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The Manufacture of Anti-Rinderpest Spleen Vaccine under Field Conditions in Tanganyika Territory.*

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DURING the latter part of 1939 it became evident that, in spite of the efforts of the Veterinary Department of Tanganyika rinderpest was continuing its spread southwards towards the Southern Highlands of that territory. The immunization campaign of 1938 had merely served to check the advance of the disease temporarily between the Great Ruaha river in the north and the Iringa escarpment in the south. This tract of cattle country is flanked on the east and west by bush, thickly infested with tsetse fly and fairly thickly populated by the larger species of wild fauna. Cattle are absent from these fly-infested bush areas but there is constant contact between cattle and game along the east and west boundaries of the cattle zone. This contact between cattle and game is assumed to be the reason for the spread of rinderpest down the western border of the cattle area subsequent to the 1938 immunization campaign, so that by the latter part of 1939 the disease had reached the outskirts of the township of Mbeya. This spread was not only a very serious threat to the thickly populated cattle areas of the Southern Highlands of Tanganyika but was fast becoming a menace to Northern Rhodesia, Nyasaland, Southern Rhodesia and ultimately to the Union of South Africa itself.

Prompt cooperative action by the Veterinary Departments of all the countries concerned was now indicated and the outcome of a series of conferences, which were concluded at Mbeya in January, 1940, was the organization of a campaign of mass immunization more or less on the lines of that conducted by the Veterinary Department of Tanganyika in 1938. This would necessitate the triple inoculation with formolized spleen pulp vaccine of all the cattle in southern Tanganyika together with all cattle along the inter-territorial border of Northern Rhodesia and Nyasaland.

Arrangements for the production of the necessary vaccine were made by establishing a small field laboratory in December 1939 at Mbosi situated south of the area of known infection. W. G. G. Peevie with M. Gillett, laboratory assistant, both of Tanganyika Territory, was placed in charge of this unit. Available buildings on the commandeered farm were hastily adapted to the needs of the laboratory and within four weeks vaccine was available for use in the field. During a period of six weeks 194,260 doses of vaccine were produced by the standard method in use at the Central Laboratory Mpwapwa. For the sake of convenience this method is referred

* This is the first of a series of articles by the late Mr. D. T. Mitchell and members of his staff in Tanganyika. (*Editor.*)

to as the "open method" in contradistinction to the "closed method", which is described in this paper and which was used for the mass production of 1,102,600 doses of vaccine.

It was early appreciated that this small unit with its meagre equipment and limited personnel could not cope with the huge demands for vaccine required for the uninterrupted progress and ultimate completion of the plan of campaign. Consequently the Department of Agriculture and Forestry of the Union of South Africa undertook to equip and staff a complete laboratory from Onderstepoort. On January 23rd, 1940, the heavy equipment was despatched by rail from Pretoria to Broken Hill, and other equipment and staff left by road in charge of D. T. Mitchell who, on arrival at Mbosi took executive charge of all laboratory services.

Originally it had been planned to establish more than one vaccine producing unit in the field but, after consultation, it was agreed that a single large unit would suffice. Consequently all equipment was delivered direct to Mbosi in the vicinity of which were a number of untenanted ex-German farms on which to establish the laboratory and to house the staff. The surplus staff and equipment was used to establish a small research laboratory to be used for the investigation of immediate problems in connection with the campaign. The activities of this unit form the basis of another report.

Personnel.—The staff was made up as follows:—

D. T. Mitchell, O.B.E., M.R.C.V.S. Executive officer in charge.

W. G. G. Peevie, M.R.C.V.S. Tanganyika Territory in charge vaccine production.

M. Gillet, Laboratory assistant Tanganyika Territory. Vaccine Laboratory.

P. R. Mansfelt, B.V.Sc. Union of South Africa. Research Laboratory.

J. F. Laubscher—Laboratory assistant. S.A. Research Laboratory.

The remainder of the staff was African some of whom were seconded from the Central Laboratory, Mpwapwa, owing to their previous training and experience in vaccine production, the others being recruited from the natives in the district. Two of the native technical assistants from Mpwapwa deserve special mention, namely Atupele Mbinde and Saidi Ismael for upon their shoulders fell much of the routine work of the laboratory and a great deal of credit is due to them for the success of the unit.

Two additional Europeans eventually were attached, one as a lorry driver to deliver vaccine to the central distributing depot at Mbeya 46 miles away. and the other in charge of maintenance of the farm roads to ensure that inter-communication was not interrupted during the heavy rains.

Establishment.

(*a*) *Slaughter house and accessories.*—In the first instance it was essential to provide accommodation immediately, on a site at least one mile from the laboratory, to house the susceptible cattle to be used for vaccine production. Two large cattle kraals, resembling large editions of the ordinary native cattle boma, were erected each with a capacity of approximately 1,000 head of cattle. All available animals were confined in these kraals at night after being herded for grazing during the day.

The slaughter house (Fig. 2) was erected at a point about 200 yards from the laboratory. The building was of simple design consisting merely of a series of pillars constructed of burnt brick, which happened to be available. supporting the beams of the thatched roof. Suspended from the beams were five blocks and tackle which were used for hoisting the carcasses to facilitate aseptic removal of spleens. The floor was made of brick with drainage channels leading from each killing space to discharge into empty oil drums.

Whenever necessary the contents of the drums were buried thus contributing greatly to the general hygiene and cleanliness.

Adjoining the slaughter house were the three quarantine bomas (Fig. 1). Two were constructed of brick but the supply of bricks was exhausted so the third was built of ordinary bush timber. These bomas housed the inoculated animals in groups varying in number from thirty to one hundred depending upon the quantity of vaccine to be prepared.

Leading from the quarantine bomas to the slaughter shed was a crush or cattle race built of wooden uprights with 1½ in. water piping wired on as horizontal bars. This crush proved invaluable for the easy control of large numbers of animals for the taking of temperatures, for preparing bloodsmears, and for shackling prior to leading the animals to the slaughter house.

Some distance away were erected a series of large cooking pots (see Fig. 10). These pots were used for cooking the meat from the carcasses in order to destroy the rinderpest virus present since cooked meat was bartered for supplies of wood fuel, and freshly cut grass or hay for feed. In addition the regular ration of cooked meat to the native personnel was an inducement to continued good work.

Finally a permanent water furrow was diverted from the nearby river through the compound so as to provide a constant and easily available source of fresh water. Water was pumped from this furrow by means of a small hand pump to reservoirs erected at suitable points e.g. to supply water to the laboratory. (See Figs. 13 and 14.)

The area covered by the slaughter house and accessory structures was approximately 1 acre. The whole was surrounded by a wooden stockade, the only entrance or exit being through a cement footbath 10 feet long and 2 feet wide. A strong solution of Jeyes fluid to a depth of 6 inches was constantly maintained in this footbath. (C.f. Fig. 18.)

(b) Vaccine Laboratory and Accessories.

1. Vaccine preparation and bottling room. This was a converted garage with brick floor and walls open at one end under cover of a galvanized iron roof. The open end was screened with mosquito gauze, with the exception of a small doorway, as a very necessary anti-fly measure (c.f. Figs 19 to 24).

2. Sterilizing room. This was a brick building previously used as a store. Suitable stands were built out of the available bricks for the autoclaves, Koch's pots and hot air oven. Paraffin burning stoves were used as a source of the requisite heat (see Figs. 15 and 16).

3. Packing shed was merely a bamboo building in which bottles of vaccine were packed in cases ready for despatch.

4. Bottle washing plant. This comprised a bamboo and thatch shed with galvanized iron roof in which two or three large galvanized iron baths were erected on stands. These large baths ensured the copious use of constantly changed fresh water the actual washing being done by a manually operated rotary brush. Beside the building three 40-gallon drums were erected on suitable stands, to serve as receptacles for boiling the bottles over wood fires. All bottles returned from the field were thoroughly washed and then boiled. As a further check measure they were reboiled and cleansed prior to sterilization as required (see Figs. 7 and 11).

5. One store was converted into a primitive workshop in which to carry out the innumerable repair and constructional jobs daily.

6. A small building was set aside to serve as a store for the packed vaccine cases. Owing to the altitude of Mbosi and the local conditions the temperature at night was seldom higher than 65° F. so that it was possible to keep the vaccine before issue at a fairly low temperature without having to resort to artificial cooling methods.

(*c*) *Equipment used for Vaccine Production.*

1. Enammel cans of ½ gallon capacity fitted with lids and provided with wire handles were used as receptacles for the excised spleens. (See Fig. 18.) A separate sterile can was used for each spleen as it was removed in the slaughter house, and the number of the animal was marked on the can with a grease pencil.

2. Large size hand operated sausage machines were used for pulping the spleens (see Fig. 19). An extension fitted with a hinged cover was welded to the mouth of the reservoir so that, with the increased capacity, an entire spleen could be covered in the reservoir for mincing. The rod of a metal plunger passed through a hole in the cover, the plunger being used for forcing material down into the spindle of the mincer. A curved large bore pipe was welded to the outlet of the machine so that pulp could be delivered direct to a receptacle without exposure to the air. Cutting plates Husq Varna—Reliance Swedish make No. 2 and 3 were used with entire satisfaction.

3. A platform scale to weigh up to 45,000 grammes (see Fig. 20) was used for weighing the spleen pulp delivered into tared pulp tanks.

4. The pulp tanks (see Fig. 16) were rectangular 45 litre copper tanks resembling a tea urn with a circular lid and two other smaller openings on top and a tap at the bottom. The rod of a plunger passed through a hole in the lid, this plunger being used for agitating the contents of the tank when necessary. Of the other openings one was used to admit the delivery spout of the mincer, the other for subsequent addition of the diluent. Inside the tank a wire gauze filter covered the opening of the delivery tap so that here the first filtration to remove gross tissue particles took place.

5. Similar tanks but each provided with an external graduated glass gauge were used to contain the vaccine diluting fluid, either 50 per cent. glycerine or water according to the type of vaccine to be prepared.

6. Seamless galvanized milk cans were used as containers for the 10 × N saline.

7. Two gallon milk cans were adapted for use as filtering and mixing reservoirs for the final product (see Fig. 17). A second filtration in addition to that provided for at the outlet of the pulp tanks was found to be essential. Consequently a wire gauze filter roughly resembling an inverted "top hat" was inserted in the mouth of each can the rim of the "top hat" being fixed in position between the flange of the mouth and the lid of the can. In each lid two holes were drilled, one at the side to allow the diluted pulp to run into the filter, and one in the centre for the shaft of a circular plunger used to facilitate filtration. The shaft of an additional plunger passed through a hole drilled in the side of the can towards the top. This plunger was used to stir and agitate the contents at the time of bottling since rapid sedimentation caused uneven distribution of pulp in the bottles, and this in turn led to blockage of needles in the field.

8. A large sterilizer, pressure autoclave and hot air sterilizer of the usual types were in constant use, heat being supplied by large Primus stoves.

9. A domestic model paraffin burning Electrolux refrigerator was essential for storing the virus infected blood.

10. Additional requirements were the usual laboratory equipment such as rubber tubing, glass tubing, hooded pipettes, Moir's clips, glass slides, stains, microscope, bottles, corks, sealing wax, twine, etc.

(*d*) *Cattle Supplies.*—Some 5,000 head of susceptible cattle were purchased in the neighbourhood of the laboratory from time to time by a European cattle buyer and drafted to the unit in droves of from one to five hundred. The cattle were mostly of the Zebu type but when the local supply was exhausted purchases were made in the Ufipa district to the north of Mbeya and east of Lake Tanganyika, where the animals were mostly of Ankole type. The Ankole type with their long horns were difficult to handle in the bomas, in the crush and on the slaughter floor. The average price paid was approximately £2 per head.

On arrival a clinical examination was carried out for the presence of intercurrent diseases, physical defects, lameness, etc., and if suitable the animals were passed into the cattle kraals.

(*e*) *Virus.*—The virus used for infection of the first two batches of animals was obtained from a clinical reactor to natural infection in the field, and was brought to the laboratory in the form of fresh citrated blood. For continuation of the virus strain, donors were selected from each group prior to slaughter, selection being made from those animals showing the most severe clinical symptoms of rinderpest accompanied by a good febrile reaction (a rise of at least 3° F. in 4 days). Blood was drawn with aseptic precautions from the jugular vein into sterile 500 c.c. bottles containing 10 c.c. of 5 per cent sodium citrate as anticoagulant, and was stored in the refrigerator until required. The dose was 2 c.c. given subcutaneously.

Methods.

As animals were required for vaccine production they were transferred from the cattle kraals to the quarantine bomas where they were branded on the rump with consecutive numbers. From that time they were fed

and watered by hand and were passed through the crush daily for recording temperatures. They were slaughtered for collection of the spleens on the 5th day after being given the virus injection. It was the policy to include all spleens from each group even though there had been no evidence of clinical infection and no febrile reaction since it is well known that the virus content of the spleen of such apparent non-reactors is high (e.g., delayed thermal reaction).

To maintain a continuous output of vaccine a detailed routine was worked out and rigidly adhered to. On Sundays, Wednesdays and Fridays groups of animals received their virus injection. The group injected on Sunday was passed through the crush on Thursday, i.e., on the 4th day, 24 hours before slaughter for the purpose of taking blood smears which were stained with Giemsa and examined microscopically to exclude intercurrent infections e.g. anthrax. As the animals were killed on the fifth day, Fridays, Mondays, and Wednesdays were devoted to slaughter, and the actual preparation of vaccine. The intervening days, Tuesdays, Thursdays and Saturdays were devoted to smear examination, general cleaning up, sterilization for future work, in addition to the numerous daily routine duties.

Sterilization.

Adequate sterilization of all plant is an essential, and under the primitive conditions of a field laboratory the attainment of complete asepsis is by no means easy.

All the pulp tanks and filtration cans after thorough cleansing and repeated rinsing were sterilized by boiling in them 2 gallons of 5 per cent. formalin in water for a period of half to one hour. All openings were plugged with cotton wool stoppers, and each stopper covered with securely tied calico. When boiling was completed residual fluid was run out of the tap which was carefully resealed.

Tanks containing the $10 \times N$ saline and also tanks containing the pulp diluting fluid were boiled for one hour, all orifices being plugged with cotton wool. The mincing machine was autoclaved for one hour at 120 lb. per square inch pressure. All rubber tubing, glassware, etc., were boiled for one hour immediately prior to use.

Sterilization of the vaccine bottles presented many difficulties owing to the colossal number that were used and the filthy condition in which many were returned from the field. After thorough cleansing a combination of 3 methods of sterilization was used:—

1. Autoclaving at 115 lb. per square inch pressure for an hour.
2. Steaming in a Koch's pot over formolized steam for an hour.
3. Drying in hot air ovens for an hour.

Technique of Vaccine Preparation.

By Sunday, March 3rd, 1940, sufficient progress had been made with the preparatory organization to permit bringing in the first batch of cattle for injection. After the routine blood smear examination on Thursday

these were slaughtered on the following Friday. Slaughtering was done by means of a Cash humane killer but for the long horned Ankole type a 0·22 Mauser was found to be more satisfactory.

The carcass was hoisted clear of the ground by the hind legs and the skin flayed back from the belly and adjacent parts. After careful disembowelling the spleen was removed with aseptic precautions (c.f. Fig 4), using sterile bulldog forceps and a scalpel, placed in a sterile can and sent to the laboratory (c.f. Fig. 18).

The mincing machine was bolted to its wooden stand, and a pulp tank adjusted on the platform of the weighing machine in such a way that the curved delivery spout entered the appropriate opening on the top of the tank without touching the sides. The tank was then balanced using small lead shot, spleen fed into the mincer and 7,000 grammes of minced pulp delivered direct into the tank. When each tank had received its quota of pulp, the mincer was stopped and 3,500 grammes of 10 × N saline run in through a hooded pipette attached by rubber tubing to the saline can. The pulp tank was then removed from the scale and placed on a shelf after the opening had been closed. On the shelf the tanks were constantly shaken for 1 to 2 hours to ensure thorough mixing of the contents and then allowed to stand for about 4 hours with intermittent agitation. Then 31,500 lb. of the diluent, either water or 50 per cent. glycerine water according to the type of vaccine to be prepared, was run in from graduated tanks through rubber tubing and hooded pipettes. This diluent it will be noted was added by volume (cf. Fig. 21). The final proportions of the constituents of the vaccine therefore were:—

Spleen pulp	7,000 gm. = 2 parts.
10 × N saline	3,500 gm. = 1 part.
Water or 50 per cent glycerine	31,500 c.cs = 9 parts.

Agitation of the contents of the tank was kept up for 3 hours by means of the metal plungers. After adding formalin to produce a final concentration of 0·2/ per cent and further thorough mixing the tanks were allowed to stand overnight.

The following morning the plunging process was repeated to disrupt the sediment which had formed on the bottom of the tanks. The contents were tapped off through rubber tubing and hooded pipettes, through the mosquito gauze "top hat" filters into the mixing cans, the filtration being expedited by the metal plunger operated through a hole in the lid (cf. Fig. 22). The can was then placed on a shelf and filled into bottles by gravity in the usual way. The filled bottles were corked with aseptic precautions, tied, sealed with paraffin wax, labelled and packed ready for despatch.

By this method 47 batches were made from 3,510 head of cattle, which were slaughtered to produce 1,102,600 doses of vaccine, each dose being 10 c.c. The yield of vaccine per animal slaughtered varied in different batches from 213 to 490 doses with an average of 314. Full details are given in tabular form in appendix I.

Appendix I.

Details of Vaccine Production.

Batch No.	Nett No. Animals Used.	Smear Results.	Weight of Pulp.	Type of Vaccine.	Total Doses of 10 c.c.	Average Yield of Vaccine per Animal Slaughtered.
			Gm.			
1	30	Negative	—	Formol-Glycerine	11,900	410
2	30	„	—	„ „	14,700	490
3	30	„	—	„ „	12,950	432
4	30	„	—	„ „	10,900	360
5	35	„	—	Formol-Saline	12,670	361
6	35	„	—	„ „	11,690	334
7	35	„	18,573	„ „	11,550	330
8	40	„	27,746	„ „	15,330	383
9	50	„	27,180	„ „	14,800	222
10	40	„	33,635	„ „	18,140	453
11	45	„	35,447	„ „	18,800	417
12	40	„	31,823	Formol-Glycerine	16,510	413
13	45	„	33,975	Formol-Saline	17,850	396
14	55	„	33,182	„ „	17,080	310
15	54	„	34,125	„ „	18,550	344
16	55	„	39,524	„ „	21,630	393
17	98	„	59,569	„ „	29,750	304
18	99	„	60,815	„ „	31,780	321
19	70	„	40,808	Formol-Glycerine	22,680	324
20	99	„	68,496	„ „	36,630	359
21	100	„	59,596	Formol-Saline	33,390	340
22	100	„	59,114	Formol-Glycerine	31,990	320
23	98	2E. C.F.	52,987	Formol-Saline	29,260	298
24	97	Negative	55,605	Formol-Glycerine	30,310	312
25	50	„	33,975	Formol-Saline	18,270	365
26	50	„	33,861	„ „	17,850	357
27	49	„	28,539	Formol-Glycerine	14,700	294
28	99	„	54,246	„ „	28,910	292
29	100	„	58,208	Formol-Saline	30,940	309
30	100	„	69,648	Formol-Glycerine	34,450	344
31	99	„	49,943	Formol-Saline	26,960	269
32	102	„	51,528	Formol-Glycerine	37,500	269
33	100	„	60,249	„ „	29,750	297
34	100	„	64,097	„ „	31,170	311
35	99	3E. C.F.	63,420	„ „	31,360	317
36	102	Negative	45,924	„ „	24,360	238
37	99	1E. C.F.	64,212	„ „	32,270	326
38	100	Negative	57,078	„ „	30,240	302
39	100	„	44,280	„ „	22,240	224
40	100	„	65,365	„ „	27,560	275
41	98	„	43,805	„ „	23,370	238
42	100	„	44,394	„ „	23,660	236
43	99	1E. C.F.	40,078	„ „	21,110	213
44	100	Negative	39,181	„ „	21,300	213
45	56	„	38,844	„ „	20,930	374
46	99	„	64,230	„ „	33,810	342
47	99	1E. C.F.	57,078	„ „	29,050	293
	3,510				1,102,600	314

Comments.

Although the method described served its purpose it is considered that the technique is too complicated. The reduction of the spleen tissue to a very fine pulp is important if blockage of needles with consequent delay and considerable annoyance in the field is to be obviated. This might be accomplished by leading the pulp through a second mincer prior to delivery into pulp tank. A more thorough mincing might make a second filtration unnecessary in spite of the large amount of fibrous tissue in the spleen particularly if a more efficient method of filtration in the pulp tanks was devised.

Too many metal taps were used in setting up the apparatus. These taps clog easily and are difficult to clean and sterilize. It is suggested that all connections should be rubber and glass which are far more easily cleaned.

Although the use of hypertonic saline subsequently brought back to normality by the addition of 9 times the volume of water may be regarded almost as a standard laboratory procedure a detailed investigation is desirable to determine whether it is actually effective in bringing about rupture of the tissue cells, more particularly when 50 per cent. glycerine water is used as the final diluting fluid. The technique was introduced because if it did not actually assist in the process it did not interfere with it. In the " open " method glass beads were shaken up with the vaccine to disintegrate the tissue.

The sterilization plant was totally inadequate to cope efficiently with the constant demands made upon it. It is essential to have large capacity autoclaves heated by paraffin burners to deal with bottles, corks and other portions of the equipment.

Fig. 1.—Quarantine boma's.

Fig. 2.—Slaughter house.

Fig. 3.—Cutting up meat.

Fig. 4. Removing spleen.

Fig. 5.—Carcasses after slaughter.

Fig. 6.—Preparing meat for cooking.

Fig. 7 Bottle washing shed.

Fig. 8.—Bartering cooked meat for firewood, fodder, etc.

Fig. 9.—Bartering cooked meat for firewoord, fodder etc.

Fig. 10.—Cooking Pots.

Fig. 11.—Pots for boiling bottles.

Fig. 12.—Washing equipment.

Fig. 13.—Water furrow and hand pump.

Fig. 14.—Water reservoir to supply laboratory.

Fig. 15.—Sterilizing room.

Fig. 16.—Sterilizing room.

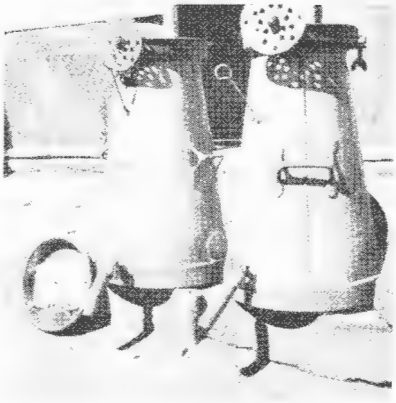

Fig. 17.—Filtering and mixing reservoirs.

Fig. 18.—Foot bath at only entrance to compound.

Fig. 19.—Interior of vaccine preparation and boiling room.

Fig. 20.—Interior of vaccine preparation and boiling room.

Fig. 21.—Interior of vaccine preparation and boiling room.

Fig. 22.—Interior of vaccine preparation and boiling room.

Fig. 23.—Interior of vaccine preparation and boiling room.

Fig. 24.—Interior of vaccine preparation and boiling room.

Fig. 25.—General view of the laboratory.

Fig. 26.—Lorry loaded with vaccine.