

THE ARTIFICIAL TRANSMISSION OF EAST COAST FEVER.

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For several years all experiments in connection with East Coast fever have had as their object its artificial transmission, and yet, since the first discovery and the ensuing experiments, all attempts failed. In the *Transvaal Agricultural Journal*, No. 4, Vol. 1, 1903, I enumerated some experiments which proved that the inoculation of the blood in doses varying from a few c.c. to 2000 c.c. failed to produce the disease in the twenty-six animals which had been treated; when exposed later, all animals died from natural infection.

In 1906 these experiments were continued, but much larger quantities of blood were used. This was done by transfusion, connecting the jugular vein of a sick animal to that of a healthy animal, and transfusing blood for about twenty minutes at a time. This was repeated, but constantly with negative results. In 1907 the experiments were recommenced, and again large quantities of blood were injected subcutaneously, in one instance amounting to 7200 c.c. In another instance the blood corpuscles were first dissolved, and the remaining parasites were injected. Finally, when all experiments with huge doses of blood failed, recourse was taken to the injection of emulsions made from the spleen, lymphatic glands, and bone marrow—also the subarachnoidal liquid was used. All this material was injected subcutaneously without any positive results. Similar experiments were made by *Professor Koch* in Rhodesia, when he carried out his studies on East Coast fever, injecting blood with an emulsion of spleen pulp and lymphatic glands. He also injected huge doses of blood subcutaneously and intrajugularly without it being noticed that the animal sickened. Experiments by injecting the pulp of the spleen were carried out by *Lichtenheld* in German East Africa, who inoculated the material intraocularly, intrathoracally, and intraperitoneally, and in every instance failed to produce the disease.

It must be remembered here that the blood of an animal suffering from East Coast fever contains the *Piroplasma parvum* in large numbers, infecting almost 90 per cent. of all corpuscles, and it was therefore a puzzle to all investigators to see negative results from the injection of material which contained what was thought to be the cause of the disease.

When recourse was taken to the inoculation of material from the spleen and from the lymphatic glands, it was done on the supposition that the peculiar bodies described as plasma bodies or blue bodies or Koch's granules stood in a certain relation to East Coast fever, representing a form of the cycle of *Piroplasma parvum*, and it was thought that the inoculation of such material would transmit the disease. It failed, as pointed out before. In some of my experiments the spleen, after having been removed under aseptic precautions, had been kept in the incubator under the supposition that these plasma bodies would develop into the piroplasm, or at least would undergo further changes which would render them fit to infect a susceptible animal.

In view of our removal from Daspoort to Onderstepoort, and owing to the absence of the necessary ticks, the experiments had to be discontinued for the time being, but were naturally taken up again after the new quarters were occupied.

Having taken into consideration that all previous attempts by inoculation with blood, spleen, and lymphatic glands had failed to transmit the disease, a new plan of operations was devised in which it was thought to find out in the first instance whether the transference of whole organs of a sick animal into the system of susceptible animals would convey the disease. These experiments were started with the transplantation of the whole spleen of an animal slaughtered in the last stage of East Coast fever. The first attempt gave positive results, and the further arrangement of experiments came as a natural sequence.

It must, however, be admitted here that the experiments received a certain impetus and a definite line of working after the possibility of puncturing the spleen of an animal *intra vitam* had been demonstrated to me. The possibility of injecting infected material into the spleen and lymphatic glands of a susceptible animal came as a natural suggestion. This turn of experiments resulted from a visit to Nairobi, British East Africa, where Mr. Sturdy, the Principal Veterinary Surgeon of that Colony, explained to me their method of puncturing the spleen of an animal during life for the purpose of obtaining material for diagnostic purposes. He also pointed out to me that he had utilized this method to inject material obtained from an East Coast fever animal into the spleen of a susceptible animal, but failed in his object to transmit the disease. The method of operation is fully explained in the Annual Report of the Principal Veterinary Surgeon of British East Africa, 1909, to which I would refer for further particulars.

In the following *exposé* the experiments are not enumerated in chronological order, but are arranged according to the site of insertion or inoculation, and are sub-divided according to material injected.

Appendices are attached at the end of the experiments showing

- (1) the reference numbers of the ticks used to test animals on their immunity, and showing that ticks of the same lot had produced the disease in other animals;
- (2) an analysis of results arranged according to experiments;
- (3) summary of results arranged according (1) to material injected and (2) to manner of injection, omitting all cases where blood was used for the experiments and all cases of animals dying of other causes (septicaemia, etc.);
- (4) results arranged according to origin and generation of the material used.

EXPERIMENT I.—INTRAPERITONEAL INSERTIONS AND INJECTIONS.

“ A ”—INSERTION OF THE WHOLE SPLEEN.

(a) *Spleen of Calf 688.*

NOTE.—Calf 688.—This animal had contracted East Coast fever in a natural way from ticks. It was killed on the eleventh day of the disease.

(1) *Bull calf* 569.—Fifteen months old; born at the Laboratory.

Treatment.—The spleen of calf 688, removed immediately after slaughter, was inserted into the peritoneal cavity of calf 569 on the 2nd June, 1909.

Result.—Between the fourth and seventh days there was an irregular temperature reaction. In the evening of the thirteenth day a high reaction started which lasted until the twenty-first day, on which date the animal was killed. During this reaction *P. parvum* was noticed; it increased in numbers, and the examination of the lymphatic glands proved the presence of plasma bodies.

Post-mortem examination.

The condition was fair; there was a small quantity of liquid in the pericardium. The left precucular lymphatic gland was swollen. In the hypogastric region was a patch measuring 20 cm. × 20 cm., with black discoloured tissue, containing in the middle a cicatrix, the size of a small apple, corresponding with another cicatrix and a fibrous patch on the peritoneum. On the rumen were a few fibrous thickenings, corresponding with the cicatrix and the new fibrous tissue on the peritoneum.

Lungs: Had not collapsed; the pleura was whitish; the interstitial tissue was slightly oedematous and hyperaemic.

Heart: The left endocardium was whitish and the right normal; the blood was coagulated in both ventricles.

Liver: Weighed 10 lb. 6 oz.; no infarcts were present. On section it appeared flesh-coloured and hyperaemic; the bile was dark-green; it had a mucus consistence. The periportal lymphatic glands were enlarged and soft.

Spleen (extracted and inserted into calf 560, *vide* Experiment 1-2): Normal size.

Stomach: The fourth stomach was swollen, the folds were hyperaemic.

Small intestines: The contents of the omasum were somewhat dry.

The duodenum: The mucosa was swollen and slightly congested.

The jejunum: The mucosa was swollen and diffusely hyperaemic.

The ileum: Large intestines; the mucosa was swollen and cross-striped.

The caecum: Had contents; its mucosa was pale and showed a few red patches.

The colon: Mucosa was swollen and slate-coloured; one nodule was found containing pus.

Kidneys: The total weight was 1 lb. 9 oz.; two large infarcts and disseminated small infarcts were seen in the right kidney; only one large infarct was present in the left kidney.

Internal lymphatic glands: Were swollen and the haemo-lymphatic glands were slightly haemorrhagic. Both tonsillae were swollen and enlarged to the size of a small apple.

Marrow of bones: In femur and humerus they were slightly yellowish and oedematous.

Diagnosis: East Coast fever.

(b) *Spleen of Bull Calf* 569.

NOTE.—Bull calf 569 was killed on the 23rd June, 1909, *in extremis*, as a result of intraperitoneal insertion of spleen (see previous animal).

(2) *Bull calf* 560.—Age fifteen months; born at the Laboratory.

Treatment.—On the 23rd June, 1909, the spleen of calf 569 was inserted into the peritoneal cavity of calf 560.

Result.—On the fourth day the animal died, and, on *post-mortem* examination, the diagnosis of acute peritonitis was made.

Summary of results obtained from the Intraperitoneal Insertions of a Whole Spleen.

(1) The insertion of a whole spleen taken from a calf, killed on account of East Coast fever, into the peritoneal cavity of a susceptible calf, was followed by a typical East Coast fever reaction differing in no way to the natural course of the disease.

(2) The operation did not succeed in a second animal, which died on the fourth day of peritonitis.

“ B ”—INSERTION OF PIECES OF THE SPLEEN.

(a) *Spleen of Heifer 684.*

NOTE.—Heifer 684 had contracted East Coast fever from the infestation of four brown adult ticks, collected from cattle suffering from East Coast fever in the Zwartkoppies Location, near Pietermaritzburg, Natal [reference number 24 (a)]. It had an incubation time of nineteen days, and was killed on the twenty-eighth day, after *Piroplasma parvum* had been found in large numbers in the red corpuscles. The spleen was removed and immediately cut into strips.

(3) *Madagascar bull 875.*—An aged beast.

Treatment.—On the 1st July, 1909, five pieces of spleen of heifer 684 were inserted into the peritoneal cavity of bull 875.

Result.—Only a slight reaction followed during the incubation time. The examination of the blood showed the presence of *P. mutans*. After an incubation period of eleven days an East Coast fever reaction set in, when *P. parvum* appeared and increased in numbers. The animal died during the night of the 16th-17th July from East Coast fever complicated with peritonitis; the presence of the plasma bodies was demonstrated in the lymphatic glands.

Post-mortem examination.

The condition was fair; in the regio hypogastrica, on the left side, was an abscess; the left prescapular lymphatic gland measured 13 cm. \times 5 cm.; the diaphragm was hyperaemic and showed fibrous filaments. From the fourth to the tenth rib ecchymoses and suffusions were noted; the pericardium was injected but did not contain liquid; the diaphragm was covered with fibrine; a portion of the peritoneum, about 50 cm., was attached to the rumen, containing two abscesses, the size of a child's head, with black liquid contents and fibrinous coagula; the diaphragm was connected with the spleen by fibrinous coagula and was covered with pus-like liquid; the sub-maxillary glands were slightly hyperaemic; the retro-pharyngeal glands were enlarged and diffusely hyperaemic.

Lungs: Had not collapsed; the margin was round and the pleura whitish; the lungs were slightly oedematous and hyperaemic; the bronchial and mediastinal lymphatic glands were soft, swollen, and congested.

Heart: Was brown-greyish in colour; the left and right endocardia were normal; there was blood coagulum in the valves; the myocardium was slightly greyish and hard; the mediastinal glands measured 11 cm. \times 3 cm.; they showed haemorrhagic infiltrations; the epicardium showed ecchymoses and petechiae along the sulcus coronarius and radix aortae.

Liver: Weighed 21 lb.; it was swollen, enlarged, and was covered with fibrine; it was of a brownish-grey colour; the bile ducts were distended; distomum hepaticum were present; the periportal lymphatic glands were enlarged and soft; the gall bladder was swollen, and showed patchy hyperaemia; the bile was of a reddish-yellow colour.

Spleen: Measured 56 cm. \times 18 cm.; it was covered with fibrine on the diaphragm side; the capsule was thickened and covered with fibrous filaments; the pulp was soft and protruded on section.

Stomach: The mucosa of the fourth stomach was slate-coloured and showed a slight oedema of the folds; there were dry contents in omasum.

Small intestines: The jejunum contained bile-stained mucus; the mucosa was slate-coloured and showed slight patchy hyperaemia; the ileum was slate-coloured and a few parasitic nodules were found in the submucosa.

Large intestines: The mucosa of the caecum was slightly swollen, the ramifications of the blood vessels showed up well, and streaky hyperaemia was present; the mucosa of the colon was slate-coloured, with a few hyperaemic patches.

Kidneys: Total weight, 21 lb. 6 oz.; the left kidney was slightly swollen and showed two typical infarcts; one lobule of the cortex was atrophied; the super-renal glands and cortex were hyperaemic; in the right kidney were ten typical infarcts protruding over the surface; the tissue surrounding the infarcts was slightly congested; the pharynx was slightly congested; the tonsillae were normal and the epiglottis was slightly congested.

Skull: The pia was slightly milky; the grey and white substance slightly yellowish. Bone marrow: That of the ribs was red and slightly gelatinous, that of the humerus was yellowish; a few hyperaemic patches were noticed in the diaphysis.

Diagnosis: East Coast fever and peritonitis.

(b) *Spleen of Heifer 686.*

NOTE.—Heifer 686 had contracted the disease after infestation with ten brown adult ticks collected from heifer 680 (reference number 174). The disease developed after an incubation time of eighteen days; the animal was killed on the twenty-sixth day, having shown the plasma granules in the lymphatic glands during life, and on *post-mortem* examination in the spleen as well.

(4) *Africander bull 615.*—Fifteen months old.

Treatment.—On the 8th August, 1909, two portions of the spleen taken from heifer 686, measuring 15 c.m. × 12 cm., were inserted into the peritoneal cavity, and attached to the peritoneal wall of bull 615.

Result.—This operation was not followed by a typical temperature reaction as would allow of a distinction between incubation time and disease. It was of an irregular nature with exacerbations to about 103° F. No examinations of the blood or glands were made, and the irregular temperatures were not considered to be typical of East Coast fever.

Immunity Test.—On the 9th January, 1910, this animal was tested on its immunity by an infestation with six brown adults collected from sick beast No. 677 (reference number 153). On the 12th January a similar number of the same lot were placed on. Thirteen days after this last infestation the temperature started to rise, and a fever reaction ensued which was in every instance typical for East Coast fever, returning to normal on about the twenty-seventh day. The blood examination during this reaction revealed the presence of only a small number of piroplasms; accordingly it was doubtful whether they belonged to the species *P. mutans* or *P. parvum*. Unfortunately, during the reaction, no search for plasma bodies was made in the lymphatic glands, and this investigation was only commenced at the conclusion of the reaction, when they were not found. On 2nd July, bull 615 was infested with brown nymphae off East Coast fever cattle Nos. 923, 917, and 700 (reference numbers 268, 335, and 309). No reaction ensued.

(c) *Spleen of Cow 830.*

NOTE.—Cow 830 had contracted East Coast fever from the infestation of brown nymphal ticks on the 24th August, 1909 (reference number 158). The disease appeared after an incubation time of about sixteen days, when plasma bodies were detected in the lymphatic glands. *P. parvum* was present in the blood daily from the eleventh day. The animal was killed on the 17th September, when plasma bodies were present in all internal organs.

(5) *Africander bull 565.*—About fifteen months old.

Treatment.—On the 17th September, directly after cow 830 was killed, a portion of the spleen was inserted into the peritoneal cavity of bull 565.

Result.—A slight fever reaction started, during which time the presence of small piroplasms, apparently *P. mutans*, was noted. From about the eleventh day the temperature reaction showed a higher elevation, reaching 104° F. as a maximum. There was, however, nothing typical of an East Coast fever reaction; but examination of the blood proved the presence of a slight anisocytosis, but parasites were absent.

The examination of the material obtained by puncture of the spleen was also negative, hence the diagnosis of East Coast fever could not be made.

Immunity Test.—On 9th January, 1910, this animal was infested with six brown adult ticks, which were collected from heifer 677 (reference number 153). Subsequently only one tick was found to be attached, and, accordingly, on the 27th January, 1910, the infestation was repeated with a similar number of ticks, this time successfully, the whole number being found attached two days later.

From the fifteenth to about the twenty-seventh day, a temperature reaction ensued, and on the seventeenth day the record was above 105° F. This reaction, however, was not quite typical for East Coast fever, the morning remissions being too low. Blood examinations during this period gave negative results. The diagnosis of East Coast fever could not be made in this instance. On the 2nd July, bull 565 was infested with brown nymphae of East Coast fever cattle Nos. 923, 917, and 700 (reference numbers 268, 335, and 309). No reaction was observed.

(d) *Spleen of Cow 592.*

NOTE.—Cow 592 contracted East Coast fever from the intraperitoneal insertion of lymphatic glands of cow 830 [*vide* Experiment II (9)].

(6) *Ox 828.*—A Transvaal animal about fifteen months old.

Treatment.—On the 24th October, 1909, a piece of the spleen of cow 592, measuring 10 cm. × 15 cm., was inserted into the peritoneal cavity of ox 828.

Result.—The animal died on the 2nd November from peritonitis.

Summary of results obtained from the Intraperitoneal Insertion of Pieces of Spleen.

Of four susceptible animals which received an intraperitoneal insertion of pieces of spleen of animals killed on account of East Coast fever, one contracted East Coast fever and died of this disease with a complication of peritonitis. In two cases the disease could not be diagnosed; one of them reacted when tested with infected ticks and recovered, the other proved to be immune to this test; the fourth one died of peritonitis on the ninth day.

“C”—INJECTION OF SPLEEN PULP.

(a) *Spleen Pulp of Cow 594.*

NOTE.—Cow 594 had contracted East Coast fever from the infestation of five adult brown ticks received from Natal (reference number 225). The disease had an incubation time of fourteen days; the animal was killed on the twenty-ninth day (15th December, 1909). *Piroplasma parvum* was frequently noticed in the blood.

(7) *Heifer 831.*—About two and a half years old; imported from the Cape Colony.

Treatment.—On the 15th December, 1909, about 50 c.c. of the spleen pulp, obtained from cow 594, was injected into the peritoneal cavity of heifer 831.

Result.—With the exception of a slight irregular disturbance, no temperature reaction occurred. Occasional examinations of the blood gave negative results.

NOTE.—This animal was used later for intralymphal and intrajugular injection [see Experiments III (10) and VI (8)] without contracting East Coast fever.

Results obtained from the Intraperitoneal Injection of Spleen Pulp.

The intraperitoneal injection of spleen pulp into one animal was not succeeded by the appearance of East Coast fever.

“D”—INSERTION OF LYMPHATIC GLANDS.

(a) *Glands of Heifer 686.*

NOTE.—Heifer 686 had contracted the disease from the infestation of ten adult brown ticks from heifer 680 (reference number 174) [*vide* Experiment I (4)].

(8) *Bull calf 458.*—Born at the Laboratory, two years old.

Treatment.—On the 8th August, 1909, four mesenteric lymphatic glands and three bronchial and mediastinal glands from heifer 686 were placed into the peritoneal cavity of calf 458.

Result.—Three days later this animal showed an irregular temperature, reaching 104° in the evening, and on the ninth day 105.4° in the evening, returning to normal on the fifteenth day. From the nature of the subsequent reaction it had to be concluded that there was nothing typical of an East Coast fever infection and the blood was not examined.

Immunity Test.—On the 12th January the animal was tested by the infestation of six brown adult ticks collected from heifer 677 (reference number 153). After an incubation time of fifteen days, a reaction ensued which in every respect was typical for East Coast fever. The puncture of the spleen on the third day of the disease, and again on the fifth day, showed the presence of the plasma bodies. *P. parvum* was noticed subsequent to the first puncture of the spleen. The animal was killed twenty-five days after the infestation, or ten days after the first rise of temperature (3rd February).

(b) *Glands of Cow 830.*

NOTE.—Cow 830 contracted East Coast fever from the infestation of brown nymphæ collected from ox 675 (reference number 158) [see Experiment I (5)].

(9) *Africander cow 592.*—Ten years old.

Treatment.—On the 17th September the supramammary and retropharyngeal glands of cow 830 were placed in the peritoneal cavity of cow 592.

Result.—There was an immediate reaction, with a temperature of 104° F. in the evening on several occasions. During this reaction small piroplasms could be detected in the blood, probably belonging to the species *P. mutans*. On the eleventh day a second rise of temperature took place which lasted up to the thirty-seventh day, on which date the animal died of East Coast fever. Small piroplasms were found corresponding with the rise of temperature, but in the course of the following days their numbers increased, and they became so numerous that there could not be any doubt as to their nature. On the nineteenth day the plasma bodies were found in the lymphatic glands, and again on the twenty-seventh day; the prescapular glands were examined, and again the presence of the plasma bodies was recorded. On the thirty-fifth day a spleen puncture was made and the plasma bodies were noted to be fairly frequent. During the reaction the animal was infested with brown larvae on several occasions, which engorged and were collected as nymphæ (reference number 153).

Post-mortem examination.

The condition was good; the subcutaneous tissue was dark-yellowish, also the fat was yellowish; there were a few ecchymoses on serosa propria of pericardium.

Lungs: Head collapsed; the pleura was whitish and folded; a pneumonia (stages 1 and 2) was present in both lungs; some lobuli had calcified necrotic contents. On section the lungs presented a mottled appearance and a slight oedema; the trachea was slightly injected; the bronchi were slightly injected and yellowish; the mediastinal glands were slightly hyperaemic.

Heart: There were ecchymoses and petechiae on the left endocardium; the right endocardium was normal; there were suffusions and ecchymoses on epicardium; the fat was yellowish.

Liver: Weighed 18 lb.; there were spots on the capsule corresponding with infarcts in the parenchyma; the parenchyma had a brownish-yellow mottled appearance; the peritoneal lymphatic glands were slightly swollen; the capsula glissoni was slightly thickened; the white spots reached the size of a cherry, often surrounded by hyperaemic areas; the gall-bladder was injected; the bile was thick and dark-green.

Spleen: Was enlarged, measuring 65 cm. × 25 cm., with the margin slightly rounded; the capsule was not folded; the pulp was dry, and the follicles were distinct.

Fourth stomach: Contained food; the mucosa was slate-coloured with some small round ulcers; the contents of the omasum were slightly liquid.

Small intestines: Peyer's patches were swollen; the mucosa of the jejunum was slate-coloured, with some small ulcers; the ileum was pale; Peyer's patches were swollen; the ileocaecal valve was of a black colour.

Large intestines: The caecum was contracted; a few small ulcers and nodules, together with hyperaemic streaks, were noted; the mucosa of the colon was slate-coloured, with some hyperaemic patches; the mesenteric glands were slightly swollen; the sinuses were slightly haemorrhagic.

Kidneys: Weighed 2½ lb. together; an infarct in the right kidney, the size of a pea, and numerous small infarcts with red areas were noted; the left kidney was also spotted with infarcts and with red areas.

Skull: The pia was slightly milky; in the frontal lobe was slight pigmentation; the white substance was discoloured; the larynx and pharynx were normal, and the tonsillae were of normal size.

Bones: Of ribs slightly oedematous; of femur and humerus strong citron-yellowish colour.

Diagnosis: East Coast fever.

(c) Glands of Cow 592.

Cow 592 contracted East Coast fever from the intraperitoneal insertion of lymphatic glands. [See previous animal, No. (9).]

(10) Cow 679.—Obtained from the Experimental Farm, Standerton.

Treatment.—On the 24th October, 1909, the following glands from cow 592 were inserted into the peritoneal cavity of cow 679:—1 suprarenary, 3 lumbal, and 1 precrucal.

Result.—On the second day after insertion there was a high rise of temperature reaching over 106° F., but descending to normal again about the tenth day, from which date a new reaction ensued resembling to a certain extent that of East Coast fever, lasting until the twenty-sixth day, with evening records averaging about 105° F. During this reaction the blood examinations proved the presence of small protozoans.

On the eighteenth day a puncture of the spleen was made and of the precapular glands, but no plasma bodies were found. Accordingly, although the temperature reaction resembled East Coast fever, there was no support to such a diagnosis.

Immunity Test.—On the 9th January, 1910, the animal was infested with six brown adult ticks from cattle 677 (reference number 153). No reaction ensued from this infestation. On the 27th January a second infestation was made with the same number of ticks of the same series, and again no reaction followed.

On the 2nd July a third infestation was made with brown nymphae collected from East Coast fever cattle Nos. 923, 917, and 700 (reference numbers 268, 535, and 309). No reaction followed.

(11) *Cow 682*.—About eight years old; received from the Standerton Experimental Farm.

Treatment.—On the 24th October, 1909, the following glands from cow 592 were inserted into the peritoneal cavity of cow 682:—1 supramammary, 3 lumbal, and 1 precrucal.

Result.—The day after the operation the temperature rose and remained high until the tenth and eleventh days, when it went over into a reaction which resembled to a certain extent that of East Coast fever, but during this time no piroplasms were noticed in the blood, and accordingly no definite diagnosis could be made.

Immunity Test.—This cow was tested on its immunity on the 9th January, 1910, by the infestation of six brown adult ticks of heifer 677 (reference number 153).

On the eleventh day after infestation the temperature rose and a typical reaction ensued, from which the animal died on the twenty-first day; that is, ten days after the rise of temperature the presence of plasma bodies was demonstrated in the prescapular glands. On the fifteenth day the same bodies were found in the spleen, and on the same date *Piroplasma parvum* was noted in the blood; the parasites increased in number and were very frequent on the day of death.

(d) *Glands of Cow 677*.

NOTE.—Cow 677 had contracted East Coast fever from the infestation of five adult brown ticks obtained from Natal (reference number 225). The disease commenced after an incubation time of thirteen days. The animal was killed on the twenty-seventh day. Parasites were frequent in the blood at the time of death, and the plasma bodies were noticed in all organs.

(12) *Heifer 895*.—Purchased in Pretoria in October, 1909, and about two years old.

Treatment.—On the 13th December, 1909, the following glands of cow 677 were inserted into the peritoneal cavity of heifer 895:—1 supramammary, 1 prescapular, and 1 subiliacal.

Result.—The day after the operation the temperature commenced to rise, and a regular reaction was noticed during the first ten days. High evening records continued, with morning remissions to normal limits. Starting from the sixteenth day another reaction was noticed, which was not so typical for East Coast fever, but the examination of the blood proved the presence of small piroplasms, probably of the *P. mutans* species. The examination of the prescapular gland did not show the presence of plasma bodies, and accordingly it was not certain whether the reaction was due to East Coast fever.

Immunity Test.—On the 10th February, 1910, this animal was tested on its immunity by the infestation of four brown adult ticks from cow 677 (reference number 153).

Only one tick became attached, and on the 3rd March, 1910, a further four adults of cow 592 (reference number 153) were placed on, when three ticks attached themselves. On the 24th March, 1910, a third test was made by placing on a large number of nymphal ticks of calf 700 (reference number 309); about 100 of these ticks

became attached. On the 2nd July, 1910, it was infested with brown nymphae off East Coast fever cattle Nos. 923, 917, and 700 (reference numbers 268, 335, and 309). No reactions succeeded these infestations.

(e) *Glands of Cow 594.*

NOTE.—Cow 594 had contracted East Coast fever from the infestation of four adult ticks obtained from Natal (reference number 225). [*Vide* Experiment I (7).]

(13) *Heifer 888.*—A heifer obtained from the Aliwal North District, Cape Colony; about one year old.

Treatment.—On the 15th December, 1909, the following glands of cow 594 were inserted into the peritoneal cavity of heifer 888:—1 supramammary, 1 prescapular, and 1 precrural.

Result.—A slight irregular reaction commenced on the twenty-sixth day after the operation, during which small piroplasms, probably of the type *P. mutans*, were noticed. Starting from the thirteenth day, another reaction began, but no high records were noticed. On the twenty-sixth day the reaction returned to normal, and a new reaction started, still with moderate rises, ending fatally on the thirty-first day. During this reaction small piroplasms were found, although only in scanty numbers, and which did not admit of a definite diagnosis.

Post-mortem examination.

The condition was fair; the muscles were pale and of a greyish colour; there were haematoma on the diaphragm; the muscles on the head were dark-red; the subcutaneous tissue showed haemorrhagic infiltrations; the intermaxillary space and the neck showed watery infiltrations; there were sugillations on the flank, sternum, and abdominal wall; on the right hind leg from knee upwards was a large haemorrhage, and similarly inside the left leg; the deep pectoral muscle was spotted with haemorrhages; the pleural cavities contained some blood-stained liquid; the mediastinum showed imbibition and ecchymoses; there were ecchymoses and petechiae on the right side of costal pleura; the pericardium showed haemorrhagic infiltrations, and contained about 5 c.c. blood-stained liquid; in the peritoneum was a small abscess, the size of an apple, containing yellowish pus; the pyogenic membrane was black; the submaxillary and retropharyngeal lymphatic glands were swollen.

Lungs: Had collapsed; the right anterior lobe showed a slight emphysema, with fibrous filaments on pleura, and there were some subpleural haemorrhages; the right middle lobe was black, and there was a fibrinous deposit on the pleura; a few lobuli showed red hepatization; there was a small haemorrhagic infarct in the right anterior lobe; the left anterior lobe was pale and showed some haemorrhagic infarcts; a slight oedema was also present.

Heart: The left endocardium showed sugillations, suffusions, ecchymoses, and sub-endocardial haematomes; the right endocardium showed imbibition and a few ecchymoses; the bronchial and mediastinal lymphatic glands showed haemorrhagic infiltration and a few white foci; the epicardium, especially the apex, showed diffuse ecchymoses.

Liver: Was slightly enlarged and had a mottled appearance; ecchymoses were noted; the bile was greenish-yellow; the mucosa of the bladder was normal; the periportal lymphatic glands were slightly swollen.

Spleen: The upper part showed ecchymoses and petechiae, and was connected with the rumen, and the latter with the diaphragm; the spleen measured 40 cm. \times 12½ cm.; the vessels were injected, and the pulp was moist; the follicles and the trabeculae were distinct.

Stomach: The mucosa of the fourth stomach was slate-coloured; the folds were slightly oedematous, and a few ecchymoses were present; the omasum had dry contents.

Small intestines: The mucosa of the duodenum and jejunum was of a black-greenish colour; that of the ileum was oedematous and showed a few sugillations.

Large intestines: The caecum was slightly swollen, partly folded, and showed some injections of the capillaries; the mucosa of the colon was slate-coloured; the ileocaecal valve was normal.

Mesentery: Showed diffuse sugillations, and also on surrounding tissue of omasum.

Kidneys: Had a greyish mottled appearance and were oedematous; pale on section; there was a slight hypostasis of the right kidney; the pericapsular tissue was strongly

oedematous, and showed haemorrhagic infiltrations; the capsule resembled that of a haematoma; the psoas muscle showed haemorrhagic infiltrations and suggillations, and the same was noted in the pelvis; the peripharyngeal tissue was oedematous; the pharynx and larynx were normal; the sublingual muscles showed strong oedematous infiltrations; the tonsillae were swollen and showed haemorrhagic infiltrations; the conchae were slightly swollen, ecchymotic, and suggillated.

Skull: The brain was pale; a slight oedema was present; a few pigments were seen in frontal lobe; on section it had a shiny appearance.

Marrow of bones: Of femur was slightly oedematous; that of humerus showed a few small red patches; that of the ribs was strongly oedematous and watery.

Cause of death: East Coast fever.

(f) *Glands of Bull Calf 458.*

Bull calf 458. This animal died of East Coast fever as a result of the intraperitoneal insertion of lymphatic glands [see Experiment I (8)].

(14) *Heifer 871.*—An animal obtained from Aliwal North District, Cape Colony; about one year old.

Treatment.—On the 3rd February, 1910, two small retro-pharyngeal glands of bull 458 were inserted into the peritoneal cavity of heifer 871.

Result.—Soon after the operation an irregular temperature commenced, in no way typical for East Coast fever. Nevertheless, the examination of blood was repeatedly made but with negative results.

NOTE.—This animal was used later for an intralymphal injection [*vide* Experiment III (8)]. Subsequently it was tested on two occasions by the infestation of infected brown nymphal ticks and proved immune.

(15) *Heifer 833.*—Received from the Aliwal North District, Cape Colony; about eighteen months old.

Treatment.—On the 3rd February, 1910, the left prescapular lymphatic gland of bull 458 was inserted into the peritoneal cavity of heifer 833.

Result.—An irregular temperature reaction commenced two days later in no way typical for East Coast fever. The examination of the blood was carried out every day for a period of twenty-two days, but with negative results.

NOTE.—This animal was used later for intralymphal injections [*vide* Experiment III (9) and III (34)].

(16) *Ox 621.*—An Africander animal; about eighteen months old.

Treatment.—On the 3rd February, 1910, the left precrucial lymphatic gland of bull calf 458 was inserted into the peritoneal cavity of ox 621.

Result.—No temperature reaction ensued in this instance, and all blood examinations were negative.

NOTE.—This animal was used later for experiments with intralymphal injections [*vide* Experiment III (5)], and at a later date was infested with infected brown nymphae, proving immune.

(17) *Ox 661.*—An animal from the Aliwal North District, Cape Colony; about one year old.

Treatment.—On the 3rd February, 1910, the inguinal gland of bull calf 458 was placed into the peritoneal cavity of ox 661.

Result.—An irregular reaction resulted during the first eleven days with a mild rise. The examination of the blood proved negative.

NOTE.—Ox 661 was used later for intralymphal injections [*vide* Experiment III (6)] and died of East Coast fever.

Summary of Results obtained from the Intraperitoneal Insertion of Lymphatic Glands.

The intraperitoneal insertion of lymphatic glands (collected from animals infected with East Coast fever) into ten susceptible animals was followed by the development of the disease and death in two animals. Two showed atypical reactions, and succumbed to the disease when tested with infected ticks. One showed an atypical reaction and died of East Coast fever as the result of a later intralymphal injection. Three showed atypical reactions and proved immune when tested at a subsequent date with ticks. One showed an atypical reaction and has not yet been tested. The tenth animal gave negative results to the insertion, but showed an East Coast fever reaction when used later for an intralymphal injection of lymphatic gland juice.

“ E ”—INJECTIONS OF LYMPHATIC GLAND JUICE.

(a) *Lymphatic Gland Juice of Heifer 884.*

NOTE.—Heifer 884 contracted East Coast fever from the intrasplenic injection of spleen pulp [*vide* Experiment II (6)].

(18) *Calf 878.*—Born at the Laboratory; about eight months old.

Treatment.—Injected intraperitoneally on the 23rd March, 1910, with 100 c.c. lymphatic gland juice of heifer 884.

Result.—Two days later a reaction commenced, during which time the presence of *P. bigeminum* and *P. mutans* was noticed. The animal died on the twenty-first day of septicaemia, and microscopical examination of the blood taken from the organs during post-mortem examination did not reveal the presence of plasma bodies.

Result obtained from the Intraperitoneal Injection of Lymphatic Gland Juice.

The only animal used in this experiment died on the twenty-first day of septicaemia.

“ F ”—INJECTION OF BLOOD.

(a) *Blood of English Heifer 923.*

NOTE.—Heifer 923 was an animal which arrived from England on the 28th December, 1909. It contracted East Coast fever from the infestation of ticks (reference number 153). Piroplasms were frequent in the blood, and plasma bodies were found before death in the lymphatic glands and in the spleen, and in all organs after death.

(19) *Heifer 1014.*—Received from Aliwal North, Cape Colony; about one year old.

Treatment.—Injected on the 22nd March, 1910, intraperitoneally with 100 c.c. defibrinated blood of heifer 923.

Result.—No reaction commenced, but nevertheless the examination of the blood was undertaken every second day with negative results in all instances.

NOTE.—This animal has not yet been tested.

Results obtained from the Intraperitoneal Injection of Blood.

The intraperitoneal injection of blood taken from a heifer suffering from East Coast fever did not produce the disease in the one experimental heifer.

EXPERIMENT II.—INTRASPLENIC INJECTIONS.

As already stated in the introductory notes, Mr. Stordy, of Nairobi, had demonstrated to me his method of puncturing the spleen, a description of which is given in his annual report for the year 1909. He also informed me that on one occasion he had tried the intralymphal injection of spleen pulp, but failed to obtain positive results.

Accordingly our experiments were again undertaken, but with larger quantities of spleen pulp than had been used by Mr. Stordy. The syringe used for the operation had rather a large canula.

“ A ”—INJECTIONS OF SPLEEN PULP.

(a) *Spleen Pulp of Cow 594.*

NOTE.—Cow 594 contracted East Coast fever from the infestation of ticks [*vide* Experiment I (c)].

(1) *Heifer 874.*—Received from Schoombie, Cape Colony; about one year old.

Treatment.—On the 15th December, 1909, this heifer received an intrasplenic injection of 50 c.c. spleen pulp of cow 594.

Result.—Two days after injection an irregular reaction commenced, during which small piroplasms (*P. mutans*) were noticed. The temperature remained high during the next nineteen days, when a distinct curve commenced; the examination of blood proved the presence of small piroplasms in rare numbers.

On the twenty-seventh day the prescapular gland was punctured and the plasma bodies were noticed to be present but rarely. The animal died on the twenty-ninth day after infection (13th January, 1910).

Post-mortem examination.

The condition was poor; there was yellowish liquid in the subcutaneous tissue and but little in the pleural cavities; the costal pleura showed a few fibrous filaments; the pericardium contained about 50 c.c. liquid.

Lungs: Had not collapsed; the left pleura was yellowish, with haemorrhagic patches. There was a strong oedema with serous liquid in the anterior lobe; there was slight yellowish foam in trachea and bronchi; the bronchial and mediastinal lymphatic glands were swollen.

Heart: There were some ecchymoses on the papillary muscle; the ventricles were empty; the right endocardium was normal; the blood was coagulated; there were some ecchymoses on sulcus coronarius.

Liver: Weighed 32 lb.; it had a granulated appearance, and on section was of a saffron colour; small abscesses, corresponding to yellow points, were present on the capsule; the periportal lymphatic glands were enlarged to the size of an apple, their sinuses showed haemorrhagic infiltrations; there were some yellowish thickenings on glissoni's capsule, and some subcapsular haemorrhages; the gall-bladder was injected, and contained thick dark-brown bile.

Spleen: Measured 52 cm. × 21 cm.; on the dorsal part was a small tumour; the capsule was slightly thickened and of a citron yellow colour; the pulp was dark red; the follicles were slightly swollen and protruding.

Stomach: There was a little food in fourth stomach; the folds were slightly oedematous, and some petechiae were present; the omasus had dry contents.

Small intestines: The jejunum was contracted and the mucosa was folded, showing some hyperaemic patches; the mucosa of the ileum was pale, with some red streaks.

Large intestines: The caecum was contracted; the mucosa was of a slight slate-colour; Peyer's patches were swollen.

Kidneys: Were of a yellowish colour; there was a distinct injection of glomerules the capsule was easily detachable.

Internal lymphatic glands: Were slightly swollen and moist.

Cause of death: East Coast fever.

(b) *Spleen Pulp of Bull Calf 458.*

NOTE.—Bull calf 458 had been injected with lymphatic glands, but did not show a typical reaction; it died of East Coast fever when infested with brown adults [*vide* Experiment I (8)].

(2) *Ox 337.*—Received from Potchefstroom—history unknown—about eight years old.

Treatment.—On the 3rd February, 1910, 30 c.c. spleen pulp of bull calf 458 was injected into the spleen of ox 337.

Result.—A slight reaction followed, but, from the twelfth day onwards, a more definite curve ensued, with remissions on the twenty-first and twenty-second days. Ox 337 was killed on the twenty-seventh day for experimental purposes.

Seventeen days after injection the plasma bodies were noticed in the lymphatic glands, and at the same time rare piroplasms were diagnosed in the blood. The number of piroplasms now increased daily, and reached a maximum on the twenty-fifth day. Two days later *P. bigeminum* was noticed. On examination of the spleen at *post-mortem*, plasma bodies were found.

Post-mortem examination.

The condition was fair; the prescapular and precrucial glands were rather large, and there was slight haemorrhagic infiltration of the sinuses.

Lungs: Had not collapsed; there were a few yellowish patches on the pleura; a strong interstitial oedema was present; the trachea and bronchi were injected and contained foam; the bronchial lymphatic glands were slightly swollen; the mediastinal lymphatic glands were swollen and soft.

Heart: The left endocardium showed a few ecchymoses on the valves; the ventricle was empty; the right endocardium was pale.

Liver: Was strongly swollen; on section it had a light brownish colour; stasis of the bile was noted; in the middle was a small hard abscess with green pus; the bile was thin and of a brownish-green colour; the vessels of the mucosa of the bladder were injected; the bile ducts were slightly enlarged; the periportal lymphatic glands were swollen; the capsule glissoni was thickened, and showed a few subcutaneous haemorrhages.

Spleen: The spleen measured 45 cm. × 14 cm.; some abscesses were noted in the dorsal part with brownish contents; the pulp was dark-brown in colour; the trabeculae and the follicles very distinct.

Stomach: There was some food in the fourth stomach; the folds were slightly oedematous and slightly hyperaemic; the omasum had dry contents.

Small intestines: The mucosa of the jejunum was swollen, and showed strong hyperaemia; the ileum had bile-stained contents; the mucosa was swollen and hyperaemic.

Large intestines: The caecum had bile-stained contents; the mucosa was swollen and showed longitudinal black streaks; patches of hyperaemia were noted; the contents of the colon were black, its mucosa was swollen.

Kidneys: Were dark-red in colour; a few infarcts, rather small, were noted in the cortex; there was a slight injection of the glomeruli.

Skull: The pia of the anterior lobes showed slight pigmentation.

Marrow of bones: Of femur and humerus were normal; the larynx and pharynx were normal; the tonsillae were slightly swollen.

Cause of death (killed): East Coast fever.

(c) *Spleen Pulp of Ox 337.*

Ox 337.—*Vide* previous animal.

(3) *Ox 620.*—Received from the Africander Stock Farm.

Treatment.—On the 2nd March, 1910, this animal received an intrasplenic injection of 30 c.c. spleen pulp of ox 337.

Result.—An irregular temperature was noticed during the first few days, and from the thirteenth to twenty-sixth day a more definite reaction could be distinguished during which the examination of the blood proved negative, and the examination of the prescapular glands