

EAST COAST FEVER.

ARTIFICIAL IMMUNIZATION AND IMMUNITY IN THEIR
RELATION TO THE CONTROL OF EAST COAST FEVER.

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OWING to conditions existing in this Colony, viz., the occurrence of East Coast fever in an endemic form; the absence of a dipping and cleansing ordinance; the difficulty of controlling illicit movements of cattle; and the wide distribution of the disease it was considered desirable that research work with a view to determining the value of artificial immunization and the clearing up of other obscure points should be carried out. Experimental work was commenced in this direction in 1924, and has been continued since. The experiments carried out and the results obtained are recorded in the Annual Reports of the Chief Veterinary Research Officer for the years 1924, 1925, 1926, 1927, and 1928.

The value of artificial immunization was originally investigated by Theiler in South Africa, who experimentally inoculated a large number of cattle and subsequently tested them on their immunity either with infected ticks or by exposing them to natural infection, and recorded the experiments and results, etc., in the second Report of the Director of Veterinary Research, Union of South Africa, 1912, as follows:—

- (a) *Immunization of Cattle in the Field.*—Of 149 head of clean cattle inoculated in various ways in the field, and exposed to East Coast fever infected veld later, 56.4 per cent. survived infection and exposure.
- (b) *Immunization of Cattle at the Laboratory.*—Of 236 cattle injected intrajugularly 59 head or 25 per cent. died of East Coast fever. Of the 172 survivors, which were tested on their immunity, 103 head or 59.8 per cent. proved to be immune.

Theiler considered that artificial immunization could be carried out by intrajugular inoculation of spleen pulp with the prospect of conferring immunity against natural infection in 56 per cent. to 60 per cent. of animals treated.

The results obtained at the Veterinary Research Laboratory, Kabete, during 1924 and 1925 were as follows:—

1924.—Of 215 susceptible cattle injected intrajugularly, 18 head, or 8.37 per cent., died of East Coast fever and other causes consequent on inoculation.

Of 87 inoculated cattle which were tested on their immunity, 64 per cent. survived.

1925.—Of 57 susceptible cattle inoculated, 49 head were subsequently exposed to infection. Of the original 57 head, 72.25 per cent. survived inoculation and exposure.

1926.—(Field inoculation) by one of us (S.H.W.).

Records were kept of immunization in the field of a herd which consisted on 17th April, 1926, of 256 head of male and female grade cattle, and in which the first case of East Coast fever was confirmed

on that date. The number of deaths, monthly, in this herd from East Coast fever up to the date of inoculation, viz., 6th August, 1926, was as follows:—

| | |
|--------------|---------|
| April | 7 head. |
| May | 27 „ |
| June | 40 „ |
| July | 33 „ |

147 head remained for inoculation on 6th August, 1926. Of these, 116 head, or 78.9 per cent., survived inoculation and prolonged exposure (12 months) on a heavily East Coast fever infected farm.

These Kabete results correspond fairly closely with the conclusions arrived at by Theiler in 1911, viz., that for the present, a practical method of inoculation which can be reasonably expected to confer immunity to the extent of 60 per cent. to 70 per cent., would consist in the injection of a quantity of not less than 5 c.c. of spleen pulp, or a mixture of spleen and gland pulp, taken from animals in the last stage of the disease, mixed with peptone and injected intrajugularly.

It is proposed in this paper to deal with immunization and immunity under the following headings:—

1. Immunization by various methods of, and immunity in—

A.—Cattle other than calves.

B.—Calves.

by—

(a) intrajugular injection of spleen pulp from a reacting East Coast fever beast.

(b) Other methods or combination of methods of inoculation of material from an East Coast fever reacting beast.

(c) Inoculation of emulsions of ticks.

2. Complications arising out of immunization.

3. The inoculation of non-infective treated spleen and gland material from a reacting East Coast fever beast as a means of producing immunity.

4. The role of the spleen in East Coast fever immunity.

1. IMMUNIZATION BY VARIOUS METHODS OF, AND IMMUNITY IN, (A) CATTLE OTHER THAN CALVES. (B) CALVES.

A.—CATTLE OTHER THAN CALVES.

(a) *Intrajugular Injection of Spleen Pulp from a Reacting Beast.*—These results are already given in the reference made to inoculations carried out in 1924, 1925, and 1926. It may be mentioned, however, that observations made over a large number of experiments have shown that varying results may be obtained. This may be due to some factor in the inoculated animals themselves or may be connected with something concerning the material inoculated. The percentage of reactions produced by the intravenous inoculation of different spleen pulps varies, and further experiments are being conducted to observe whether or not more constant results might be obtained. These are being especially directed towards determining whether transmission occurs more frequently at some particular stage of the life cycle of the parasite in the animal host as distinct from the tick host.

Experiments conducted by Theiler on the effect of treatment of the spleen pulp with drugs, such as hydro-quinine, before inoculation, gave no definite results, except that pulp saturated in a 10 per cent. and stronger solution of hydro-quinine, did not produce reactions or immunity in inoculated cattle.

Experiments at Kabete on similar lines and with additional drugs did not give any conclusive results.

Further experiments on the intrajugular injection of spleen pulp are included in the following tables.

(b) *Other Methods or Combination of Methods, and Materials, from an East Coast Fever Reacting Beast.*—This work was undertaken with a view to determining—

- (1) Whether inoculation of material other than spleen pulp would transmit East Coast fever.
- (2) Whether these methods or combination of these methods, and materials, in one inoculation or more than one inoculation, would produce a larger percentage of reactions and confer a higher resistance to natural infection.

1. A summary of these transmission experiments is set out in the following table:—

| Method. | Per cent. of Reactions with Koch's Bodies in Gland Smears. | Per cent. of Temperature Reactions only. | Per cent. of Non-reactors. | Per cent. of Mortality. |
|--|--|--|----------------------------|-------------------------|
| <i>Blood—</i> | | | | |
| Subcutaneous..... | 0 | 14 | 85 | 0 |
| „ and Intracutaneous..... | 12.2 | 26.8 | 60.9 | 2.7 |
| „ and Intravenous..... | 0 | 0 | 100 | 0 |
| „ and Intrasplenic..... | 0 | 0 | 100 | 0 |
| <i>Spleen Pulp—</i> | | | | |
| Subcutaneous..... | 50 | 16.6 | 33.3 | 16 |
| „ and Intracutaneous..... | 0 | 0 | 100 | 0 |
| „ and Intravenous..... | 14.2 | 14.2 | 71 | 14.2 |
| <i>Blood Mixed with Spleen Pulp—</i> | | | | |
| Subcutaneous..... | 16.6 | 33.3 | 50 | 0 |
| „ and Intracutaneous..... | 25 | 12.5 | 62.5 | 0 |
| „ and Intravenous..... | 25 | 25 | 50 | 0 |
| „ Intracutaneous and Intravenous..... | 27.2 | 36.3 | 36.3 | 9 |
| <i>Spleen Pulp—</i> | | | | |
| Intravenous and Blood Intracutaneous and Subcutaneous..... | 34.4 | 17.2 | 48.2 | 6.8 |
| <i>Spleen Pulp mixed with Blood—</i> | | | | |
| Subcutaneous and Intracutaneous and Spleen Pulp Intravenous..... | 30.6 | 56.4 | 12.8 | 10-12 |
| <i>Spleen Pulp Intravenous.....</i> | 32.3 | 20.7 | 46.9 | 6.7 |
| <i>Spleen Pulp mixed with Blood Intravenous..</i> | 42.8 | 28 | 28 | 7 |
| <i>Gland Juice Intravenous.....</i> | 16.6 | 0 | 82.5 | 0 |
| <i>Gland Juice Subcutaneous and Intracutaneous</i> | 0 | 16.6 | 82.5 | 0 |

CONCLUSIONS.

1. East Coast fever is transmissible by various methods or combination of these methods, with the following materials from an East Coast fever reacting beast:—

- A.—Blood.
- B.—Spleen pulp.
- C.—Blood mixed with spleen pulp.
- D.—Gland juice.

The various methods of inoculation of these materials included intracutaneous, subcutaneous and intravenous inoculations.

2. None of these methods invariably produced a reaction.

3. The largest percentage of reactions result from the intravenous inoculation of spleen pulp, and simultaneously subcutaneous and intradermal inoculation of spleen pulp mixed with blood. With the exception of gland juice (dose 1 c.c. which produces 16 per cent. of reactions) blood alone gives the smallest percentage of reactions (average 28 per cent.). Inoculation of spleen pulp produces reactions varying from 0 to 87 per cent.

4. The percentage mortality varies in different lots of cattle from nil to 16 per cent.

(Blood alone inoculation, average mortality 1.6 per cent. Spleen alone or spleen mixed with blood inoculation, average mortality 7.5 per cent.)

2. *Whether these methods or combination of these methods and materials, in one inoculation, or more than one inoculation, would produce a larger percentage of reactions and confer a higher resistance to natural infection.*

A number of susceptible cattle were originally inoculated by one of the following methods, and subsequently re-inoculated, and in some instances subsequently exposed to natural infection:—

- (a) Blood subcutaneously and intradermally.
- (b) Blood subcutaneously.
- (c) Blood mixed with spleen pulp, subcutaneously and intradermally.
- (d) Spleen pulp subcutaneously and intradermally.
- (e) Spleen pulp subcutaneously.
- (f) Blood subcutaneously and intravenously.
- (g) Gland juice intravenously.
- (h) Gland juice subcutaneously and intracutaneously.
- (i) Blood intracutaneously and intrasplenically.

The following tables give the summaries of the results obtained:—

| | In Ex- peri- ment. | Reacted. | Non- reactors. | Mor- tality. |
|---|--------------------------|----------|-------------------|-----------------|
| THREE INOCULATIONS. | | | | |
| (a) 1st Inoculation.—Blood subcutaneously and intradermally | 8 | 0 | 8 | 0 |
| 2nd Inoculation.—Blood mixed with spleen pulp, intravenously and intradermally | 8 | 0 | 8 | 0 |
| 3rd Inoculation.—Blood mixed with spleen pulp, intravenously and subcutaneously | 8 | 6 | 2 | 1 |
| * Exposed to natural infection | 7 | ? | ? | 1 |
| (b) 1st Inoculation.—Blood subcutaneously and intradermally | 4 | 1 | 3 | 0 |
| 2nd Inoculation.—Spleen pulp subcutaneously (non-reactors) | 3 | 0 | 3 | 0 |
| 3rd Inoculation.—Spleen pulp intravenously and subcutaneously (non-reactors) | 3 | 1 | 2 | 0 |
| Exposed to natural infection (non-reactors) | 3 | 1 | 2 | 0 |
| (c) 1st Inoculation. Blood subcutaneously | 4 | 0 | 4 | 0 |
| 2nd Inoculation.—Spleen pulp subcutaneously .. | 3 | 0 | 3 | 0 |
| 3rd Inoculation.—Spleen pulp subcutaneously and intravenously | 3 | 0 | 3 | 0 |
| Exposed to natural infection | 2 | 2 | 0 | 0 |
| (d) 1st Inoculation.—Blood mixed with spleen pulp, subcutaneously and intradermally | 8 | 2 | 6 | 0 |
| 2nd Inoculation.—Blood mixed with spleen pulp and gland pulp intravenously | 8 | 2 | 6 | 0 |
| 3rd Inoculation.—Spleen pulp intravenously | 8 | 0 | 8 | 0 |
| Exposed to natural infection | 8 | 8 | 0 | 2 |
| (e) 1st Inoculation.—Spleen pulp subcutaneously and intradermally | 4 | 0 | 4 | 0 |
| 2nd Inoculation.—Spleen pulp subcutaneously and intradermally | 4 | 1 | 3 | 0 |
| Exposed to natural infection— | | | | |
| Reactors | 1 | 1 | 0 | 0 |
| Non-reactors | 1 | 0 | 0 | 0 |
| 3rd Inoculation.—Spleen pulp subcutaneously and intravenously | 1 | 0 | 1 | 0 |
| Exposed to natural infection (non-reactors) | 1 | 1 | 0 | 1 |

* Not under daily observation—Exposed on owner's estate.

| | In Ex- peri- ment. | Reacted. | Non- reactors. | Mor- tality. |
|---|--------------------------|----------|-------------------|-----------------|
| TWO INOCULATIONS. | | | | |
| (a) 1st Inoculation.—Blood subcutaneously and in- travenously | 6 | 0 | 6 | 0 |
| 2nd Inoculation.—Blood and spleen pulp subcu- taneously and intradermally | 5 | 0 | 5 | 0 |
| Exposed to natural infection | 5 | 4 | 1 | 0 |
| (b) 1st Inoculation.—Blood subcutaneously and in- tradermally | 10 | 4 | 6 | 0 |
| 2nd Inoculation.—Blood plus spleen pulp subcu- taneously and intravenously | 8 | 2 | 6 | 0 |
| Exposed to natural infection | 7 | 5 | 2 | 1 |
| (c) 1st Inoculation.—Blood subcutaneously and in- tradermally | 6 | 2 | 4 | 0 |
| 2nd Inoculation.—Blood mixed with spleen pulp subcutaneously and intradermally | 6 | 5 | 1 | 0 |
| Exposed to natural infection | 6 | 5 | 1 | 1 |
| (d) 1st Inoculation.—Spleen pulp and blood subcu- taneously | 6 | 6 | 0 | 0 |
| 2nd Inoculation.—Spleen pulp and blood subcu- taneously and intravenously | 6 | 0 | 6 | 0 |
| Exposed to natural infection (non-reactors) | 1 | 1 | 0 | 0 |
| (e) 1st Inoculation.—Spleen pulp subcutaneously | 3 | 1 | 2 | 1 |
| 2nd Inoculation.—Spleen pulp subcutaneously and intravenously | 2 | 0 | 2 | 0 |
| Exposed to natural infection (non-reactors) | 2 | 2 | 0 | 2 |
| (f) 1st Inoculation.—Gland juice 1 c.c. intravenously | 6 | 1 | 5 | 0 |
| 2nd Inoculation.—Spleen and gland pulp intra- venously | 5 | 5 | 0 | 0 |
| Exposed to natural infection (1 reactor, 5 non- reactors) | 6 | 1 | 5 | 0 |
| (g) 1st Inoculation.—Gland juice 1 c.c. subcuta- neously and intradermally | 6 | 1 | 5 | 0 |
| 2nd Inoculation.—Spleen and gland pulp intra- venously | 6 | 4 | 2 | 0 |
| Exposed to natural infection | 6 | 2 | 4 | 0 |
| (h) 1st Inoculation.—Blood subcutaneously and in- tradermally | 4 | 1 | 3 | 0 |
| 2nd Inoculation.—Spleen and gland pulp intra- venously | 3 | 1 | 2 | 1 |
| Exposed to natural infection | 3 | 1 | 2 | 0 |

The following results were obtained from one inoculation and a subsequent exposure:—

| | In Ex- peri- ment. | Reactors | Non- reactors. | Mor- tality. |
|---|--------------------------|----------|-------------------|-----------------|
| ONE INOCULATION. | | | | |
| (a) Spleen pulp subcutaneously | 3 | 3 | 0 | 0 |
| Exposed to natural infection | 3 | 3 | 0 | 2 |
| (b) Spleen pulp intravenously | 24 | 21 | 3 | 1 |
| Exposed to natural infection | 19 | 8 | 11 | 0 |
| (c) Spleen pulp intravenously and subcutaneously... | 7 | 2 | 5 | 0 |
| Exposed to natural infection | 6 | 3 | 3 | 2 |
| (d) Blood and spleen pulp intravenously, subcuta- neously, and intradermally | 1 | 0 | 1 | 0 |
| Exposed to natural infection | 1 | 1 | 0 | 0 |
| (e) Blood, subcutaneously and intradermally | 6 | 3 | 3 | 0 |
| Exposed to natural infection | 6 | 5 | 1 | 3 |

CONCLUSIONS.

1. An original inoculation does not always confer a resistance against re-inoculation. An original inoculation with blood gives less protection against re-inoculation than an original intravenous inoculation with spleen pulp; or spleen pulp intravenously, and simultaneously subcutaneous and intradermal inoculation of blood mixed with spleen pulp.

2. Re-inoculation does not confer a higher resistance to natural infection.

3. Intravenous inoculation with spleen pulp and simultaneously subcutaneous and intradermal inoculation of blood mixed with spleen pulp, produces a larger percentage of reactors and a greater resistance to natural infection, in comparison with an original and subsequent inoculation by other methods or combination of methods.

4. A percentage of susceptible cattle which do not react to an original inoculation and one or two subsequent inoculations are not protected against a reaction and death from East Coast fever when exposed to natural infection.

5. On estates where losses are occurring from East Coast fever, and no facilities exist for controlling the disease, an original inoculation by the method which produces the larger and more marked percentage of reactions, and most resistance to natural infection, viz., the intravenous inoculation of spleen pulp and simultaneously subcutaneous and intradermal inoculation of spleen pulp mixed with blood, is indicated.

(c) *Emulsions of Ticks.*—These experiments were conducted to observe the transmissibility of East Coast fever from ticks fed on infected cattle and to note immunity, if any, possessed by cattle so inoculated.

The experiments with results are as follows:—

1. *Intravenous inoculation of 20 cattle with emulsion of larvae which engorged on East Coast fever infected cattle.*

RESULT.—No East Coast Fever reaction produced, and all the cattle on experiment subsequently proved to be susceptible to East Coast fever.

2. *Intravenous, subcutaneous, and intracutaneous inoculation respectively of nymphal ticks which engorged as larvae on an East Coast fever reacting beast and which were fed for 96 hours prior to emulsifying. Eight cattle on experiment.*

RESULT.—(a) *Subcutaneous Inoculation.*—Five cattle on experiment. One reacted and survived exposure. Remainder did not react and were subsequently proved to be susceptible.

(b) *Intravenous Inoculation.*—One animal on experiment. Reacted and slaughtered *in extremis*.

(c) *Intracutaneous Inoculation.*—Five cattle on experiment. None reacted and were subsequently proved to be susceptible.

3. *Inoculation of emulsion of nymphs which engorged as larvae on an East Coast fever beast, and which were fed for 96 hours prior to emulsifying, and simultaneously blood of a beast reacting to East Coast fever.*

Four cattle on experiment.

RESULT.—One animal reacted and died of East Coast fever.

CONCLUSIONS.

1. Transmission of East Coast fever is not effected by larvae or nymphal ticks which are emulsified and inoculated immediately after engorging on an East Coast fever beast, and no immunity is conferred in such inoculated cattle.

2. East Coast fever is variably produced by subcutaneous and intravenous inoculation respectively of an emulsion of nymphal ticks which engorged as larvae on an East Coast fever beast, and fed for 96 hours prior to emulsifying. (Intracutaneous inoculation of the same emulsion did not produce East Coast fever. One in experiment.)

3. The immunity conferred in animals which react to inoculation is not complete against natural infection.

4. Subcutaneous inoculation of emulsions of nymphs which engorged as larvae on an East Coast fever beast and fed for 96 hours prior to emulsifying, and simultaneously intracutaneous and subcutaneous inoculation of blood of an East Coast fever beast *variably* produces East Coast fever.

5. The inoculation of nymphal ticks which engorged as larvae on an East Coast fever beast and which were fed for 96 hours prior to emulsifying does not offer better prospects of producing a reaction and immunity to natural infection than intravenous inoculation of spleen pulp, or intravenous inoculation of spleen pulp and simultaneously subcutaneous and intradermal inoculation of blood mixed with spleen pulp.

B.—CALVES.

(a) *Intrajugular injection of spleen pulp from a reacting beast.*

- (1) 46 weaned calves put on experiment. One inoculation. Mortality from inoculation and exposure 45.92 per cent.
- (2) 45 weaned calves put on experiment. Two inoculations. Mortality from inoculation and exposure 84.37 per cent.

(b) *Other methods of inoculation.*

28 weaned calves were inoculated intrasplenically with spleen pulp from a reacting East Coast fever beast.

- (1) 16 calves on experiment. Mortality from inoculation and exposure 39.56 per cent.
- (2) 12 calves on experiment. Mortality from inoculation and exposure 58.33 per cent.

Summary of (a) and (b) results:—

| | |
|-------------------------|-----|
| Total inoculated | 119 |
| Total exposed | 97 |

Average percentage of mortality from all causes 57.4 per cent.

(c) Exposure of 10 non-inoculated weaned calves. Mortality from exposure 90 per cent.

(d) Exposure of 4 non-inoculated unweaned calves. Mortality from exposure 75 per cent.

(e) Exposure of 4 non-inoculated unweaned calves.

(f) Infestation of calves with infected ticks. Of 15 unweaned grade calves infested by hand with nymphal ticks which engorged on an animal reacting to East Coast fever five or 33.3 per cent, reacted to East Coast fever and one reacted and survived, but subsequently reacted to East Coast fever on exposure.

Ten, or 66.6 per cent., did not react. Eight of these reacted to East Coast fever on exposure, with five deaths and three recoveries. Two out of the 10 calves did not react on exposure.

Several observations made during these experiments are not detailed above but are included in the following summary:—

- (1) Artificial immunization of calves by any of the methods employed does not give very satisfactory results.
- (2) As may be expected, immunization is more frequently complicated with other tick-borne diseases, Redwater-Anaplasmosis, Spirillosis, etc., than is the case with adult undipped cattle.
- (3) The duration of the disease from the commencement of the temperature reaction varied from 13 to 24 days—average 18 days.

NOTE.—This period is usually 9 to 10 days in cattle of 20 months to 2 years.

- (4) It would appear that calves react to East Coast fever more than once, in a greater percentage of cases in comparison with older cattle, and that the immunity in calves in some cases which survive original natural infection, is built up by natural re-infection.

2. COMPLICATIONS ASSOCIATED WITH IMMUNIZATION.

Apart from occasional deaths occurring as the result of emboli and purulent pneumonia following the intravenous inoculation of material, the co-existence or super-imposition of other tick-borne diseases, viz., Redwater, Anaplasmosis, and Spirillosis with East Coast fever consequent on inoculation, or natural infection during exposure, is responsible for an increase in the death rate. This, of course, is of great consequence in cattle not immune to these infections, and is responsible for increased mortality in the immunization of calves.

The incubation period from tick infestation in these diseases, may be given as follows:—

- (1) East Coast fever, average 13 days.
- (2) Redwater, average 15 days.
- (3) Spirillosis, average 17 days.
- (4) *Theileria mutans*, average 50 days.
- (5) Anaplasmosis, average 50-70 days.

Redwater and Spirillosis infections may be concurrent with the East Coast fever reaction as a result of inoculation of these infections to susceptible cattle, with the East Coast fever infected material, the Redwater reaction usually commencing about five days before the East Coast fever reaction. Since the incubation period from inoculation in the case of Anaplasmosis is usually 30-35 days, this infection is more likely to be associated with East Coast fever exposure reactions. It must be borne in mind, however, that any of these infections may occur as "breakdowns" in Redwater, Anaplasmosis, etc., recovered cattle reacting to East Coast fever from inoculation.

On exposure, however, all these infections may be transmitted to susceptible cattle, and as will be seen from the above-mentioned incubation periods from tick infestation, Redwater, and Spirillosis infections may occur simultaneously with East Coast fever exposure reactions, while *Theileria mutans* and Anaplasmosis infections will generally appear subsequent to an East Coast fever reaction.

3. THE INOCULATION OF NON-INFECTIVE TREATED SPLEEN AND GLAND MATERIAL FROM A REACTING EAST COAST FEVER BEAST AS A MEANS OF PRODUCING IMMUNITY.

Experiments were conducted during 1927 and 1928 to observe whether extracts prepared by treating East Coast fever infected spleen and gland material with either toluol or formalin, have any value as an immunizing agent on inoculation to susceptible cattle either prior to exposure, or during an East Coast fever reaction, or both.

The cattle put in experiment included:—

- (a) Animals which had been inoculated previously for East Coast fever and were reacting to natural infection on exposure. Three cattle in experiment. They were inoculated with the treated spleen and gland material immediately after the commencement of the East Coast fever reaction.

RESULT.—All three cattle recovered.

- (b) Animals which had been inoculated previously for East Coast fever and subsequently exposed to natural infection, the treated spleen and gland material being inoculated at intervals commencing from the date of exposure and continued during the East Coast fever exposure reactions. Three cattle in experiment, one of which died of East Coast fever. One animal failed to react on exposure.
- (c) Animals not previously inoculated for East Coast fever inoculated with treated material—
 - (1) at frequent intervals prior to exposure and after exposure. Five cattle in experiment;
 - (2) at intervals after exposure prior to East Coast fever reaction, and after the reaction commenced. Three cattle in experiment.

RESULT.—All eight cattle died of East Coast fever.

CONCLUSION.

The material, as prepared, proved of no value as an immunizing agent to prevent infection, or mortality, either inoculated prior to exposure, or during an East Coast fever reaction, or both. The recoveries in cattle previously inoculated for East Coast fever were, in all probability, not due to inoculation of treated material, but owing to the resistance acquired from artificial immunization.

4. SPLENECTOMY AND EAST COAST FEVER IMMUNITY.

These experiments were undertaken by one of us (S.H.W.) with a view to observing whether splenectomy had any influence on immunity to East Coast fever, or course of the disease. Observations were made on splenectomized cattle as follows:—

- (1) Splenectomy of six 2 to 3 year old East Coast fever recovered cattle.
- (2) Splenectomy of ten 2 year old East Coast fever susceptible cattle. This lot was divided as follows:—
 - (a) 5 cattle splenectomized 7-10 days prior to exposure to East Coast fever.
 - (b) 2 cattle splenectomized 1-2 days prior to first rise of temperature to East Coast fever reaction.
 - (c) 3 cattle splenectomized immediately on first rise of temperature to East Coast fever reaction.

The results obtained were as follows:—

1. The six East Coast fever recovered cattle were kept under observation for some time after operation, and daily examination of blood and gland smears failed to show any breakdown to East Coast fever, in so far as Koch's bodies did not reappear in gland smears. They all showed a severe recrudescence of *Theileria mutans* in the red blood cells. This infection amounted to 100 per cent. of red cell invasion on different dates in all cattle, more than one parasite per cell being frequently seen. Reappearance of Redwater parasites in blood cells occurred in all cases, subsequently causing the death of four out of the six cattle. Reappearance of *Anaplasma marginale* occurred, but not in great numbers.

Five out of the six cattle were exposed to re-infection to East Coast fever. Of these, one showed Koch's bodies "rare" in gland

smears on the 28th day after exposure, and recovered. Such re-infection with East Coast fever on re-exposure of East Coast fever recovered cattle being not rare, this re-infection had probably nothing to do with splenectomy.

NOTE.—It may be mentioned, in passing, that repeated examination of gland smears from these cattle taken either before, during, or after the heavy redblood cell infection with *Theileria mutans*, failed to show the presence of any bodies resembling the characteristic Koch's blue body of East Coast fever. This observation would appear to throw some doubt on the suggestion made by Theiler, namely, that the parasite previously known as *Gonderia* or *piroplasma mutans*, is really a *Theileria*, and has a stage in its development in the animal body, which corresponds to the Koch's body of East Coast fever and is indistinguishable microscopically from that body. Moreover, no such bodies as described by Theiler and Graf (13th and 14th Reports of Director of Veterinary Education and Research, South Africa, 1928), have been observed during the examination of routine smears submitted to the laboratory for examination, except in cases of what appeared to be undoubtedly East Coast fever.

2. Splenectomy of East Coast fever susceptible cattle:—

(a) The five cattle splenectomized 7-10 days prior to exposure, became infected on exposure, the period of incubation being 13-14 days. The disease appeared to run a normal course in each case and ended fatally in all five cattle.

(b) and (c) The infection in these five cattle appeared to run the usual course and ended fatally in all cases.

CONCLUSIONS.

1. Splenectomy of East Coast fever recovered cattle does not give rise to a breakdown to East Coast fever with reappearance of Koch's bodies in gland smears, either before or after exposure to East Coast fever infection.

2. Splenectomy of East Coast fever susceptible cattle does not influence either the susceptibility to infection or course of the disease.

3. Splenectomy of Redwater, Anaplasmosis, and *T. mutans* recovered cattle does give rise to a marked re-infection of red blood with these parasites, particularly in the cases of Redwater and *T. mutans*.

DISCUSSION.

With the knowledge so far gained, it would appear that, with the present methods, the artificial immunization of cattle against East Coast fever, has limited application, and especially in relation to the control and eradication of the disease.

The percentage of reactions to inoculation is variable, as is also the percentage of inoculated cattle which survive exposure. The greater the percentage of reactions to inoculation, the greater the number of inoculated cattle which survive exposure.

It has been our experience that susceptible non-inoculated cattle invariably react to the exposure to which they are submitted at Kabete with a mortality of approximately 95 per cent. We accept, therefore, that a percentage of inoculated cattle which do not visibly react to the inoculation (as determined by temperature and smear examination), have acquired some immunity from such inoculation, and survive exposure.

This variability in producing reactions or immunity, or both, as a result of inoculation may be due to some factor in the inoculated animals themselves, but, more probably, is associated with some factor concerning the material inoculated. This variability has been noted by Theiler in South Africa, and by Montgomery and the writers in Kenya.

The inoculation of calves gives less satisfactory results than is the case with older cattle, not only as a result of the complications which may be associated with inoculation and exposure, but also on account of the prolonged course and difficulty in producing a reaction in these animals and the acquiring of complete immunity.

The method of control of East Coast fever by inoculation, therefore, must be considered to have the following disadvantages:—

1. *Mortality from the Inoculation itself.*—These include—

- (a) occasional deaths from embolus and septic pneumonia from intravenous inoculation;
- (b) East Coast fever;
- (c) Redwater, Anaplasmosis, Spirillosis, etc., as complications.

2. *Mortality from Exposure* including East Coast fever and other tick-borne diseases, viz., Redwater, Anaplasmosis, Spirillosis, Heart-water, etc.

3. *The maintenance of the above-mentioned tick-borne infections* on farms where the inoculations and exposures are carried out.

4. *The effect of any of these diseases on cattle* which have reacted and recovered.

5. *The effect of tick infestation* itself on undipped cattle.

The method, however, might prove of value in certain instances such as the control of infection, or the saving of as many cattle as possible exposed to infection, on farms where dipping, etc., cannot produce immediate results, or where dipping facilities do not exist. It might also be made use of for the production of an immune herd for exposure on undipped, infected farms.

With regard to immunity in East Coast fever, it would seem possible that there is an immunity and a disease producing stage in the cycle of *Theileria parva* in the animal host; and that, whereas cattle inoculated with material containing the early stages of the parasite may react, a larger percentage will react with higher mortality when inoculated with material from an animal in the later stages of the disease. It would appear that the immunity and death producing stages are included in the same spleen in the later stages of East Coast fever, but the immunity producing stage occurs in the early stages of the disease.

It is generally recognized that susceptible cattle exposed to tick infection invariably contract the disease with a high percentage of mortality, the inference being that from the transmitting stage in the tick all stages develop, whereas the early stages of *T. Parva* in cattle do not variably develop to the death producing stage.

Further work is necessary to work out definitely the life cycle of the East Coast fever parasite both in the tick and in the animal host, and to determine the existence or otherwise of an immunity producing stage as distinct from a disease producing stage.

In diseases such as Redwater, Anaplasmosis, *P. mutans* infection, etc., the spleen has been shown to have some function associated with immunity to these diseases.

Although a larger percentage of reactors are likely to be obtained when large doses—5 to 10 c.c. of material—are injected, the experimental inoculations showed that even a 1 c.c. dose of gland juice collected in the early stages of the disease, is capable of transmitting the disease.

There are limitations to immunity in nature, particularly in calves and also in cattle born and reared on clean farms which recover from an original exposure.

In cattle, other than calves, which have been exposed to natural infection since birth in an endemic area, there is no evidence to show that immunity is not complete, in which case it seems immunity has been built up by natural re-infection. The limitations to immunity in inoculated cattle recovered from inoculation are greater than in non-inoculated cattle recovered from natural infection.

The limitations to immunity in inoculated cattle, particularly in calves, are greater than in non-inoculated cattle recovered from natural infection.

The limitations in inoculated cattle, particularly in calves, which have been subsequently exposed and recovered are greater than in non-inoculated cattle recovered from a natural attack of the disease.

It should be noted also that the best results in artificial immunization are obtained when cattle are exposed as soon as reactions from inoculation appear. This would indicate that the immunity obtained from artificial inoculation may in some cases be shortlived.

So far it has not been determined by direct methods, e.g., such as examination of material, transmission by ticks, that the recovered East Coast fever animal is a reservoir of the virus, but judging from the results of investigations by indirect methods such as splenectomy and lowering of the vitality by various methods, injections of drugs and setting up other infections, it would seem that the virus does not exist in recovered animals and is not a factor in the maintenance of infection.

Lichtenheld claims to have demonstrated complement fixing bodies in the serum of recovered animals but this observation has so far not been confirmed.

Immune serum has so far been found to have no protection or curative properties and the spleen of a recovered beast no prophylactic value. The process of immunity and maintenance of immunity in the immune animal is not known.

Under field conditions animals which have recovered from one infection after natural exposure and would be considered immune, may, as such, constitute a danger in the spread of infection inasmuch as they may not really be immune, although considered immune.

Moreover, since re-infection may occur and escape notice, it would be difficult to determine when the last case occurred unless the cattle were on daily temperature. Under the circumstances it is possible for cattle and particularly calves to maintain infection on a farm without such being observed.

Under conditions where facilities do not exist for control of the disease, immunization may be used as a temporary measure for the purpose of saving a percentage of the stock under the worst conditions.