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Further Investigations into Immunization of Cattle against Rinderpest.

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Introduction.

In a subsequent paper by Mitchell and Mansfeld, on work carried out at a temporary field station at Mbosi, it will be pointed out that certain results were obscured and certain conclusions might be invalidated by a doubt as to the full susceptibility to rinderpest of some of the available cattle used for experimental purposes. Whether this resistance to infection was due to natural variation in the susceptibility of the local animals, or to immunity developed as a result of contact with the disease in an enzootic area, could not be determined. Since additional and confirmatory evidence on various aspects of immunity and immunization was urgently required for the formulation of a scheme to be applied to the rapid elimination of any outbreak of rinderpest, which might occur among the fully susceptible cattle of the Southern African states, it was decided to continue the work in an area where the results would not be open to question. Eventually, the Government of Northern Rhodesia agreed to permit the work to be carried out in the northern portion of their territory and Col. Gore-Browne, a member of the Government, generously offered to provide a site, facilities for the erection of the requisite kraals, and accommodation for staff on his estates at Shiwa Ngandu.

The experimental animals used were drawn from several sources.

1. Col. Gore-Browne again generously offered to loan part of his herd of cattle for the work. These animals referred to in the text as Shiwa cattle, were grade stock (South Devon-Shorthorn-Zebu crosses).

2. The Northern Rhodesia Government supplied animals from their herds at District Commissioner's headquarters at Mpika some 65 miles south of Shiwa (referred to as Mpika boma cattle) and from Chunga about 150 miles north east of Shiwa (referred to as Chunga cattle).

These three herds of cattle were isolated groups in local tsetse fly free areas surrounded at some distance by fly belts. No rinderpest had been encountered in the area for at least 30 years. The natives in the vicinity of Shiwa owned some goats but no cattle, so that the risk of spreading infection was reduced to a minimum.

Procedure.

The experimental work was carried out at Shiwa and Mpika in two main stages; firstly from September to November 1940 (two months), and secondly, in June and July 1942 (one month). The test inoculations at Chunga were carried out during November, 1941.

While the experimental work was in progress the animals were kraaled at night, and were temperatured at sunrise before being turned out to graze in appropriate groups. Some modification of this general procedure had to be made in the case of the animals in the transmission tests but it must be emphasized that a temperature record was kept of each individual whether in an actual experiment at the time or not.

When the first stage of the work was discontinued in November, 1940, all the animals were rebranded for future identification, the Shiwa herd being kept in reserve by Col. Gore-Browne, the remainder being controlled by the Veterinary Department of Northern Rhodesia. On resuming the work in June, 1942, it was found that a large number of the animals had died of intercurrent disease, or for other reasons were no longer available. This is the explanation for the unfortunate gaps in the protocols and is an indication of the difficulties under which this work was carried out.

To minimize the risk of spreading infection only goat virus was used in the tests. This Kabete type goat virus (referred to as K.G.V.) was supplied by the Veterinary Research Laboratory, Kabete, Kenya. It was sent to Mpika by air mail, where it was placed on ice in a Thermos flask, transported by car and stored in a refrigerator. Unless specifically stated to the contrary, K.G.V. refers to a fresh emulsion of this stored desiccated spleen virus.

Experimental.

1. *To repeat the investigation previously carried out at Mbosi on the possible transmission of K.G.V. from reacting to susceptible animals.*

(*a*) *Close contact.*—On 17/10/40 a group of 23 head of cattle was placed in a small boma (kraal). Ten, to comprise the infected group, received an injection of K.G.V. All, with one exception, No. 18, reacted severely, five showing severe clinical symptoms of diarrhoea with nasal and lachrymal discharges. The non-reactor, No. 18, which resisted infection on two subsequent occasions, is discussed later. Arrangements were made for this group of infected and in-contact animals to be fed and watered by hand. In addition to a plentiful supply of food, salt was fed *ad. lib.* in an open trough. They were not allowed out to graze but were retained continuously in the kraal for a period of 3 weeks by which time the reactors had fully recovered and were allowed out to grass. The in-contacts then received an immunity test of K.G.V. the dose being 2 c.c.; all reacted severely, 12 showing marked clinical symptoms.

Result.—Infection was not transmitted to 13 susceptible cattle during close contact with 9 reactors throughout the entire period of the rinderpest reaction to K.G.V.

(*b*) *Open grazing.*—On 17/10/40 75 head of cattle on open grazing received an injection of K.G.V. A total of 50 susceptible animals were maintained in the infected area and reacting herd as in-contacts from October 20th to November 8th, 1940, i.e. for a period of 19 days.

Results.—(*a*) During the period of exposure 8 of the in-contacts showed a fluctuating rather than a definite rise of temperature. The highest recorded temperature was only 102·8° but this was several degrees higher than the average of the remaining animals.

Blood was collected from these 8 animals for subinoculation into goats. As the number of available rinderpest susceptible goats was limited, 4 goats each received the pooled blood of two cattle. All the goats showed a slight febrile reaction (maximum temperature 105·2°) between the 6th and 13th day. The reactions in the goats were all delayed 3-5 days beyond the normal incubation period of rinderpest in goats and no clinical symptoms developed. On testing with virus all the susceptible cattle, including the 8 mentioned above, reacted at the normal period showing in some cases marked clinical symptoms.

These doubtful reactions in the 8 cattle were not considered to be due to rinderpest infection as the cattle were still susceptible twelve days after the first rise of temperature. If these doubtful reactions were caused by rinderpest virus, some, if not all, would have failed to react to the K.G.V. immunity test inoculation.

It was concluded that some other virus, transmissible to goats, had been picked up from the cattle. Had time permitted, these goats would have been tested with K.G.V. for immunity to rinderpest but the experimental work was then closing down and this could not be done. (When the immunity tests were conducted at Chunga in June, 1941, a second rise of temperature followed the normal K.G.Virus reaction in some of the animals and blood from these animals caused a slight rise in temperature, similar to that observed at Shiwa, in two subinoculated goats. Lack of time once again prevented further investigation of the virus).

(*b*) One in-contact animal (No. 103) commenced a thermal reaction on the 14th day of contact, the temperature rising to a maximum of 104·6° on the 16th day and slowly returning to normal on the 23rd day. Smear examination showed the presence of a heavy infection of *Theileria mutans* and the development of anaemia. Blood was collected on the 4th day of the reaction and 10 c.c. was subinoculated into a goat (No. 26). A febrile reaction commenced on the 3rd day rising to 106° on the 6th day and returning to normal on the 13th day. Blood from goat 26 was subinoculated into a second goat, 28, on the 4th day of the febrile reaction. Again a febrile reaction commenced on the 3rd day rising to 105·4° on the 6th day and returning to normal on the 8th day. In none of the animals could any clinical symptoms of rinderpest be detected.

Conclusion.—It is not possible to draw any definite conclusion from the experiment. It is quite apparent that nine of the in-contact cattle became infected with a febrile condition transmissible to goats. In the case of 8 animals this febrile condition was evidently not due to the transmission of K.G.V. as the animals proved susceptible to an immunity test dose twelve days after the first rise of temperature. In the case of the ninth animal, (No. 103), there is a possibility that the K.G.V. was transmitted. From the nature of the reaction and the absence of well marked clinical symptoms, however, the opinion is held that the K.G.V. was not transmitted.

(*c*) *Drenching and inoculation.* (i) *Urine.*—On 25/10/40 urine was collected from 5 reactors to K.G.V. immediately after slaughter on the 5th day of the reaction. Two head of cattle were each drenched with 20 c.c. of the fresh urine immediately after collection and a further two each received 10 c.c. subcutaneously.

Result.—None of the animals showed any reaction and, on testing their immunity 15 days later, all reacted.

(ii) *Faeces.*—Fresh faeces were collected from two reactors showing profuse blood-stained diarrhoea on the 8th day after infection with K.G.V. Immediately after collection 2 cattle were drenched with 20 c.c. of the pooled material. A further sample was passed through a Berkefield candle, the process of filtration taking several hours at a high room temperature. Two animals each received 10 c.c. of the filtrate subcutaneously.

Result.—One animal which was drenched with faeces developed a febrile reaction with clinical symptoms indicative of rinderpest, and failed to react to an immunity test given 15 days later. The other 3 animals failed to react and on testing their immunity 15 days later were found to be susceptible.

Conclusion.—From this series of experiments it would appear to be safe to conclude that transmission of attenuated goat virus under conditions of close contact or open grazing, if it does occur, is exceedingly rare. However the possibility that such transmission may occur by the ingestion of food contaminated with faecal material from reacting animals is proved by the single positive transmission obtained by drenching infected faeces.

2. *To determine the duration of immunity produced by vaccination with formolized spleen vaccine.*

(*a*) During November all the cattle in the estate herd at Shiwa received a single injection of 10 c.c. of Mbosi formol-glycerine vaccine as additional security against the possibility of disseminating infection. A group of 6 heifers from this herd was selected in July, 1941, i.e., 8 months after immunization and they were given an immunity test in the form of the subcutaneous injection of 5 c.c. of fresh goat blood virus collected from donors reacting to the standard injection of K.G.V.

Result.—All 6 heifers showed marked febrile reactions indistinguishable from those produced in unvaccinated controls.

Conclusion.—After an interval of 8 months the immunity conferred by a single injection of formol-glycerine vaccine had disappeared completely.

(*b*) A group of 9 cows and 10 calves was purchased for test from Mbesuma ranch 200 miles north-east of Shiwa (Rumsey's cattle) where triple vaccination with freshly prepared formol-saline vaccine had been carried out 8 months previously. The calves, whose history is somewhat obscure, had not been vaccinated but may have developed some transient immunity as a result of the ingestion of antibody contained in colostrum. The cows and calves were given an immunity test of K.G.V.

Result.—Marked febrile reactions were produced in 4 cows and 7 calves, the cows showing clinical symptoms of rinderpest. None died.

Conclusion.—After an interval of 8 months the immunity produced by triple vaccination with formol-saline vaccine had diminished considerably, the decrease apparently being correlated with an idiosyncrasy of individual animals.

(*c*) A group of 64 Chunga cattle was triple vaccinated with freshly prepared formol-saline vaccine in February, 1940. On October 2nd, 1940, these cattle received a reinforcing injection of 10 c.c. of Mbosi formol-glycerine vaccine. In June, 1941, i.e., 17 months after the original triple vaccination and 9 months after the second single injection a standard immunity test of K.G.V. was given.

Result:—

No reaction	45
Doubtful febrile reaction	8
Febrile reaction	11
Clinical reaction	0

Conclusion.—Triple vaccination followed by a single reinforcing injection of vaccine 9 months later is followed by an immunity which persists for 9 months. This conclusion is justified by the finding that 45 of the 64 animals were solidly immune and that a considerable degree of immunity had persisted in the 11 reactors since the febrile reactions were exceedingly mild. This result is of prime importance and affords a striking contrast to the decrease in immunity observed 8 months after triple vaccination alone [cf. previous experiment (b)].

To determine whether the immunity had been sufficient to block the reaction to the K.G.V. completely and thus prevent the development of a durable active immunity, the entire group of 64 cattle were given a second immunity test of K.G.V. in November, 1941, i.e. 5 months later.

Result.—All the previous reactors and doubtful reactors proved to be immune. Of the 45 non-reactors 43 were immune and two showed very mild febrile reactions.

Conclusion.—A durable immunity had been produced in the animals.

3. *Vaccine-Virus Immunization Tests.*

Using the transient immunity produced by formolized spleen-virus vaccine to control the reaction produced by a fully virulent or partially attenuated virus in an endeavour to develop a durable active immunity, is a standard procedure requiring no elaboration. Since the application of the method to immunization against rinderpest has received little attention, a detailed investigation was started at Shiwa. Unfortunately a true appreciation of the results is negatived by the fact, as stated previously, that a number of the animals were not available for the subsequent immunity tests.

For the experiments the vaccine used was formol-glycerine vaccine prepared at Mbosi on 12/6/40, stored at Iringa for use in Tanganyika and is referred to in the text as Iringa vaccine. A further consignment of the same batch of vaccine had been sent from Iringa for use in the Sumbawanga area. Some of this vaccine was obtained from Sumbawanga for use at Shiwa and is referred to in the text as Sumbawanga vaccine. This vaccine had been stored at Sumbawanga in a grass hut for four months.

The virus used was the fresh emulsion of desiccated Kabete Goat virus, previously referred to. Dose of vaccine 10 c.c. Dose of virus 2 c.c. subcutaneously.

In the tables the interval stated refers to the time that elapsed between the injection of vaccine and the virus. These injections were commenced in October, 1940. The immunity tests, which consisted of 5 c.c. of freshly drawn blood obtained from goats reacting to K.G.V. given subcutaneously, were carried out in June, 1942.

TABLE 1.

Vaccine-Virus Immunization.

	Interval in Days.	No. of Cattle.	Febrile Reaction.*			Clinical Reactions.	Immunity Test.			
							Non-Reactors.		Reactors.	
			Neg.	Mild.	Marked.		Pos.	Neg.	Pos.	Neg.
Shiwa grade cattle	6	10	—	6	4	4	—	—	—	—
	7	15	4	7	4	3	—	—	0	4
	11	15	10	4	1	—	3	2	—	—
	15	15	11	2	2	—	6	0	—	—
	19	15	14	1	—	—	7	1	1 (*d*)	2
	19 (*a*)	11	3	5	3	6				
	22	15	11	3	1	—	5	1	—	—
Mpika Zebra type cattle	7 (*b*)	10	6	4	—	1	0	5	1 (*d*)	3
	7 (*c*)	15	9	4	2	5	0	10	0	3
Controls.....	—	2	—	—	2	2	—	—	0	2

* In this and other similar tables reactions are divided into two classes:—

(1) Febrile reactions (Negative, Mild and Marked) as indicated by the record of daily temperatures.

(2) Clinical reactions, where a diagnosis could be made from the symptoms. It is apparent, therefore, that the clinical reactors are included amongst the febrile reactors.

(*a*) The test on this 19 day interval group was carried out at a different time during the rains, when the cattle were showing signs of diarrhoea and severe reactions.
(*b*) Sumbawanga vaccine.
(*c*) Iringa vaccine.
(*d*) Very mild and possibly doubtful reactions.

Results.—Consideration of the reactions indicated in Table I shows that, as the interval between the injection of vaccine and of virus increased, so the severity of the resultant reactions decreased and the number of non-reactors increased. An exception is the second batch of 11 animals in the 19 day interval group. These animals were treated after the rains had begun and it was a constant observation that as soon as the new grass began to appear a large percentage of cattle showed diarrhoea and all lost condition. Any rinderpest reactions which developed during this period were markedly accentuated.

As regards the immunity produced it is indeed regrettable that all survivors of the experiment were not available for test, since it is of paramount importance to ascertain whether a non-reactor to an attenuated virus develops a permanent immunity or not. Since the immunity tests were carried out after an interval of 20 months, i.e. at a time when practically all residual immunity conferred by the vaccine should have disappeared (see above) any immunity must have resulted from a reaction to K.G.V. It is worthy of note, therefore, that in the case of the Mpika boma cattle, where the interval between vaccine and goat virus was 7 days, all the non-reactors developed a solid immunity. In the case of the Shiwa grade cattle none of the 7 day group non-reactors were tested but 3 out of 5 of the 11 day interval group were resistant. Non-reactors from the longer interval series were not immune in the great majority of instances (16 out of 18).

Conclusion.—As the interval between a single injection of vaccine and K.G.V. increases so the severity of the reactions as well as the percentage of reactors decrease. When the interval is 7 days it can be expected that both non-reactors and reactors, i.e., all animals, will develop a solid persistent active immunity. When the interval is longer than 7 days the immunity produced by the vaccine is sufficient not merely to control the reaction produced by the attenuated virus but to block it completely; development of an active immunity will be prevented and the transient immunity produced by the vaccine after rising to a peak in an undetermined interval will decline until it is no longer effective after 8 months.

4. *The efficacy of formol-glycerine spleen prepared from Kabete goat virus.*

It has been shown that there is an almost negligible danger of Kabete goat virus spreading from reacting to susceptible cattle whereas there is a very real danger of the spread of ordinary cattle virus. In any extensive immunization campaign involving the preparation of large quantities of formolized spleen-vaccine it is frequently necessary to establish the vaccine production plant in close proximity to the source of susceptible cattle, i.e. outside the immune zone or belt. This necessitates the adoption of vigorous precautions to prevent further spread of the very disease whose elimination is aimed at, and there always exists an unpleasant feeling of doubt that a new focus of infection is being established. It is an accepted phenomenon that the vaccine produced from a different genus of animal from that which is to be immunized is less effective than vaccine produced from the same species. Consequently it was considered inadvisable to investigate the value for cattle of spleen-vaccine prepared from goats but some information on the antigenic value of cattle spleen-vaccine prepared from K.G.V. would be of interest.

The K.G.V. vaccine used in the following experiments was prepared at Shiwa from the pooled spleens of 10 oxen killed at the height of the reaction on the 5th day after infection with K.G.V. The method of preparation of vaccine was identical with that used at Mbosi, the percentage of formalin being 0·25 per cent. The vaccine was prepared in two batches, the first batch being used on 21 Mpika boma cattle, the second batch on 15 Shiwa cattle. The reactions and results are shown in Table 2.

TABLE 2.

K.G.V. Vaccine-Virus Immunization.

Cattle.	No.	Interval in Days.*	Febrile Reaction.			Clinical Reaction.	Immunity Test.			
							Non-Reactors.		Reactors.	
			Neg.	Mild.	Marked.		Pos.	Neg.	Pos.	Neg.
Mpika.....	21	7	4	6	11	13	0	2	1 (*b*)	15
Boma.....										
Shiwa.....	15	8	0	6	9	7 (*a*)	—	—	—	—

* Interval refers to time between injections of vaccine and virus.

(*a*) 1 died; rinderpest.
(*b*) Very mild or doubtful reaction.

Results.—The reactions produced should be considered in conjunction with those reported with ordinary cattle spleen-vaccine in Table 1. The reactions were far more severe, in fact one of the Shiwa grade animals actually died of rinderpest. On the other hand only two Mpika boma non-reactors whose immunity was tested 20 months later were both solidly immune.

Conclusion.—It may be concluded that formol-glycerine spleen-vaccine prepared from cattle reacting to attenuated Kabete goat virus has a lower antigenic value for cattle than a similar product where virulent cattle virus is used as the source of infection.

5. *Duration of Immunity.*

All animals which showed a febrile reaction to Kabete goat virus were subsequently found to be immune. Under the experimental conditions the limit of interval between infection and immunity test was 22 months.

DISCUSSION.

Although every effort was made to bring a series of carefully planned experiments on fully susceptible grade cattle in a rinderpest free area to their logical conclusion it was found that some of the difficulties under which the work was carried out could not be overcome. This is exemplified by the fact that the requisite immunity tests on many of the most important animals in various groups could not be carried out simply because these animals had either succumbed to intercurrent disease or for other reasons were no longer available when finally required. Nevertheless the results and the justifiable conclusions drawn indicate the necessity for careful investigation into immunity production in a rinderpest free area on grade cattle whose full susceptibility to rinderpest is not open to question. It must be noted, however, that even though these two requirements appeared to be fulfilled at Shiwa yet a single animal, No. 18, was encountered

which was completely refractory to infection. It is certain that this animal had not been in contact with the disease previously, yet on three occasions it failed to react to fully active virus. No explanation of this phenomenon can be given.

The first conclusion that may be drawn from the whole series of experiments is that Kabete goat virus is not sufficiently attenuated for use on the average type of animal to be found in the Southern African states. Although only a single death was recorded, the reactions produced in the control animals in the various groups were severe and were frequently accompanied by well-defined clinical symptoms of rinderpest. This point should be borne in mind when any extensive campaign is planned in the future.

Insufficient work was carried out to determine the best method of controlling these severe reactions; for instance, the simultaneous use of hyper-immune serum and K.G Virus was not investigated. However, it should be borne in mind that at least in the Union of South Africa the initiation of a campaign of control would probably be a matter of extreme urgency and great speed, and consequently, a supply of serum would not be available in adequate amount. For this reason the results produced by the use of formolized spleen-vaccine are important.

Vaccine produced from cattle spleens using Kabete goat virus as the infecting agent for the donors possesses inferior antigenic properties and cannot be recommended. However, the combination of what may be termed standard formolized vaccine with attenuated goat virus appears to hold out considerable promise. Since the term ' standard vaccine ' has been used with set purpose it is necessary at this stage to emphasize that no technique has yet been evolved to determine the antigenic value of different batches of formolized cattle spleen-vaccine. Vaccine is prepared by a particular technique from a sufficiently large number of spleens to justify the hope that the antigenicity of different batches will vary only within very narrow limits, but this cannot be controlled by any rapid *in vitro* method so that a comparison of the results of different experiments, particularly by different workers, may not be justified.

From the results of the experiments under consideration it appears that the rapid production of immunity by formolized spleen-vaccine may be used to control the reaction produced by goat virus, and a factor of extreme importance is the interval between the injection of vaccine and virus. If the interval is less than 7 days sufficient immunity will not have been produced and a high percentage of severe reactions will occur subsequently. If the interval is greater than 11 days the immunity may be sufficient to block out the reaction to goat virus completely and a permanent immunity will not be produced. An interval of 7 days appears to be the optimum for adequate control of the attenuated virus and the development of the highest percentage of animals with a lasting active immunity.

As far as the dissemination of the disease is concerned the use of Kabete virus appears to be reasonably safe. No case of transmission under conditions of close contact was recorded and only one doubtful case on open grazing. The record of a febrile condition transmissible from cattle to goats indicates the great care that must be taken in evaluating the results of work of this nature. No dogmatic denial of the possibility of transmitting goat virus

from reacting to susceptible cattle can be made since a positive transmission was obtained by drenching infected faeces. However, under natural conditions the danger of transmission appears to be remote.

The decrease in immunity produced by spleen-vaccine after an interval of 6 months is in accordance with current opinion but the rapid stimulation of a more durable immunity by a single reinforcing injection of vaccine 9 months after triple vaccination is a finding which merits further investigation.

Summary.

1. Kabete goat virus was not transmitted from reacting to susceptible cattle under conditions of close contact.

2. A single doubtful transmission was recorded under conditions of open grazing.

3. A febrile condition of unknown aetiology transmissible from cattle to goats was encountered.

4. Urine from reacting animals was non-infective, but faeces in one out of two cases was infective by drenching.

5. Immunity produced by a single injection of formol-glycerine spleen-vaccine had completely disappeared after 8 months.

6. Immunity produced by triple vaccination with formol-saline vaccine had diminished considerably after 8 months.

7. Triple vaccination followed by a single injection of formol-glycerine spleen vaccine 9 months later produced an immunity which persisted for at least 20 months.

8. The rapid production of immunity induced by a single injection of formol-glycerine spleen-vaccine could be used to control the reaction to K.G.V. An interval of 7 days between vaccine and virus appeared to be the optimum.

9. Spleen-vaccine prepared from cattle reacting to K.G.V. has an inferior antigenic potency.

10. The reaction produced by K.G.V. in grade cattle (British breeds of cattle × Zebu) are severe but usually non-fatal. A durable immunity follows the reaction.

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