SPORADIC OUTBREAK IN CATTLE RESEMBLING TETANUS

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A Sporadic Outbreak in Cattle Resembling Tetanus.

By

Raymond LeRoy Conklin.

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Introduction.

During the past two years the writer has been called upon to investigate the cause of serious losses among Ayrshire cattle on a farm located in the eastern part of the Province of Quebec. As far as could be ascertained losses began in the year 1918, and still continue. Among the early losses, there were seven adult animals and a few calves. Lately, only animals under the age of three years have died, with a mortality as high as ninety-five (95) per cent. Once the symptoms appear, age has little to do with the prognosis of the case. Of the animals which have shown symptons only two have survived.

Records of the herd show that in 1921 twelve animals were lost, and in 1922 sixteen. Previous years have shown about the same rate of mortality. The initial losses from the outbreak have been estimated at approximately $15,000. The potential losses would be even much greater as the herd was pure-bred and free from tuberculosis.

The disease has persisted and has been confused with other diseases of a contagious and infectious nature. The clinical symptoms have indicated forage, arsenic, and strychnine poisoning, blackleg, anthrax, hemorrhagic septicemia, tetanus and "vibrion septique". Early in the outbreak chemical and bacteriological examinations did not assist in determining the causal agent.

EXTENT AND SCOPE OF THE WORK.

The investigation of the disease includes the following:-

(a) Post-mortem examination of the animals dead of the disease.

(b) A study of the cultures made from the bodies of the calves, from the ears which were tagged, navels, livers, spleens, kidneys, heart blood, brains, and the fluids in the body cavities.

(c) Isolation of the causal organism.

(d) Detection of the source of the organism.

(e) Experimental work with rabbits, cats, guinea pigs, dogs and small pigs.

(f) Comparison of the disease with those with which it has been confused.

(g) Comparison with the organisms producing other diseases.

(h) Comparison of Bacillus suberis with B. tetani and the many pseudo-tetani organisms.

(i) Production of the toxin, and

(j) Agglutination tests.

The following points have been given consideration:-

(a) Is this organism a tetanus organism with peculiar cultural characteristics?

(b) Is it a pathogenic pseudo-tetanus organism?

(c) What was its origin?

(d) What are the means by which death is produced?

TECHNIQUE EMPLOYED.

In all post-mortem examination, the regular routine was followed with special attention to aseptic precautions in the taking of all cultures. The tissues were seared with a spatula,

incised with a sterile scalpel, and cultures were taken with a platinum loop 4 mm. in diameter, a platinum needle or portions of the tissue were placed directly into the media to be used.

The media consisted of the following:- 1. B. P. broth, 2. B. P. agar, in stab, slope, and plate, 3. lactose, saccharose and maltose broths, 4. Dunham's solution, 5. Cohn's solution, 6. Nitrate broth, 7. Litmus and plain milk, 8. Loeffler's blood serum, 9. Gelatin.

Anaerobic growths of the organism were obtained by the use of the following:-

(a) The media seeded with B. suberis were placed into larger tubes containing 10 grs. of pyrogallic acid and 10 cc. of a 10% solution of KOH., the tubes were then sealed and placed in the incubator at 37° C. (Burris method)

(b) A second method was to place about an inch of sterile paraffin oil above the surface of the seeded media, seal the tube and incubate at 37° C.

All animal inoculations are described in another part of this paper. In these experiments subcutaneous, intramuscular, interperitoneal, and interpleural injections were employed. Aside from direct inoculations, artificial infection of wounds and feeding experiments were tried.

CLINICAL SYMPTOMS OF THE DISEASE.

The onset of the disease has been very insidious. No symptom has been noticed by the herdsman until a short time before death. The first thing to be observed is the bleating of the animal, and its forced movements. The animal continues to run about the stall, circling in the same direction, until it

falls to the ground. The ears are erect, the pupils dilated, the tongue protruding from the side of the mouth; excessive salivation, and also changes in the respiration. The head is always drawn upward and backward.

The animals are apparently blind, for any object may be brought into contact with the cornea without causing a blinking of the lids provided the eye-lashes are not touched. The ears are constantly moving as is the case with a blind animal.

The pulse is very rapid, weak, and almost imperceptible. The mucosaeare congested and the extremities are cold. The heart, upon auscultation, is found to be irregular, beating very rapidly, and forcibly. When the animal is near death the sound of the beat can be heard easily by one standing near the animal.

Respiration, while very dyspneic, is shallow and painful. The character of the respiration also varies with the nature of the other symptoms manifested by the disease. In some cases there appears a frothy, bloody nasal discharge. This is especially true in the acute forms of the disease. (i.e. if the disease exsists for twelve hours or over before death.)

In the case of the digestive tract we notice a chain of symptoms, several of which always exist, but not necessarily the same in all cases. The presence of anorexia, the cessation of peristalsis, tucked-up appearance of the abdomen and diarrhoea are usually present. The drooling of a thick, stringy, viçid saliva is always seen. In the early stages there is a constant champing of the jaws, which gives the animal the appearance of yawning. As the disease progresses the jaws become set, and the animal is unable to take either food or water. It is impossible to open the mouth by force after they have reached this stage.

Many cases exhibit an extensive bloody diarrhoea, and a protrusion of the rectum.

The temperature remains normal throughout the condition. This is shown by the case records of numbers, 9, 10 and 11, described on pages 6 to 10.

Hyperthesia is observed from the time that the animal exhibits the first symptom. Any external noise, rubbing the hair in the wrong direction, offering of food, or palpation causes the animal to go into convulsions. The running into objects, bleating, the twitching of the muscles, falling down in spasms, and the blindness are all nervous symptoms. The arching of the back and the position of the head come also in this group of symptoms.

There are no areas of subcutaneous swellings to be found upon palpation.

Death takes place in the position assumed some time previous to the end. In cases which have shown symptoms for several hours, there is no final struggle, the animal appears to have passed into a comatose condition. Animals sick for only a short time,die in great agony, having tonic and clonic convulsions.

Recovery leads to a marked chronic debilitated condition, atrophy of the muscles, and extensive nervous disturbances.

The three animals which were exhibiting the symptoms of the disease, at the time of the second visit to the farm on November the sixth, (Nov. 6th. 1922.) will be called numbers 9, 10 and 11. All of these animals began exhibiting symptoms of the disease in varying degrees, about the same time. The animals numbered 6, 7 and 8, were observed at this time also, and they were

dead by the time that the writer arrived, and were autopsied immediately.

Case No. 9. Ayrshire heifer about 2½ years old, in advanced pregnancy. Sick 21 hours when first viewed.

This animal began to exhibit symptoms about midnight on the 5th., of November. Many violent attacks, in the form of forced movements, running into objects, bleating, convulsions and the drooling of abundant saliva, followed rapidly after this. A complete history of the case follows.

Physical Examination:- Temperature, 101.2° F, respiration 40, irregular and dyspneic, moist crepitant rales upon auscultation over the upper portion of each lung. The lower lobes were consolidated. The animal was extremely sensitive to precussion.

Pulse:- 140, weak and irregular. The heart could be heard by one standing beside the animal.

The hair coat was rough, dry and starry. All four feet were drawn very closely together.

The nose was extended, the head drawn up and backward, ears erect and constantly moving, and objects placed before the eyes could not be seen. The jaws were set and could not be opened by manual force. A frothy, sticky, viscid saliva was issuing from the corners of the mouth. The tongue was protruding from the mouth. Anorexia was observed.

Peristalsis had ceased. There was neither diarrhoea nor bloat.

While the animal had shown signs of cerebral excitement before the writer arrived at the farm, all violet symptoms of this nature had passed away. The animal gave symptoms of hyperesthesia when the hair coat was rubbed in the wrong direction and when the hands were clapped, convulsions appeared.

Manual examination proved the bladder to be full, but this condition was remedied by slight pressure through the rectum.

From knowledge of the manner in which the disease had acted in experimental animals, and considering the symptoms of the individual, a favorable prognosis was given. The following directions as to the care of the animal were given to the farm manager. "Keep the animal quiet, warm and in a darkened place or at least out of the direct sunlight. When the animal begins to eat, feed lightly upon a diet of mild laxative food such as roots, bran mashes, molasses, and very small quantities of roughage. Give a small dose of Epsom salts as soon as possible. Keep a daily temperature record."

As the result of the cooperation with the farm manager, the following report of the condition of the animal for the succeeding days is available.

Nov. 7th.,

7 A. M. Temperature 101.2° F.

11 A. M. Temperature 101.5° F.

3 P. M. Temperature 101.2° F., standing with drawn to the right side.

8 P. M. Resting more easily, sick for 56 hours.

Nov. 8th.,

8 A. M. Temperature 101.° F., more natural position, tries to nibble food, moves slightly.

12 A. M. Lying down for the first time.

6 P. M. Temperature 101.6° F., down, quiet, eyes closed, breathing more natural.

Nov. 9th.,

Temperature 101.4° F., sick 80 hours, standing and appears about normal, nosing about for food, drinks, ears have

ceased to twitch, able to open the mouth and attempts to pick up food. No swellings on the body, normal bowel movement.

Nov. 10th.,

9 A. M. Temperature 101.6° F., eats roots and drinks a little. ~~Acts~~ Appears more normal and comfortable.

8 P. M. Temperature 101.4° F., bright, eats some.

Nov. 11th.,

9 A. M. Temperature 101.6° F., eats and drinks but does not see as yet.

Nov. 12th.,

Improving.

Nov. 13th.,

Doing nicely, can see a little, very sensitive to noise and to touch.

Nov. 14th.,

Eats better and drinks an abundance of water, but remains sensitive to external stimuli.

Case No. 10., Ayrshire heifer 1½ years old.

The symptoms were first observed in this heifer about 9 P. M., on the fifth of November. Symptoms were not as violent as those observed in the preceeding case. When examined by the writer, at 9 P. M., on November sixth, the animal was down upon her knees and was unable to arise when given assistance.

The clinical symptoms in this case were the same as those observed in case No. 9 in respect to the following points:- hair coat, eyes, drooling, pulse, respiration, heart sounds, anorexia, cessation of peristalsis, and hyperthesia. This heifer also had a bloody discharge from the anus.

Due to the presence of this bloody diarrhoea, the prognosis

in this case was very bad.

Nov. 7th.,

7 A. M. Temperature 101.8° F.

11 A. M. Temperature 102.2° F.

3 P. M. Braces the fore legs and balances herself upon them for a short time.

8 P. M. Down on knees again with all body weight upon them.

Nov. 8th.,

6 A. M. Has turned herself in stall by tipping over head first.

8 A. M. Temperature 101.8° F., remains the same.

12 A. M. Temperature 101.4° F.

6 P. M. Temperature 101.8° F., same general symptoms balances herself on fore legs.

9 P. M. Standing by bracing against the stanchion.

Nov. 9th.,

Temperature 101.2° F., about the same as yesterday, but gets up and down, no body swellings.

Nov. 10th.,

Temperature 101.4° F., growing weaker, has made no attempt to eat as yet.

8 P. M. Temperature 101.6° F., weaker.

Nov. 11th.,

9 A. M. Temperature 101.6° F., weaker, does not eat or drink.

6 P. M. Down, resting easily.

Nov. 12th.,

6 A. M. Found dead in the position which she occupied when last seen on the 11th. Carcass burned.

Case No. 11. Ayrshire heifer 1½ years old.

Clinical symptoms:- Temperature 100.8° F., pulse 110, respirations accelerated, dyspneic, and shallow, anorexia, no peristalsis, rough coat, blind, hyperthesia, back arched, nose pointed out, drooling a bloody exudate, and exhibits mild convulsions.

The prognosis in this case was unfavorable.

Nov. 7th.,

7 A. M. Temperature 100.8° F.

11 A. M. Temperature 100.0° F.

6 P. M. Temperature 101.2° F., down on knees, standing on hind legs, respirations loud, rapid, and dyspneic, increase in drooling and in convulsions.

8 A. M. Temperature 102.0° F., eyes closed, same symptoms as previously observed.

Nov. 8th.,

6 A. M. Down and weak, but living.

8 A. M. Temperature 100.4° F., down and in convulsions which occur very rapidly, blood streaked drool has increased, and animal suffers greatly.

12 A. M. Temperature 101.0° F., weak, bloody fecal excreta.

3 P. M. Down, breathes much easier.

6 P. M. Temperature 102.2° F., down, stretched out with head about the post of stanchion, nose elevated, dyspneic respirations, legs extended and convulsions are observed.

9 P. M. Appears to be suffering greatly, groaning, dyspneic, panting respirations, head drawn backwards as far as is possible.

Nov. 9th.,

6 A. M. Still down in the position occupied last night, appears not to be suffering, no struggling no swellings.

9 A. M. Temperature 101.0° F.

12 A. M. Temperature 101.8° F., has not moved to-day.

3 P. M. Found dead in the position occupied since 9 P. M. of the 8th.

PRELIMINARY EXAMINATION OF CARCASSES AND STABLE.

Many carcasses of animals from this farm had been forwarded to other laboratories for examination but, due to the great distance carried in hot express cars, they were so decomposed that no results could be obtained from the post-mortem. The first carcass, (case No. 1.) received from this source was in a very bad condition. In making a report, the owners were advised as to the proper method of forwarding a body for autopsy, in order to obtain the best results.

On October the sixteenth (Oct. 16th., 1921) another carcass, (case No. 2.), was received in good condition. It was an Ayrshire female calf, about five weeks old, and had been ear-tagged by the Health of Animals Branch.

The navel, at the time of post-mortem, was thickened. There were numerous subcutaneous, subserous and submucous hemorrhages. A sero-hemorrhagic exudate was found in the thoracic and abdominal cavaties. All parenchymatous organs showed petechia and degeneration. The pupils were dilated, the neck was drawn backward, an exudate from the nose and mouth which was of a bloody, fetid nature. All the muscles had the appearance of cooked meat. The ear in the area about the tag, was swollen and inflamed.

Case No. 3. January fifth, (Jan. 5th. 1922), Ayrshire male, six weeks old. Arrived in good condition. This calf had not been ear-tagged, and the navel had apparently healed normally.

Autopsy revealed the following:-

(a) A few subcutaneous hemorrhages.

(b) An area of caseated material within the abdominal cavity, and about the navel.

(c) Congestion and degeneration of all the parenchyma.

(d) Slow clotting of the blood.

Examination of the Stable:- A visit to the farm was made on twenty-fifth of January, 1922, for the purpose of obtaining further information regarding the disease and existing conditions. The barn was a modern dairy barn, well lighted, well disinfected, well kept and ventilated. The cattle were in fair condition. The floors were of concrete, and in two maternity stalls, cork-brick was used above the concrete.

The maternity stalls were in the corner of the barn not receiving direct sunlight. An iron grating separated the two stall each having individual mangers, drinking cups, and drains. The outside of the stalls were made of brick. The walls, about six feet high, isolated these stalls from the remainder of the stable. Straw was provided for litter. The manure from these stalls had been used upon various parts of the farm. Since the outbreak of the disease, disinfectants have been used very liberally in these stalls, but of no avail.

It was discovered that, with the exception of five yearlings, all animals dead to this date have died in one or other of these two maternity stalls. These stalls were used for the isolation of new stock, for maternity cases, and for any case of sickness. When the first animals were lost it was stated in a report on file in the owner's office that the deaths were due to ptomains received from the milk of cows which had died with symptoms of strychnine

poisoning. Since that date they have continued to lose animals in these stalls. With the exception of an adult bull, and a two year old heifer, (case No. 9 of this paper), no animals showing symptoms have survived.

The bull mentioned above was purchased in the west, brought to the farm, and placed in one of these stalls. Three days after his arrival he began to exhibit the same chain of symptoms shown by all animals which had previously died. A veterinarian was called from a nearby town, who diagnosed tetanus, and stated that nothing could be done for the animal. He did, however, give the animal a narcotic and a half pint of whiskey. The next day the symptoms began to abate, the convulsions were lessened, and within a month the animal had apparently recovered.

The five yearlings which were lost during the spring of 1921 had been pastured upon a field where the manure from these stalls had been placed. All exhibited typical symptoms.

Note. Preliminary recommendations.

The manager was advised:-

(a) To remove all cork-brick from the stable and burn or otherwise destroy the same.
(b) To burn all litter from the infected stalls.
(c) To place at least three inches of unslacked lime over the concrete, which had formed a support for the cork-brick.
(d) To place a temporary plank floor three inches above the lime, and to keep it disinfected daily.
(e) To take precautionary methods with all wounds.
(f) To allow the pasture, occupied by the five yearlings mentioned above, to stand idle for the season.

CASE RECORDS.

Case No. 4. May 11th., 1922. Ear tag No. 7961, Ayrshire calf three years old, good condition, born in the pasture, brought to the stable, and fed from a pail.

The usual pathological symptoms were observed:- subcutaneous hemorrhages, cooked appearance of the muscles, fluid of a sero-

hemorrhagic nature in the abdominal cavity, slough of the mucous menbrane of the small intestines, petechia and degeneration of all the parenchymatous organs, distention of the bladder, a pneumonic condition of the lungs, heart devoid of blood, myocardium soft, flabby, hemorrhagic and degenerated, no areas of oedema, or emphysema and no local lesion.

Case No. 5. October 20th., 1922.

Ear tag No. 8831, Ayrshire calf, 10 months old, fair condition. This calf was first seen by the writer on January 25th., 1922, when a trip was made to the farm. At this time the calf was occupying one of the infected stalls. On March 2nd., we were informed by mail that this calf was exhibiting symptoms of the disease. She had been ear-tagged only four days previously, (Feb. 26th.). At this time the animal was down upon its knees and was unable to rise. When assisted to its feet it could not stand. There was no diarrhoea, no swelling of the navel, no cough, and no discharge of any nature. The temperature remained normal. Convulsions and hyperthesia were observed.

March 10th., the calf was apparently paralized in all four legs. The animal took no food, the head was elevated, and sudden noises caused a twitching of all muscles. Violent convulsions were seen when the animal was approached by strangers.

March 29th., the animal began to eat, and was extremely nervous to touch and to external sounds. Atrophy of the muscles of the limbs, and emaciation were seen. Aside from these factors the animal apparently made an uneventful recovery from this date, although it was a very slow process. As a result of this illness the animal did not make the same growth as other animals of her age.

In October, after being in the pasture all summer, this

same calf was noticed to be lame. The lameness gradually increased, debility became marked, hair coat was rough, starry, and dry, nose evelated, back arched, anorexia developed and death followed.

The carcass was received on the 20th. of October in good condition. The body showed extreme emaciation probably due to the length of time which the animal had not taken food, and to its lowered resistance. In the post-mortem, aside from the usual changes observed, an area termed a local lesion was found below the left carpal joint and beneath the tendons. This lesion was about the size of a small walnut, or about one and a half inches in diameter. The center of the lesion was caseated, the outer part showed an attempt at encapsulation, with a definite tract leading from the skin to the center of the lesion.

On Octover 9th. another calf was born in the open field and brought to the stable, where it died at the age of three weeks. The symptoms were the same as in the case of previous calves and no effort was made to have the carcass post-mortemed.

On the sixth day of November a telephone message was received from the farm manager stating that several animals which had been recently brought in from the fields were exhibiting symptoms of the disease. It was also stated that one had already died. It was impossible for the writer to arrive at the farm until nine o'clock in the evening, about twelve hours after receiving the message. Upon arrival three animals were found to have succumbed to the disease and three others were exhibiting symptoms. The symptoms in these cases were interpreted by two other Veterinarians, as "blackleg" All animals on the farm that were under four years of age were treated with "Blackleg Filtrate" on the following day by one of these

Veterinarians.

The affected animals were all under the age of three and a half years, and some of them were well advanced in pregnancy. They had been in open pasture all summer and were placed in the building a week previous to death. The flooring in this building was of cork-brick. It was a modern well-equipped barn which had been built for a piggery.

The post-mortem findings on the dead animals were as follows:-

Case No. 6. Ear tag No. 444590, senior two year old Ayrshire, well advanced in pregnancy, and in good condition. Dead 12 hours.

Autopsy revealed:- slight bloat, bloody exudate from the nose and anus, a sticky and gelatinous saliva from the mouth, pupils dilated, jaws set, and no areas of oedema or emphysema. Subcutaneous, submucous, and subserous hemorrhages were observed.

A sero-hemorrhagic exudate was found in the abdominal cavity. Petechia and parenchymatous degeneration were present in all of the parenchyma. The bladder was distended. A sloughing of the mucosa of the small intestines was observed.

The thoracic cavity:- The lungs showed a hemorrhagic pneumonia, the heart had stopped in auricular diastole, and the myocardium was greatly degenerated, flabby, and presented a cooked appearance. The pericardium exhibited petechia, and the blood was very dark and clotted slowly.

Brain and meninges:- Congestion and a collection of a sero-hemorrhagic fluid were observed.

Cultures were made from the following sources:- (a) fluid about the brain, (b) heart blood, (c) heart muscle, (d) liver, (e) kidney, and (f) the spleen. The media used consisted of

Loeffler's blood serum, B. P. agar, and broth. Aerobic cultures only were made.

B. Suberis was obtained from all these cultures.

Case No. 7, Ear tag No. 91, Ayrshire calf, two year old, pregnant, and in good condition. Dead for four hours.

There was no bloating after death, no discharge was seen from the nose but there was a bloody exudate from the anus, The saliva exuding from the mouth was sticky, frothy, and abundant.

The post-mortem findings in this case were the same as in the fore-going case. Cultures were made as before, which also gave B. Suberis.

Case No. 8, Ear tag No. 44473, Senior two year old, in good condition. Dead about fifteen minutes.

The changes were identical with those of the previous cases but not so marked.

B. Suberis was obtained in the laboratory from cultures.

Case No. 9. Material taken By another veterinarian, from a calf which had died of the same disease on Dec. 25th. 1922. In this case the samples were duplicates of those forwarded to another laboratory. Portions of the liver, spleen, and thymus were received Cultures were made as in the case of all autopsy material. Positive results were obtained, and B. suberis was isolated from all organs mentioned.

In carrying out these post-mortems, another Veterinarian was present to observe them and assisted in the taking of the cultures upon direction. In the examination of the cultures in the laboratory, and in the testing of the same upon experimental animals, a Bacteriologist checked the results. The results of work with B. suberis, in animal experiments are given in another part of this paper.

Case number 10April 21st,1923. Calf born from cow #25 on Jan, 25th,1923.

March 25th,1923- animal recieved 15c.c's.of blood from case #9 in the subcutaneous tissue of the neck.

The animal began to exhibit symptoms of the disease on April 20th, and was first seen by the writer on the following day at 1:30 P.M. The temperature at this time was 101.2°F.,respiration 30,and the pulse 110. Hyperthesia,excitability,and blindness together with posterior paralysis were observed. There were no body swellings,the animal ate and its bowel movements appeared normal.

The animal was killed by bleeding,April,21st. The following post-mortem changes were noted- degeneration of the parenchyma,a slight amount of serous exudate was present in the abdomenal and the thoracic cavity,the lungs were normal,the brain was congested and degenerated whilst the subarachnoid space was distended by a serous fluid. The mucous menbrane of the small intestine was sloughed in areas.

Cultures were made from the fluid in the subarachnoid space,the liver,and the kidney. B.suberis was recovered from these sources.

Cases number 11 and 12. History- A small herd of Jerseys were brought from Plattsburgh,N.Y.,to the farm about July 1st,1923. Since they could not be placed directly with the main herd until aftervthe period of isolation required under the "accredited herd plan",they were kept separate on non-infected soil. When this segregation became a burden on account of the extra work,they were put in a field where other animals had become infected.

Exactly three weeks from the day that they were placed in this infected pasture,the two animals numbered 11 and 12 together with one other individual began showing symptoms of the disease and which had been observed in all previous cases.

On July 31st.,1923,a few hours after the first symptoms had been

observed, the writer saw these animals. One of them died within a few minutes after his arrival and the second died within an hour. The third animal remained in a comatose condition for about three days before death took place.

The ante and post-mortem symptoms of these animals were the same as the cases previously described. Cultures were made as in previous cases and B. suberis was recovered from all of them.

HISTOLOGY AND PATHOLOGY.

The histo-pathological changes are as follows:-

(a) Brain and Meninges, Acute hemorrhagic and degenerative meningitis.

(b) Heart, Acute sero-hemorrhagic and degenerative myocarditis.

(c) Liver, Acute sero-hemorrhagic and degenerative hepatitis.

(d) Spleen, Acute hemorrhagic and degenerative splenitis.

(f) Small Intestines, Acute hemorrhagic and diptheritic enteritis.

(g) Local Lesion (when one was located) exhibited a necrotic and purulent condition with a proliferation of tissue.

Detailed changes given below:-

Liver:- Passive congestion, arteries empty, cells are granular and pale, cloudy swelling, few nuclei stain, cells swollen, hemorrhage between the cells, many polymorphonuclear leucocytes in the cells.

Spleen:- Many areas of cloudy swelling, granular fragmented cells, few nuclei stained, extensive hemorrhage, and little serous exudate.

Heart:- Separation of the muscle fibers by a serous exudate, granular, swollen appearance of the cells, few nuclei stain and those only slightly, extensive areas of hemorrhage and serous exudate.

Kidneys:- Bowman capsules show an extensive disquamation and degeneration of the cells; the glomeruli exhibit a hemorrhagic condition, and the cells are very pale and do not take the nuclear stain. The collecting tubules show a cloudy swelling and a collection of fibrinous exudate within the same. Hemorrhagic

areas are present throughout the parenchyma.

Small Intestines:- Cloudy swelling of the mucosa and a peeling of the same. Submucous hemorrhages and a serous exudate are observed.

Local Lesion:- Connective tissue is swollen by an infusion of a serous and purulent exudate. There is a degeneration of the surrounding muscle fibers, hemorrhage, and a productive imflammation of the area.

The extent of these various lesions depends upon the duration of time which it required for the organism to cause death of the individual and also the length of time which the animal had been dead previous to the performing of the autopsy. In animals which have succumbed within the three day period, after the first symptom is observed, all of the above changes are to be found. In chronic cases the changes may be more marked, while subacute or peracute cases show very little upon post-mortem.

ISOLATION OF THE ORGANISM FROM AUTOPSIED ANIMALS.

From case No. 2 cultures were taken from the parenchymatous organs, abdominal fluid, fluid in the thoracic cavity, heart blood, and the navel. The ear was brought to the laboratory for examination. Cultures were made upon the various media as noted previously, and under aerobic and anaerobic conditions.

The following results were obtained from calf No.2:-

In 24 hours, a diplococcus and a short rod appeared in cultures made from the heart blood and the navel. The other cultures showed a short rod and a coccus.

In 48 hours, in a culture from the ear, a short rod was seen

which had produced terminal round endospores. This organism resembled B. tetani, but proved to be Gram negative. Pending further study the owners were advised to take sanitary precautions with all wounds, and to properly sterilize their ear punch before using it again.

Since this organism was not obtained in a pure culture, steps were taken to insure the fact that this was the organism sought. A mixed broth culture was injected into Rabbit No. 1, (page 41), which caused the animal's death and the organism was again recovered. Following this proceedure the culture was plated out on B. P. agar, and colonies were picked and transferred to broth and other liquid media. After the above, the "Kitasato method" of isolating B. tetani, was employed. To follow this method a 72 hour broth culture was used, heating it at 80° C in moist heat for 1/2 hour. This method was used to kill all vegetative forms and leave the spore free to produce new growth.

Spores so isolated were seeded in broth, milk, agar (in the stab and on the slope), and Loeffler's blood serum.

From case No. 3, cultures were taken from the parenchymatous organs, heart blood, fluid about the meninges, and from the navel.

All cultures, except those from the navel, were discarded upon finding B. suberis.

Case No. 4, cultures were made in the same manner as from previous animals. In this case, however, all cultures were examined over a 7 day period. The organism was recovered from all tissues. B. suberis from this case was used upon experimental animals Nos 23, 27-A and 30.

Case No. 5, cultures were made from the parenchyma, and from the local lesion. Growths from these cultures proved to be

B. suberis and pathogenic.

Case No. 6, fluid about the brain, heart blood, heart muscle, liver, kidneys, and spleen were cultured by aerobic methods.

Case No. 7 and case No. 8, were cultured the same as from case No. 6, and also gave B. suberis.

Case No. 9, cultures were made from the material received and B. suberis was obtained.

ISOLATION OF B. SUBERIS FROM THE SOIL.

On August the twenty-fourth, 1922, samples of the soil from the farm were received. The samples were taken from the following sources:-

(a) from an old barnyard,

(b) from the open pasture,

(c) from the soil over the carcass of an animal which died during the summer of 1922,

(d) from soil above the carcass of a heifer which had been buried in 1921,

(e) from litter from one of the maternity stalls.

Cultures were made from the above materials upon B. P. broth, B. P. agar, and milk, later subcultures were made upon other media such as, Loeffler's blood serum, Dunham's solution, and gelatin.

While the organism was not found in a pure culture, it was present in all of the above materials. When obtained in a pure culture, by use of the "Kitasato method", B.suberis so isolated proved to be pathogenic for experimental animals.

ISOLATION OF B. SUBERIS FROM CORK-BRICKS.

At the time of the first visit to the farm, January 25th

1922, after a thorough investigation of the stable, it was suspected that the floor might be the source of the infection. In order to confirm or deny this supposition samples of the cork-brick then in use were taken from three different parts of the stalls.

These portions of the cork-brock were brought to the laboratory and cultured in broth and milk. Later subcultures were made upon other media. These cultures all gave positive results and the organism so obtained proved pathogenic for experimental animals.

Cultures from these bricks were made by heating a knife over a flame and cutting off small shavings from the brick directly into the media used. Aerobic cultures only were used. Mixed cultures were obtained but the organism was easily isolated by the "Kitasato method" previously described.

After finding this organism in the used cork-brick it was thought necessary to examine brick from other sources in order to determine the origional habitat of the organism. In this investigation cork-bricks were obtained from:-

(a) the infected farm, unused bricks which had been exposed to the weather for four years,

(b) an office, where a sample brick had been stored for 10 years,

(c) a loft, where an unused brick had been stored for 10 years,

(d) the manufacturers, who forwarded 3 year old samples of their brick for horse and cow stalls,

(e) used brick from another farm in the Province of Quebec,

(f) a farm in the State of New York, where the brick had been in use for a number of years.

B. suberis was obtained from all of the above material.

ISOLATION OF B. SUBERIS FROM CORK.

Having cultured the cork-bricks with positive results, the next step was to ascertain which of the constituents of the cork-brick acted as the habitat for this particular organism. As asphalt and granulated cork are the products used in making the bricks, the cork was naturally suspected.

In culturing this product, small pieces of the cork were dropped into liquid media (broth and milk), and subcultures were made upon other media.

Cork was obtained from the following sources:-

(a) insulating cork from the manufacturers of the cork-brick,

(b) insulating cork from an English firm,

(c) granulated cork used in the packing of grapes, obtained from two different sources,

(d) granulated cork used in the manufacturing of the cork-brick.

B. suberis was isolated from the cork obtained from all sources mentioned above.

ISOLATION OF B.SUBERIS FROM EXPERIMENTAL ANIMALS.

In the case of all experimental animals which died following the inoculation with cultures of B. suberis, the organism has been recovered. The results of these experiments are shown in another part of this paper.

Inoculation of other experimental animals with culture of B. suberis isolated from others dead of the disease produced by inoculation proved such cultures pathogenic. By these means Koch's postulates have been proven. (see pages 45-57.)

BACILLUS SUBERIS.

In 24 hours cultures at 37°C inoculated from the diseased

animal tissue, diplococci forms are found; 48 hour broth cultures show bipolar forms when stained with methylene blue; 72 hour cultures exhibit a bacillus with terminal spores resembling B. tetanus. Subcultures on agar show spore formation and bipolar forms in 24 to 36 hours. The bacilli have rounded ends and seldom unite in pairs or chains. In 12 to 18 hour broth cultures the bipolar forms appear, and they were observed in milk up to 8 days. With methylene blue the organism does not stain deeply but at one or both poles, and sometimes in the centre of each rod, metachromatic granules are found, which take a reddish or purplish tint. Jordan and Harris (1) in a paper on "Milksickness", reports an organism with similar reddish granules.

24 to 36 hour agar slope at 37° C show vegetable cells from 0.5 μ. to 1.0 μ. in width, and average 4, 5, μ. in length. The organism is motile with peritrichous flagella, Gram negative, aerobic, and facultative anaerobic.

CULTURAL CHARACTERISTICS.

B. P. agar slope at 37° C. Growth in 24 hours is rapid, spreading, glistening, and of a dirty greyish color.

B. P. agar plate at 37° C. (a) Surface colonies in 24 hours are spreading, irregular, smooth, flat, contoured, and grey to white in color.

(b) Surface colonies of a second type are circular to amoeboid, smooth, slightly raised, and of greyish white color. Colonies in 24 hours are 2-3 mm. in diameter.

(c) Under low power the second type is entire, irregular, dark centered, coarsely granular, and greyish to white in color.

(d) Submerged colonies are amoeboid, floccose, greyish to white in color, and up to 1 mm. in diameter.

B. P. broth, at 37° C. In 24 hours, clouding, no pellicle, and a heavy precipitate.

B. P. gelatin stab at 20° C. Faint filiform growth on deep line of puncture. Distinct cup or funnel shaped liquifaction in 4 days.

B. P. gelatin plate at 20° C. Glistening, entire, waxy, surface colonies of 0.5 to 1.0 mm. in diameter appear after 72 hours. Submerged colonies are smaller, and about 0.5 mm. in diameter, yellowish in color and yeast like in growth. Under low power these are entire, conglomerate, finely granular, and of a yellow color. Liquefaction of surface colonies in 6 days, which is cup-shaped and has distinct concentric rings. It is a colony of 3 to 6 mm. in diameter.

Dunham's solution at 37° C. Heavy pellicle, medium clear, the pellicle falls upon slight disturbance but forms again.

Dunham's solution at 37° C with the addition of 1% of lactose, saccharose, maltose, dextrose, and mannit and 1% of Andrade's indicator are negative for acid and gas in 48 hours.

Dunham's solution at 37° C gives positive indol test with the Ehrlich's test in 72 hours.

Plain milk at 37° C. In 6 days partial coagulation, in 7 days partial coagulation and digestion.

Litmus milk at 37° C. In 6 days partial coagulation and reduction of litmus, in 8 days reduction almost complete with partial digestion and coagulation.

Nitrate broth at 37° C. Slight clouding in 72 hours with no change to nitrites.

Cohn's solution at 37° C. No change in 72 hours.

Potato at 37° C. Moderate, raised, spreading, dirty yellowish growth in 24 hours.

TOXIN.

B. suberis produces an extracellular toxin. The methods used in the production of this toxin are as follows:-

(a) Giltner's (22) method for the preparation of tetanus toxin.

(b) anaerobic cultures, by the aid of pyrogallic acid and KOH solutions, with the organism grown at varying periods of time and at 37° C,

(c) aerobic cultures of B. suberis and B. cereus grown for varying times at 37° C,

(d) the aerobic growth of B. suberis alone for varying periods of time.

The time as stated above, varied from 4 days to 21 days. Cultures were then passed through a Berkefeld filter to remove all of the organisms. The filtrate was tested by microscopical and cultural means for purity. The toxin thus produced was then used upon experimental animals. The results of these experiments are shown under the section of the paper dealing with experimental animals.

PATHOGENICITY.

The organism (B. suberis) herewith described has proven pathogenic for cattle, rabbits, small pigs, cats, dogs, and guinea pigs. The average period of incubation has been three days. In some individuals the disease may assume a chronic form leading to recovery in three or four weeks, with cachexia, general debility, and extreme nervous disturbances.

The toxin, or the transfer of a portion of the local lesion, usually produces death within 20 hours.

Of the artifical media, milk produces the most virulent growth of the organism.

The minimum lethal dose has been determined for rabbits with average weight of 1800 grains and is found to be 3 cc., of a broth culture 72 hours old at 37° C.

AGGLUTINATION TESTS.

Agglutination test of serum of a recovered heifer gave a positive test in dilutions up to 1:320. In the case of an adult bull which recovered some three years ago, a positive test was furnished in dilutions up to 1:80. Preliminary tests indicate the presence of agglutinins in the blood of recovered animals.

The technic of the microscopic and macroscopic tests was carried out according to "Kolmer". (23).

GENERAL REVIEW OF DISEASES AND ORGANISMS RESEMBLING THIS OUTBREAK.

It is not necessary to go into detail regarding the history of the organisms producing the diseases which have been confused with that produced by B. suberis, except in the case of those which are very similar morphologically. In this class of organisms the tetanus and the many pseudo-tetanus organisms must receive attention.

The organism which is most similar to B. suberis is B. tetani. Tetanus has been known to exist since the time of Apsyrtus, (25) in the 4th., century. Long before the causative organism was discovered by Nicolaier (2), in 1884, it was considered a wound infection disease.

The forms in which tetanus appears were understood very well by Thomson (3), who gave a paper on these conditions in England in 1862. He described the form of tetanus which is due to wound infections, and the type called idiopathic.

While Nicolaier succeeded in obtaining the organisms from infected wounds, he did not secure a pure culture. Kitasato, in 1889, obtained a pure culture of B. tetani by means of the method now bearing his name. He made a very careful study of the organism from cultural, morphological, and pathological view points.

As an explanation of idiopathic tetanus, Tarozzi (4) gives the finding of the spores of B. tetani in the blood and the parenchymatous organs. Sanchez et al. (5) believe that these spores enter the body after death.

Francis (6) has thoroughly demonstrated that spores injected into the tissues are rapidly attacked by the phagocytes.

The sources of B. tetani are shown to be:-

(a) soil from certain localities, (7)

(b) from feces, (8)

(c) from wounds of many types,

(d) contamination of biological products,

(e) burrows of crabs (9),

(f) hay dust (10),

(g) mud at the bottom of Lake Geneva (11),

(h) and from the intestinal canal of animals (8),

Tetanus causes death by the production of an extracellular toxin, .004 cc. of which is sufficient to kill a man of 175 pounds. The organism does not multiply within the body. The toxin acts upon the central nervous system. It is taken up by the peripheral nerves and conducted to the medullary centers where it combines with the motor nerves. Death results from a paralysis of the respiratory muscles, (24.)

A means of treating tetanus was first discovered by Kitasato and Behring (12), and it is due to their work that we have the tetanus antitoxin.

COMPARISON OF B. TETANI AND B. SUBERIS.

B. tetani.		B. suberis.
Optimum temperature	20 to 37 °C.	Same
Thermal death point	100 °C for 15 minutes	100 °C for 30 minutes
Conditions of growth	strictly anaerobic	aerobic & facultative anaerobic
Broth, glucose,	cloudy, precipitate, gas	no pellicle, or gas
Agar, glucose,	anaerobically only, gas	aerobic or anaerobic
Gelatin liquefied	yes and gas formed	yes, no gas formed
Loeffler's serum	liquefied	not liquefied
Isolation	"Kitasato method"	same
Gram stain	Positive	negative
Habitat	soil, infected wounds	cork-brick, cork, soil infected wounds
Pathogenic	yes	yes
Spore formation	round endospores	same
Flagella	Peritrichous	same

Rosenthal (13) states that B. tetani may assume life under aerobic conditions. He describes three stages in this change from a strict anaerobe to an aerobe. In the beginning the organism is intact chemically, biologically and pathogenically. These characters are later lost and can only be recovered under strictly anaerobic conditions.

Assuming that it may be possible to cultivate B. tetani, as has been stated by Rosenthal, there are still many factors remaining in which the organisms vary. These differences may be summed up as follows:-

(a) B. tetani is always Gram positive, although this fact has been disputed by Kymer (14) who states that not all

cultures of B. tetani take the Gram stain and that some take it only feebly.

(b) B. tetani produces gas under anaerobic conditions and B. suberis does not.

(c) B. suberis produces indol,

(d) B. tetani is activated by the use of lactic acid, as this substance inhibits the action of the leucocytes; it has no effect where B. suberis is employed. It has been used in sufficient quanaties to produce the sloughing of the skin from the base of the head to the shoulders without the organism producing any of the symptoms of the disease. Rabbits inoculated with B. suberis alone, used as checks in these cases, died.

(e) B. tetani has a definite period of incubation in experimental animals while with B. suberis it is variable. With B. tetani, Bosanquet (15) gives the period of incubation for rabbits as 18 to 36 hours, while with B. suberis it varies from 2 days to 2 weeks, depending upon the age of the culture used, and the amount injected. The toxin or the transfer of a portion of a local lesion with B. suberis produced death within a few hours.

HISTORICAL ACCOUNT OF THE PSEUDO-TETANI ORGANISMS.

The literature describes a number of organisms which are similar to B. tetani and B. suberis. The greatest difference between these pseudo-tetanus organisms and B. tetani lies in the non-pathogenicity of the former. Culturally and morphologically they are closely allied to B. tetani.

The main factors involved in their similarity are:-

(a) the production of round endospores with the drumstick formation, (b) and the liquefaction of gelatin.

Among the pseudo organisms described are the following:-

(a) Bain (16), found an organism in wounds from blank cartridges which was non-pathogenic, and Gram negative.

(b) Bushnell (17) isolated an organism from a case of fistulous withers, which produced oval spores, and was non-pathogenic.

(c) B. pseudo-tetanicus (Kruse) Migula 1900, was also described by Neide in 1904 under the name of B. sphaericus (18). It was Gram positive, non-pathogenic, and did not liquefy gelatin.

(d) Bienstock (19) in 1899-1901, discovered an organism present in contaminated agar plates, which he called B. putrificus. Rettger (2) 1906, also worked with the same organism. It was strictly anaerobic and difficult to isolate, not easily grown on artificial media, and non-pathogenic.

(e) Another group of pseudo-tetanus organisms are known as the tetanus group, and they liquify gelatin and produce endospores (21).

(1) B. pseudo-tetanicus (Sanfelice) which is less toxic, is Gram positive, produces gas, pathogenic to guinea pigs, and mice.

(2) B. cuneatus is found in milk and is non-pathogenic.

(3) B. saccharobutyricus is similar to B. suberis in staining reactions only.

(4) B. lubinskii (Kruse) is found in abcesses, is Gram positive and pathogenic for rabbits in 24 hours. It produces gas.

(5) B. longus is found in the soil, is Gram negative, produces gas, and is non-pathogenic. Spore not terminal.

(6) B. taveli, is found in abcesses, produces oval non-terminal spores, is non-pathogenic, and produces gas.

(7) B. pseudotetanicus var. aerobicus, (Kruse) grown at ordinary temperature only. It was isolated from a case of tetanus.

COMPARISON WITH THE PSEUDO-TETANUS ORGANISMS.

In the comparison of the many pseudo-tetanus organisms which are mentioned in the literature, with B. suberis it is necessary to consider only those similar in the following respects:

(a) those which produce terminal endospores,

(b) those which are Gram positive,

(c) non-gas producers,

(d) anaerobic or facultative anaerobic organisms,

(e) those not producing acid,

(f) those which liquefy gelatin, and

(g) those which are pathogenic.

The pseudo-tetanus organisms belonging to the tetanus group, previously mentioned, are similar to B.suberis in that they grow anaerobically, liquefy gelatin, and produce terminal endospores. They differ from B. suberis in staining, gas production, inability to grow aerobically, and with the exception of B. lubinskii they are non-pathogenic.

In another group of the tetanus type, B. sublanatus, B. lacteus, B. leichenformis, B. pseudotetani (Bain), and B. pseudotetanicus (Bushnell) appear. These organisms are all aerobic and facultative anaerobic, produce terminal endospores, are Gram negative, and they differ from B. suberis in that they are non-pathogenic.

B. saccharobutyricus, is found in milk and is non-pathogenic, produce gas and acid, and is similar to B. suberis in that the rods contain granules which may be seen in stained preparations.

While there are some organisms which are similar to B. suberis in staining, morphology, or in cultural characteristics,

the literature fails to show one organism which coincides in most respects.

DIFFERENTIATION FROM DISEASES CONFUSED WITH THAT PRODUCED BY B. SUBERIS.

Aside from these previously mentioned diseases, produced by B. tetani and the pseudotetanus organisms, the following diseases have been confused with the one produced by B. suberis; namely,-

(a) blackleg,

(b) anthrax,

(c) hemorrhagic septicemia,

(d) "vibrion septique",

(e) milk sickness.

Incorrect diagnosis of these various diseases have been made by veterinarians who have seen animals affected with the disease, and who have held and witnessed post-mortem examinations. Errors have not been confined to the clinical and pathological examinations, for some laboratory workers to whom material was sent, stated that the disease was anthax or "Vibrion Septique", or hemorrhagic septicemia. Whilst the diseases may be somewhat puzzling a differential diagnosis is possible.

The clinical symptoms in which the diseases differ from that produced by B. suberis, are as follows:-

1. Blackleg has been the most common diagnosis by those who have seen the animals affected with the disease. With blackleg there is always an elevation of the temperature, an edematous, emphysematous, and crepitating area about the infected part of the body. Cerebral excitement and hyperthesia are not observed. Blindness does not occur in blackleg.

2. Anthrax always causes a very high elevation of the temperature, a greatly enlarged spleen, and a tarry consistency of the blood.

3. Hemorrhagic septicemia exhibits a high temperature, external body swellings, and bloody exudates.

4. "Vibrion septique" produces a high temperature, and a spreading edema and emphysema about the infected part.

5. Milk sickness causes an edema of the lungs, and enlarged kidneys.

6. Tetanus does not give rise to blindness, nor to the pathological changes observed in the parenchyma with this disease.

The chart shown on page 37, shows the comparison of the ante-mortem symptoms, of the above mentioned diseases.

A summary of the symptoms and post-mortem findings of the diseases which have been confused with the disease produced by *B. suberis*, are given below.

COMPARISON OF ANTE-MORTEM SYMPTOMS OF DISEASES.

	Disease produced by *B. suberis*.	Blackleg. (*B. chauvei*)	Anthrax. (*B. anthracis*)	Hemorrhagic Septicemia (*B.bovisepticus*)	"Vibrion septique (*B. oedematis maligni*)	Milk sickness. (*B. lacto-morbi*)
Age affected	all	6mo.4yr	all	all	all	all
Temperature	normal	E.	E.	E.	E.	subnormal
External exudates	P.	P.	P.	P.	P.	P.
Edema	N.	P.	N.	P.	P.	lungs
Local lesions	P.&N.	P.	N.	N.	P.	N.
Dyspnea	P.	P.	P.	P.	P.	P.
Pleuritis	N.	N.	N.	N.	N.	P.
Pneumonia	P.	N.	P.&N.	P.&N.	N.	N.
Anorexia	P.	P.	P.	P.	P.	P.
Drooling	P.	N.	N.	P.	P.	N.
Suspended rumination	P.	P.	P.	P.	P.	P.
Colic	N.	N.	P.	P.	N.	N.
Diarrhoea	P.&N.	P.&N.	P.	P.	P.	N.
Emesis	N.	N.	N.	N.	N.	P.
Excitement	P.	N.	P.	N.	N.	P.
Blindness	P.	N.	N.	N.	N.	P.
Hyperthesia	P.	N.	N.	N.	N.	N.
Dullness	N.	P.	N.	P.	P.	N.
Vertigo	N.	N.	N.	N.	N.	P.

Note:- N = negative, P = positive, E = elevated.

1. Blackleg	Diseases produced by B. suberis.
Bloat quickly at death.	Do not bloat quickly.
Emphysematous and crepitating swellings over local lesion.	No such swellings are observed.
Gas and odor in infected tissues	No gas or odor.
Petechia and parenchymatous degeneration of all parenchyma	Same.
Collection of sero-hemorrhagic fluid in the body cavaties.	Not always present.
Gas in liver.	Not found.
Lungs seldom affected.	All stages from congestion to consolidation.
Exudates from external orifices.	May or may not be present.
2. Anthrax.	
Blood dark, not readily coagulated.	Dark but coagulated.
Extravasation of blood from nose and anus.	Not always seen.
Lympnatic tissue swollen, hemorrnagic, and edematous.	Sometimes observed.
Parenchyma hemorrhagic and degenerated	Same.
Lungs, hyperemia, and edema.	All stages from congestion to consolidation.
Brain, hyperemia, and edema.	Same.
Mucosa of small intestines, swollen, dark, and necrotic.	Sloughing of the mucosa.

3. Hemorrhagic Septicemia.	Disease produced by B. suberis.
Bloody exudate from body openings.	Same in some cases.
Sero-hemorrhagic exudate in body cavaties.	Sometimes observed.
General hemorrhagic condition of the subcutaneous, subserous and submucous tissue.	Usually found.
Lungs, stages from congestion to consolidation.	Same.
Pericardial hemorrhages	Not usually seen.
Meningeal lesions.	Same.
4. "Vibrion Septique".	
Local lesion, crepitating, swollen, emphysematous, and spreading.	No swelling on body.
Sero-hemorrhagic fluid in the abdominal cavity.	Sometimes seen.
Parenchyma, hemorrhagic and degenerated.	Same.
Heart muscle, soft and flabby.	Same.
5. Milk Sickness.	
Sero-hemorrhagic exudate in body cavities.	Same sometimes observed.
Edema of lungs, serous pleural exudate.	Lungs from congestion to consolidation.
Parenchyma hemorrhagic and degenerated, fatty degeneration of the liver, enlargement of the kidney.	Parenchyma, hemorrhagic and degenerated. No fatty degeneration of liver, no other change in the kidney.

CULTURAL CHARACTERISTICS AND PHYSIOLOGY.

Organism.	Organism found in.	Staining.
B. suberis.	Animals dead of the disease, cork-brick, granulated cork, soil.	Gram negative. Reddish granules in rods from young cultures when stained with methylene blue.
B. chauvei.	Animals dead of the disease, and soil.	Gram positive.
B. anthracis	Animals dead of the disease, soil, hides, infected streams.	Gram positive.
B. bovisepticus	Respiratory tract of animals dead of the disease.	Gram negative.
B. "vibrion septique"	Soil, dust, and putrid material of animals dead of the disease.	Gram negative.
B. lactomorbi	Infected soil, plants, grains and from animals dead of the disease.	Gram positive.

Organism.	Growth on broth.	Growth on agar.
B. suberis.	No pellicle, cloudiness of media, heavy precipitate No gas or acid upon sugar broths.	Surface round, raised, glistening, submerged spreading.
B. chauvei.	Gas and acid on sugar media.	growth along needle track, gas and sweet odor.
B. bovisepticus	Media cloudy, no gas or acid in 7 days.	smooth shiny colonies 2 mm. in diameter.
B. anthracis	Pellicle, precipitate, clear media.	greyish surface colonies.
B. "vibrion septique"	Uniform cloudiness.	radial filaments along needle track.
B. lacto-morbi	48 hours pellicle, precipitate and cloud-iness.	grey, moist, smooth veil like surface growth.

Organism.	Gelatin.	Milk.	Properties.	Pathogenic
B. suberis	liquefied	coagulation partial digestion in 7 days.	facultative anaerobe. Opt. Temp. 20-37°C, it produces indol, no gas nor acid on broth. Toxin produced.	Cattle, calves, pigs, cats, rabbits guinea pigs, and dogs.
B. chauvei	liquefied		anaerobe, Opt. Temp. 37°C toxin acid and gas are produced.	young cattle, sheep.
B. anthracis	liquefied	slowly coagulated	facultative anaerobe, Opt. Temp. 37°C, spores in presence of oxygen, capsules in blood.	Cattle, sheep, and man.
B. boviseptious			facultative anaerobe, Opt. Temp. 30-37°C, does not form indol.	cattle and laboratory animals
B. "vibrion septique"	liquefied	slowly coagulated	obligate anaerobe. Opt. Temp. 37°C. does not form indol gas and odor given off.	horse, dog, sheep and pig.
B. lactomorbi	liquefied gas formed	no change	obligate aerobe Opt. Temp. 30-37°C.	man, dog, cattle.

BACTERIOLOGICAL RESULTS.

Morphology of the various organisms which produce the diseases previously mentioned, and of B. suberis, follow:-

1. B. suberis,- From young agar cultures the bacilli appear as motile rods, with peritrichous flagella, measuring 0.5 - 1.0 μ by 4.0 to 5.0 μ. The bacilli form terminal round endospores which measure 1.5 u in diameter. Many forms are observed which vary from a diplococcic form to a bipolar form, and the drum-stick formation when the spore is attached. The bipolar form contains two or more granules which stain with a reddish tint with methylene blue.

2. B. Chauvei,- This organism is a motile rod, which produces spores forming a slostridium. The organism measures 0.5 - 1.0 μ by 3.0 - 5.0 μ. and appears in pairs and chains, never in filaments.

3. B. anthracis,- Is a non-motile rod, measuring 1.0 - 2.5 μ by 5.0 - 10.0 μ. Spores are formed only in the presence of oxygen, and are in the centre of the organism. Capsules are formed in the blood.

4. B. boviseptieus,- This is a short non-motile, bipolar organism with rounded ends. It measures 0.5 - 0.7 μ by 1.0 - 2.0 μ in size.

5. B.oedematis maligni,- This is a motile rod, producing oval spores which cause a spindle like form of the organism. It measures 1.0 μ by 3.0 - 8.0 μ.

6. B.lactomorbi,- This is a long, motile, slender rod, which forms round endospores, has peritrichous flagella.

10 to 15 in number. The bacilli appear in pairs, chains, and filaments.

In the preceeding pages the cultural characteristics, the staining reactions, pathogenicity etc., have been considered. As a result of these facts being known it is possible to arrive at a differential diagnosis between the disease produced by B. suberis and those diseases with which it has been confused. As a basis for such a diagnosis, the factors mentioned under each disease must be considered.

1. Blackleg, eliminated by comparison of the following:-

(a) morphology and spore formation,

(b) reaction to Gram's stain,

(c) use of methylene blue stain,

(d) B. Chauvei is an obligate anaerobe,

(e) no indol produced,

(f) growth on agar,

(g) growth in broth,

(h) animal inoculation,

2. Anthrax, the following:-

(a) examination of blood of animals dead of anthrax, should show the capsules about the organisms,

(b) morphology and spore formation,

(c) reaction to Gram's stain,

(d) use of methylene blue stain,

(e) no production of indol,

(f) animal inoculation,

3. Hemorrhagic Septicemia, by,

(a) morphology,

(b) use of methylene blue stain,

(c) growth on broth,

(d) no production of indol,

(e) animal inoculation.

4. "Vibrion Septique" by

(a) obligatory anaerobe,

(b) growth on various media,

(c) use of methylene blue stain,

(d) animal inoculations.

5. Milk Sickness by,

(a) its strictly aerobic nature,

(b) reaction to Gram's stain,

(c) non-production of indol.

6. Tetanus and the various pseudo-tetani organisms have been dealt with in another part of this paper and will not be discussed here.

It will be observed that B. suberis, which produces the disease herein described, differs from the diseases mentioned in the following points:- (a) morphology, (b) staining with methylene blue, (c) production of an extracellular toxin, (d) production of indol, and (e) in cultural characteristics.

The effect of B. suberis upon experimental animals, the ante-mortem and post-mortem symptoms, are very characteristic.

EXPERIMENTAL ANIMALS.

No. 1. White rabbit. Nov. 19th., 1921 at 3 P. M.
Injected intraperitioneally, 2 cc., 72 hour broth culture (mixed) from calf case No. 2.
Found dead at 8 A. M., Nov. 20th., 1921.
Autopsy:- Hair was scratched from the abdomen, bloody diarrhoea and salivation were observed externally.

A necrotic area about the site of injection was called a local lesion. Internally, a purulent peritonitis, and a degeneration of all of the parenchyma were observed.

Cultures were taken from the local lesion, the exudate in the abdominal cavity, and the parenchymatous organs. The B. suberis was recovered.

No. 2. Brown rabbit, Nov. 20th., 1921, at 11 A. M.

Injected subcutaneously with .5 cc. of purulent exudate from the abdomen of rabbit No. 1.

No change on Nov. 25th., and was returned to pen.

No. 3. Grey rabbit, Nov. 24th.,

Inoculated subcutaneously with 2 cc. broth culture, 14 days old, from local lesion of rabbit No. 1. Died on Dec. 6th., without showing any pronounced external symptoms. Anorexia, a champing of the jaws, and slight diarrhoea were present from the second day after the inoculation. On autopsy a local lesion was found at the site of injection, and internally all the parenchyma were found to be hemorrhagic and degenerated. No fluid was found in the abdominal cavity.

B. suberis was recovered from the local lesion and the parenchyma.

No. 4. White rabbit, Nov. 24th., 1921.

In this case an intramuscular injection of 1 cc. of a broth culture, 72 hours old, from calf case No. 2 was used. Previously to the injection the culture was heated for 30 minutes at 80°C.

Found dead on Nov. 28th., at 8 A. M.

Autopsy:- Pupils were dilated, the jaws were set, a sticky saliva had caused the hair about the mouth and on the chest to become matted. A local lesion about the size of a pea was found at site of injection. The internal changes were not marked, but an exudate was present in the thoracic cavity.

Smears were made from all of the parenchyma and the fluid in the thoracic cavity. Cultures were made from the above and from the local lesion. B. suberis was recovered from all cultures.

No. 5. Brown rabbit, Nov. 28th., at 3.45 P. M.

Local lesion from rabbit No. 4., was placed under the skin of the hip.

Convulsions were observed at 7 P. M. At 8 P. M., the animal was very uneasy and was dragging the left hind leg. At 10.30 P. M., rapid convulsions brought on by the slightest noise, dyspnea, a chattering of the teeth, a frothy salvia exuding from the mouth, pupils dilated, and a drawing to the side of the head were observed. Animal unable to arise from this time. Death took place at 11.05 P. M. Duration 7 hours and 5 minutes.

Cultures were made from the local lesion only, and B. suberis was recovered.

No. 6. Fawn and white rabbit, Dec. 6th., 1921.

Intramuscular injection of 1 cc. of the toxin produced by the growth of B. cereus and B. suberis., 7 days old. Six hours later convulsions were seen, the animal rises upon its haunches rythmatically, and constantly champs its jaws. An excessive amount of saliva exuded from the mouth

and caused the hair to become matted.

Dec. 7th., the animal showed typical symptoms, the muscles were rigid, the respiration was dyspneic, diarrhoea, which has matted the hair about the anus and thighs was also present. The animal was unable to stand when placed upon its feet. The temperature remained normal. Death took place about noon.

No autopsy.

No. 7. White rabbit, Dec. 6th., 1921.

Injected subcutsneously with .5 cc. of the 7 day old toxin.

No results were obtained.

No. 8. White rabbit, Dec. 6th., 1921.

.5 cc. of the toxin was injected intraperitoneally. A large abcess developed over the site of injection but it disappeared and recovery had apparently taken place in 24 days.

No. 9. Brown rabbit, Dec. 6th., 1921.

Inoculated subcutaneously with 2 cc. of toxin produced by the 4 day growth of B. suberis.

No results. Animal returned to pen Dec. 10th.

No.10. Grey and white rabbit, Dec. 12th., 1921. 10.45 inoculated subcutaneously with 2 cc. broth culture, 72 hours old, from local lesion of rabbit No. 6.

Observations:- Dec. 13th., at 8 A. M., the animal was moving but dragging the leg in which the injection had been given. Slight cunvulsions, champing of the jaws, and salivation were also seen. At 11 A. M., the animal could not arise, it was upon its haunches, weaving, and continuing the symptoms exhibited earlier. Dyspneic respirations were observed with a panting for breath. Animal died at 1.45 P. M., with no further symptoms.

The local lesion was cultured and B. suberis recovered.

No. 11. Brown rabbit, Dec. 13th., 1921.

2 cc. of an emulsion from the local lesion of rabbit No. 10, were injected subcutaneously.

Dec. 14th., animal had backed into a corner, did not eat, and was rising upon its haunches as the others had done. There was a champing of the jaws and a drooping of the ears. No temperature.

Dec. 15th., it was unable to draw its limbs under the body, or to arise. Dyspnea and salivation were seen. When placed upon its legs, the animal moves with very great difficulty.

Dec. 19th., the animal began to eat again, and the other symptoms have receeded. Animal apparently recovered.

No. 12. Black and white rabbit. Dec. 21st. 1921.

Injected subcutaneously with 2 cc. of the toxin of B.suberis, made after eighteen days incubation.

Found dead on Dec. 22nd.

Autopsy showed no marked changes.

No. 13. Tan and white rabbit. Jan. 6th., 1922.

Inoculated intramuscularlly with 2 cc., of 14 hour broth culture from navel of calf case No. 3. (mixed culture).

Died on Jan. 8th., and B. suberis was recovered from the local lesion.

No. 14. Brown rabbit, Jan. 17th., 1922.

Inoculated subcutaneously with 3 cc., of a culture of an organism which produced gas, and was isolated from the navel culture used above. (Rabbit No. 13).

No results. Animal returned to pen.

No. 15. Grey rabbit, Jan. 25th., 1922.

Intramuscular injection of 2 cc. of a washed 48 hour agar culture of B. suberis, isolated from a rabbit. Slight symptoms were observed on the third day but the animal recovered.

No. 16. White rabbit, Jan. 27th., 1922.

Intramuscular injection of 2 cc., of a broth culture 48 hour from the cork-brick flooring.

No results. Returned to pen.

No. 17. Tan and white rabbit, Jan. 31st., 1922.

Inoculated subcutaneously with 2 cc., washed agar plate 72 hour old, from the floor culture.

Animal died on Feb. 6th., showing positive symptoms, and a well defined local lesion. B. suberis was recovered in cultures.

No. 18. Cat, Feb. 7th., 1922.

Castrated with the instruments used in the previous autopsy. Exhibited characteristic symptoms and died in three days. Autopsy gave the usual post-mortem findings. Cultures were made from the scrotum, and a mixed infection containing B. suberis , was found.

No. 19. Brown rabbit, Feb. 10th., 1922.

Incision upon the thigh was made with a scalpel which had been dipped in an old broth culture of B. suberis. Clean wound gave no results.

No. 20. White rabbit, May, 11th., 1922.

Inoculated subcut neously with 3 cc., solution from the navel of calf case No. 4.

Died May 15th., with typical symptoms. Autopsy exhibited the usual changes.

B. suberis was recovered from the cultures made.

No. 21. Fawn rabbit, May 12th., 1922.

Inoculated subcutaneously with 2 cc. of an anaerobic culture made Nov. 29th., 1921, produced death on May 16th. Autopsy revealed a local lesion of a triangular shape and which measured about 6 cm. on a side. This lesion showed caseation necrosis and hyperemia surrounding it. B. suberis was recovered from cultures made from this lesion.

No. 22. White rabbit, May 12th., 1922.

Inoculation of 2 cc. of a 7 day old culture of B. suberis produced death in 21 hours when injected at the base of the brain.

No autopsy was held.

No. 23. Dog, 2 months old, weight 20 pounds, May 16th., 1922.

Transferred a portion of the navel of calf No. 4, to the dog, by making an incision through the skin over the hip. After the tissue had been placed in the wound it was sutured.

May 17th., the dog was found dead.

Only slight post-mortem changes were observed. The organism was recovered from the tissue which had been placed under the skin.

No. 24. Dog, 3 months old. June 10th., 1922.

Inoculated subcutaneously with 3 cc. of broth cultures of B. suberis as used in the previous rabbits.

June 16th., injected 5 cc. lactic acid and 1 cc. of Gram's solution, in one thigh. Injected 5 cc. of a broth culture 72 hours old of B. suberis into the other thigh.

June 21st., another subcutaneous injection of 2 cc. of Gram's solution and 15 cc. of a 72 hour old culture, of B. suberis were given intraperitioneally.

The following observations were made:-

(a) complete anorexia for several days,

(b) an enormous swelling about the sites of injection,

(c) a stage of coma, during which it relaxed all muscles, evacuations and voiding of the urine taking place involuntarily.

(d) increase of the respirations and dyspnea,

(e) marked emaciation and atrophy of the muscles, probably due to disuse.

The animal did however, make a good recovery within three weeks from the time of the last injection.

No. 25. Brown rabbit, June 15th., 1922.

Inoculated subcutaneously with 1 cc. broth culture 72 hour old, from calf case No. 4.

10 drops of lactic acid were given by subcutaneously in the neck, on the following day.

Results:- Extensive sloughing of the skin in the region of the neck. This healed within two weeks and the animal showed no other bad effects of the injections.

No. 26. Spotted rabbit. June 15th., 1922.

Inoculated subcutaneously with 10 drops of lactic acid and 1 cc. of a broth culture of B. suberis (incubated on Dec. 10th., 1921).

The lactic acid was injected 5 days after the culture of B. suberis.

Results:- Slight convulsions on June 25th., and a slight slough of the skin of the neck. Recovery on July 3rd.

No. 27. White rabbit. June 27th., 1922.

Inoculated subcutaneously with 2 cc. of a 48 hour old broth culture B. suberis and 5 cc. lactic acid.

June 30th., skin over the entire neck had sloughed, exposing the ligamentun nuchae which was covered with a purulent exudate. Anorexia and stiffness were observed.

July 3rd., since there were no further symptoms of the disease, the wound in the neck was cleaned up and healing started.

No. 27-A. White rabbit, June 27th., 1922.

2 cc. of the same broth culture as used on rabbit No. 27, was injected subcutaneously into the thigh.

June 29th., animal exhibited symptoms of the disease and was found dead on the morning of June 30th.

Autopsy:- local lesion about 3 cm. in diameter was found under the site of injection. Slight internal changes.

B. suberis was recovered from the local lesion.

No. 28. Grey rabbit. June 30th. 1922.

Inoculated subcutaneously with .5 cc. of milk culture of B. suberis from calf case No. 4., and 14 days old.

No results.

No. 29. Brown rabbit, June 30th., 1922.

1 cc. of milk culture as per No. 28.

No results.

No. 30. Black and white rabbit, June 30th., 1922.

Inoculated subcutaneously with 2 cc. milk culture of B. suberis 14 day old.

Found dead on July 2nd., at 8 A. M.

Autopsy:- Animal had died in the position assumed the previous day. The pupils were dilated, the hair ruffled, a fetid diarrhoea was observed, and a sticky saliva was present about the mouth. A local lesion was found under the site of injection, which was about 2 inches in diameter and covered the thigh. It had a purulent and iedematous appearance. The parenchyma were congested and degenerated. No fluids were found in the body cavities.

Cultures from the local lesion gave B. suberis.

No. 31. White rabbit, July 2nd., 1922.

Inserted under the skin a portion of the local lesion from rabbit No. 30.

At 6 P. M., the respiration were accelerated, and the body was drawn towards the side inoculated. The tissue was removed from inoculation and the wound was painted wit with iodine.

July 3rd., at 10 A. M., the body movements were stiff and cramped, edges of the wound were clean, but a slight exudate of pus was flowing from the wound.

Aug. 8th., the animal was found dead. A well developed and circumscribed local lesion was found. B. suberis was recovered.

No. 32. Grey rabbit. July 2nd.,1922.

Inserted a portion of local lesion of rabbit No. 30 subcutaneously in the tail. The tail was removed at its base three hours after the insertion of the tissue.

Animal lived.

No. 33. Small spotted rabbit, July 4th., 1922.

Intramuscular, 2 cc. of 14 day old milk culture of B. suberis.

July 5th., pronounced typical symptoms.

July 6th., death had taken place during the night.

Autopsy:- A well developed local lesion, and the usual internal changes were found.

No. 34. Brown rabbit, Oct. 2nd. 1922.

Inoculated subcutaneously with 2 cc., 72 hour old broth culture from an unused cork-brick. Two days later the animal exhibited slight symptoms of the disease, but recovered.

No. 35. Black and white rabbit, Oct. 3rd., 1922.

Culture of agar slope 7 days old was used, the growth being washed from the surface with a saline solution. No definite symptoms were seen until five days later, when lameness, anorexia, debility, and nervous symptoms were observed. The animal died on the 20th., of October. B. suberis was recovered.

No. 36. White rabbit, Oct. 3rd., 1922.

Inoculated with 2 cc. of a broth culture 72 hour old of B. suberis isolated from the soil of the infected farm were injected subutaneously.

Positive symptoms were exhibited on the second day after the inoculation and death occurred on the third day. Autopsy showed the usual changes and the organism was recovered.

No. 37. Brown rabbit, Nov. 2nd., 1922.

Dried carrots were saturated with 20 cc. of a 24 hour

growth of B. suberis, isolated from an animal. The carrots, so treated, were fed to a rabbit. No symptoms of the disease were visible for 10 days and the rabbit was returned to the pen.

No. 38. Fawn rabbit, Nov. 3rd., 1922.

Inoculated with 2 cc. of an agar subculture 96 hour old made from B. suberis isolated from granulated cork used in the manufacturing of the brick.

Symptoms were seen on the 5th., of November.

Death took place on the night of the 6th., day of Nov.

The organism was recovered.

No. 39. Grey and white rabbit. Nov. 9th., 1922.

Inoculated subcutaneously with 3 cc. of broth culture, 72 hour old taken from calf case No. 6. (brain culture). Typical symptoms and death followed. Autopsied on Nov. 14th., and B. suberis was recovered. Photo, fig. 9.

No. 40. White rabbit, Nov. 9th. 1922.

Inoculated subcutaneously with 2 cc. broth culture 48 hour old made from unused brick from the manufacturers. On the second day after the injection the animal exhibited marked lameness, anorexia, and nervous symptoms. Blindness was noted. The hair coat was ruffled, and matted about the mouth and anus by the discharges. Convulsions were not noted. The champing of the jaws continued for several days. Emaciation and weakness were observed for two weeks.

After the second week the animal began to show signs of recovery. The symptoms receeded gradually.

No. 41. Brown rabbit, Nov. 9th., 1922.

Inoculated subutaneously with 2 cc. of 72 hour old broth of B. suberis from an unused cork-brick, from same source as above.

No marked symptoms were seen, but the animal assumed a position in the pen with its head in the corner, back arched, and its hair coat ruffled. Anorexia was observed from the first day. On the third day, the animal was to be moved, and was found to be dead in the position which it haf assumed on the first day. (Photo figure 8).

The organism was recovered and used in the following case.

No. 42. White rabbit, Nov. 13th., 1922.

Inoculated subcutaneously with 2 cc. of a 24 hour old broth culture of B. suberis as isolated from the above animal.

Death occurred in 4 days. and no autopsy was held.

No. 43. Grey rabbit, Jan. 22nd., 1923.

This rabbit had not been used in any of the preceeding experiments. Its blood was drawn under aseptic conditions to be used for agglutination experiments.

Results with its blood were negative.

SUMMARY OF RESULTS WITH EXPERIMENTAL ANIMALS.

The animals used in this work consisted of rabbits, the average weight of which was 1800 grams, guinea pigs, dogs of two and three months of age, one cat weighing about 15 pounds, and suckling pigs weighing about 900 grams.

Broth cultures of B. suberis 48 hours old failed to produce uniform and fatal results. Transfers of portions of the local lesion from one animal to another or the inoculation with

To follow Expt. animal #43 p 57.

Experimental Work With Calves and An Immune Serum.

During July of 1923 four calves purchased from adjoining farms were placed in one of the infected stalls. They were nursed by one cow which had served as a nurse cow and in whose milk B.suberis had been found. These calves were young and weighed from 85 to 95 pounds each.

Calf #1 recieved no treatment.

Calf #2 recieved 10c.c. of blood from case number 9, subcutaneously

Calf #3 recieved 20 c.cs of the immune serum from case #9, whilst calf #4 recieved 30c.c's. of the serum subcutaneously.

Calf #1 sickened and died on the 14th., day. Calf #2 died after 28 days. The other two remained alive and are alive as yet. Other calves born on the farm were given 30 c.c's. of the serum at birth and no further fatalaties have occurred among animals so treated.

The two calves mentioned above which died were autopsied in the presence of another doctor, cultures were made from the various parts of the carcass and subsequently studied.

B.suberis was isolated from the cultures from these animals. In view of the above facts, it would appear that definite proof had been obtained as to the pathogenicity of B.suberis, and that it is also possible to prevent the disease by the use of a serum from an animal which had recovered from the disease.

the toxin made from a 14 to 21 day growth of B. suberis produced fatal results within 24 hours. The lethal dose of the toxin (filtered broth, 14 days old) was from 1 to 2 cc., depending on age and size of rabbit.

The artificial infection of clean wounds with B. suberis did not prove injurious to the animal. When wounds which were contaminated were infected with B. suberis, death followed in three days, and it was possible to recover B. suberis from the animal when autopsied.

The fatal dose was determined for rabbits. 3 cc. of a 72 hour old broth culture inoculated subcutaneously produced typical symptoms and death in three days. B. suberis was recovered from all of these animals.

B. suberis isolated from the various sources mentioned proved to be pathogenic for all the animals named and all animals gave the same post-mortem changes. The most virulent cultures of B. suberis were those isolated directly from animals dead of the disease produced by the organism.

Koch's postulates were proven by the obtaining of B. suberis from animals dead of the disease produced by it, growing it, in pure cultures, reproducing the disease by inoculating experimental rabbits, and recovering the organism from the post-mortem examination of the experimental animals.

SUMMARY.

Bacillus suberis, which produces the disease described in this paper, has been given this name provisionally, due to its presence in cork obtained from numerous sources. Bacillus suberis has been isolated from:-

(a) animals dead of the disease,

(b) experimental animals inoculated with the organism,

(c) cork-brick used in the infected stable floor,

(d) cork-brick used in many stables,

(e) cork-brick three and ten years old, manufacturers' samples,

(f) insulating cork from two different firms,

(g) granulated cork used in making cork-brick,

(h) cork dust used in packing of grapes.

Bacillus suberis shows granular or bipolar forms which stain with a reddish tint with methylene blue. These forms are best observed in milk cultures six to eight days old at 37° C.

Terminal endospores are formed which give the organism the appearance of B. tetani. It is Gram negative and facultative anaerobic. B. suberis produces an extracellular toxin, not so virulent as that of B. tetani.

Bacillus suberis is agglutinated by various dilutions of serum from animals which have recovered from the disease. Not only the organisms isolated from the animals dead from the disease, but organisms obtained from the numerous sources mentioned above, all gave positive results to microscopic or macroscopic agglutination tests.

A presumptive clinical diagnosis may be made by the absence of temperature, and by the presence of blindness in the individual, but should be followed by a bacteriological examination in all cases.

Pathological examination is of little value. It has been shown that this disease has been confused with many diseases of the contagious and infectious type, although in all cases some slight difference may be observed, but these are not sufficiently diagnostic.

Bacteriological examination is the only method of absolutely determinating the diagnosis.

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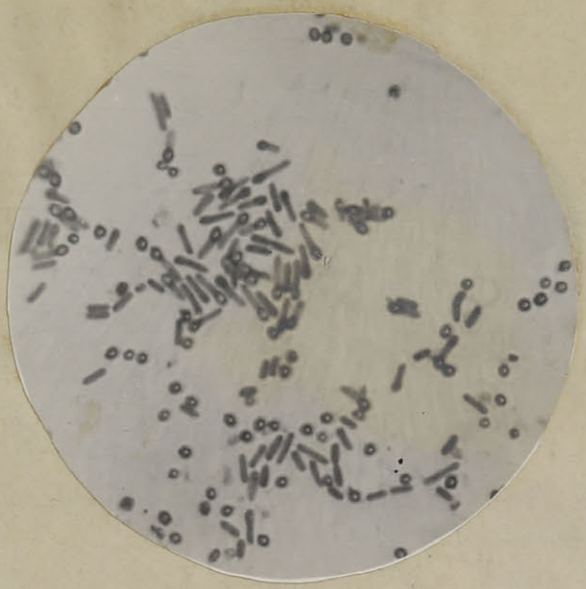

Figure 1. B. suberis from an agar slope, 72 hours old at 37°C, stained with Gram's stain.

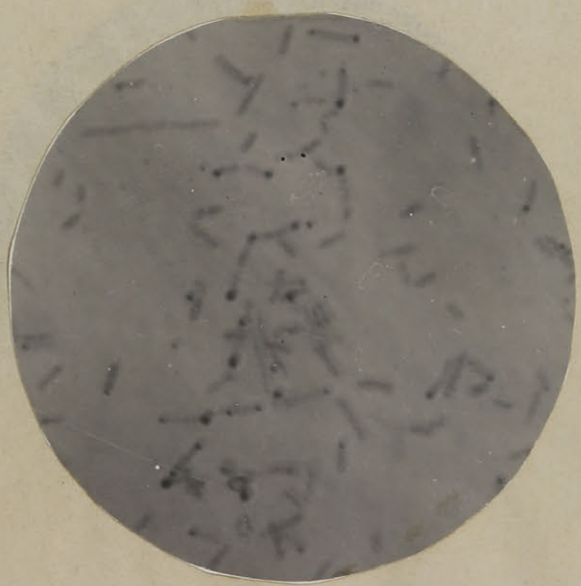

Figure 2. B. suberis from a 48 hour old milk culture at 37°C, stained with methylene blue to show the reddish granules.

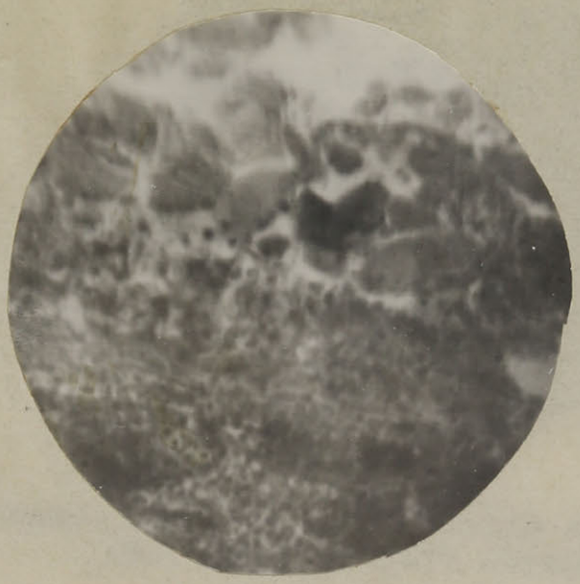

Figure 3. Local Lesion. Note the infiltration of leucocytes and the serous exudate separating the muscle fibers. The muscle is undergoing degeneration.

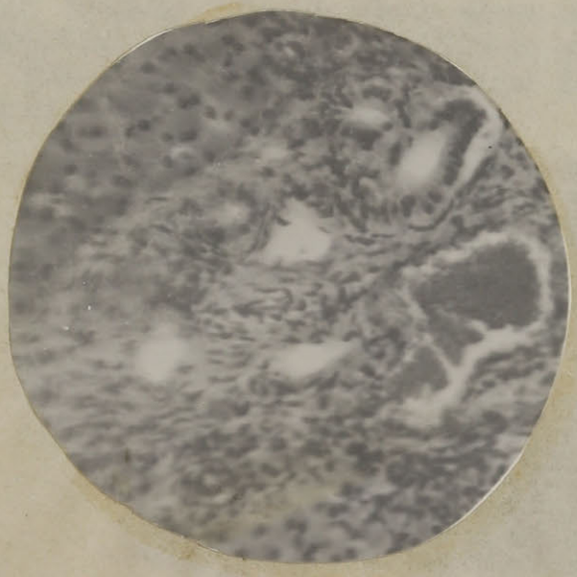

Figure 4. Liver. This photo shows the extensive degeneration of the cells and the hemorrhagic condition of the organ.

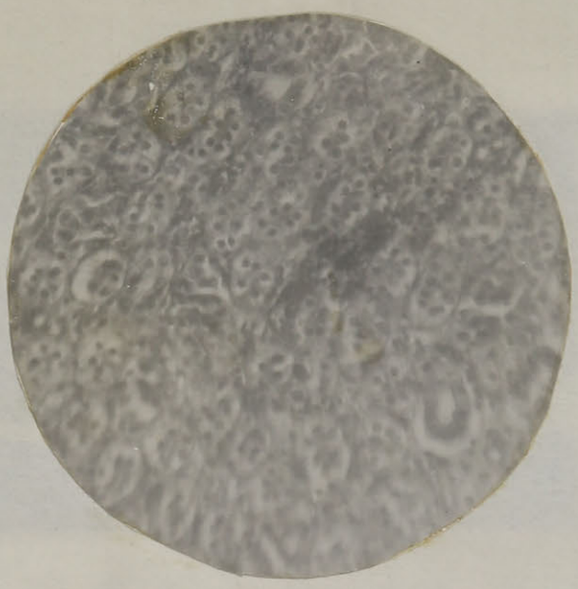

Figure 5. Kidney. Note the hemorrhagic condition of all parts, and the extensive degeneration of the organ.

Figure 6. Case number 11, as seen soon after the symptoms were observed. Note the condition of the hair coat, position of the head and ears, and the slight exudate from the mouth.

Figure 7. Same case as above after 48 hours of sickness. Note the changes in hair coat, arching of the back, position of head, and the apparent stiffness.

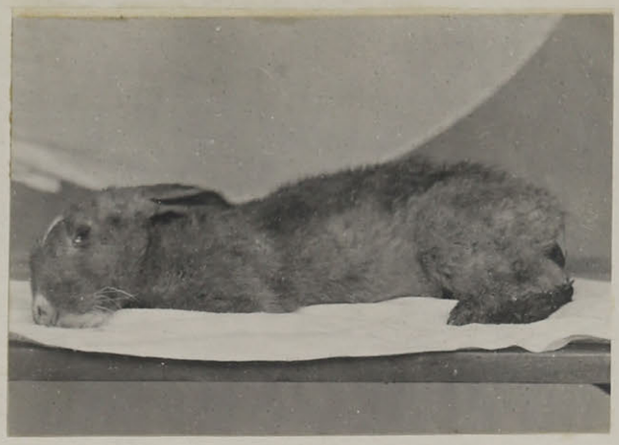

Figure 8. Rabbit inoculated with a 72 hour old broth culture of B.suberis isolated from cork dust used in the packing of grapes. Note the characteristic position.

Figure 9. Rabbit exhibiting typical symptoms. Inoculated with a 72 hour old culture of B. suberis isolated from a calf,which had died of the disease produced by B.suberis. Note the position of the head,ears, and limbs.

Figure 10. Rabbit inoculated with a 72 hour old broth culture of B. suberis isolated from cork brick. Note the usual position and the typical external symptoms such as the change in hair coat,diarrhoea,position of limbs etc.