

to find any more bones. Cow 2844 was the first animal to lie down. She was followed by heifer 3856 and tollie 3903 and a little later by heifer 3910 and tollie 3930. Some of the remainder went to the salt-box, viz., cow 2968, heifer 3900, heifer 3936.

11.7.19: Shortly after 9 a.m. the remaining twelve animals entered the paddock. Cow 2844, tollies 3903 and 4105 found something to munch, but in a few minutes they had finished.

Subsequently the cattle stood quietly in the bone place, only one moved to the salt-box (heifer 3900) but returned shortly after 10 a.m. By this time all cattle had settled to rest and only cow 2968 was standing and ruminating. Tollie 4029 was the first animal to lie down.

12.7.19: The cattle were driven into the paddock about 9.20 a.m. To-day cow 3853 was added to this experiment. On arriving at the bone place, tollie 3903 sniffed about, and on two occasions found a piece of skin. It was taken away. Tollie 4105 and heifer 3905 sniffed about on the old place and found something to munch for a few minutes. The cattle then stood quietly for a while, and one by one lay down in the following order:—Cow 2844, heifer 3905, tollie 4029, tollie 3930, heifer 114, heifer 3936, tollie 4105, heifer 3910, heifer 3856, and heifer 3900. At 10 a.m. only cow 2968 was standing amidst the herd. Cow 3853 kept aloof from the commencement.

13.7.19: The cattle were driven into the paddock at about 10 a.m. and they soon scattered about. Two were seen sniffing about at the old-bone place; tollie 4105 picked up a molar tooth, but dropped it when approached; tollie 4129 found something to munch for a few minutes. Heifer 114 was the first to lie down and did so soon after arrival, but subsequently she rose of her own accord. Some cattle went to the salt-box.

14.7.19: Discontinuation of experiments.

#### SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

(1) The results of the three experiments (5th and 6th report of the Director of Veterinary Research) in which cattle had been exposed in a paddock containing carcasses now find an explanation, in as much as the animals that contracted the disease must have been eating bones or carrion, although no observations at the time to this effect were made.

(2) Similarly the results of experiment 4 (5th and 6th report of the Director of Veterinary Research) in which three cases of lamsiekte occurred in the enlarged paddock containing the identical carcasses must be explained in the same way.

(3) Of special interest is the fact that one heifer contracted the disease twice, the second attack three and a half months after the first one (heifer 3614. Exp. 4, 5th and 6th report of the Director of Veterinary Research).

(4) The results of experiment 5 (5th and 6th report of the Director of Veterinary Research), although occurring in a paddock from which the carcasses were excluded must also be interpreted as above, bones having been scattered about, of which no notice had been taken at the time.

(5) It is not a mere coincidence in the above experiment that the cattle which contracted lamsiekte did so practically at the same

date or only shortly after each other. They had probably been feeding from the same material at the same time.

(6) The results of experiments 2 and 7 (5th and 6th report of the Director of Veterinary Research) in which cattle did not contract lamsiekte, although exposed to bones, can now be explained by the fact that the cattle in the first lot were freshly imported and, therefore, not yet craving for bones. The cattle in the second lot were fed on supplementary rations and thus craving was prevented.

(7) Also the grazing down experiments 1 and 6 (5th and 6th report of the Director of Veterinary Research) find an explanation. The paddocks concerned were in close proximity to the carcass paddock and scattered bones were subsequently found.

(8) The failure of the experiments commenced on the 9.12.18 (this report), in which eight head of cattle were exposed in a carcass paddock without contracting the disease, can be explained by the fact that the cattle were not bone-eaters at all, having been fed on a ration that prevented the craving.

(9) The cattle acting as control to the carcass paddock lot also remained free of the disease. They too had been fed on supplementary rations.

(10) The cattle which were picked out of the herd, having been recorded to chew bones, proved to be bone-eaters when placed in a paddock containing bones. These cattle had, however, preference for bleached bones and would not touch putrid bones.

(11) At the beginning of the experiment the cattle were eating bleached bones very eagerly and continued for a considerable length of time. Subsequently some of the cattle discontinued whilst others continued to chew bones.

(12) One cow died as the result of bone-eating; she had consumed so much bone that the reticulum was over filled, and it burst the diaphragm, forming a hernia in the pleural cavity and displacing heart and lungs (3146).

(13) Some of the control cattle in the paddock not containing bones, also selected bone-eaters, were occasionally noted to chew bones, which they had probably picked up whilst going and coming from the water, bones, which had been carried out of the carcass paddock and then dropped by the bone-eaters. But since these cattle had no access to bones in their paddock their behaviour was quite a different one, grazing or lying down, whilst the carcass paddock cattle with free access to bones were chewing eagerly.

(14) Generally the cattle were chewing bones whilst standing; rarely, however, did they do so when in the recumbent position.

(15) Cattle, which were bone-eaters, would pick rotten bones to a very limited extent only when the bones originated from a carcass that had recently undergone decomposition. They would not pick green bones, but some would pick boiled bones. Bone-eaters were thus partial to some particular bone, whilst all would eat bleached bones.

(16) The putrid bone, generally speaking, was not the bone which was liked by all cattle.

(17) The possibility that cattle learn eating bones by imitation must be excluded.

(18) Bone-eating cattle that had free access to bleached bones would continue to search for bleached bones after such bones had been removed and would even pick small splinters and lick the ground for such splinters in preference to rotten bones lying near by. Cattle would even pick stones in the search for such bones.

(19) Cattle with no access to bleached bones but to cooked fresh bones would show preference for these and did not care for fresh green bones. Only a few would pick old rotten and still fewer new rotten bones.

(20) When cattle had no longer access to bleached bones, and were brought to rotten bones daily, some of them would commence to pick these bones and gradually develop a taste for such bones.

(21) The bones which were supplied to the cattle were taken from carcasses, the bones of which in another feeding experiment (trough feeding experiment) had proved to be toxic. In tests subsequently undertaken, however, not all bones proved to be toxic.

(22) Of the cattle which consumed putrid bones, a number developed lamsiekte, viz., 3910, 3583, 2600, 3905, 3903, 3930, 3856, 2968. It is interesting to note that these cattle belonged to the lot that had been eating bleached bones for some time previous to feeding on rotten bones and that none died of the disease. Indeed, lamsiekte in some of these instances was of an exceedingly mild type; whilst of cattle that had been added to the herd in the course of the experiments and that previously had not been feeding on bleached bones, a number died of acute lamsiekte. (Bull 2600, heifer 169, heifer 172.) Of these cattle, not all contracted the disease from the same lot of bones. The disease could be traced to two different distinct lots of bones. Bones of the first lot (original carcass paddock) produced the disease in 3910, 3583, 2600, 3903, 3905, 3930, 3856, 2968. Bones of the second lot produced the disease in 169 and 172. Of the former lot, only one animal died (2600). It had been added to the herd six days before taking ill, and was a confirmed bone-eater. All the other animals had a mild disease, and it would almost appear that the continuous consumption of putrid bones gave these animals some resistance against the disease. None of the animals contracted the disease a second time, although they continued to eat bones.

(23) In the course of the experiment it appeared that bones which were refused in the earlier period would be eaten in the later period of the experiment.

(24) A regular supply of salt, to which the cattle had access had no influence on the craving for bones. Cattle would lick salt and eat bones or vice versa.

(25) Cattle, whilst suffering from lamsiekte would continue to chew bones, unless they were too ill to keep on their legs.

(26) The bones tested at intervals for toxicity by drenching cattle with a weighed crushed quantity produced lamsiekte in some instances, and not in others. Positive results were obtained from the tests with cow 3805, drenched on 1.5.19 with one pound of crushed bones. Negative results were obtained from the tests with cow 4178, drenched on 16.5.19 with one pound of rotten bones; with tollie 4095 drenched on 3.6.19 with the same bones as used for cow 4178; with heifer 181, drenched on 22.5.19 with twelve ounces old bones; with cow 3516 drenched on the 10.7.19 with dry rotten flesh.

## APPENDIX.

## CARCASS-CAMP EXPERIMENTS.

## CASUISTICS.

1. Cow 3146 (BLUE SCHIMMEL): 20.4.19: This morning, soon after entering the kraal, the cow was noted to lie down. This was an unusual occurrence in this animal, since for a long time it had not been noticed to pick bones. At 12 noon, when the herd was driven to the water, she was unable to rise. Food and water was then brought to her. In the evening, the cow was standing at the gate, and when let out joined the herd, walking with good strides. A peculiar grunting noise was heard at regular intervals whilst walking. The abdomen was conspicuously distended on both sides, which observation had been made for some time. The cow was placed in the stable.

21.4.19: The cow was found in sterno-costal position and feeding a little. At times the grunting noise was heard. About 80-90 pulsations of the jugular vein were counted. The muzzle was dry. The examination of a blood-smear gave negative results.

22.4.19: The cow was found in the same place where left the previous afternoon. She was reported not to have been drinking water. She did not feed during the period of observation. The faeces were copious and soft. The muzzle was moist and the eyes bright. Head and ears were carried well. The cow had made no attempt to rise. In the afternoon she rose by herself, and when allowed to go to the camp she again walked freely; the grunting noise could still be heard. She was noticed to lie down whilst out of the stable.

23.4.19: This morning the cow was found in sterno-costal position. She was reported not to have been drinking water and was not seen feeding. In the evening she was again grunting.

24.4.19: The cow was still down. An attempt was made to lift her, but she would not support herself. The faces were dry, blackish, and spiral-shaped. The head and neck were carried well, and the muzzle was dry. The cow was grunting all day long. A trembling of the muscles in the forequarters was noticed. At times the cow made an attempt to shift the forelegs, but was unable to move them. The pulsation of the jugular vein was rather well pronounced, and the recurrent waves were noted to roll back high up in the neck, which was carried erect.

25.4.19: The cow was still in the same position as yesterday; apparently she felt uncomfortable in this position, since she moved the forelegs from time to time. The grunting noise was still present and was accompanied by a movement of the nostrils. She was reported not to have been drinking. The ears were drooping, but the head was carried well. No improvements were noticeable in the afternoon.

26.4.19: The cow was placed in a more comfortable position this morning. She maintained the sterno-costal position, but she now doubled the head back to the near side. In the afternoon she was stretched out flat on the ground. She died at 3 p.m.

*Post-mortem Report of Blue Schimmel Cow 3146:* The autopsy was made soon after death. Rigor mortis was absent. The condition was somewhat poor. The abdomen was much distended. The integument of both carcases showed fresh decubitus. The natural openings were closed. The pupillae of the eyes were distended. The visible mucous membranes showed no abnormalities. The blood was somewhat thin; the flesh slightly pale; the intramuscular connective tissue was in parts infiltrated with a jelly-like substance, as were also some of the superficial fasciae and aponeuroses. The subcutaneous tissue was devoid of fat, and jelly-like patches were present in several places. On cutting the parotid the knife produced a harsh noise. An increase of the interlobular connective tissue was evident. All superficial lymph nodes, with the exception of the superficial cervical, appeared rather small, but watery and embedded in gelatinous connective tissue. The superficial cervical was especially rich in watery substance. The internal lymph nodes of the trunk were also rather small. There were no changes in the tongue, pharynx, and oesophagus. In the peritoneal cavity some straw-coloured liquid was present. Both visceral and parietal serosa were smooth and glistening. The cranial portion of the rumen could not be removed from the abdominal cavity, it being diffusely attached to the diaphragm. The reticulum could not be found in the abdominal cavity, and the situs viscerum only became clear after the

opening of the pleural cavity (*vide infra*). In the pleural cavity the position of the organs was also abnormal. Behind the heart was found a tumour-like round body in size that of a man's head. This turned out to be the reticulum. It was attached to the caudal portion of the pericardium, to the costal pleura behind the heart, and to the caudal mediastinum, and part of the lung. The heart was pushed up cranio-dorsally, and the base was near the dorsum thoracis; the lungs were also pushed dorsally. The right apex and the left main lobe were attached to the opposite costal pleurae, but could easily be detached. There was a little liquid in the pleural cavities. The parietal and visceral pleurae of the non-attached parts were smooth and glistening. The pharynx contained some ingesta in the recesses; the mucosa was otherwise of usual appearance. The larynx also contained ingesta. In the cervical trachea some froth was present. The lungs were in the expiratory stage, the right lobe was full of blood, the left almost bloodless. The consistence was elastic, with the exception of the right apical lobe, which was consolidated and on section appeared white, granular, and full of liquid. The rest of the lung-tissue was slightly moist. The eparterial bronchus and its ramifications contained fibrinous plugs. The bronchi of the rest of the lungs showed no abnormalities. The intima of the pulmonary veins and arteries appeared smooth. One echinococcus cyst was found in one main lobe containing the crumbled-up cuticle of the parasite.

The pericardium contained a small quantity of clear liquid. The parietal serosa was smooth and glistening. The right ventricle and atrium were dilated but empty; those of the left heart were not dilated. The epicardium showed no changes. Some fat was present at the base of the heart. The myocardium appeared slightly opaque. A small button-like white tumour, in size that of a bean, was present on the septum cordis. The coronary arteries and the vena magna cordis showed no changes.

The periportal lymph nodes were somewhat small and conspicuously dark in colour. The parietal serosa of the ventral portion of the liver was partly attached to the diaphragm. Otherwise the capsule was smooth and glistening. The parenchyma on section was light red-brown and smooth. It was hard on pressure, and the knife caused a harsh noise whilst cutting it. Echinococcus cysts were present in the parenchyma. The gall-bladder was distended with brownish mucous bile, which, on opening the bladder, escaped as a semi-solid mass. The ductus choledochus was open; the hepatic ducts showed no changes. The pancreas appeared somewhat pale. The atrial lymph nodes were of normal size. The spleen (48 by 12) showed the capsule somewhat shrivelled; the parenchyma appeared dry and poor in pulp, the trabeculae were distinct. The suprarenal glands were rather small and unusually yellow in the cortex. The adipose capsule of the kidney was of gelatinous consistence. The fibrosa stripped easily. The kidney was rather moist and dark brown in colour. On section, all zones were distinct; the cortex was somewhat striated. The pelvis was gelatinous. A cyst was present in the cortex of one organ. In order to remove the stomachs, the diaphragm had to be cut. The cranio-dorsal portion of the rumen was diffusely attached to the diaphragm, and the reticulum was in front of it, the walls of the reticulum had blended with the diaphragm, and were much thickened by fibrous organizations. In one place a canal was present with a blackish lumen containing a piece of wire, ending in a blind alley. The rumen was greatly distended with ingesta; these did not appear to be fresh, and were somewhat dark in colour. The reticulum was filled with hard and dry ingesta, which contained numerous fragments of bones and much sand, all tightly packed. The oesophageal groove was open. The omasum was almost empty, and the remaining contents were watery. The abomasum contained watery ingesta; the mucosa showed no changes. In the duodenum mucous contents were present. The mucosa of the jejunum and ileum was reddened in some parts and cross-striped in others. The stripes were haemorrhagic. The mucosa of the large intestines was normal. The mesentery was somewhat watery, the lymph nodes of usual size and appearance. The urinary bladder was distended with clear urine. The mucosa was normal. The uterus showed no abnormalities. The brain was of usual appearance.

*Pathological Anatomical Diagnosis.*—Hernia of the reticulum in the pleural cavity. Impaction of the reticulum with foreign bodies (bones and sand); impaction of the rumen. Translocation of the heart and lungs. Broncho-pneumonia in right apex. Pleuritis and peritonitis adhaesiva fibrosa. Cirrhosis of the liver. Echinococcosis. Catarrhal enteritis. Atrophy of the spleen. Oedema of the kidneys. Anaemia. Hydraemia.

2. BULL 2600: 3.5.19: The bull was reported to be lying down before noon and was unable to rise. When visited in the afternoon he was in sterno-costal position, and rose as soon as he was approached. His gait appeared very clumsy. He walked for a short distance and then lay down. He then received an intrajugular injection of 500 c.c. defibrinated blood of cow 2790 and tollie 4341. Both animals had recovered from lamsiekte. After the injection the bull rose again and walked a short distance and lay down. He died during the night.

*Post-mortem Report of Bull 2600.*—Full mouth. Fair condition. Rigor mortis was present in the hindquarters and absent in front. The integument, with the exception of the operation wound, was intact. The mouth was open, the tongue inside. The pupilla was distended. The anus was closed. The blood was coagulated; it stained well. The flesh was dark in colour. The subcutaneous tissue was rather poor in fat. The salivary glands showed no changes. The supporting connective tissue in some places was watery. The superficial cervical lymph node appeared slightly enlarged, moist, and of red colour. The tongue, pharynx, and oesophagus showed no changes. In the pharynx a small bolus of chewed grass was present. The peritoneal cavity showed no foreign contents. The parietal and visceral serosa appeared smooth and glistening. A portion of the rumen and the reticulum and part of the liver were connected with the diaphragm by fibrous adhesions, which were strongly developed between reticulum and diaphragm and serosa of the xiphoid region, where it formed thick layers. On severing it, abscesses were opened, in size that of a fowl's egg; they were situated in the walls of the rumen and the reticulum. No foreign contents were present in the pleural cavities. The parietal pleura was smooth and glistening.

The larynx and trachea showed traces of ingesta; froth was present. The bronchi of the right lung also contained ingesta. The mucous membrane was normal. The lungs were in the expiratory stage. The tissue was elastic, and a little moisture was present. The pulmonary veins contained some coagulated blood. The pleura was smooth and transparent. The pericardium contained 300 c.c. straw-coloured liquid. The parietal serosa was smooth and glistening. The right ventricle and atrium were distended. The left ventricle was contracted. The left auricle showed a diffuse hæmorrhagic infiltration. The endocardia showed no changes. The myocardium in parts appeared streaky white. The thoracic aorta showed no changes. The abdominal aorta showed pits of the intima arranged in longitudinal streaks. The periportal lymph nodes were of usual size. The serosa of the parietal face of the liver in the central parts was attached to the diaphragm by fibrous thickenings, otherwise the capsule was smooth and glistening. The liver appeared slightly enlarged and rich in blood. It was moist on section, and the cut surface appeared smooth. The consistence was somewhat soft. The gall-bladder contained a moderate amount of liquid green bile. The ductus choledochus was open. The atrial lymph nodes appeared slightly enlarged. The spleen (70 by 17 cm.) was long and drawn out, being attached almost in its whole length to the rumen and the diaphragm. The colour of the parenchyma was brown. The trabeculae were visible; the follicles were not seen, the consistence was fairly firm. The suprarenal glands were of usual size. The cortex showed yellow streaks. The adipose capsule of the kidney was somewhat poor in fat; the fibrosa stripped easily. The kidney was dark brownish red in colour. On section the intermediary zone appeared almost black; in the cortex some striation was noticeable. The consistence was firm. The rumen had ingesta of usual consistence and appearance. The reticulum contained pieces of bones, wire, and nails. The wall of one side was thickened, and on cutting into it a black fistula was opened, which was empty. The omasum contained dry ingesta. The contents of the abomasum were liquid; the mucosa showed no change. The mucous membrane of the small intestines was slightly thickened throughout the whole length and covered with a viscid whitish mucus; the duodenum and first portion of jejunum showed red cross stripes; more caudally red patches were present, and in the portion approaching the ileum it was diffusely reddened. At one place a patch of superficial necrosis of the mucosa was present, and the adjacent portion was deeply injected. The rectum showed no change. Some fat was present in the mesentery. The mesenteric lymph nodes were somewhat enlarged. The bladder was distended with normal urine. The mucosa showed no changes. The sexual organs showed no abnormalities. The brain was of usual appearance.

*Pathological Anatomical Diagnosis:* Peritonitis fibrosa of the rumen, reticulum, liver, and diaphragm. Hypertrophica of the spleen. Foreign bodies in the reticulum. Enteritis catarrhalis et hæmorrhagica.

*Diagnosis of Disease:* Lamsiekte.

3. **BLACK HEIFER 169.**—Arrived at Armoedsvlakte on 3.3.19; and running on pasture since 7.4.19. Transferred to putrid bone experiment (trough) on 23.4.19, and to control carcass paddock on 20.6.19.

8.7.19: The heifer, when standing, was observed to be restless, moving both front and hind legs frequently. At times she moved forward in a clumsy manner, placing the front legs in abduction. She soon lay down. When disturbed after a while, she rose. Some difficulty was noted in raising the hind quarter. She again moved forward very clumsily and lay down almost at once. In the afternoon the heifer accompanied the herd to the homestead, and returned to the hospital camp subsequently.

9.7.19: This morning the heifer was found in sterno-costal position, carrying head and neck well and pricking the ears. The hind legs were slightly pushed backwards and were flexed, as if the animal had just failed in an attempt to rise. To judge from the disturbances in the ground and the position of droppings, she must have been down for some time. She made two attempts to rise, but was unable to raise the hindquarters. She moved slightly forwards, however. The droppings were somewhat dry, and had the shape of balls. The animal was then carted into the stable. The heifer received an intrajugular infusion of acacia gum. On examination of a blood-smear, negative results were obtained.

10.7.19: The heifer was lying in sterno-costal position, and at the same place where she was left the previous day. She looked fairly bright and had a wet muzzle. The faeces were black and dry, partly spiral-shaped.

11.7.19: The heifer was found in the same place and in the same position as the previous day. She carried head and neck well. The muzzle was dry. The nostrils showed the presence of a sero-mucous discharge. The faeces were black and dry. The heifer did not feed.

12.7.19: The heifer was lying in right sterno-costal position. She had shifted from her former place during the night; she carried head and neck well; she was slightly salivating. She was reported to have been feeding and drinking.

13.7.19: The heifer was lying in right sterno-costal position; she carried the head low. The muzzle was dry, and around the nostrils some dirt was sticking. The mandible was moving very slowly, and grinding of the teeth could be heard. A slight salivation was present. Subsequently she doubled the head back on to the near side. Some mucus was present in the inner canthi of both eyes. At one time a feeble attempt was made to rise. The infusion with acacia gum was repeated. In the afternoon the animal went over into lateral position. When put back into sterno-costal position she was able to maintain it.

14.7.19: The heifer had shifted from her place over night, but was still in sterno-costal position. She was feeding slowly. A loss of condition during the last five days was fairly marked.

15.7.19: The heifer was lying stretched out in lateral position. She was not able to maintain the sterno-costal position when put back, and had to be propped up. Muzzle and nostrils were dry and scaly. The flanks were hollow.

16.7.19: The animal was lying on the left side. The head was resting on a bag of grass, placed there the previous night. The right nostril was filled with froth. The respiration was quiet; the heifer never moved her limbs. She was lying quietly all day long. When approached, she turned the eyes in the direction of the approaching visitor.

17.7.19: The heifer was lying in the same place and in the same position. The bedding between the legs was still undisturbed. No movements were noted, except those of the eyeball when approached. There was much froth in and outside the right nostril. The respiratory movements were slightly more accentuated and slightly increased in number. In the evening the heifer was turned over into the right lateral position.

18.7.19: The heifer was in the same place and position as last evening. She was still lying very quietly. Froth was present in the nostrils. She occasionally moved the ear. The respiration was quick.

19.7.19: The heifer was killed this morning.

*Post-mortem Report of Heifer 169:* A four-tooth black heifer in fair condition. The autopsy was commenced ca. 3 hours after death; rigor mortis was absent. The integument was intact; the abdomen slightly distended. The tongue was hanging out. In the nostrils a thick mucus was present. The visible mucous membranes showed no changes. The blood was coagulated and stained well. The flesh had a healthy colour. The subcutaneous tissue of the right side showed the presence of some red patches diffusely injected; in the

left hypochondric region a patch of jelly-like infiltration was present. The superficial lymph nodes were of usual size and aspect, except the two superficial cervicals, which were much enlarged. The peritoneal cavity showed no foreign contents. There was no change in the situs viscerum; the serosa was smooth and glistening. The omentum was conspicuously rich in fat. The diaphragm was convex forwards. The pleural cavities showed no foreign contents. The costal pleura was smooth and glistening. The tongue showed no changes. In the left tonsilla a small focus containing a yellow substance was present, in circumference that of about a sixpence. The oesophagus was empty. The mucosa showed no changes. The parotids were firm; the submaxillary showed the usual consistence. The larynx and the cervical trachea were filled with froth. The thyroidea showed no change. The lungs were in the inspiratory stage. The pleura was smooth, glistening, and transparent. The right main lobe was purple, due to the collapse of the superficial lobules under the pleura. The parenchyma was elastic, fairly rich in blood, and on section was moist. The bronchi and trachea were filled with froth. The pericardium contained about 30 c.c. clear liquid. The parietal serosa was smooth and glistening. In the grooves of the heart a fair amount of fat was present. A few ecchymoses were present in the epicardium of the left ventricle. The right ventricle was distended with well coagulated blood; the left ventricle was contracted. The endocardium showed no changes. The myocardium was reddish brown, moist, and shiny. The vessels showed no changes. The periportal lymph nodes showed no changes. The liver was of usual size, the borders of the left lobe were fairly sharp. The capsule was smooth, glistening, and transparent. The colour was dark reddish-brown, the consistence firm, the section smooth. The gall-bladder contained thick greenish bile; its mucosa was brownish discoloured and ramified injected. The ductus choledochus was open. The pancreas was of red greyish colour, and somewhat rich in blood. The dimensions of the spleen were 43 by 12 cm. Some fat was present in the capsule. The pulpa protruded; on section it was dark red brown. The trabeculae and follicles were not distinct. The consistence was fairly firm. The suprarenal glands were light brown. The adipose capsule of the kidney was rich in fat, the fibrosa stripped easily. The surface of the kidney was smooth. The colour of the parenchyma was dark reddish brown; it was rich in blood. The zones were distinct. The rumen contained coarse ingesta and a bone in the dorsal sack; behind the rumeno-reticular fold numerous amphistomas were present. The reticulum was empty. The contents of the omasum were soft; those of the abomasum were of watery consistence. The mucosa of the latter showed slightly pink-stained patches between the folds.

The mucosa of the small intestine was slightly reddened and covered with mucus; some deeper red-stained patches were present. The mucosa of the caecum and of the first portion of the colon was slightly reddened and folded. The rectum contained soft faeces. The mesentery was rich in fat. The mesenteric lymph nodes were rather small and conspicuously black. The bladder contained a small quantity of clear urine. No changes were found in the brains.

*Pathological Anatomical Diagnosis:* Tonsillitis ulcerosa. Cholecystitis acuta. Foreign body and parasites in rumen. Oedema pulmonum. Enteritis catarrhalis.

*Diagnosis of Disease:* Lamsiekte.

4. BLACK HEIFER 172.—Arrival at Armoedsvlakte on 30.3.19. She had been running in the veld since 7.4.19. She was transferred to rotten bone experiment on 23.4.19 and to the carcass-control paddock experiment on 20.6.19.

10.7.19: This morning the heifer was found lying in the hospital camp and was unable to rise. A number of attempts to stand up must have been made to judge from the tracks left in the ground and the heaps of faeces deposited in several places. She had been moving in a circle. When approached, she again made an attempt to rise, bringing the head down almost violently on to the ground. In so throwing the weight on the forequarters she just managed to lift the hindquarters a little and thus shifted slightly forward. She carried the head well and had a somewhat wild look. She was carted into the stable. An examination of a blood-smear gave negative results.

11.7.19: The heifer had shifted about a good deal during the night. She was in sterno-costal position. She was noted to make an attempt to rise, being able to lift the hindquarters slightly, and in so doing pushed the body forward, whilst the flexed hind legs gave way backwards. She was feeding, and carried the head and neck well. A mucous discharge was present in the



nostrils, and the muzzle was soiled with food debris and dust. Examined in the afternoon, she again had shifted from her former place. She did not feed. She was dosed with diluted acetic acid solution.

12.7.19: The heifer was in right sterno-costal position. She had shifted from her place during the night. She was reported not to be feeding or drinking. The faeces were black but well-formed and soft. She carried the head well. She was dosed again and placed into left sterno-costal position.

13.7.19: The heifer was found in the same position, carrying the head well. Muzzle and nostrils were dry and scaly. Mucus was present in the inner canthi of both eyes.

14.7.19: The heifer has again shifted from her former place during the night. She was not feeding. Loss of condition had become most marked during the last few days. During the morning the heifer was found stretched out on left side. She was placed into sterno-costal position and was able to maintain it.

15.7.19: The heifer had again shifted a few yards during the night and had left black spiral-shaped faeces in her trail. She was not feeding. The skin on muzzle and nostrils was scaling.

16.7.19: The heifer was found in left lateral position fully stretched out, and lying quietly. The respiration was quiet. In the afternoon she was lying in the same position and no movements were noted, except of the eyeballs when the animal was approached.

17.7.19: The heifer was in the same position where left last night. The bedding between the legs was slightly disturbed. There was no struggling of the legs—they were occasionally moved slowly. The respiration was quiet. In the evening the heifer was turned into the right lateral position.

18.7.19: The heifer was in the same position where left last night. Froth was present in the nostrils. At times the heifer was moaning. She occasionally moved the ears and the legs.

19.7.19: The heifer was killed this morning.

*Post-mortem Report of Heifer 172:* A two-tooth black heifer in moderate condition. The autopsy was commenced two hours after death. Rigor mortis was absent. The integument was intact, the abdomen relaxed. Mouth and anus were closed. The visible mucous membranes showed no changes. The blood was coagulated and stained fairly well. The flesh showed a healthy colour. In the subcutaneous tissue, in some places gelatinous infiltrations were present. The lymph nodes of the trunk were rather small, except the superficial cervicals, which were enlarged and red; those of the head were also larger than usual. The peritoneal cavity showed no foreign contents. The serosa was smooth and glistening. The dorso-cranial portion of the rumen was attached to the diaphragm by fibrous adhesions. The diaphragm was convex forward. The pleural cavities showed no foreign contents; the parietal pleura was smooth and glistening. The tongue, pharynx, and oesophagus showed no changes. The larynx showed no abnormalities. The ventral aspect of the cervical tracheal mucosa was covered with a mucous deposit, and, when removed, a hæmorrhagically infiltrated and injected surface appeared. The lungs were contracted. The central portion of the right main lobe was of purple colour due to collapse of the lobules under the pleura. The consistence of the lung parenchyma was elastic. It was moist on section. The thoracic trachea also contained a mucous deposit which covered longitudinal hæmorrhagic streaks and low tufts of granulation tissue. The pleura was smooth and glistening. No liquid was present in the pericardium; the parietal serosa was smooth and glistening. The right ventricle was distended with well-coagulated blood; the left was empty and contracted. Fat was present at the base of the heart. The endocardium showed no changes. The myocardium was shiny and light brown in colour. The vasa cordis showed no changes. The periportal lymph nodes were somewhat moist and conspicuously dark in colour. The liver appeared slightly enlarged; the left border was somewhat blunt. The capsule was smooth and glistening, except for a scar on the parietal surface of the left lobe. The parenchyma was reddish-brown and fairly rich in blood. The section was smooth and shiny. The consistence was firm. The gall-bladder was filled with yellow liquid bile, its mucosa was yellow-discoloured, brown-pigmented, and ramified-injected. The ductus choledochus was open. The pancreas was somewhat whitish. The atrial lymph nodes were very much enlarged and moist. Dimensions of the spleen were 38 by 14 cm. The pulpa was red brown and somewhat soft. The follicles and trabeculae were visible. The adipose capsule of the kidney showed a small amount of fat. The fibrosa stripped easily; the surface of the kidney was smooth. The colour was light brown.

On section all three zones were distinct. In the dorsal portion of the rumen the mucosa showed a fairly extensive area of necrosis. The necrotic surface membrane could be removed *in toto*. Around this area, smaller patches of identical description, and in size that of a shilling, were present. After the removal of the membrane, a rough surface appeared, which was partly injected with blood. The reticulum was empty. The contents of the omasum were moist; the abomasum contained moist ingesta. The mucosa of the fundus was slightly pink; that of the duodenum and jejunum was slightly reddened and covered with mucus. The mucosa of the caecum was slightly swollen; that of the colon showed no changes. The rectum contained soft faeces; the mucosa was covered with mucus. In the mesentery some fat was present; the lymph nodes were slightly enlarged and conspicuously black. The bladder was distended with clear urine. The uterus was pregnant, and a yellow body was in the left ovary. The brain showed no changes.

*Pathological Anatomical Diagnosis:* Ruminitis mortificans. Enteritis catarrhalis. Tracheitis mortificans hæmorrhagica. Oedema pulmonum.

*Diagnosis of Disease:* Lamsiekte.

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### SUB-SECTION D.—THE PRODUCTION OF LAMSIEKTE BY MEANS OF MIXED CULTURES PREPARED FROM TOXIC MATERIAL.

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#### 1. SELECTION OF SEED MATERIAL.

The positive experiments with crushed bones and fleshy remains of putrid carcasses, by means of drenching, naturally led to the conclusion that the toxin responsible for the disease was produced by bacteria and the similarity of the symptomatology in lamsiekte with that of botulism of the human, as well as that of animals, suggested similar organisms, viz., anaerobic, sporebearing bacteria, to be the cause. The examination of the remains of a carcass (ox 2519) revealed the presence of a black, somewhat viscous, substance that could easily be scraped off, attached to some bones of the skeleton, from which all flesh had disappeared. When placed under the microscope, it appeared as an amorphous débris, which contained a large number of bacterial spores. It was decided to test whether such material, when injected into bovines, would give rise to lamsiekte.

Cow 4191 was selected for this purpose.

BLACK Cow 4191.—Arrived from Grahamstown on 10.5.18 and was running in camp B.

2.5.19: Injected subcutaneously 10 grammes black débris suspended in 50 c.c. water. The material had been scraped from bones of the carcass of an ox (2519) which had undergone decomposition.

3.5.19: A swelling at the seat of inoculation was noted, which was neither painful nor hot. The animal was feeding well.

4.5.19: The cow was down in sterno-costal position this morning and unable to rise, having difficulty to carry the head. Salivation was fairly profuse. She died during the morning.

*Epicrisis:* The cow contracted and died of lamsiekte as a result of the injection of the débris. The injected substance, which was rich in bacterial spores, apparently also contained sufficient toxin to produce an acute disease. The incubation period in this case lasted two days; the disease of a peracute course lasted only a few hours. (Post-mortem report of cow 4191, *vide* Appendix, page 1031.)

CONCLUSION.—The result of this experiment showed clearly that the toxin must have been of a very potent nature, killing in small quantities when applied subcutaneously. Accordingly it was expected that such toxin could be produced by means of cultures when applying anaerobic methods, since it had to be accepted that the toxin producing bacteria belonged to the botulism type of organisms, or if of the putrefactive group would be growing only under such conditions.

For this purpose liver bouillon containing liver tissue was utilized. Pieces of liver of an otherwise healthy ox were placed in test tubes containing liver broth and sterilized in the autoclave. Into such test tubes was placed the seed material. The seed material was taken from several sources:—

- (1) The black débris off a bone of a dead ox, containing numerous spores.
- (2) The intestinal contents of pycnosoma larvae.
- (3) Pycnosoma larvae crushed.
- (4) Putrid bones.

## 2. PRODUCTION OF THE DISEASE BY MEANS OF TOXIN OBTAINED IN ANAEROBIC CULTURE USING AS SEED MATERIAL THE BLACK DÉBRIS OFF A BONE OF A CARCASS HAVING UNDERGONE PUTREFACTION.

The seed material was heated for half an hour at 65-70° C. This was primarily done to destroy all non-sporulating germs and to restrict growth only to spores. Incidentally in this way also any toxin (as later experiments proved) was inactivated. A small quantity of the black material was suspended in bouillon in a test tube, and with a fine pipette some of the bouillon was transferred to a second tube and so diluted for a second time; the procedure was repeated a third time, and the test-tube with the third dilution was placed into the incubator. Growth took place and gas developed, and after a few days a deposit had formed that contained spores. There was no further growth subsequently.

*Inoculation of the Culture:* On 9.5.19 the original culture was injected into a bovine, red and white heifer 3659, in the quantity of 12 c.c. subcutaneously.

*Result:* 12.5.19: In the evening the heifer refused to feed and salivation was present. 13.5.19: This morning the animal again appeared to be normal.

Subsequently the heifer was transferred to a drenching experiment and died of lamsiekte.

*Epicrisis:* The heifer had a slight illness which, from the symptom of salivation, had to be interpreted as a mild case of lamsiekte. The incubation period lasted three days; the disease only a few hours. The original material from which the culture was made proved to be very toxic. Not so this culture. It is of interest to note that this slight attack of lamsiekte did not produce any immunity, since in a subsequent drenching experiment the animal died of lamsiekte.

## 3. PRODUCTION OF THE DISEASE WITH TOXIN FROM A CULTURE WHICH WAS OBTAINED, USING INTESTINAL CONTENTS OF A PYCNOSOMA LARVA AS SEED MATERIAL.

In order to exclude extraneous infection, the larva was first well rinsed in saline solution and dried between blotting paper. It

was then dipped into absolute alcohol and kept there for a short while. It was then placed into the flame of a spirit lamp, and the alcohol burned off. The charred and shrunken larva was transferred to a sterilized Petri dish and torn asunder with sterile forceps; the intestinal canal was squeezed out by slight pressure and by means of a platinum needle placed in the test tube containing the medium. The cultures were made on 10.4.19.

Already after twenty-four hours the bouillon showed the presence of intensive bacterial growth, gas being formed vigorously. After the lapse of another twenty-four hours, gas production ceased.

#### *Tests.*

On 3.5.19 the liquid was tested on two guinea-pigs, 1 c.c. being injected subcutaneously. Guinea-pig 1 died on the morning of the 5th, not having been noticed ill the previous day. The post-mortem examination showed the presence of a slight enteritis catarrhalis, some atelectasis in ventral border of left lung. The spleen was normal. The place of injection was diffusely reddened.

Guinea-pig 2 was found dead on the morning of the 6th. It had not been noticed ill the previous day. On post-mortem it showed at the place of injection a whitish somewhat hard thickening of the tissue, in the circumference of which there was much injection of the blood vessels. There were no alterations noted in the internal organ, and in particular the spleen was normal.

8.5.19: Two tubes of cultures were tested on guinea-pigs and cattle, two guinea-pigs, 3 and 4, and bovine 149, with the contents of one tube; and two guinea-pigs, 5 and 6, and bovine 120, with the contents of the other. The guinea-pigs received 1 c.c. subcutaneously and the cattle 20 c.c.

*Result:* 9.5.19: Guinea-pig 3 died during the night. There was no infiltration at the place of injection. A hyperaemia of the lungs and a sanguinolent liquid in the pleural cavity were noted. The heart ventricles and auricles were distended with blood. The intestines were normal. The microscopic examination of the blood was negative. In the spleen a bacterium was found.

Guinea-pig 5 died during the night. There was a slight infiltration of the subcutaneous tissue at the place of injection and a localized necrosis was also noted. There was a hyperaemia of the lungs, the pleural cavity contained a sanguinolent liquid and there was dilatation of the ventricles and atria. The examination of blood and spleen smears gave negative results.

Guinea-pig 6 died during the night. There was a slight infiltration at the place of injection; a very small necrotic focus, hyperaemia of the lungs, and sanguinolent liquid in the pleural cavity, dilatation of the heart, and a slight reddened mucous membrane of the intestines were also present. Some serous liquid was present in the peritoneal cavity. The examination of smears of blood and spleen was negative.

Guinea-pig 4 died during the day. The post-mortem showed infiltration around the place of injection. There was sanguinolent liquid in the peritoneal cavity. Bacteria were found on microscopic examination both in spleen and blood, but they were few.

8.5.19: HEIFER 120.—Injected subcutaneously 20 c.c. liver bouillon culture No. 4 (10.4.19).

13.5.19: *Result*: The heifer had not been feeding well since last night. This morning (6 a.m.) she was down and looking very dull and hollow in the flanks. She was, however, able to rise when forced to do so, and walked somewhat wobbly. After rising, much saliva escaped from the mouth. She walked to the shed and attempted to eat. The prehension of food was very slow, the masticatory movements were weak and slow. Three hours later the heifer was found stretched out flat. The nostrils were dirty, dry mucus was adhering to the muzzle. She was placed in sterno-costal position, and was able to maintain it all day long feeding very slowly.

14.5.19: The heifer was found in sterno-costal position and feeding slowly. She maintained this position during the day.

15.5.19: The heifer died during the night. (Post-mortem report *vide* Appendix, page 1032.)

*Epicrisis*: The symptoms noted in heifer 120 after an incubation period of five days were those of lamsiekte. The disease lasted two days. It must be concluded that the animal died as a result of the injection of toxin contained in the injected anaerobic liver bouillon culture.

8.5.19: HEIFER 149.—Injected with 20 c.c. of liver bouillon culture No. 3, made on 10.4.19.

13.5.19: *Result*: The heifer had not been feeding well since last night. She was down this morning (6 a.m.) with the head doubled back on the left flank. She was unable to rise. She made an attempt to do so when forced, but was unable to lift the hind-quarters. The heifer was then carried into the shed and placed in sterno-costal position. Attempts to rise were now voluntarily repeated, but these failed, the hind-quarters being raised just a little, the forequarters not at all. Subsequently (9 a.m.) the animal was noted to feed, the masticatory movements were slow, the nostrils were soiled with dry mucus, the muzzle was dry. In the afternoon the heifer was found in recumbent position with the head doubled back. She did, however, at times carry it normally. She was noted to eat, the masticatory movements were very slow.

14.5.19: The heifer was found stretched out in lateral position. When propped up in sterno-costal position, she stretched the head out on the ground, apparently unable to carry it. Abdominal breathing was fairly accentuated. The heifer died in the afternoon. (For post-mortem report *vide* Appendix, page 1032.)

*Epicrisis*: The symptoms which heifer 149 developed after an incubation period of five days, were those of lamsiekte. The disease lasted for two days after which the animal died. The disease was undoubtedly caused by the injection of the toxin contained in the culture.

CONCLUSIONS.—The cultures made in liver bouillon under anaerobic conditions produced a toxin that caused death in six guinea-pigs and two cattle. The disease in cattle was lamsiekte. There was a definite incubative period and there were the symptoms noted in cases of lamsiekte produced by drenching with or feeding on putrid material.

The culture utilized was the original obtained from the intestinal canal of a pycnosoma larva. It may be argued that it contained still some of the toxin that must also have been present in the larva.

Since, however, the intestinal canal is a small organ and only a portion of it was used, this objection may safely be ruled out. The bacteria so transmitted multiplied, produced a vigorous growth that showed itself in the production of gas and they gave rise to the toxin.

#### 4. CULTURE INJECTION OF THE FOURTH GENERATION.

The original culture utilized in the previous experiments and which proved to contain the toxin organisms was subcultured. On each occasion the same procedure was adopted, viz., diluting down with bouillon through three test tubes, putting the last one into the incubator to grow. The first culture was made on the 29.4.19, the second on the 7.5.19, the third on the 20.5.19, and the fourth on the 26.5.19. It is this fourth culture that was utilized. In order to obtain large amounts of toxin, a mass culture in flasks was made.

*Inoculation of Bovines*: 9.7.19: Injected 100 c.c. subcutaneously.

TOLLIE 181: 10.7.19.—The tollie to-day refused to feed. In the afternoon it went down. In the evening it was standing and feeding lucerne-hay, but apparently it had some difficulty in the prehension of the food, the mastication of which was very slow. Much saliva and froth were hanging about the mouth.

11.7.19: The tollie was down and unable to rise. It was moved into the shed and placed in the right sterno-costal position. It was able to maintain this position, but doubled the head back on the near side. The animal was very listless, not taking notice of what happened around it, never moving head, ears, or eyelids.

13.7.19: The tollie was lying stretched out flat on the left side, breathing very quietly. White mucus was present on the inner canthus. The muzzle was dry and dirty. The surface of the body was still warm. No struggling was noted, nor were there any traces of such. The tollie was injected intrajugularly 500 grammes magnesium sulphate in 500 c.c. water. It died soon after the injection. (For post-mortem report, *vide* Appendix, page 1033.)

*Epicrisis*: The tollie contracted lamsiekte as the results of the injections of a bouillon culture injected on the 2.7.19, i.e. after an incubation period of eight days. The disease had lasted three days when the tollie died, probably from shock, as a result of an intrajugular injection of magnesium sulphate. There can be no doubt that the disease was caused by the toxin contained in the culture.

RED AND WHITE COW 3708.—Born at Armoedsvlakte in 1916. Had been running on the veld in different experimental batches and was used in a previous experiment without results.

2.7.19: Injected 80 c.c. of culture 4 (4), 26.5.19, subcutaneously.

9.7.19: The cow was noted to go down at noon and refused to feed. She had a somewhat staring look. Saliva was dropping from the mouth. When the tail was pinched the cow flinched, but made no attempt to rise.

10.7.19: There were no changes in the condition of the animal.

11.7.19: The cow was found lying quietly stretched out on the right side. There was some frothy liquid at the mouth. The respiration was slightly laboured. The cow died at one o'clock. (For post-mortem report, *vide* Appendix, page 1034.)

*Epicrisis*: The cow developed lamsiekte after eight days. The disease lasted one day and was of a very acute character. There can be no doubt that the disease was caused by the toxin contained in the injected culture.

CONCLUSION.—A bouillon culture of the fourth generation that had been injected subcutaneously into the two bovines proved to be toxic and caused lamsiekte in both animals. If in the previous experiments there could have been any doubt as to the toxicity of the culture, which was of the first generation, this doubt could no longer be maintained after the culture had passed three generations. It is evident, therefore, that given a suitable medium and anaerobic conditions, subcultures of the organism originally obtained from the intestinal canal of a pycnosoma larva propagate and produce the toxin that causes lamsiekte.

#### 5. CULTURES MADE BY USING CRUSHED PYCNOSOMA LARVAE COLLECTED FROM A CARCASS UNDERGOING PUTREFACTION.

The culture was made as a mass culture by placing 80 grammes of crushed half-mature larvae in three litres of bouillon containing pieces of cattle liver. The larvae had been collected from the putrid carcass of a horse. The culture was made on 9.7.19. The larvae were thoroughly washed in saline solution before they were crushed.

At various intervals this culture was tested for toxicity, viz.:—

- (1) 25.7.19 on heifer 128.
- (2) 6.8.19 on cow 2886 and heifer 3507.
- (3) 17.9.19 on cow 2315 and (4) heifer 4152.

1. BLACK HEIFER 128.—Arrived from Pretoria on 30.4.19 and had been stabled since 18th July.

25.7.19: Injected subcutaneously 100 c.c. 9.7.19 liquid of the pycnosoma mass culture.

2.8.19: The heifer was found down this morning. She was put on to her feet and was able to walk into the shed, but went to lie down at once.

3.8.19: The heifer was found in the same place where she lay down the previous day. She refused to feed. She carried the head well.

4.8.19: The heifer was found in the same place where left yesterday. She was in sterno-costal position, but not feeding. She made a feeble attempt to rise. In the afternoon she was put on to her feet. In the first attempt she was able to stand, she then suddenly turned round as if to charge and soon collapsed. The second time she tried to walk a few steps and then again collapsed. The third time she stood for a while and then made an attempt to charge, but again collapsed.

5.8.19: The heifer was in sterno-costal position. After nine hours she was found in left lateral position. When put back into sterno-costal position, she was able to maintain it.

6.8.19: In the morning the heifer was found in the right lateral position and when placed into sterno-costal position was able to maintain it. In the afternoon she was found stretched out on her side. She was put into sterno-costal position twice, but was too weak to maintain it.

7.8.19: The heifer was still alive this morning, breathing slowly and superficially. She was killed at 9 o'clock. (For post-mortem report, *vide* Appendix, page 1035.)

*Epicrisis*: The heifer was in poor condition when selected for this experiment. The symptoms of lamsiekte were present during life. The incubation period in this case lasted eight days and the disease five, when the heifer was killed. The autopsy revealed the presence of an extensive cachexia.

2. BLACK Cow 2886 had been running on the farm since 1916 and had been used in different experiments.

6.8.19: Injected subcutaneously 100 c.c. bouillon culture of the pycnosoma mass culture, 9.7.19.

14.8.19: At 3 p.m. the cow was noted to be ill. She was not feeding and had a dull expression. There was copious salivation. The diagnosis lamsiekte was made and the cow submitted to treatment. After removing the cud, 50 grammes salol dissolved in one litre 10 per cent. alcohol were given. The cow stood the drenching badly. She coughed several times and swallowed with difficulty. As nearly half the drench was spilled, another dose of 50 grammes was administered at once. This was given very carefully. Eventually all was taken. No further ill-effects were noted and the cow was seen masticating half an hour later. At 8 p.m. she was standing, still salivating profusely.

15.8.19: The cow showed about the same condition as yesterday; she ate a little bran and mealies. She was dosed again after removing several handfuls of cud from the mouth. The first mouthful of the drench was coughed up, then the cow tried to swallow, but great difficulty was experienced and the cow became very restless and excited. The dose was swallowed only with great difficulty. After drenching the cow was very distressed; lying down on one side, the respiration was laboured and hurried. Half an hour later, the cow was standing and seemed none the worse. No change was noted during the day; the flow of saliva was marked.

16.8.19: Profuse salivation was present. The cow rose of her own accord. She was feeding a little bran and mealies. She again was drenched with 25 grammes salol in  $\frac{1}{2}$  litre 10 per cent. alcohol. The drench was taken well. In the evening the cow refused to rise. She did not feed nor drink. A little later she was found to have changed her place. The respiration was jerky. Salivation was still marked.

17.8.19: The cow was found dead in the morning. (For post-mortem report, *vide* Appendix, page 1036.)

*Epicrisis*: The culture injected in the quantity of 100 c.c. caused lamsiekte. The disease was typical lamsiekte. The difficulty experienced in drenching this cow for treatment was due to a partial paralysis of the pharynx, and some of the liquid passed into the lungs. This apparently had no ill effects. The incubation period in this case lasted eight days and the disease two and a half.

3. BLACK HEIFER 3507.—Arrived from Pretoria on 28.4.17.

6.8.19: Injected subcutaneously 100 c.c. bouillon of pycnosoma mass culture 9.7.19.

14.8.19: At 3 p.m. the heifer was noted to be ill. She was feeding at the time of the first observation, showing froth at the mouth. At 8 p.m. she was salivating profusely.



15.8.19: Salivation was very profuse and the heifer was feeding. Subsequently she masticated continuously.

16.8.19: Salivation was marked. The heifer was feeding mealies and bran. She was standing most of the time and was attentive. In the evening she drank a bucket of water; deglutition was slow. Later she had a good feed of mealies, bran, and a little lucerne. Salivation was less marked.

17.8.19: The heifer was feeding bran and mealies and a little lucerne hay. She was lying down most of the time during the day.

19.8.19: Salivation was not so marked. The dribbling of saliva from the mouth had ceased, but frothing was still present. Boluses of ingesta were found on the ground where the heifer had been standing.

20.8.19: The heifer had lost considerably in her condition and had become weak. When chewing she was still frothing slightly at the mouth. More boluses of food were found lying about her. She ate a little maize and bran.

21.8.19: The heifer rose when disturbed. Salivation had disappeared. She was feeding and drinking.

22.8.19: Salivation had entirely ceased. The heifer was feeding and drinking normally.

*Epicrisis*: The heifer contracted lamsiekte and recovered. The disease showed itself after an incubation period of eight days and lasted eight. A characteristic of the disease was the paralysis of the throat; the heifer for two days was apparently unable to swallow the boluses of food, these being thrown out.

4. BLACK COW 2315 had been running on the farm since August, 1915. being used in various experiments. On the 17.7.19 she was stabled.

17.9.19: Injected subcutaneously 100 c.c. of pycnosoma mass culture (9.7.19).

24.9.19: The cow went down, and was unable to rise by herself. She was an old cow and in a poor condition. She was lifted daily during the following few days. These symptoms were put down to extreme poverty and old age.

27.9.19: To-day it became apparent that the cow was suffering from lamsiekte. She salivated much. There was no bolus found in the mouth.

28.9.19: The cow was lying in lateral position flatly stretched out. She died the same night. (For post-mortem report, *vide* Appendix, 1036.)

*Epicrisis*: The cow died of lamsiekte. The symptoms, during life, were somewhat obscured by the poor condition at the time of the injection of the culture. The incubation period lasted seven days and the disease four.

5. BLACK HEIFER 4152.—Arrived from Pretoria on the 10.1.19, and was stabled on the same day. She was subsequently used in experiments with negative results.

17.9.19: Injected subcutaneously 100 c.c. bouillon of pycnosoma mass culture, 9.7.19.

18.9.19: Painful swelling on seat of injection and stiffness of limb.

24.9.19: The heifer showed symptoms of illness since the previous evening. She was lying in sterno-costal position, and slightly frothing at the mouth. Subsequently she was noted to move the mandible as if chewing, but at times stretching the tongue in an attempt to remove something from behind. She was noted to turn the tip of the tongue backwards, apparently to remove something. Salivation was profuse.

25.9.19: This morning the same movements were noted and salivation was still profuse. In exploring the mouth, chewed grass, forming a fairly large bolus, was found at the root of the tongue. It was removed. The heifer was then drenched 50 grammes salol in 500 c.c. water. The drench was swallowed but coughing was noted. At 2 p.m. another bolus was removed and the heifer was again drenched with the same quantity. Drenching produced a slight cough, and some of the liquid returned via the nostrils. Some of the liquid was, however, noted to pass down the oesophagus. After drenching, a bucket of water was placed before the heifer; she drank this very slowly, the water passing down the oesophagus in small quantities. Considerable time was taken to drink a very small quantity. The heifer walked with head carried low and the movements in the front legs were distinctly stiff. She walked about fairly freely, however, when let loose.

26.9.19: The heifer was lying in sterno-costal position and was frothing at the mouth; saliva had collected on the ground. Subsequently she rose by herself. Her gait was still stiff, she walked carrying head and neck low. The mouth was explored and a bolus was found at the root of the tongue. In the afternoon the heifer was down again and frothing at the mouth. Water was given and she drank very slowly for a long time, only a small quantity being taken in. The passage of the liquid bolus was faintly noticeable in the left jugular groove. The heifer had not been feeding all day; the flanks were deeply sunken.

27.9.19: The heifer was lying in a right lateral position. She was lifted on to her legs and was able to stand and walk. She salivated slightly. She again had a bolus of grass at the root of the tongue. It was removed. Subsequently the heifer lay down in a right sterno-costal position and doubled her head back.

28.9.19: She was found in this position early this morning. She was then killed. (For post-mortem report, *vide* Appendix, page 1037.)

*Epicrisis*: The animal developed symptoms of lamsiekte after an incubation period of seven days. The disease had lasted four days when the heifer was killed. The first symptom noted was the paresis of the pharynx. The heifer was able to swallow water with some difficulty. She was able to masticate the food and to form a bolus, but could not swallow it.

CONCLUSION.—The bouillon culture was made by suspending about 80 grammes of crushed pycnosoma larvae into 3 litres of bouillon or about 2.66 grammes larval substance per 100 c.c. It may be said that the culture also contained the original toxin in or adhering to the larvae which had leaked out by the maceration of the crushed substance. Since, however, the quantity of blow-fly larvae with which we were able to produce the disease was never less than  $\frac{1}{2}$  oz.,

and sometimes failed with 3 oz., it may safely be concluded that the bacteria contained or attached to the larvae were growing and responsible for the production of the toxin in the bouillon.

#### 6. CULTURES MADE WITH PUTRID BONES.

For this purpose a small quantity of bones was crushed and suspended in 3 litres of liver bouillon containing pieces of liver of an otherwise healthy ox.

The culture was used both for subcutaneous injection in one animal (150) and for drenching (1 litre) in another (156).

13.10.19: TOLLIE 150. Injected subcutaneously 100 c.c. bouillon culture.

16.10.19: The tollie showed to-day profuse salivation, the saliva was running from the mouth. The animal had a dejected appearance, when walking it carried the head low. The gait was stiff. This was partly due to the swelling caused by the injection of the culture. On exploring the oral cavity with the hand, a bolus was found at the root of the tongue.

17.10.19: This morning the tollie was lying in left sterno-costal position; it carried the head low; the ears were half drooping; the eyes half closed, the mouth open, and the tongue hanging out for a distance of about 3 inches; salivation was present and profuse, the saliva was hanging in strings mixed with débris of food. A mucous discharge was present in the nostrils; the muzzle was wet. The respiration was superficial and slightly hurried. When lifted, the tollie was able to stand and walked with a stiff gait, carrying the head and neck low. It soon, however, went to lie down. The tollie died at about 9 o'clock. (For post-mortem report, *vide* Appendix, page 1038.)

*Epicrisis*: After an incubation period of three days, the tollie showed symptoms of lamsiekte, viz., paralysis of the mandible, paralysis of the pharynx, paralysis of the tongue and paresis of the locomotor muscles. The disease lasted one day. The lesions found on post-mortem were those that were usually found in cases of naturally contracted lamsiekte.

13.10.19: TOLLIE 156.—Drenched 1 litre bouillon culture from rotten bones.

17.10.19: The tollie was standing and feeding bran. Mouth, muzzle, and nostrils were noted to be soiled with bran. Subsequently fairly profuse salivation was present. The look was bright. In the afternoon the tollie showed much improvement. It was still somewhat wild, but standing and feeding. Frothing at the mouth had disappeared.

18.10.19: The tollie appeared to be recovering.

19.10.19: The tollie was found in sterno-costal position, but jumped up at once and ran away. It still had a somewhat wild look and attitude. It was feeding well and salivation had disappeared.

*Epicrisis*: The tollie had a very slight attack of lamsiekte. It would appear that the bouillon culture made from the rotten bones, was only slightly toxic, since 1 litre of it only produced a mild disease. The incubation period lasted four days, the disease about one.

CONCLUSION.—The culture was made by suspending putrid crushed bones into liver bouillon, producing a toxin that, when injected subcutaneously, gave rise to typical lamsiekte. The same culture, when given per os, gave rise to a mild case of lamsiekte, from

which the animal recovered. This might be interpreted that the culture contained but little toxin. In view of the fact that the subcutaneous injection produced a disease from which the animal died, it would appear that the drenched animal possessed a great resistance.

#### GENERAL CONCLUSIONS CONCERNING CULTURE EXPERIMENTS.

The few experiments undertaken to grow the toxigenetic bacterium in artificial culture have been successful. It appears that a toxin can be obtained from several sources. All material that proved to be toxic, when ingested, gave rise to a culture that produced a toxin causing the typical disease.

It would appear, therefore, that, provided suitable media are selected anaerobic conditions observed, lamsiekte toxin is comparatively easily obtainable, and for this purpose a pure culture of the pathogenetic organism is not an essential factor.

#### SUB-SECTION D.—APPENDIX.

##### POST-MORTEM REPORTS.

1. POST-MORTEM REPORT OF COW 4191.—An aged black cow in poor condition. The post-mortem was made eight hours after death. Rigor mortis was absent in fore and present in hind legs. The abdomen was relaxed. The integument was intact. The natural openings were closed. The visible mucous membranes showed no changes. The blood was coagulated, staining fairly well. The flesh was dark in colour. The subcutaneous tissue was devoid of fat. In front of left shoulder at the place of injection was a swelling of about 15 by 5 cm. The parotid was very hard. The maxillary salivary glands showed no change. The superficial lymphatic glands were very small and embedded in jelly-like connective tissue. The tongue was normal. The oesophagus was empty. The pharynx showed a small ulcer in the right wall. The peritoneal cavities contained a little straw-coloured liquid. The situs viscerum was normal. The diaphragm was convex forward. The pleural cavities contained a small quantity of straw-coloured fluid. The costal pleura was smooth and glistening. The trachea and bronchi showed no change. The lung was in expirum, the lung tissue elastic. The pleura was translucent. The arteries and veins were normal. The mediastinal lymphatic glands were somewhat watery. The pericardium contained about 30 c.c. clear liquid. The right ventricle of the heart was slightly distended and contained a clot of blood. The left ventricle also contained some coagulated blood. The endocardium and valves, as well as intima of arteries and veins were normal. The epicardium showed some ecchymoses of the left ventricle. The myocardium showed no change. The periportal lymph glands appeared normal. The capsule of the liver was smooth and glistening. The parenchyma was dark brown, smooth on section; the consistence was normal. The gall-bladder contained yellowish-green bile. The ductus choledochus was open. The bile ducts contained bile. The pancreas showed nothing unusual. The atrio lymphatic glands were rather small. The spleen (46 by 12 cm.) showed the capsule somewhat wrinkled. The pulpa was somewhat dry and dark brown in colour. The trabeculae were distinct, the follicles not. The suprarenal glands were small, the cortex dark brown. The adipose capsule of the kidneys was poor in fat; the fibrosa stripped easily. The parenchyma was dark red-brown. The intermediary zone appeared very dark. The rumen contained normal ingesta. The reticulum contained liquid ingesta and pieces of bone. The omasum had fairly soft contents. The abomasum had liquid contents. The mucous membranes were normal. The mucosa of the small intestine was covered with viscid mucus, brown yellowish stained, and in parts showed red cross stripes and red patches. Some places were much injected. The mucosa of the caecum was injected, that of the colon showed red patches. The rectum was normal. The mesentery was poor in fat. The mesenteric lymph glands were somewhat enlarged and moist. The bladder was filled with clear urine. The sexual organs were normal. The brain showed no change. On examination the blood-smear was negative.