Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 22, Number 2, April, 1949.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Investigation into Immunization of Cattle against Rinderpest in Tanganyika Territory.

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In a previous article (Mitchell and Peevie, 1945) a detailed description is given of the manufacture of antirinderpest spleen vaccine under field conditions in Tanganyika for use in the campaign of 1940. While the immunization campaign was in progress it was apparent that the veterinary officers in the field would welcome additional information on a number of points connected with immunity production. Since experimental animals were available in fairly large numbers it was decided to carry out a number of field tests at Mbosi. The results of these tests form the basis of this report.

Field Tests at Mbosi (Tanganyika).

A. *Experimental Conditions.*

The research section was erected on a Coffee farm in the Mbosi area, which is in the central part of Southern Tanganyika. The object was to control the efficiency of the vaccine produced at the vaccine section; to assist in the diagnosis of rinderpest outbreaks and to clear up difficulties encountered in the field.

Other duties were the routine examination of smears of animals to be killed for vaccine production and the staff had to be available for work on the vaccine unit when so required.

The time spent on this work was just over four and a half months, i.e. 18th February, 1940 to the beginning of July, 1940.

(a) The Animals.

The cattle used consisted of two main types or breeds, i.e. locally bought humped Zebu, and long horned, humpless Ankole or Ufipa cattle, originating from the South-Western area of the territory.

Bulls were in the majority, only a few cows or heifers being used in the work. The ages varied from 2 to 6 years; with the larger number under four years of age.

All cattle were serially branded with numbers either on the croup or horns.

Received for publication on 15th November, 1945.—Editor.

(b) Grazing and Environment.

Grazing was abundant at the commencement of the experiments, and lasted up to the middle of May, but thereafter there was a marked drop in the condition of the cattle.

Although the grazing areas were not completely fenced we were able to prevent the spreading of disease from one group to the other by rigid precautions, as we had a large area at our disposal which was subdivided by a road, a stream and coffee plantations. Natives were employed to herd the animals during the day and they were kraaled at night. Three main subdivisions were made as follows: unvaccinated available, vaccinated available and cattle under virus test.

East Coast fever is enzootic in the area and the locally bought Zebu cattle were found to be highly resistant, while the Ankole cattle from the Ufipa area were very susceptible. A careful watch was therefore kept and smears examined from all animals dying on the station. At post mortems, a thorough check was kept on East Coast fever.

The rainy season lasted up to the end of April and it was never very hot.

(c) Vaccine and Virus Used.

The vaccine was either Formol saline or Formol glycerine spleen vaccine, prepared as described by Mitchell and Peevie (1945). Except in one experiment all the vaccine was produced at Mbosi, and is indicated as F/G or F/S according to whether it contained glycerine saline or saline alone.

Virulent bovine virus is indicated as "O" virus, and was obtained from the vaccine unit, i.e. ex Mpwapwa laboratory.

The donors were the vaccine production animals, the blood being taken on the 5th day, just before killing for vaccine, and used fresh as citrated blood.

Two "types" of attenuated goat virus were employed and they are referred to as Dickinson (D./G.V.) or Kabete (K.G.V.). Both were obtained from Kabete Laboratory in the desiccated form.

As a standard 1 gram of desiccated goat virus was emulsified in 500 c.c. normal saline, the preparation was done shortly before use and 2 c.c. employed as a dose per animal unless otherwise indicated.

(d) Evaluation of Reactions.

This was based on early morning temperatures. The occurrence of clinical symptoms was found to be very erratic, frequently mild and of so short a duration that it was difficult to differentiate from other causes like non-specific ophthalmia, digestive disturbances or vegetable poisoning. The drop in temperature between the 6th and 10th day, however, frequently coincided with a diarrhoea, the intensity of which showed marked variations. Only a few cases, showing a foetid bloodstained diarrhoea were met with, and in these animals it was associated with an extremely rapid loss of condition.

Buccal and nasal lesions were also very indefinite.

During the rainy months, animals which reacted subacutely to rinderpest and recovered developed pustules on the skin. These pustules later ruptured, and caused matting of the hair. Crusts formed and the animals lost their hair in patches, particularly on the head and behind the shoulders giving them a " mangy " appearance.

Post mortem examinations were performed on all animals dying on the station.

Experiment 1.

To ascertain:—

(*a*) Whether F/G organ vaccine produced from Zebu Cattle would efficiently immunise both Zebu (local) and Ankole (Ufipa) breeds of cattle.

(*b*) The comparative degree of immunity produced by single, double and triple vaccinations with 10 c.c. F/G vaccine at weekly intervals.

(*c*) The relative safety of attenuated goat as compared to " O " virus.

Number of Animals.—126 head of cattle, 63 Ufipa and 63 Zebu, were subjected to the virus tests. The grouping was done as evenly as possible, according to sex, size and condition.

Vaccine.—Formol/glycerine saline vaccine produced at Mbosi was used. Vaccination was commenced on 18th February, 1940, and the groups completed as follows:—

3 vaccination group: 18th February, 1940, 25th February, 1940 and 3rd March 1940.
2 vaccination group: 25th February, 1940, and 3rd March, 1940.
1 vaccination group: 3rd March, 1940.

Virus.—2 c.c. " O " virus and 2 c.c. Dickinson attenuated Goat virus, Kabete batch D 196 of 11th March, 1940. Virus test of all groups on 16th March, 1940.

Results.

Breed of Cattle.	No. in Group.	No. of Vacci-nations.	Virus.	Reactions to Virus.			
				Acute.	Mild.	Neg.	Mortality.
Ufipa.....	8	One	D.G.V.	4	3	1	0
Ufipa.....	11	One	O.	4	2	5	**1**
Zebu.....	10	One	D.G.V.	4	3	3	**0**
Zebu.....	10	One	O.	3	2	5	**0**
Ufipa.....	12	Two	D.G.V.	0	3	9	**0**
Ufipa.....	12	Two	O.	1	0	11	**0**
Zebu.....	8	Two	D.G.V.	0	0	8	0
Zebu.....	10	Two	O.	1	1	8	**0**
Ufipa.....	10	Three	D.G.V.	1	3	6	1
Ufipa.....	7	Three	O.	0	1	6	0
Zebu.....	9	Three	D.G.V.	0	2	7	0
Zebu.....	10	Three	O.	0	0	10	0
Ufipa.....	2	Control	D.G.V.	2	0	0	1
Ufipa.....	1	Control	O.	1	0	0	1
Zebu.....	3	Control	D.G.V.	3	0	0	0
Zebu.....	3	Control	O.	3	0	0	3

Remarks.

(i) The Ufipa cattle were highly susceptible to East Coast fever which is enzootic in the area and some deaths occurred from East Coast fever subsequent to vaccination while awaiting the virus tests.

(ii) East Coast fever cases have also been eliminated from the final grouping of reactions.

Conclusions.

(i) There was no appreciable difference in the degree of immunity produced in Ufipa (Ankole) and local Zebu cattle, by F/G vaccine made from the local Zebus.

(ii) The onset of temperature reaction was delayed in all vaccinated groups.

(iii) The difference in degree of immunity produced is greater between one and two vaccinations, than between two and three vaccinations.

This obvious conclusion may, however, be misleading as the virus test was done on the 20th and 27th day after the first vaccination in the two and three vaccination groups as compared to 13 days in the one vaccination group. The time taken for immunity development may have been a factor if it takes more than 13 days to reach its peak. Should the time interval be the only factor, then the experiment indicated that optimum immunity was reached somewhere between the 13th and 20th day after vaccination.

Practically, it would appear that 3 vaccinations at weekly intervals are not necessary unless the 3rd vaccination increased the duration of the immunity.

Another possibility was that the differences could be due to the larger amount of vaccine injected in the latter groups.

(iv) There was no appreciable difference in the number of reations of vaccinated animals to D.G.V. and O. virus. The controls, however, show both breeds to be susceptible to " O " virus and D.G.V. but the Zebus had a higher degree of resistance to attenuated goat virus in as much as the animals recovered although they reacted severely. One of the two Ufipa cattle died in the D.G.V. reaction, and the one animal died in the " O "-virus control group.

Experiment 2.

Object.—To test the immunity produced, by one and two vaccinations with F/S vaccine in highly susceptible grade cattle from Northern Rhodesia.

Remarks.

(i) The animals were vaccinated during January, 1940 and subjected to D.G.V. and " O " virus test on 16th March, 1940.

(ii) Seventeen of the original 20 died of East Coast fever, before and after virus inoculation. The one survivor in the single 10 c.c. vaccination group reacted to D.G.V. and recovered. In the 2 vaccination group two animals survived East Coast fever and reacted only mildly to " O " virus.

Experiment 3.

Object.—To test the duration of immunity following triple vaccination with F/S vaccine in the field and to compare it with the natural resistance of Zebu cattle.

Number of Animals.—19 *head of Zebu cattle.*

Vaccine.—F/S vaccine produced at Mbosi. Vaccination completed on 14th January, 1940.

Virus Test.—Against 2 c.c. K.G.V. and " O " on 14 May, 1940 and 2nd June, 1940 respectively.

Results.

No. of Animals.	Virus.	Date.	Interval.	Reactions to Virus.			
				Acute.	Mild.	Negative.	Mortality.
7.....	K.G.V.	14/5/40	120 days	2	2	3	0
6.....	O.	2/6/40	138 days	2	3	1	0
6.....	O.	2/6/40	No vaccine	5	1	0	2

Remarks.

The Zebu " O " virus controls of Experiment 1 lingered some time before they died and we felt that the number had to be increased for determination of the resistance of Zebu cattle to " O " virus.

Conclusions.

(i) The results indicated that immunity from triple vaccination was not high, 120 and 138 days after vaccination.

(ii) Local Zebu cattle possess some degree of natural resistance against rinderpest.

Experiments 4 and 5.

Objects.—(*a*) To compare the degree of immunity produced by larger doses of F/G vaccine given in one injection, viz., 20 c.c. and 30 c.c. doses.

(*b*) To compare the immunity produced by F/G vaccine produced at Mpwapwa Laboratory in local Zebu and Zebu cattle from the Njombe area, i.e. importance of area of origin.

Remarks.

The effect of larger doses of vaccine had to be tested, because of the hypothesis that the vaccine action depended on its containing infinitesimal quantities of live virulent virus, i.e. the larger doses of vaccine given in any one injection might convey a higher degree of immunity.

Number of Animals.—25 small local Zebu and 17 well nourished Njombe Zebu.

Vaccine.—Vaccinated on 29th March, 1940, F/G vaccine produced at Mpwapwa dated 31st January, 1940.

Virus.—D.G.V. and "O" used in doses of 2 c.c.

Results.

Breed.	No. of Animals.	Dose of Vaccine.	Interval.	Virus.	Reactions to Virus.			
					Acute.	Mild.	Negative.	Mortality.
Local Zebu....	10	20 c.c.	17 days	D.G.V.	0	2	8	0
Local Zebu....	10	20 c.c.	17 days	O.	1	1	8	0
Local Zebu....	2	Control	Control	D.G.V.	2	0	0	0
Local Zebu....	3	Control	Control	O.	3	0	0	1
Njombe Zebu..	4	20 c.c.	17 days	D.G.V.	1	1	2	0
Njombe Zebu..	4	20 c.c.	17 days	O.	1	2	1	0
Njombe Zebu..	4	30 c.c.	17 days	D.G.V.	1	1	2	0
Njombe Zebu..	4	30 c.c.	17 days	O.	0	1	3	0
Njombe Zebu..	1	Control	Control	O.	1	0	0	0

Conclusions.

(i) There is no appreciable difference in the degree of immunity produced by doses of 20 c.c. and 30 c.c. of F/G vaccine, when tested 17 days after vaccination against D.G.V. and "O" virus. Unfortunately no direct comparison could be made with the one vaccination group of Experiment 1, as the time interval was 4 days longer.

(ii) Complete immunity against the virus used had not been established in all the animals 17 days after vaccination. The reactions were, however, controlled to such an extent that no mortality occurred and suggested the possibility of enhancing organ vaccine immunity with live goat virus, should the animal reacting to the virus not transmit the disease to susceptible incontacts.

(iii) There would appear to be a difference in the immunity produced by Mpwapwa vaccine in local Zebu and Njombe Zebu cattle. The latter group showed a higher percentage of acute reactions, but the total number of cattle was considered too small for drawing definite conclusions, except that the field staff were advised of the possible danger of using goat virus in the Njombe area, as the animals might have a very low resistance to rinderpest.

Experiment 6.

Object.—To ascertain if Kabete attenuated goat virus could be transmitted when used in triple vaccinated (F/G) cattle, 15 days after the last vaccination.

Number of Animals.—10 vaccinated and 10 susceptible cattle of the Zebu breed.

Vaccine.—F/G produced at Mbosi on 3rd April, 1940 from Ufipa cattle. Triple 10 c.c. doses weekly as from 8th April, 1940.

Virus.—Kabete attenuated goat virus—first injection on 6th May, 1940.

Method.—The 20 cattle were kept in a kraal (boma) day and night from 6th May, 1940, i.e. when the 10 vaccinated animals received the goat virus injections. All animals were previously on temperature record and this was continued in both the vaccinated and susceptibles.

Water was given in one trough, and fresh grass was cut and fed daily. Stomoxys flies were abundant and it was considered to be ideal conditions for contact transmission.

Unfortunately none of the animals inoculated with Kabete goat virus reacted, and although injections were repeated with larger doses on three occasions the vaccine immunity appeared to completely " block out " the virus reaction.

The animals were kept in contact for 26 days, i.e. up to 2nd June, 1940, and no rise in temperature was recorded in the susceptible animals. The animals were then transferred to Experiment 20.

Experiment 7.

Object.—To determine whether F/G vaccine produced from Ufipa (Ankole) cattle would immunise Zebu cattle.

Remarks.

This experiment was started at the same time as No. 6 as results were immediately required for practical application in vaccine production.

Number of Animals.—12 local Zebu.

Vaccine.—F/G produced at Mbosi from Ufipa cattle on 3rd April, 1940.

Virus.—2 c.c. " O " virus injected 14 days after vaccination.

Results.

No. of Cattle.	Dose of Vaccine.	Interval.	Virus.	Reactions.			
				Acute.	Mild.	Negative.	Mortality.
5.....	10 c.c.	14 days	O.	0	1	4	0
5.....	20 c.c.	14 days	O.	1	1	3	0
2.....	Control	Control	O.	2	0	0	1

Conclusion.

(i) A single dose of 10 c.c. vaccine made from Ufipa cattle protects Zebu against 2 c.c. " O " virus, 14 days later.

(ii) 20 c.c. of the same vaccine gives no better protection, and again seems to point to the fact that the time required for immunity to develop and not the dose of vaccine above 10 c.c., is the important factor.

(iii) There appears to be no ground for the hypothesis that the antigenic value, for other breeds of cattle, of the vaccine is determined by the breed from which it is made.

Experiments 8 and 16.

Object.—To test the viability of virus in Formol/saline spleen vaccine under room temperature storage conditions, the formalin content being 0·15 per cent.

Number of Animals.—12 local Zebu.

Vaccine.—10 c.c. doses of F/S Mbosi vaccine produced on 15th April, 1940. Batch No. 17.

Immunity Test.—2 c.c. D.G.V. on 5th June, 1940, i.e. 46 to 49 days after vaccination.

Results.—Commenced on 16th April, 1940.

No. of Cattle.	Hours after Formalinization.	Reactions.				Virus Test on 5/6/40.	Reactions.		
		Acute.	Mild.	Neg.	Mort.		Acute.	Mild.	Neg.
2.....	21	0	2	0	0	2 c.c. D.G.V.	0	0	2
2.....	34	0	1	1	0	D.G.V.	0	1	1
2.....	40	0	0	2	0	D.G.V.	0	2	0
2.....	45	0	1	1	0	D.G.V.	1	0	1
2.....	64	0	1	1	0	D.G.V.	0	1	1
2.....	85	0	0	2	0	D.G.V.	0	0	2

Remarks.

During the period of storage the minimum temperatures recorded varied between 50° F. and 54° F. in the shade, while the maximum temperatures ranged between 82° F. and 86° F.

Conclusions.

(i) Formol/saline vaccine was not safe to use within 64 hours after formalinization when stored at room temperature.

(ii) The virus in the vaccine was either attenuated or killed between the 64th and 85th hours.

(iii) The immunity produced in non-reactors was not solid against D.G.V. between 46 and 49 days later.

Experiments 9 and 18.

Objects.—(i) To determine the rate of development of immunity following vaccination with F/G and F/S vaccine.

(ii) To compare the rate of development following on doses of 5 c.c. and 10 c.c. of vaccine, i.e. to determine whether it is only the time factor concerned when using doses of vaccine not smaller than 5 c.c.

Number of Animals.—21 Zebu in Experiment 9 and 12 Zebu in Experiment 18.

Vaccine.—Experiment 9. Vaccinated on 23rd April, 1940 with F/G vaccine and Batch 19 of 19th April, 1940.

Experiment 18. F/S vaccine—vaccinated on 26th May, 1940.

Virus.—" O " virus. 2 c.c.

Results.

EXPERIMENT 9.

No. of Animals.	Dose of Vaccine.	Virus Test: Days After Vaccine.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
2	5 c.c.	4	2	0	0	0
2	10 c.c.	4	2	0	0	0
2	5 c.c.	7	1	0	1	0
2	10 c.c.	7	2	0	0	0
2	5 c.c.	10	0	0	2	0
2	10 c.c.	10	0	0	2	0
2	5 c.c.	13	0	0	2	0
2	10 c.c.	13	0	0	2	0
2	5 c.c.	20	0	0	2	0
2	10 c.c.	20	0	0	2	0
1	Control	Control	0	0	1	0

EXPERIMENT 18.

No. of Animals.	Dose of Vaccine.	Virus Test: Days After Vaccine.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
2	5 c.c.	10	1	0	1	0
2	10 c.c.	10	1	0	1	1
2	5 c.c.	15	0	0	2	0
2	10 c.c.	15	0	0	2	0
2	5 c.c.	20	0	0	2	0
2	10 c.c.	20	0	0	2	0

Remarks.

The indication in the previous experiments had been that optimum immunity was developed after the 13th day after vaccination, and in Experiment 9 no reactions were noted in the 10 day group. The control animal did not react and it was therefore considered advisable to repeat the latter half of the experiment as soon as animals were available.

To avoid any doubt, fresh pooled citrated blood was used on each occassion in Experiment 18. The same blood was being used for inoculating vaccine production animals at the vaccine production unit and the reactions there served as control to each sample used.

Conclusions.

(i) Immunity development was gradual and attained its optimum after the 10th day after vaccination.

(ii) Within limits (5 c.c. and 10 c.c.), the dose of vaccine had no particular influence on immunity production.

(iii) The results indicated that it may be possible to augment formalinized organ vaccination immunity with live virus, even in highly susceptible animals, by varying the time interval between vaccine and virus inoculations. The basis of assumption that the immunity would be augmented was that an animal contracting the disease retains a life long immunity. The danger of spreading had, however, to be kept in mind.

Experiment 10.

Object.—To note the protection given by a small initial dose of 5 c.c. of vaccine followed 7 and 14 days later by a dose of 20 c.c. The immunity tested 14 days after the second dose of vaccine.

Number of Animals.—22 Zebu cattle.

Vaccine.—Vaccination commenced on 30th April, 1940 with F/G vaccine produced at Mbosi on 19th April, 1940.

Virus Test.—2 c.c. " O " virus, 14 days after last vaccination.

Results.

No. of Animals.	Interval between Vaccinations.	Reactions to Virus.			
		Acute.	Mild.	Negative.	Mortality.
10	7 days	0	0	10	0
10	14 days	1	1	8	0
2	Controls	2	0	0	0

Conclusions.

(i) 5 c.c. of vaccine followed 7 days later by 20 c.c. gave a solid protection against 2 c.c. " O " virus 14 days after the second vaccination.

(ii) From the results of this experiment it appeared that when the interval between vaccinations was increased the herd immunity was not as solid. However, in Experiment 18, a dose of 5 c.c. gave a solid immunity in 2 animals 13 days after vaccination. This variation could therefore not be explained on this basis, the probability being individual variations in the susceptibility of the experimental animals.

Experiment 11.

Object.—To ascertain if Kabete Goat Virus could be transmitted to susceptible in-contacts when used without prior vaccination with formalinized organ vaccine.

Number of Animals.—5 local Zebu, 2 of which injected with Goat Virus and 3 susceptible in-contacts.

Commenced.—6th May, 1940.

Remarks.

On 2nd June, 1940, 10 cattle from Experiment 17, reacting to K.G.V. were introduced into the kraal.

Method.—Identical to Experiment 6, only a different kraal or boma.

The two animals inoculated with K.G.V. gave good temperature reactions, but only 1 showed mild and, therefore, doubtful symptoms. It is for this reason that the 10 reacting bovines of Experiment 17 were introduced and contact was maintained up to 30th June, 1940., i.e. 54 days.

Conclusions.

K.G.V. could not be transmitted from reacting Mbosi Zebu cattle to susceptible animals of the same breed and origin when placed in intimate contact for 54 days.

Experiment 12.

Object.—To determine the antigenic value of Formol/saline vaccine at different periods after production.

Number of Animals.—9 local Zebu.

Vaccine.—F/S produced at Mbosi on 29th April, 1940. B. 23. Formalin content 0·15 per cent. The vaccine was bottled in a series of bottles containing 100 c.c. each and kept in the shade on an open veranda pending use. Maximum temperature recorded 92° F. and minimum 50° F.

Virus Test.—2 c.c. " O " virus at stated intervals.

Results.

Age of Vaccine.	No. of Animals.	Interval before Virus Test.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
9 days.....	1	14 days	0	0	1	0
13 days.....	2	21 days	1	0	1	0
20 days.....	2	22 days	2	0	0	1
27 days.....	2	21 days	2	0	0	1
34 days.....	2	21 days	1	1	0	0

Conclusions.

The test confirmed the procedure adopted of using F/S vaccine before the 10th day after production.

Experiment 13.

Object.— (i) To determine the safety of vaccine plus K.G.V. immunisation in Zebu cattle when used at the convenient interval of 7 days between inoculation.

(ii) To determine whether the combination of different doses affected the safety.

Number of Animals.— 46 local Zebu.

Vaccine.—F/G produced at Mbosi.

Results.

No. of Cattle.	Dose of Vaccine.	Dose of Virus.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
10	5 c.c.	2 c.c.	6	3	1	1
10	10 c.c.	2 c.c.	3	4	3	0
10	5 c.c.	5 c.c.	6	3	1	0
10	10 c.c.	5 c.c.	5	2	2	0
3	Control	2 c.c.	2	1	0	0
3	Control	5 c.c.	3	0	0	0

Remarks.

(i) The animals were starting to lose condition.

(ii) One animal died from other causes, probably secondary to rinderpest, e.g. (*a*) Chronic pleuritis, epicarditis, and pericarditis. The other one (*b*) Fasciola hepatica and rinderpest.

(iii) Clinical symptoms were not marked except in the 1 animal that died from rinderpest.

Conclusions.

(i) The percentage of reactions was high in all the groups and it appears that a 5 c.c. dose of vaccine in combination with 2 c.c. or 5 c.c. K.G.V. borders on the danger line.

(ii) The reactions in the 10 c.c. vaccine groups were more controlled and the percentage of reaction was 70 per cent. and above.

(iii) Compared with the control animals of the experiment it is clear that the vaccine inoculation subdued the virus reactions in ratio of the dose of vaccine. This is not in absolute agreement with conclusions from the previous experiments.

Both time and dose of vaccine, therefore, appear to influence the degree of immunity produced 7 days after vaccination.

Experiment 13a and 19.

Object.—To determine the safety of vaccine K.G.V. immunisation in Ufipa (Ankole) cattle, the interval being extended to the next convenient time, i.e. 14 days, as it was assumed that Ufipa cattle were more susceptible to rinderpest.

Remarks.

There had been no confirmed reports of rinderpest in the Ufipa area for many years and the cattle were considered to be as highly susceptible as animals in the areas south of the border.

Number of Animals.—10 Ankole type from Ufipa area.

Vaccine.—Vaccinated with F/G vaccine, batch 32, on 29th May, 1940.

Virus.—2 c.c. K.G.V. on 12th June, 1940.

Results.

No. of Animals.	Dose of Vaccine.	Interval.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
5	5 c.c.	14 days	1	1	3	0
4	10 c.c.	14 days	1	1	2	0
1	Control	Control	1	0	0	1

Conclusions.

The percentage reactions was 40 per cent. and 50 per cent., which was lower than that obtained in Experiment 13. This might have been due to the longer period, but it had to be tried as any other interval between 7 and 14 days would result in disorganisation when applied in the field where the cattle owners are all natives.

Experiment 14.

Object.—To ascertain the virus content of brain tissue of animals reacting to rinderpest for purposes of inclusion of brain material in vaccine production.

Number of Animals.—4 Zebu cattle.

Commenced.— 21st May, 1940.

Method.

Fresh brain tissue was obtained from an animal killed at the height of temperature reaction, i.e. 5th day, at the vaccine section. A portion of the brain was emulsified with a measured quantity of normal saline after being ground in a sterile mortar with sterile sand. Further dilutions were made to convenient doses containing the amounts of brain tissue given in the table..

The material was injected subcutaneously.

Results.

No. of Animals.	Dose of Brain.	Reactions.			
		Acute.	Mild.	Negative.	Mortality.
1	0·001 gm.	0	0	1	0
1	0·002 gm.	0	0	1	0
1	0·005 gm.	0	0	1	0
1	0·01 gm.	0	0	1	0

Conclusions.

No reactions were obtained and brain tissue was therefore considered not to contain enough virus to be of antigenic value in vaccine production.

Experiment 15.

Object.—To control Experiment 9, the immunity of the animals used from the 10th day onwards was tested 30 days after vaccination against K.G.V. and " O " virus.

Number of Animals.—12 Zebu transferred from Experiment 9 and re-grouped according to quantity of vaccine received.

Viruses.—2 c.c. of K.G.V. or " O " virus. Tested on 23rd May, 1940.

Results.

No. of Animals.	Dose of Vaccine.	Virus.	Reactions to Virus.			
			Acute.	Mild.	Negative.	Mortality.
3	5 c.c.	K.G.V.	0	0	3	0
3	5 c.c.	'O'	2	0	1	0
3	10 c.c.	K.G.V.	0	0	3	0
3	10 c.c.	'O'	1	0	2	0

Conclusions.

(i) The " O " virus used in the 10 day and later groups of Experiment 9 was inert.

(ii) The immunity conferred to Zebu cattle by 5 c.c. and 10 c.c. of vaccine when tested 30 days later was solid against K.G.V. but could be broken down with 2 c.c. " O " virus in 50 per cent. of the animals, which, however, did not die.

Experiment 17.

Object.— Due to the fact that Pfaff (1938) had increased the safety of attenuated goat virus vaccination in buffaloes by decreasing the dose and secondly the theory that formalinized vaccine conferred immunity because it contained minimum quantities of live virus, it was decided to determine the effect of varying doses of Kabete attenuated goat virus.

Number of Animals.—10 Zebu.

Virus.—Desiccated K.G.V. emulsified in normal saline.

Commenced.—24th May, 1940.

Remarks.

The animals were in very poor condition, and one in the 0·005 gram dose group died from poverty.

Results.

No. of Cattle.	Dose of Virus.	Reactions.			
		Acute.	Mild.	Negative.	Mortality.
2	0·12 gm.	1	1	0	0
2	0·04 gm.	1	0	1	0
2	0·02 gm.	2	0	0	2
2	0·01 gm.	1	0	1	0
2	0·005 gm.	0	0	2	0

A feature in this experiment was that the animals showed definite rinderpest symptoms. These symptoms varied from mouth lesions to diarrhoea, but could not be correlated to the dose of virus received.

Conclusions.

(i) The degree of reaction in Zebu cattle does not appear to depend on the dose of virus injected.

(ii) Animals in poor nutritional condition appear to be less resistant to rinderpest, i.e. clinical symptoms during the temperature reactions are more common in animals in poor condition.

Experiment 20.

Object.—To test the immunity produced by triple vaccinations of 10 c.c. F/G vaccine followed by K.G.V. 16 days after the last vaccination. The animals did not show a temperature reaction to K.G.V. even when 2 more injections were given with larger doses.

Number of Animals.—10 Zebu transferred from Experiment 6.

Vaccinations.—Completed on 21st April, 1940.

Viruses.—(i) K.G.V. on three occasions, i.e. 6th May, 1940, 14th May, 1940 and 23 May, 1940.

(ii) Immunity tested with 2 c.c. "O" virus on 3rd June, 1940.

Results.

None reacted to the "O" virus.

Conclusions.

All the animals had a high degree of immunity and in the light of the previous experiments, where up to then the formalinized vaccine immunity could be broken down with "O" virus, it appeared probable that the vaccine immunity might have been augmented by the K.G.V. in spite of the fact that the animals did not show a temperature reaction.

Experiment 21.

Object.—To ascertain the transmissability of D.G.V. from unvaccinated reactors to susceptible cattle in close contact.

Number of Cattle.— 15 Zebu local cattle.

Commenced.—5th June, 1940.

Method.—Same as described in Experiment 6. The identical kraal being used. Nine animals were injected with D.G.V. and 6 untreated susceptible animals placed in contact with them.

Results.

No. of Cattle.	Dose of Virus.	Reactions to Virus.			
		Acute.	Mild.	Negative.	Mortality.
9	2 c.c.	4	4	1	3

Remarks.

One susceptible in-contact showed a rise in temperature and *A. marginale* in a blood smear from the ear. As a double check subinoculations were done into two other animals, which, however, did not react.

Conclusions.

D.G.V. has not been transmitted by contact.

Experiment 22.

Object.—(i) To determine the herd immunity against K.G.V. of cattle in the Mbosi area after having been triple vaccinated in the course of the campaign during February and March, 1940.

(ii) The K.G.V. used in this experiment was fresh citrated blood collected from a goat at the height of temperature reaction to rinderpest. It was anticipated that during the later stages of the campaign it would be necessary to carry out goat virus inoculations in areas where the facilities for rapid transport or cold storage would not be available and the virus would have to be maintained in goats.

Number of Animals.—23 Zebu's were bought from the area around the laboratory. That they had been triple vaccinated was proved by the brands put on at the time.

Virus Test.—3 c.c. K.G.V. on 21st June, 1940.

Results.

No. of Cattle.	Reactions to Virus.			
	Acute.	Mild.	Negative.	Mortality.
23	7	6	10	0

Remarks.

No clinical symptoms were observed.

Conclusions.

(i) The immunity in Mbosi cattle could now be safely augmented with K.G.V.

(ii) K.G.V. could be maintained in goats of the Mbosi area.

Experiment 23.

Object.—In continuation of the previous experiment it was decided to ascertain the variations which might occur in the temperature and clinical reactions of cattle as a result of different doses of K.G.V. in the form of fresh citrated goat blood.

Number of Animals.— 14 Njombe Zebu cattle.

Results.

No. of Cattle.	Dose of Virus.	Reactions.				Remarks.
		Acute.	Mild.	Negative.	Mortality.	
2.....	0·005 c.c.	2	0	0	0	1 diarrhoea.
2.....	0·04 c.c.	2	0	0	0	2 diarrhoea.
2.....	0·01 c.c.	2	0	0	0	1 diarrhoea.
2.....	0·1 c.c.	0	1	1	0	1 diarrhoea.
2.....	0·5 c.c.	2	0	0	0	2 diarrhoea.
2.....	1 c.c.	2	0	0	0	1 diarrhoea.
2.....	40 c.c.	2	0	0	0	No diarrhoea.

Conclusions.

(i) The dose of K.G.V. could not be correlated with the severity of reactions, and the differences in reactions in the groups could only be explained on the grounds of difference in individual resistance.

(ii) Fresh citrated blood when taken at the height of the temperature reaction, from a goat reacting to K.G.V., was a suitable vehicle for goat virus vaccination.

Discussion of Results.

The primary object of the research work was achieved, namely to provide experimental data for the guidance of the staff carrying out, simultaneously, the work of immunisation of cattle in Southern Tanganyika, and increasing use of experimental results were made in the field as the immunisation work neared its conclusion.

Briefly, the results will be discussed in regard to their application under practical field conditions in Southern Tanganyika at the time.

A. *The Comparative Virulence of Viruses Used.*

The virulence of the three types of virus used could be compared in the control groups of the different experiments. Unfortunately owing to the small numbers of Njombe Zebu and Ufipa Ankole cattle subjected to virus only, the virulence could not be further correlated with the breeds of cattle.

For the sake of brevity the results are given in tabular form.

(a) "O" Virus.

Exp. Ref.	Breed.	Origin.	No. of Cattle.	Reactors.	Mortality.
1	Zebu	Mbosi	3	3	3
3	Zebu	Mbosi	6	6	2
4	Zebu	Mbosi	3	3	1
7	Zebu	Mbosi	2	2	1
9	Zebu	Mbosi	1	1	1
10	Zebu	Mbosi	2	2	0
TOTAL LOCAL ZEBU			17	17	8
5	Zebu	Njombe	1	1	0
1	Ankole	Ufipa	1	1	1

(b) D.G. Virus.

Exp. Ref.	Breed.	Origin.	No. of Cattle.	Reactors.	Mortality.
1	Zebu	Mbosi	3	3	0
4	Zebu	Mbosi	2	2	0
21	Zebu	Mbosi	9	8	3
TOTAL LOCAL ZEBU			14	13	3
1	Ankole	Ufipa	2	2	1

(c) K.G. Virus.

Exp. Ref.	Breed.	Origin.	No. of Cattle.	Reactors.	Mortality.
11	Zebu	Mbosi	2	2	0
13	Zebu	Mbosi	6	6	0
17	Zebu	Mbosi	10	6	2
TOTAL LOCAL ZEBU			18	14	2
23	Zebu	Njombe	14	13	0
19	Ankole	Ufipa	1	1	1

The comparative virulence of the "types" of virus in Mbosi Zebu cattle showed up as follows:—

Virus.	No. of Cattle.	Reactors.	Per Cent. Reactors.	Mortality.	Per Cent. Mortality.
'O'	17	17	100	8	47
D.G.V.	14	13	93	3	21
K.G.V.	18	14	78	2	11

When the figures in regard to mortality are considered it should be remembered that towards the end of the experiments the animals were in poor condition and reactors died which may have survived the same reaction when in good condition. This was particularly noticeable in the K.G. Virus group of Experiment 17.

The conclusions drawn from these results are that Zebu cattle used in the experiments had a relatively high basal resistance to rinderpest and that there was a definite difference in the degree of virulence of the three " types " of virus employed, Kabete Goat virus being more attenuated than Dickinson's Goat virus. In the determination of the degree of immunity produced by formalinized vaccine with these viruses it should therefore be kept in mind that the severity of the test depended on the " type " of virus used. The time taken for optimum immunity to develop as tested by virus would therefore also be either short or long depending on the virulence of the virus used.

B. *Vaccine Immunity.*

The experiments confirmed the observations of previous workers in regard to the viability of the virus in Formol/saline vaccine, and the retention of its antigenic properties after storage. Futhermore, sufficient evidence has been obtained that formalinised spleen vaccine is a safe method of conferring immunity to cattle.

From the results obtained it appeared that the antigenic value of the vaccine was not dependent on its containing minimal quantities of virulent live virus. The probability that the virus was attenuated by the F/S or F/G is great.

The breed or type of cattle from which the vaccine was made did not influence the immunity produced (engendered) in other breeds as long as the animals for vaccine production were killed at the height of reaction.

(*a*) *Development of Immunity.*

The quantity in single doses of vaccine, varying from 5 c.c. to 30 c.c., administered to animals in these experiments did not appear to have much influence on the rate of immunity development and degree of immunity produced. Immunity appeared to develop relatively slowly, reaching its peak between the 10th and 15th day. This was, however, also found to be subject to individual variation.

The effect of two and three doses of vaccine given at intervals of a week on the rate of immunity development could not be determined because the first dose had had sufficient time to confer immunity and it was not possible to evaluate the influence of the 2nd and 3rd injections in this connection.

(*b*) *Degree of Immunity.*

The degree of herd immunity produced by formalinized vaccine, as determined by subsequent virus inoculation, is high after the 15th day but not absolute in all animals. Some of the experimental animals still showed temperature reactions although sufficiently protected against death.

Complete " blocked out " reactions in whole groups were only recorded as follows:—

Exp. Ref.	No. of Animals.	Dose of Vaccine.	Interval between Vaccinations.	Interval before Virus Test.	Virus.
1.....	8	10 c.c. × 2	7 days	20 days after 1st	D.G.V.
1.....	10	10 c.c. × 3	7 days	27 days after 1st	" O "
8.....	2	10 c.c.	Nil	48 days after 1st	D.G.V.
18.....	2	5 c.c.	Nil	15 days after 1st	" O "
18.....	2	10 c.c.	Nil	15 days after 1st	" O "
18.....	2	5 c.c.	Nil	20 days after 1st	" O "
18.....	2	10 c.c.	Nil	20 days after 1st	" O "
10.....	10	5 & 20 c.c.	7 days	21 days after 1st	" O "
12.....	1	10 c.c.	Nil	14 days	" O "

It was therefore concluded that the immunity conferred by formalinized spleen vaccine was strong but not 100 per cent. in all animals.

The fact that the vaccine was produced in batches from the pooled spleens of 35 to 100 cattle eliminated the possibility of great variations in the antigenic value of such vaccine as the result of low virus content in individual spleens. Secondly the antigenic value may have been influenced by the period of storage before use, in which case the individual animals with " blocked out " reactions, in the same groups, remain unexplained.

Early on in the experiments it occurred to us that the variations might be due to the amount of spleen pulp injected into the animals, the vaccine being inclined to form a sediment of the pulp in the bottom of the bottles. Frequent shaking while inoculating and increase dosage from 5 c.c. to 30 c.c. made no appreciable difference.

The difference in the degree of the immunity produced by single, double and triple vaccinations did not appear to be significant when tested between the 15th and 30th day after the first injection of formalinized spleen vaccine.

(c) Duration of Immunity.

Owing to the short time at our disposal this important aspect was not thoroughly investigated.

According to the results obtained in Experiments 3 and 22, the degree of herd and individual immunity was not high 4 to 4½ months after triple vaccinations. When comparing Experiment 22 it should be kept in mind that K.G.V. was used for the immunity test.

The significant fact was that, although the percentage of reactors was high, the immunity was still strong enough to protect against death. Superficially it appeared that the immunity was tailing off but no definite statement can be made as most of our experiments did not show a 100 per cent. herd immunity.

Further research into this side of the question seems necessary, particularly on immunity following on single, double and triple vaccinations with formalinized spleen vaccine.

(*d*) *The safety factor.*

The experimental results showed that F/S and F/G spleen vaccine were safe and effective methods of conferring immunity to cattle in Southern Tanganyika. F/S vaccine should only be used between the 3rd and 10th day after production.

It has already been indicated that there has been no agreement on the duration of formalinized spleen vaccine immunity and that the general evidence is against it lasting for more than a year. Our results show that there may be a decline in the immunity at 4 to 4½ months.

In contrast the evidence on the duration of immunity following on attenuated goat virus inoculation, is in favour of it persisting for at least 2 years. There is, however, a definite reaction to inoculations which under certain circumstances may make it dangerous to use as shown by Lowe (1940).

Our problem then was to augment the spleen vaccine immunity so as to protect the Southern States for a longer period. The obvious possibility was by using attenuated goat virus after vaccination and at a stage where the vaccine immunity was sufficient to protect the animals against fatalities resulting from attenuated goat virus. Furthermore, we had to determine whether goat virus would not spread from inoculated animals reacting to it under Southern Tanganyika conditions.

Close contact experiments at Mbosi with attenuated goat virus proved that it could not be transmitted from reacting animals. Our results also showed that Kabete attenuated goat virus when used in Zebu cattle did not cause mortality comparable to the results obtained in the Tukuya trials of Lowe.

The possibility of death occurring in more susceptible breeds of cattle, particularly when in poor state of nutrition, and under adverse weather conditions had to be borne in mind. Initial resistance and naturally or artificially acquired immunity together with concurrent protozoal disease were also recognised as possible limiting factors.

Our experiments showed that a combination of spleen vaccine followed by Kabete Goat virus at an interval of 7 to 10 days was a safe method of producing immunity against rinderpest. Unfortunately we did not have the time to determine the duration of the resultant immunity.

In these experiments the object was to use the goat virus at such an interval after vaccination where a high percentage of animals would show controlled temperature reactions without symptoms and risk of death. The idea behind this was that immunity following after temperature reaction might be of longer duration.

Experiment 20 seemed to indicate that even without a temperature reaction following the injection of K.G.V., the degree of vaccine immunity was augmented by the goat virus. More work should, however, be done before reaching a definite conclusion.

A significant fact was observed in that, by increasing the time interval between vaccination and goat virus inoculation the risk of mortality could be decreased.

In regard to practical application our experiments showed that Kabete goat virus could be maintained in Mbosi goats and their blood used for inoculation in areas where it would be difficult to maintain a supply of desiccated goat virus in iced thermos flasks. The dose of goat virus was also proved to have no influence on the degree of reaction.

The application, in the field, of results obtained at Mbosi can not be better indicated than the following summary of statistics of inoculations performed during the rinderpest campaign in Southern Tanganyika in 1940. The figures are extracted from Reid's (1940) report on the campaign.

1. 284,847 head of cattle immunised by triple vaccination with three 10 c.c. subcutaneous inoculations of Formol/Saline or Formol/glycerine vaccine at weekly intervals.

2. 143,508 head of cattle treated with one 10 c.c. subcutaneous inoculation of vaccine followed by 2 c.c. subcutaneous injections of goat virus at 7-10 day intervals.

3. In 14,527 head, the interval between the above doses of vaccine and virus was increased to 14 days.

4. 17,200 cattle treated with 20 c.c. immune serum and 2 c.c. goat virus simultaneously.

5. 412,506 cattle received goat virus only. The animals were of the northern areas which proved to have a high resistance to rinderpest as a result of possibly triple vaccinations done in 1938 and the endemic existence of the disease.

The officer in charge reported that mortality as result of reactions was negligible in all the different areas.

Summary.

Experiments were done at Mbosi, in Southern Tanganyika, to determine the efficiency and safety of formalinized spleen vaccine and attenuated goat virus as agents of conferring immunity to cattle in the area. The results, conclusions and practical application of these experiments have been recorded.

Under five months was spent on the work and a number of aspects, particularly on duration of immunity, remained uninvestigated.

Acknowledgments.

We owe a debt of gratitude to the Directors of Veterinary Services of the Union of South Africa, Southern Rhodesia, Northern Rhodesia, Tanganyika and Kenya for their consent to do this work and the assistance received from them.

Further, we wish to express appreciation of the assistance and full co-operation given by Mr. N. Reid, Officer in Charge of the special rinderpest campaign.

Thanks are also due to Mr. Laubscher, who in the capacity of Technical Assistant, carried out his onerous duties faithfully, concientiously and efficiently.

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