091.

Result.—A temperature reaction from 17/7/25 to 24/7/25, maximum 105° Fahr., ending in recovery. No clinical symptoms noted.

NOTE.—1,091 reacted to virus ex 1,035 and died of swine fever, 7/8/25 with 20 c.c. blood, in equal parts of citrate solution, ex pig 1,113.

Result.—No temperature reaction and no clinical symptoms.

NOTE.—Pig 1,113 gave a protracted temperature reaction, viz., from 5/8/25 to 20/8/25 to subcutaneous injection of 1 c.c. blood in O.C.G. solution ex pig 1,125 of 18/5/25 and died of swine fever on 20/8/25.

NOTE.—Blood of 1,125 was collected on 17/8/25 and proved virulent.

Blood of 1,125 was collected on 26/8/25 and proved non-virulent.

29/9/25, with 60 c.c. of a mixture of equal parts of blood and citrate solution, ex pig 1,121 of 28/9/25.

Result.—On the 19th day after inoculation, viz., 18/10/25, a temperature reaction commenced to swine fever lasting till death on 25/10/25, maximum 107.2° Fahr. 23/10/25.

NOTE.—Pig 1,121 was inoculated on 26/8/25 with 2 c.c. fresh blood of pig 1,125 and did not react, but subsequently contracted swine fever by contact from a pig reacting to the same strain of virus.

SUMMARY.

Pig 1,125 reacted and recovered to an original injection of virulent blood, and when tested on its immunity with the same virus did not react; when re-inoculated with 10 c.c. blood of the same strain of virus, after passage through a susceptible pig, it reacted and recovered. On subsequent re-inoculation with 10 c.c. blood, of the same strain of virus after passage through another susceptible pig no reaction occurred, but on re-inoculation with 30 c.c. of blood of the same strain of virus after passage through another susceptible pig, a reaction occurred, commencing on the 19th day and ending in death from swine fever on the 26th day.

Blood of pig 1,121 was collected in equal parts of O.C.G., on 18/5/25, and utilized on various dates between 18/5/25 and 22/2/26 for the inoculation of susceptible pigs destined for anti-swine-fever serum production, with the following results:—

SUMMARY OF RESULTS.

Reacted and recovered but died of other causes	2	= 4.6 %
Reacted and died of swine fever	13	= 30.2 %
Reacted and recovered but died of swine fever when tested on their immunity	4	= 9.5 %
Indefinite reaction and recovered and when tested on their immunity died of swine fever	10	= 23.2 %
Reacted but not tested on their immunity	5	= 11.6 %
No reaction, died of swine fever when tested on their immunity	9	= 20.9 %
TOTAL	43	

The results obtained show—

(1) that blood collected during the reaction from a domestic pig which reacted and recovered to the injection of blood of a naturally infected pig, after passage, produces inconstant results, as regards reactions and mortality, in susceptible pigs;

(2) pigs which reacted and recovered, when subsequently tested on their immunity with blood of another pig which reacted to the same strain of virus or which were put in contact with pigs reacting to the same strain of virus again reacted and died of swine fever;

(3) pigs which reacted and recovered and again reacted and recovered to re-inoculation with the same strain of virus, after passage, again reacted and died, when re-inoculated with virus of the same strain or when put in contact with a pig reacting to the same strain of virus.

Further work in this direction was continued in 1928 and 1929 with the same strain of virus passed through domestic pig No. 789.

The history of 789 is as follows, viz., inoculated on the 22/2/26 with 5 c.c. of blood of pig 1,125 of the 18/5/25 diluted 1/10 saline.

Result.—A temperature reaction commenced on the 13th day and continued till the 26th day, maximum 105.4° Fahr., 22nd day. 789 was tested on its immunity on the 14/4/26 with 5 c.c. blood diluted 1/20 in saline ex pig 1,113 collected on the 7/8/25.

Result.—Temperature reaction commenced on the 16/4/26. Animal died on the 3/6/26 of swine fever. (Blood was collected in O.C.G. mixture on the 19/4/26 and stored.)

NOTE.—Pig 1,113 was inoculated with 1 c.c. blood of Pig 1,125 on the 28/7/25 and died of swine fever on the 21/8/25.

Blood of pig 789 collected on the 19/4/26 in O.C.G. mixture was inoculated to the following pigs on the following dates:—

No.	Date.	Result.	Immunity Test.		Result.
			Date.	Inoculated with.	
1204	7/2/29	Died of Swine Fever	—	—	—
1205	14/2/29	Reacted and recovered	1/5/29	Blood *ex* 1213 of 27/3/29	No reaction.
1210	11/3/29	" "	"	" "	"
1217	31/3/29	" "	"	" "	"

Blood of pig 789 collected, on 19/4/26, passed through 1,205, and blood of latter collected on 25/2/29 and inoculated to susceptible pigs and their blood passed through susceptible pigs.

No.	Date.	Inoculated with.	Result.	Immunity Test.		Result.
				Date.	Inoculated with.	
1201	25/2/29	Blood *ex* 1205 of 25/2/29	Reacted and recovered.....	1/5/29	Blood *ex* 1213 of 29/3/29	No reaction.
1212	12/3/29	,, 1205 of 12/3/29	Died of Swine Fever.....	—	—	—
1208	23/3/29	,, 1212 of 12/3/29	,, ,, ...	—	—	—
1213	27/3/29	,, 1208 of 27/3/29	Reacted and destroyed *in extremis* for virus	—	—	—
1219	31/3/29	,, 1213 of 31/3/29	Reacted and destroyed *in extremis* for material	—	—	—
1215	2/4/29	,, 1205 of 25/2/29	Died of Swine Fever.....	—	—	—
711	2/5/29	,, 1213 of 31/3/29	,, ,, ...	—	—	—

Summary.

Seventy-five per cent. of the pigs inoculated with passage virus (strain 1,125) stored in the ice-box at 4-10° Cent. or at room temperature for approximately 34 months, reacted and survived; and when tested on their immunity with passage virus (strain 1,125) collected 33 days previous, were resistant. Four in experiment.

Eighty-five per cent., approximately, of the pigs inoculated with passage virus (strain 1,125) immediately after collection, or up to 32 days after collection, died of swine fever or were destroyed *in extremis* for collection of virus or material. Seven in experiment.

Prophylactic Vaccination with Tissue Extract.

Experimental prophylactic vaccination with tissue extract was commenced in 1927. The spleen of reacting pigs was collected and mixed with saline after mincing, and then treated with toluol, or formalin.

The following is a summary of the results obtained:—

(1) 1 in 1,000 formalin spleen extract stored at 0° C. for 7 days, and then centrifuged, and the supernatant fluid inoculated, produced swine fever and death from swine fever.

(2) 1 in 100 toluol spleen extract incubated for 48 hours and stored for 3 days at 0° C., produced swine fever and death from swine fever.

(3) 1 in 100 toluol spleen extract incubated at 38° C. for 4 days and normal saline in the proportion of 1 of spleen to 9 of saline; a further lot of toluol added to make 1 per cent. of the total bulk, and the extract stored at 0° C. produced swine fever and death from swine fever.

(4) Extract to which toluol to make 3 per cent. of the total bulk was added and the extract incubated for 48 hours at 38° C. and stored at 0° C. produced an irregular temperature reaction, but did not protect against a subsequent subcutaneous inoculation of 1 c.c. blood ex 1,059 of 16/11/25 (attenuated virus).

(5) Extracts of 20/9/27 in normal saline, 1 per cent. spleen to 9 per cent. saline, to which toluol to make 3 per cent. of the total bulk was added, then incubated for 48 hours and stored at 0° C. produced a temperature reaction in a susceptible pig, 1,180; when re-inoculated with virus, 1,059 of 16/11/25 (attenuated virus) and simultaneously 10 c.c. extract, an irregular temperature reaction occurred, maximum 102.6° Fahr., when 1,180 was subsequently tested on its immunity with virus, 1,184 of 26/11/27, a reaction to swine fever and death from swine fever resulted.

Inoculation of extract prepared by Method 5, and simultaneously virus 1059 of 16/11/25, produced a temperature reaction in one susceptible animal, viz., 1,179 (blood collected from 1,179, and inoculated to a susceptible pig, 1,183 produced a mild temperature reaction, but no immunity was conferred in 1,183 against virus 1,184 of 26/11/27).

The remaining pig inoculated with virus 1,059 of 16/11/25, and simultaneously extract prepared by Method 5, gave an irregular temperature reaction, but no immunity was conferred to virus 1,184 of 12/11/27.

Two inoculations with extract, prepared by Method 5, at an interval of 12 days, and a third inoculation 10 days later with the extract and simultaneously attenuated virus 1,059 of 16/1/25, produced no reaction; and immunity was not conferred against virus 1,184 of 26/11/27.

Inoculation of extract prepared by Method 5, did not protect against virus 1,184 of 26/11/27, simultaneously inoculated.

(6) Inoculation of spleen extract in normal saline, 1 of spleen to 9 of saline, to which toluol to make 3 per cent. of the total bulk was added, and the extract then incubated for 48 hours at 38° C. produced a temperature reaction in 1,183 from the 3rd day. Animal died of swine fever on the 7th day.

NOTE.—1,183 was inoculated on 12/11/27 with blood of 1,179, (see above). On post-mortem examination, 1,183 showed only chronic swine fever bowel lesions probably result of the reaction from the original inoculation.

Prophylactic vaccination was continued during 1928 and 1929.

Details are given in the tabulated sheet.

Tissue Used.	Method of Treatment of Tissue.	No. of Pig Put in Expt.	Date.	Dose.	Result.	Immunity Test.		Result.	Remarks.
						Date.	Method.		
Spleen, liver and kidney *ex* pig 1191	Minced, 28/9/28, and mixed with normal saline 1 gramme to 1 c.c. Chloroform added to make 3% of the total bulk	1181	19/12/28 27/12/28	15 c.c. 20 ,,	No reaction ,,	7/2/29	Put in contact with reacting pigs	Died of Swine Fever, 8/3/29	Pig 1181 bled, 2/3/29, and blood inoculated to pig 1203. 1203 died of Swine fever, 12/3/29.
,, ,,	,, ,,	1186	12/12/28 19/12/28 4/1/29	10 ,, 10 ,, 10 ,,	Slight temperature reaction	7/2/29	,, ,,	Reacted and recovered..	Pig 1186 bled, 2/3/29, and pig 1202 inoculated. 1202 died of Swine Fever, 11/3/29. Pig 1186 re-tested on its immunity, 1/5/29, blood *ex* 1213 of the 31/3/29. Result—No reaction.
Spleen *ex* pig 1131	Minced, 13/3/29, and mixed with saline 1 gramme to 1 c.c. Toluol added to make 3% of the total bulk. The mixture then incubated for 48 hours and then stored in ice-chest	1216	19/3/29 26/3/29	10 ,, 10 ,,	Reacted & recovered	1/5/29	1 c.c. blood of 1213 of the 31/3/29	No reaction.....	—
,, ,,	,, ,,	1218	31/3/29 8/4/29	10 ,, 10 ,,	,, ,,	1/5/29	,, ,,	,, ,,	—
,, ,,	,, ,,	1214	6/4/29 13/4/29 20/4/29	5 ,, 5 ,, 5 ,,	No reaction	28/4/29	Put in contact with reacting pigs	Reacted from the 11th day of contact and died of Swine Fever on 17/5/29	1214 also got blood of 1213 of the 31/3/29, on the 10/5/29.
,, ,,	,, ,,	1222	6/4/29 13/4/29 20/4/29	15 ,, 15 ,, 15 ,,	,,	30/4/29	1 c.c. blood of 1213 of the 31/3/29	Reacted.....	—
,, ,,	,, ,,	1223	18/4/29 25/4/29 2/5/29	7 ,, 7 ,, 7 ,,	,,	10/5/29	,, ,,	Reacted and died of Swine fever, 21/5/29	—
,, ,,	,, ,,	1224	18/4/29 25/4/29 2/5/29	20 ,, 20 ,, 20 ,,	,,	10/5/29	,, ,,	Reacted and died of Swine Fever, 22/5/29	—

Tissue Used.	Method of Treatment of Tissue.	No. of Pig Put in Expt.	Date.	Dose.	Result.	Immunity Test.		Result.	Remarks.
						Date.	Method.		
Liver and spleen *ex* pig 1219	Minced, 6/4/29, saline added in the proportion of 1 gm. of tissue to 4 c.c. of saline and commercial formalin to make 1% of the total bulk added	1236	11/4/29 20/4/29	10 c.c. 10 ,,	Reacted & recovered	1/5/29	1 c.c. blood of 1213 fo the 31/3/29	Reacted.....	—
,, ,,	,, ,,	1211	11/4/29 20/4/29	10 ,, 10 ,,	,,	10/5/29	,, ,,	,, ,,	1211 was bled on the 20/4/29, and inoculated to pig 1226. Result—1226 reacted and recovered. 1226 tested on its immunity, 10/5/29, 1 c.c. blood of the 31/3/29. Result—Reacted.
,, ,,	,, ,,	1196	18/4/29 25/4/29	15 ,, 15 ,,	Reacted & recovered	10/5/29	,, ,,	Reacted in extremis, 22/5/29. Died 25/5/29	—
,, ,,	,, ,,	1237	18/4/29 25/4/29	5 ,, 15 ,,	,,	10//529	,, ,,	Reacted.....	Pig 1237 bled, 25/4/29, and inoculated to 1225. Result—Mild reaction. 1225 tested on immunity, 10/5/29, 1 c.c. blood *ex* 1213 of 31/3/29. Result—Reacted.
Control for virus 1213 of the 31/3/29	—	711	2/5/29	1 c.c. virulent blood *ex* 1213 of 31/3/29	Reacted	—	—	—	—

Summary.

1. Tissue (spleen, liver, and kidney) pulped and mixed with saline in the proportion of 1 gramme to 1 c.c. saline and chloroform added to make 3 per cent. of the total bulk and the treated tissue stored for 85 days in the ice-chest at approximately 4° Cent. Two inoculations of the above-treated tissues did not protect a pig, which gave no definite reaction to the vaccine, against contact infection and death; three inoculations protected a pig, which reacted slightly to vaccination, against a severe reaction and death from contact infection. Two in experiment.

2. Spleen pulped and mixed with saline (1 gramme to 1 c.c. of saline) and toluol added to make 3 per cent. of the total bulk and the treated tissue incubated for 48 hours and then stored in the ice-chest produced swine fever when inoculated on the 6th and 18th days respectively after the date of preparation. Two in experiment. Both recovered and when tested on their immunity with virulent blood, were protected.

The same lot of treated spleen stored for 24 days did not set up swine fever or confer immunity. Four in experiment.

3. Pulped liver and spleen (mixed) ex pig 1,219 and mixed with saline, 1 gramme to 4 c.c. of saline and commercial formalin, added to make 1 in 500 of the mixture and stored in the ice-chest at approximately 4° Cent. and inoculated five days after preparation produced swine fever; animal when subsequently tested on its immunity with virulent blood reacted severely. One in experiment.

4. Pulped liver and spleen of the same pig, viz., No. 1,219, mixed with saline (1 in 4) and commercial formalin added to make 1 in 100 of the mixture and stored in the ice-chest for approximately twelve days at approximately 4° Cent. produced swine fever. Three in experiment. All recovered and when subsequently tested on their immunity with a dose of virulent blood reacted severely.

Conclusions.

(1) Virulent blood collected in equal parts of O.C.G. mixture becomes, after a period of storage, attenuated and produces in susceptible pigs a definite reaction. A large percentage of pigs inoculated with attenuated virus recover and survive inoculation with the same strain of virus recently passed through a susceptible pig.

(2) Virulent blood attenuated by storage increases in virulency by passage through susceptible pigs and produces a high percentage of mortality.

(3) Attenuation of the virus is also possible by treating tissues (spleen, liver, or kidneys) collected from a reacting pig, with toluol, chloroform, or formalin; susceptible pigs inoculated with non-inactivated extract, but in which virus is attenuated, react and recover. Some of the survivors are completely protected against inoculation with virulent blood; others are protected against a severe reaction and survive; in others no protection is conferred against a severe reaction and death.

(4) Inactivated extract confers little or no protection. The strain of virus used for testing the pigs on their immunity after treatment with inactivitated extract would appear to be responsible for the small percentage which survive an immunity test with virulent blood.

As a result of the experimental immunization work, a number of recovered pigs are now available for hyperimmunizing with a view to the production of an anti-serum, and work in this direction is being continued.

Paper No. 33.

RABIES IN SOUTH AFRICA.

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For many years the Union of South Africa has been considered free of rabies.

1. Port Elizabeth Outbreak, 1893.

The last authentic outbreak to occur was at Port Elizabeth in 1893. The disease had been introduced into the country with an Airedale terrier which was landed at Port Elizabeth in September, 1892. This dog took ill soon after arrival and exhibited symptoms which were very suspicious of rabies: " he first became unaccountably savage, attacked and fought with every dog he met, and barked and howled incessantly for a day or two before he died." The next case was observed in January, 1893, and this was followed by numerous cases until the disease was diagnosed by the local Government Veterinary Officer Britton in April, 1893.

The diagnosis was confirmed by subinoculation into rabbits by Edington and Hutcheon at the Laboratory in Grahamstown.

Steps were immediately taken to deal with the outbreak. A Rabies Act was passed by Parliament and regulations were issued which prescribed the measures to be enforced. In Port Elizabeth all dogs had to be muzzled and tied up. Stray dogs were to be destroyed; and in less than a year about 2,000 had been dealt with in this way.

The disease also spread to the surrounding districts of Uitenhage, Jansenville, Willowmore and Albany, and in these areas also large numbers of ownerless dogs were destroyed.

The measures adopted were entirely successful and *a year after the first outbreak the disease had disappeared completely.* No mention is made of rabies in the subsequent annual reports of the Colonial Veterinary Surgeon, and at no time since 1893 has the disease again made its appearance in dogs in the Union of South Africa.

Before leaving this outbreak the following significant statement which occurs in the Annual Report for the year 1893 of Colonial Veterinary Surgeon, Dr. Hutcheon, may be quoted: " I was in great