

under serum alone, which, however, is a costly method. Vaccination with inactivated spleen extract is now being used to temporarily immunize pure-bred stock and calves.

5. IMMUNIZATION OF IMPORTED CATTLE.

With a view to controlling the rinderpest reaction in highly susceptible imported cattle, large index doses of anti-serum from 45 c.c. upwards per 100 kilos body weight are generally used, but in some instances even when fresh virulent blood or fresh nasal secretion has been simultaneously inoculated, animals failed to react and were subsequently proved susceptible. In other cases, larger doses of anti-serum may fail to control the reaction.

With a view to reducing the risk of active immunization, owners may, if they desire, vaccinate with an inactivated spleen extract, which confers a temporary immunity.

6. *Various Obscure Points in connection with the Simultaneous Inoculation of Cattle under Serum have been cleared up, viz.—*

(1) Do cattle under serum treatment react to simultaneous inoculation of fresh virus and anti-serum?

(2) Does other than fresh blood of a reacting virus-maker transmit rinderpest to susceptible cattle under serum?

(3) Does fresh blood and blood collected when the temperature of the virus-maker has dropped to normal and stored at room temperature for 20 hours, transmit rinderpest? The results obtained show that cattle under—

- (i) serum treatment react to simultaneous inoculation of fresh virulent blood and the prescribed doses of anti-serum;
- (ii) other than fresh blood (40 hours old) does not always produce rinderpest in cattle under serum treatment;
- (iii) fresh blood collected from a virus-maker at the termination of the temperature reaction where the temperature has fallen to normal produced rinderpest, whereas blood of the same bleeding stored for 20 hours at room temperature did not set up infection.

SERUM POTENCY TESTS.

240 cattle—61 grades and 179 highly susceptible Ugandas—were inoculated with fresh virulent blood and anti-serum at 15, 30, and 45 c.c. All reacted, except three, which proved to be immune. Remainder when tested were found immune.

Generally speaking, increased doses of anti-serum produced a decrease of mortality and milder reactions and a temperature reaction of shorter duration. A higher death rate occurred in poor-conditioned than in good or fair-conditioned animals, and a greater rate if poor-conditioned animals are exposed to rain.

In view of the above, an increased index dose of serum was recommended, viz., 20 c.c. for cattle of average susceptibility and for highly susceptible cattle up to 30 c.c. or more per 100 kilos body weight according to grade, susceptibility and field conditions, weather, and condition of the animals.

The serum prepared at Kabete thus does not block out a reaction at the doses generally employed in the field up to 35 c.c. per 100 kilos body weight, but a percentage of high-grade cattle have failed

to react to simultaneous inoculation of blood kept at approximately 4° C. and used up till 24 hours after collection, and a large dose of anti-serum 55 c.c. per 100 kilos body weight—total dose 250 c.c.

SERUM ALONE INOCULATION.

Susceptible cattle—grade and Ugandas, 9 of each—got from 7.5 c.c. per 100 kilos up to 22.5 c.c. per 100 kilos at intervals of a fortnight and were kept in contact with rinderpest. All the grades contracted rinderpest and recovered. All the Ugandas contracted the disease and one inoculated at the 7.5 c.c. dose died.

Recommendations were made that the dose be increased for highly susceptible cattle, and that large doses be given in the early phase of the temperature reaction.

Observations as to the period animals under serum may be exposed and escape infection are shown below.

21 highly susceptible Cape cows, weighing from 335-435 kilos, were put under serum and kept in a stable in the laboratory boma. In the latter rinderpest exists throughout the year, and there was contact by native attendants between the boma and stables. Each animal was inoculated on 24/3/29 (date put on experiment) with 100 c.c. of serum and on 8/4/19 and 14/4/19 respectively, with 40 c.c. of anti-serum, and thereafter at fortnightly intervals with 40 c.c. Some, in addition, received 1,000 c.c. in the early phase of the temperature reaction. The following is a summary of the results:—

RÉSUMÉ OF SERUM ALONE TREATMENT.

(Experiment No. 1 of 24/3/19.)

Of 21 susceptible cattle put under serum treatment on 24/3/19 and kept stabled since then in a boma in which rinderpest existed, but not in immediate contact—

1	contracted rinderpest on the	12th day;
1	” ” ” ”	21st day;
1	” ” ” ”	22nd day;
1	” ” ” ”	23rd day;
1	” ” ” ”	27th day;
1	” ” ” ”	29th day;
1	” ” ” ”	41st day;
1	” ” ” ”	48th day;
4	” ” ” ”	49th day;
1	” ” ” ”	50th day;
1	” ” ” ”	51st day;
1	” ” ” ”	52nd day;
1	” ” ” ”	54th day;
1	” ” ” ”	58th day;
1	” ” ” ”	61st day;
1	” ” ” ”	62nd day;
1	was destroyed on the day after being put in experiment;	
1	did not contract rinderpest.	

Total 21

Of the 19 which contracted rinderpest, 10 recovered = 52 per cent. approximately; of these which recovered, 8 were treated with large doses of serum in the early phase of the disease; 2 were not.

Nine died of rinderpest = 47 per cent. approximately; of these, 8 were not treated with large doses of rinderpest serum, the remaining animal was, but died of rinderpest complicated with redwater and septic metritis (abortion).

IMMUNIZATION WITH TISSUE EXTRACT.

Some years ago Dr. W. Boynton, of the Insular Bureau of Agriculture, Phillipine Islands, introduced a heated glycerized and phenolized mixture of finely ground organs and blood, collected from an animal in the acute stages of rinderpest for protecting cattle and carabaos. After preparation, the treated mixture is kept in the refrigerator for from one, two, or three months or until it no longer produces rinderpest. This inactivated tissue extract has been largely used in the control of rinderpest in the Phillipine Islands. It would seem that by this method of preparation the mixture does not retain its immunizing properties for long after it becomes inactivated, and a large quantity cannot be kept ready for issue, and that the output cannot be quickly increased to meet large demands at short notice.

Capt. R. A. Kelsner, V.C., United States Army Medical Department Research Board, working in co-operation with the Insular Bureau of Agriculture, Phillipine Islands, prepared an inactivated tissue extract, and in 1927 published the details of preparation. Briefly, this consists of a mixture of finely-ground spleen, liver, and kidneys, testicles, and lymphatic glands (except the mesenterics) collected from an animal in the acute stages of rinderpest just when the temperature begins to drop. To each gramme of tissue is added 1 c.c. normal saline, and choloform is then added to each bottle to make a concentration of 0.75 per cent. The bottles are then tightly stoppered and the contents well shaken, after which they are stored in the refrigerator until required for use. The chloroform is said to inactivate the virus in several hours, and the tissue extract is said to retain its potency for a long time in the refrigerator. Three injections are given at intervals of seven days, each dose being 20 c.c., representing 10 grammes of tissue per dose or 30 grammes for the three inoculations. Kelsner, as the result of his experimental inoculation, conceived the possibility of reducing the dosage and number of injections, and Rodier, Pathologist, Bureau of Agriculture, Manila, with a view of obtaining more data hereon, investigated immunization with a single dose of the chloroform treated extract and published in August, 1928, the result of his experiments and conclusions, which were as follows:—(a) That for ordinary purposes with cattle under field conditions, a single injection of chloroform-treated vaccine prepared with spleen, lymph glands, and liver will undoubtedly protect against natural infection. However, as carabaos are highly susceptible to rinderpest, if the preparation of the vaccine is limited to the use of spleen, lymph glands, and tonsils the objections arising from the use of a dose too large to be practical for field use can be overcome by eliminating the less potent liver when preparing a vaccine for use on carabaos. (b) That several tests, in which twenty of the cattle and twenty-seven of the carabaos were inoculated with virulent virus, definitely show that a satisfactory immunization can be produced against a *heavy* artificial infection with a single dose of vaccine prepared from spleen, lymph glands, and tonsils. (c) That a dosage of 20 c.c. for carabaos and 10 c.c. for cattle is in every way entirely satisfactory for field use.

In 1927, the writer commenced experimental inoculations with inactivated tissue extract and recorded the results obtained during 1927 and 1928 in the Report of the Chief Veterinary Research Officer for 1927 and 1928, which are embodied in the Annual Report of the Agricultural Department, Kenya Colony, for these years.

Method of Preparation of the Extract.

Spleens are collected on the fifth or sixth day of the temperature reaction from virus-makers after they are bled out for virus for the hyperimmunizing of serum-makers. To one part of the pooled pulped spleens, four parts of normal saline are added, and to the mixture toluol is added to make 1 per cent. of the total bulk. The extract is then incubated at 37° C. for 72 hours, treated with 1 in 1,000 commercial formalin, and stored in the ice-box (48 hours' incubation at 37° C. inactivated the virus, but as a precautionary measure incubation is extended to the 72nd hour).

The advantages of this method are:—

- (1) Inactivated extract is available shortly after preparation, and may be inoculated without setting up rinderpest.
- (2) *Keeping Properties.*—Tissue extract prepared by the above method and stored in the ice-box at a temperature which varied from 4-10° C. (depending upon the quantity of ice available) was tested on its potency by three inoculations at intervals of 7 days, doses from 10 c.c. upwards.

The following test was made:—

(Extract prepared on 22/12/28.)

Cattle Nos.	Dose.	1st Inoc.	Dose.	2nd Inoc.	Dose.	3rd Inoc.	Immunity Test with 2 c.c. virulent blood and virulent nasal secretion. 9/4/29.
	cc.		cc.		cc.		
3477.....	10	4/3/29	10	21/3/29	10	28/3/29	No reaction.
3483.....	15	14/3/29	15	21/3/29	15	28/3/29	No reaction.
3470.....	30	12/3/29	30	19/3/29	30	26/3/29	No reaction.

This lot of extract was thus potent for at least approximately 3 months. A large quantity can thus be prepared and stored and tested on its potency before use. Briefly, the results obtained in 1927 showed (1) that two inoculations at 7 days' interval of spleen-pulp, plus toluol (as prepared above), but mixed with saline 1-9 instead of 1-4, dose 30 c.c., each inoculation, representing a total quantity of 6.66 grammes of tissue for the two inoculations, did not give in all cases complete protection on the seventh day after the last inoculation. Others were protected on the seventh day, but, when re-tested 97 days later, reacted mildly. (2) Inoculation of toluol extract did not protect against simultaneous inoculation of virulent blood. (3) Spleen-pulp treated with toluol, not incubated, but stored in the ice-box at 5° C. for five days was not completely inactivated and produced a slight temperature reaction. The animal was subsequently proved protected against virulent blood. (4) Toluol spleen extract, not incubated, but stored in the ice-box at 5° C. for seven days, did not produce a reaction, and the animal was proved susceptible on immunity test thirteen days later. A comparison of extracts of various tissues, e.g. spleen, liver, small intestines, muscle showed that spleen possesses high potency, and liver comparatively little potency.

Investigations were continued during 1928 and 1929, and the following is a summary:—

1. Three inoculations of the toluol extract at seven days' interval, completely protected highly susceptible cattle at a dose of from 10 c.c. each injection, which represents 2 grammes of tissue for each injection, or 6 grammes for the series. For use in the practice, a dose of 15 c.c. for each inoculation is issued, provided the lot of extract of which it consists has been tested and 6 grammes found to protect against a dose of 2 c.c. fresh virulent blood on the 14th day after the last inoculation.

2. *To Determine the Protective Properties of Spleen of a Hyper-immunized Animal.*

Two cattle which received three inoculations at seven days' intervals, dose 20 c.c. and 40 c.c. each inoculation respectively, of a treated spleen extract from a *hyperimmunized* serum-maker were completely protected against 2 c.c. virulent blood nine days after last inoculation of extract. One beast which received two inoculations, each dose 60 c.c. was not completely protected, but reacted and recovered.

3. *Protective Properties of Spleen of a Recovered Virus-maker.*

Three cattle inoculated each with 2 doses of toluol treated spleen extract of a recovered virus-maker (recovered approximately 2 months prior to collection of spleen), one at 20 c.c., one at 40 c.c., and one at 60 c.c. each dose, were not protected against contact infection and death.

4. Inactivated spleen and intestines, dose 60, 28, and 12 grammes respectively, partially protected against 2 c.c. fresh virulent blood injected 6 days later (three in experiment).

5. *The Efficacy of Two Inoculations at Increased Doses of Toluol Spleen Extract, Incubated for 72 hours.*

Two cattle, Nos. 3322 and 3326, were inoculated twice at seven days' intervals, dose 80 and 100 c.c. respectively each inoculation. No. 3322 was tested on its immunity on sixteenth day after last inoculation of extract, and No. 3326 on the tenth day after last inoculation of extract with 2 c.c. fresh virulent blood.

RESULT.

Both were completely protected.

6. *Spleen treated with Chloroform.*

Spleen extract of a virus-maker in the later stages of the disease, just when the temperature had fallen, mixed with equal parts of saline, and chloroform added to make 0.75 per cent. concentration.

One beast was inoculated twice at seven days' interval, each dose 20 c.c. Animal reacted and recovered to 2 c.c. fresh virulent blood injected nineteen days after last inoculation of extract. One beast inoculated with 40 c.c. chloroform extract and one beast which received 60 c.c. also reacted, but recovered to inoculation of 2 c.c. virulent blood nineteen days after last inoculation of extract.

7. *Inoculation of Unweaned Calves with Inactivated Toluol Spleen of a Reacting Virus-maker (5/3/29).*

Fourteen unweaned calves, varying in age from two to five months, were treated with toluol treated spleen. (Three inoculations at intervals of seven days.)

Seven got 20 c.c. subcutaneously each dose.

Seven got 30 c.c. subcutaneously each dose.

RESULT.

None reacted.

Immunity Test (1/4/1929).

Each got subcutaneously 2 c.c. fresh citrated virulent blood.

RESULT.

None reacted.

These animals will be re-tested on their immunity after weaning and at subsequent dates.

DURATION OF IMMUNITY CONFERRED.

As mentioned above, tests made in 1927 showed that some cattle which were protected by 2 doses of the minimum dose of extract at seven days' interval, each dose representing 3.33 grammes of tissue, against 2 c.c. virulent blood inoculated seven days later, were protected, but when re-tested ninety-seven days later, against 2 c.c. virulent blood, gave a slight temperature reaction, the resistance thus being partial, but not complete.

During 1929 further tests were carried out to determine the duration of immunity conferred in cattle by three inoculations of spleen extract at higher doses.

RESULT.

Cattle No. 3316 received three inoculations of extract, each of 60 c.c. on 24/12/28, 31/12/28, and 7/1/29 respectively. When tested on its immunity on 18/1/29 against 2 c.c. virulent blood, animal was completely protected.

Cattle No. 3326 received two inoculations, as above, on 24/12/28 and 31/12/28, each dose being 100 c.c. When tested for immunity on 10/1/29 against 2 c.c. virulent blood, there was no reaction.

Both animals were re-tested against virulent blood on 2/5/29. Neither reacted.

Several hundred head of susceptible cattle have been experimentally inoculated in the field with toluol spleen extract at 15 and 20 c.c. doses, representing 9 and 12 grammes of tissues respectively for the three inoculations. The animals have been branded for future identification, and the immunity will be tested at varying intervals up to a year at least.

CONCLUSIONS.

(1) For conferring an active immunity by the simultaneous method, the use of fresh blood is of fundamental importance; the dose of anti-serum is a factor; non-reactors and non-conferring of immunity sometimes results when large doses of anti-serum are simultaneously inoculated with fresh blood.

(2) Simultaneous inoculation of blood kept at a temperature of 0-4° C. and used up till twenty-four hours after being bled; and a high index dose of anti-serum was responsible for a high percentage

of non-reactors and non-conferring of immunity. Cattle which fail to react to simultaneous inoculation may contract rinderpest from the reacting cattle of the herd with some mortality.

Further work is necessary to determine the efficacy of blood kept at 0-4° C. and used up till 24 hours after being bled and injection of the dose of anti-serum 24 hours after the virus.

(3) Nasal secretion collected in citrate in the early stages of the temperature reaction (second day), if used fresh, may be substituted for fresh blood for infecting virus-makers without the risk of setting up redwater and anaplasmosis, and for the simultaneous inoculation of cattle susceptible to redwater and anaplasmosis, such as imported cattle, but they should be kept under close observation, when blood free of these infections is not available. Owing to the difficulty of obtaining large supplies and maintaining the virulency, its use is restricted to small numbers of cattle.

(4) Blood of a recovered redwater-anaplasmosis beast did not produce an apparent reaction to either infection in sheep, and blood of the inoculated sheep did not transmit either infection to susceptible calves.

(5) Blood of sheep and goats respectively reacting to inoculation of virulent bovine blood (first passage) is unsuitable for simultaneous inoculation.

(6) Peritoneal washings are virulent, but usually contain some red cells, and are thus unsafe to use for virus for inoculating dipped stock.

The virus of rinderpest is filterable through the Berkefeld and Seitz filters and a virulent filtrate free of red cells may be obtained by peritoneal washings. The filtrates of treated nasal secretion are virulent. (Attempts to obtain a virulent filtrate from virulent bovine blood were unsuccessful.) Owing to the variability of obtaining virulent filtrates and the difficulty of maintaining the virulency, the filtrates of peritoneal washings and nasal secretion are of no practical utility.

(7) Cattle born and reared in tick-free stables may be successfully utilized when of a suitable age after weaning, for the production of "clean" virulent blood for simultaneous inoculation of regularly dipped stock. To avoid the risk of infecting them with redwater and anaplasmosis, they should be inoculated with fresh nasal secretion free of red cells.

(8) On some farms where dipping is regularly carried out, particularly when combined with hand-dressing, a large percentage of the stock escape redwater and anaplasmosis, and may be used after their blood has been tested on newly-born calves to exclude, or otherwise, these infections, and, if found clean for virus-makers, for the inoculation of dipped stock.

(9) Simultaneous inoculation of unweaned calves, the progeny of immune mothers, does not confer an active immunity in a varying percentage; simultaneous inoculation after weaning is necessary to ensure an active immunity.

(10) Simultaneous inoculation of calves (the progeny of immune mothers), when reared on dairy farms, may be responsible for some losses; the mortality is higher in calves, the progeny of susceptible mothers.

The losses from simultaneous inoculation in calves (the progeny of immune or susceptible mothers) reared under ranching conditions is usually not serious.

(11) Active immunization of imported cattle recently recovered from redwater and anaplasmosis is likely to produce a recurrence of both infections and some mortality. Large doses of anti-serum do not always control the reaction to rinderpest in highly susceptible cattle, but a large dose of anti-serum, 500 c.c. upwards, injected in the early stages (second day) of the thermal reaction is usually effective.

(12) Inactivated spleen extract confers protection. Three inoculations of 15 c.c. each, at intervals of seven days, of pooled spleen extract, each dose representing 3 grammes of tissue or a total of 9 grammes, protected on the tenth day after the last injection against 2 c.c. fresh virulent blood. Three inoculations of 5 c.c. each (1 gramme of tissue) partially protect.

Two inoculations, at intervals of seven days, of not pooled spleen extract, each dose representing 3.33 grammes of tissue, partially protect some and completely protected others on the seventh day after last injection of extract. Of the latter, some are partially protected on the 97th day after the date of the original immunity test; others are still completely protected.

Two inoculations at larger doses, viz., 80 c.c. and 10 c.c. respectively, representing a total dose of 48 and 60 grammes tissue, completely protects against 2 c.c. virulent blood on the tenth day. (When re-tested on their immunity 102 days afterwards were still completely resistant to 2 c.c. fresh virulent blood.)

Inoculation of spleen extract does not protect against simultaneous inoculation of virulent blood.

A single inoculation partially protects against contact infection (three cattle on experiment).

Spleen of a recovered animal which had been hyperimmunized for serum-making purposes, protected at three inoculations and partially at two inoculations.

Spleen of a recovered virus-maker (two inoculations) does not protect.

Chloroform extract, two inoculations and one inoculation respectively, conferred protection against a severe reaction and death (two on experiment).

(13) Spleen-pulp stored for five days at 0° C. was not completely inactivated.

(14) The duration of a period of immunity conferred by three inoculations of pooled extract is being investigated. For this purpose several hundred head of cattle have been inoculated with spleen extract at doses varying from 15-20 c.c. each dose, representing 3 and 4 grammes of tissue respectively, and have been branded for future identification. These cattle will subsequently be tested with fresh virulent blood at varying intervals.

(15) Inactivated spleen extract is now being utilized for experimental inoculation of dairy cattle, cows heavy in calf, pure-bred and high-grade cattle, calves, and, in cases where it is considered not desirable to simultaneously inoculate.

Vaccination in the practice as a substitute for simultaneous inoculation will be considered when further data on duration of immunity is available. Further research is necessary to determine whether cattle treated with spleen may be successfully immunized later by simultaneous inoculation.

(16) Inactivated spleen extract is still potent 3 months after preparation. In view of its keeping properties, a large supply could be prepared and stored.

(17) Variations occur in potency of spleens. The pulp of several spleens should be pooled to obtain an extract of high potency, and each lot of prepared extract tested on its potency prior to issue.

(18) Further work is necessary to determine whether spleen-pulp inactivated by a few days at incubator temperature (37° C.) is as effective as spleen-pulp treated with various chemicals, such as toluol, chloroform, formalin; if so, the addition of an antiseptic which would sterilize the extract of bacterial and protozoal infections without affecting its potency would only be necessary.

(19) Experiments show that redwater infection is not transmissible by inactivated spleen prepared from a beast reacting to redwater, but further work is necessary to determine the effect of the various chemicals which have been used for making extracts, e.g. toluol, chloroform, formalin on anaplasma parasites and period contact required for these to sterilize the extract of anaplasma infections.

Paper No. 6.

PROPHYLACTIC VACCINATION AGAINST RINDERPEST.

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RESEARCHES directed towards the preparation of a satisfactory immunogen from virus-containing tissues have, in recent years, been stimulated by certain defects in both the serum and serum-virus methods of immunization. These defects were discussed in the attached paper, and it is not proposed to do more than mention them here as an indication of the need for further research into methods of immunization.

The serum-alone method suffers from a grave disadvantage in the shortness of duration of the immunity conferred, and in the consequent trouble and expense involved by the repeated inoculations that are necessary to maintain animals in an immune state during an epizootic.

The serum-virus method, which is adopted by most countries where the disease is enzootic, involves the creation of new centres of infection where immunization is being carried out. The relative importance of this factor depends partly upon the dose and titre of the serum used in the inoculation, and partly also upon the