CORYNEBACTERIA STUDIES IN SHEEP

by

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INTRODUCTION

Diseases caused by corynebacterium species are very important economic problems to the food animal industry. A variety of diptheroid organisms have been isolated from various tissues in man and animals (3, 7, 10). They have been isolated from obviously pathogenic conditions and on occasion the tissues from which the organisms were isolated appeared normal (2).

Based on available information, it is difficult to determine whether these organisms have, or have not, played a significant pathogenic role in many cases, and in some cases they have not been studied in sufficient detail.

The purpose of this study was to induce corynebacterial infection in adult sheep with Corynebacterium renale, C. equi, and C. pyogenes to gain information regarding the specific lesions produced and differential features if any produced by these three organism species in sheep.

CORYNEBACTERIA STUDIES IN SHEEP

Paper I: A Study of the Pathogenecity of *Corynebacterium renale*, *C. equi*, and *C. pyogenes* in Sheep.

INTRODUCTION

The genus Corynebacterium is composed of several species of bacteria which are found in man and animals (7, 10). Many of these species are nonpathogenic, being isolated primarily from normal appearing mucous membranes (3). The pathogenic species are associated with both chronic and acute diseases of man and animals (2).

The most important species from the viewpoint of human health is C. diptheria, the cause of diptheria in children (10). Among the domestic animal species, four have been found to be of greatest importance. These are C. pyogenes, C. renale, C. pseudotuberculosis, and C. equi (2, 3, 4).

Members of the group other than C. diptheria are frequently referred to as diptheroids (10). All diptheroid bacilli are gram positive, and show a rather high degree of pleomorphism (7). Pathogenic species of corynebacterium are non-motile (1). Most species when stained with polychrom dyes or Methylen blue, have granules and beading as morphologic features (3, 7). The cells often appear club like and are swollen at one or both ends (3, 7). In both tissues and cultures, they tend to form masses with the cells arranged in parallel or palisade formations (3). The cells of many species may morphologically resemble some of the short acid-fast bacteria. The diptheroid organisms are not acid-fast (3, 7).

Corynebacterium pyogenes is the most frequently recovered pus forming bacterium reported in domestic ruminants (9). In sheep, it has been isolated from mastitis, chronic purulent pneumonia, bronchopneumonia, and from joint infections (3, 9).

Corynebacterium renale is found mainly in cattle and is responsible for pyleonephritis and cystitis (2, 6). It has been isolated from kidney abscesses in swine, and has also been reported in sheep (3).

Corynebacterium equi causes suppurative bronchopneumonia in foals, with scattered small abscesses throughout the lungs and medistinal lymph nodes (6, 7).

MATERIALS AND METHODS

Animals

Twenty western crossbred ewes two to four years of age were divided into four test groups (16 ewes), and one control group (4 ewes), and kept in isolation quarters. The sheep were fed alfalfa pellets. The body temperature of each ewe was recorded twice daily for two days prior and each day post inoculum until death.

Corynebacterium Strains

C. renale (strain no. 19412) and C. equi (strain no. 6939) were each purchased from the American type culture collection, Rockville, Maryland. The C. pyogenes strain was isolated from a clinical case of bovine mastitis admitted to the large animal clinics, College of Veterinary Medicine, Kansas State University.

Preparation of Inoculum

C. renale, C. equi, and C. pyogenes inocula were 72 hour cultures in brain heart infusion (BHI)*. The absorbance of the cultures was read at 420 mu in a Bausch and Lomb Spectronic 20. Triplicate plate counts were also made in nutrient agar. C. renale gave an absorbance of 0.84 and a count of 4.9×10^8/ml, C. equi gave an absorbance of 0.75 and a count of 4.6×10^8/ml, and C. pyogenes gave an absorbance of 0.185 and a count of 4.6×10^7/ml.

Experimental Design

Each ewe in groups one and two was inoculated with 2 cc of C. renale inoculum intravenously (I.V.) into the left jugular vein. Each ewe in group three was inoculated (I.V.) with 2 cc of C. equi inoculum into the left jugular vein. Each ewe in group four was inoculated (I.V.) with 15 cc of C. pyogenes inoculum directly into the left jugular vein. The control ewes were inoculated (I.V.) with 15 cc of B.H.I. broth, and were treated identically to those inoculated with Corynebacterium.

Necropsy

All four inoculated groups were euthanized with pentobarbital sodium (Barb-Euthal) given intrapericardially and necropsied at:

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*Difco laboratories, Detroit, Michigan.

Group One	45-50 days	post inoculum
Group Two	90-100 days	post inoculum
Group Three	90-100 days	post inoculum
Group Four	90-100 days	post inoculum
Controls	90-100 days	post inoculum

Tissue specimens were collected at the time of necropsy from the lungs, liver, kidneys, urinary bladders, and brain routinely. In addition, specimens were collected from any other tissue containing gross lesions. All tissues were fixed in 10 % phosphate buffered formalin. The tissues were processed and embedded in parafin. They were cut at 6 u, and stained routinely with hemothoxylin and eosin (H & E).

Microbiologic Studies

Specimens were taken from gross lesions and the adjacent normal appearing areas from the lungs, livers, urinary bladders, and the leg joints of each ewe. These specimens inoculated into 5 % ovine blood agar, and inoculated at 37° C. Two blood agar plates were used for each specimen, one was incubated aerobically at 37° C for 48 hours, and the second anaerobically at 37° C for 48 hours. Plates having bacterial growth were subjected to microscopic and differential chemical tests for specific identification of the bacterial species.

RESULTS

Clinical Observations

Group one (1). A febrile response (104.0 to 105.0° F) was observed in all four ewes (Nos. 800, X, 825, and 180) in group one. The elevation in body temperature occurred one to four days post inoculum (P.I.). All four ewes presented clinical signs of depression and anorexia. Two ewes (No. 800 and 180) each developed coughs and a nasal discharge of mucous consistency on day 30 and 33 post inoculation.

Group two (2). All four ewes (Nos. 832, 898, 796, and 815) developed a febrile response (104.0 to 105.0° F). The elevation in body temperature occurred one to four days post inoculum (P.I.). Two of the four ewes (Nos. 898 and 832) in this group presented no overt clinical signs of illness other than the elevated body temperatures reported above. Ewe (No. 796) showed some signs of hypersensitivity to sounds and movements from day 33 post inoculation, she was visibly depressed and had anorexia. She was unable to stand on day 45 (P.I.), and died on day 46 (P.I.). Ewe (No. 815) was visibly depressed from day 31 (P.I.), and developed a cough from day 45 (P.I.). She became progressively weaker, and had anorexia until the time of necropsy.

Group three (3). All four ewes (Nos. 892, 812, 814, and 833) presented a febrile response (104.0 to 106.0° F) from

day 1 through 3 (P.I.). Ewe (No. 892) showed some signs of hypersensitivity to movements and sound from day 23 (P.I.), and developed a yellowish-brown, adhesive feces on day 28 (P.I.). She developed high temperature (103.5 to 104.5° F) from day 25 (P.I.) until day 36 (P.I.) when she died. Ewe (No. 812): no clinical signs of illness were recorded in this animal. Ewe (No. 814): other than elevation in body temperatures, no other overt sign of illness was detected in this ewe. Ewe (No. 833) was visibly depressed from day 62 (P.I.), and developed a cough on day 68 (P.I.).

Group four (4). All four ewes (Nos. Z, 926, 934, and 841) had a febrile response (103.5 to 106.0° F) from day 1 to 4 (P.I.). Other than the elevation in body temperature, no other overt signs of illness were detected in this group. Ewe (No. 926) was found dead on day 38 (P.I.) without prior clinical signs of illness.

Controls. No clinical signs of illness were detected in this group.

Macroscopic Findings

Group one (1). Two large (5 x 5 x 3 cm) thickly encapsulated abscesses were found in the subcutaneous tissues of the ventral abdominal region of ewe (No. 800). There were purulent adhesions between the liver, the diaphragm, and the lungs. The lungs contained multiple large (2 to 4 cm) and small (0.5 to 1 cm) abscesses distributed through all lobes.

Ewe (No. X) had enlarged bronchial lymph nodes containing numerous foci of (0.5 x 0.5 cm) yellowish inspissated exudate. One large (2 to 4 cm) abscess was localized between the liver and diaphragm, extended through the diaphragm and included the right diaphragmatic lobe of the lungs. The liver included several widespread small abscesses. The lungs were edematous and congested and included some areas of ventral consolidation in diaphragmatic and apical lobes. Ewe (No. 825): in this animal, all tissues, organs, systems and body fluids appeared grossly normal. Ewe (No. 180): there were large bilateral consolidated areas in diaphragmatic, cardiac, and apical lobes of the lungs. The bronchial lymph nodes were enlarged (2 x 4 cm), and contained yellowish inspissated granular exudate. There was no significant gross lesion in the central nervous systems of this group.

Group two (2). No significant gross lesions were observed in ewes (Nos. 898 and 832). Ewe (No. 796): the lungs were congested and the liver was yellow and of a fatty consistency. No other significant gross lesions were recognized. Ewe (No. 815): the lungs were focally adhered to the parietal pleura particularly the apical and diaphragmatic lobes. The lung paranchyma included multiple abscesses (0.5 to 2 cm). A few moderate size abscesses were scattered over the ventral surface of the liver. The left kidney was enlarged (15 cm in length and 10 cm in width), pale, and contained multiple large (2 to 3 cm) and small (0.5 to 1 cm) abscesses. The medulla and cortex

were both observed to be involved. The right kidney was essentially normal in size (10 cm in length and 5 cm in width), but contained two medium size abscesses (1 to 2 cm), one at each pole, which were visible grossly. No significant gross lesion was observed in the brains of the four ewes.

Group three (3). Ewe (No. 892): the lungs were congested, and the liver was greasy and pale. The kidneys were grossly normal. A large mass of dry feces and wool bundles were localized in the mid-colon, resulting in severe impaction and necrosis of the local colonic tissue. Ewe (No. 812): all lobes of the lungs had extensive fibrous adhesions to the parietal pleura. There were no significant changes in the liver and kidneys. Ewe (No. 814): fibrous adhesions involved all lobes of the lungs. On cut surface, a small firm nodule was found in the right diaphragmatic lobe. This nodule contained dark yellow inspissated material. The liver and kidneys were normal in appearance and consistency. Ewe (No. 833): severe adhesions involved all lobes of the lungs. There were no gross lesions in the liver and kidneys. The central nervous systems of this group appeared normal.

Group four (4). Ewe (No. 926): the lungs, the liver, and the kidneys of this animal had no significant gross lesion. Two large (3 x 3 x 5 cm), encapsulated abscesses were found in the subcutaneous tissue of the ventral abdominal area. These abscesses were localized along the midline about 5 cm posterior

to the diaphragm. The abscesses contained a yellowish-green inspissated material. Ewe (No. Z): focal areas of consolidation were observed in the ventral portions of the diaphragmatic lobes of both lungs. There were emphysematous areas in the diaphragmatic lobes. The liver and kidneys had no gross significant lesion. There was an excessive amount of tenacious yellowish-brown fluid (about 3 cc) in the carpal joint of the right fore limb. Ewe (No. 934): multiple variable sized abscesses were scattered throughout all lobes in the lungs. The liver and kidneys appeared grossly normal. Ewe (No. 841): the lungs contained numerous abscesses similar to those observed in ewe (No. 934). Bronchial lymph nodes were enlarged and contained yellowish inspissated material. There were no significant gross lesions in the brains of the animals.

Controls. Three ewes (Nos. 910, 861, and 932) had multiple encapsulated abscesses in the lungs. Ewe (No. 910) also had a few caseated abscesses in the liver. Ewe (No. Y) had no gross significant lesion.

Microbiological Findings

Group one (1). Ewe (No. 800): C. renale was recovered from the tarsal joint of the left rear leg. Ewe (No. X): C. renale was not recovered. C. pseudotuberculosis was recovered from the lungs and liver. Ewe (No. 825): Corynebacteria species were not recovered from the cultured tissues. Ewe (No. 180): C. renale was recovered from the carpal joint of the left front leg.

Group two (2). Ewe (No. 898): C. renale was recovered from the lungs. Ewe (No. 832): C. renale was recovered from the carpal joint of the left front leg. Ewe (No. 796): all tissues cultured were negative for Corynebacteria. Ewe (No. 815): C. renale was recovered from the carpal joint of the left front leg in this animal. C. pseudotuberculosis was recovered from the liver, kidneys, the urinary bladder, and the bronchial and medistinal lymph nodes.

Group three (3). Ewe (No. 892): all tissues cultured were negative for Corynebacteria species. Ewe (No. 812): all tissue cultured was negative for C. equi. C. pseudotuberculosis was recovered from the bronchial lymph nodes. Ewe (No. 814): C. equi was recovered from the lungs and the tarsal joint of the right rear limb. Ewe (No. 833): C. equi was recovered from the tarsal joint of the left rear leg.

Group four (4). Ewe (No. 926): all tissues cultured were negative for Corynebacteria species. Ewe (No. Z): C. pyogenes was recovered from the right kidney. Ewe (No. 934): all tissues cultured were negative for C. pyogenes. C. pseudotuberculosis was recovered from the lungs. Ewe (No. 841): all tissues cultured were negative for C. pyogenes. C. pseudotuberculosis was recovered from the lungs.

Controls. C. pseudotuberculosis was isolated from the liver of ewe (No. 861) and also from the lungs of ewe (No. 932).

Microscopic Findings

Group one (1). Ewe (No. 800)--Lungs: large areas of suppuration, necrosis, and mineralization were observed. The individual foci were surrounded typically by a thick fibrous capsule, and accumulations of inflammatory cells, neutrophils, mononuclear phagocytes, lymphocytes, and plasma cells. The neutrophils were infiltrating and accumulated in the bronchi and alveolar spaces adjacent to the necrotic areas. Liver: there were wide spread fatty changes in the hepatic cord cells, and proliferation of fibrous tissue in portal traids. Non-suppurative inflammatory cells (lymphocytes and mononuclear cells) had accumulated around the intrahepatic bile ducts. Some caseated debris was present in a few bile ducts. Kidney: there were multiple focal collections of mononuclear cells and lymphocytes in the subcapsular and cortical areas. Multiple focal areas of mineralization were present in both the cortex and medullary portions.

Ewe (No. X)--Lungs: thickening of the alveolar wall by proliferation of fibrous tissue and severe congestion was observed. There were microscopic abscesses containing necrotic debris and mineralized material. The liver contained multiple large and small circumscribed abscesses containing necrotic and mineralized debris, each surrounded by thick fibrous capsules. There were moderate infiltrations of mononuclear cells and lymphocytes around bile ducts in the portal traids. Kidneys: there were a few focal mineralized areas in the medulla of the kidneys.

Ewe (No. 825)--Lungs: the alveolar walls were slightly thickened around the bronchi and were congested. Liver: there was widespread fatty changes in the hepatic cord cells. Kidneys: the medullary portions were severely congested and contained multiple small areas of mineralization. The urinary bladder wall was thickened by proliferation of fibrous tissue and edema.

Ewe (No. 180)--Lungs: there were multiple focal and diffuse granulomas around the bronchi and bronchioles as well as thickened alveolar walls. The infiltrating cells were mostly mononuclear phagocytes. The bronchial tree and the alveolar spaces in the consolidated areas contained phagocytes and polymorphonuclear leukocytes. Liver: the sinusoides were dilated and congestion around the central veins were observed. Kidneys: multiple small mineralized circumscribed areas were seen in the medulla. Lymph nodes: the bronchial lymph nodes contained numerous foci of suppuration and areas of necrosis. No significant microscopic changes were seen in the brain tissues.

Group two (2). Ewe (No. 898)--Lungs: the alveolar walls were uniformly thickened by proliferation of fibrous tissue about the pleural surfaces. Liver: there were widespread fatty changes in the hepatic cord cells. Kidneys: the medullas contained multiple small foci, consisting of accumulated non-suppurative inflammatory cells. In addition, there were multiple small mineralized areas in the medulla. The urinary bladder wall was thickened by proliferated fibrous tissue and moderate edema.

Ewe (No. 832)--Lungs: there was a thickening of the alveolar walls adjacent to the pleural surfaces by a proliferation of fibrous tissue. Liver: mild fatty changes were observed in the hepatic cord cells. Kidneys: the medullary portions were severely congested and contained small focal areas of mineralization. The urinary bladder wall was slightly thickened by edema and proliferated fibrous tissue.

Ewe (No. 796)--The lungs had no significant microscopic lesion and appeared normal. Liver: the hepatic cord cells had undergone extensive fatty changes. Kidneys: most convoluted tubular cells contained fat; also, there was crystals in the tubules both in the cortex and medulla. The urinary bladder wall was slightly thickened by fibrous tissue.

Ewe (No. 815)--Lungs: multiple widespread encapsulated abscesses with central necrosis and mineralization were observed. There were focal and diffuse infiltrations and accumulations of polymorphonuclear leukocytes and mononuclear phagocytes. Liver: large encapsulated abscesses and multiple small granulomas were observed in the liver. There were accumulations of neutrophils and mononuclear cells in the portal traids and around abscesses. There was extensive proliferation of fibrous tissue in portal areas, and replacement of hepatic cords by fibrous tissue associated with parenchymal collapse. Kidneys: multiple small and large abscesses were localized both in the cortex and medulla. These abscesses were surrounded by a thick fibrous capsule, and contained necrotic and mineralized material.

There were focal and diffuse infiltrations and accumulations of neutrophils and mononuclear phagocytes around abscesses. Urinary bladder: there were slight accumulations of mononuclear cells in the subepithelial areas and thickening by proliferation of fibrous tissue. The brain tissues appeared normal in all four ewes.

Group three (3). Ewe (No. 892)--Lungs: there were no significant microscopic changes. Some thickening of the alveolar walls were observed close to pleural surfaces. Liver: fatty changes involved the hepatic cord cells. Mononuclear cells in moderate numbers had accumulated around the portal bile ducts. Kidneys: some refractile crystals were found in the tubules both in the medulla and cortex. There was some congestion and focal mineralization in the medulla.

Ewe (No. 812)--Lungs: there were diffuse thickenings of the alveolar walls by a proliferation of fibrous tissues, and mononuclear cells in moderate numbers had accumulated in the thickened alveolar walls. Liver: there was a mild fatty change in the hepatic cord cells and some congestion of the sinusoides. Kidneys: there was a mild congestion of the arteries in the medulla. A small number of mineralized tubules were observed in the medulla.

Ewe (No. 814)--Lungs: the alveolar walls were thickened, and there was congestion of the blood vessels of the lungs. The thickened areas were concentrated close to the pleural

surfaces. Liver: the centralobular areas were congested. Kidneys: there was moderate congestion of the medullary arteries.

Ewe (No. 833)--Lungs: the alveolar walls were thickened by a proliferation of fibrous tissues particularly in the subpleural areas. Most of the arteries in the lungs were congested. There were a few mononuclear cells localized in the thickened alveolar walls. Liver: mild fatty changes were observed in the hepatic cord cells. Kidneys: both the cortex and medulla appeared to be normal. The brain tissues in this group appeared normal.

Group four (4). Ewe (No. 926)--Lungs: no significant microscopic changes were seen. Liver: mild fatty changes were found in the hepatic cord cells. Kidneys: no significant changes.

Ewe (No. Z): there were areas of emphysema and atlectasis in the diaphragmatic lobes of the lungs. Liver: no significant lesions were observed. Kidneys: both kidneys contained multiple circumscribed round cell nodules in the pelvic area.

Ewe (No. 934)--Lungs: encapsulated abscesses with central necrosis and mineralization were present in both lungs. There were diffuse and focal infiltrations and accumulations of polymorphonuclear leukocytes and mononuclear phagocytes. Liver: there was a moderate number of mononuclear cells around the portal bile ducts. Kidneys: no significant lesions were observed.

Ewe (No. 841)--Lungs: there were multiple areas of abscessation with central areas of necrosis and mineralization.

There were infiltrations and accumulations of the neutrophils in the adjacent bronchial tree elements and alveolar spaces. Liver: there were mild fatty changes in the heaptic cord cells. Kidneys: moderate numbers of mononuclear cells had accumulated focally in the pelvic portions of both kidneys. The brain sections from all four animals appeared normal.

Controls. There were multiple areas of abscessation with central necrosis in the lungs of ewes (Nos. 910, 861, and 932). There were also a few thickly encapsulated abscesses in the liver of ewe (No. 910). No other significant microscopic lesions were observed in the tissues of the four ewes in this group.

DISCUSSION

The etiological role of Corynebacteria species as a pathogen in several species of animal is well documented in the literature. While C. pseudotuberculosis and C. pyogenes are recognized as common causes of diseases in sheep, there have been very few reports incriminating C. renale or C. equi. The first portion of this report describes the recovery of C. renale from the lungs of one and the limb joints of four experimentally infected ewes.

Eight ewes comprising two groups of four each were inoculated intravenously with C. renale strain. Group one was held for 45 days (P.I.), and the second group was held for 90-100 days (P.I.). A febrile response was detected in all

eight ewes. Three ewes (No. 800, X, and 180) in group one, and one ewe (No. 815) in group two each developed chronic abscesses in the lungs throughout all lobes. The lesions in the lungs of ewe (No. 180) were a granulomatous type pneumonia with abscessation. Liver abscesses were observed in two ewes (No. X) in group one and (No. 815) in group two. Kidney abscesses were observed in one ewe (No. 815) of group two. The lesions consisted of a severe bilateral abscessation involving both the cortex and medulla, and were more advanced in the left kidney.

C. renale was recovered from the tarsal joint of the left rear leg of ewe (No. 800), and the carpal joint of the left front leg of ewe (No. 180) in group one. In group two, C. renale was recovered from the lungs of ewe (No. 898), and carpal joints of the left front limbs of ewes (Nos. 832 and 815). The abscesses observed in the lungs, livers, and the kidneys did not yield C. renale.

In group three, four ewes were inoculated intravenously with the C. equi strain and held for 90-100 days. All four ewes showed a febrile response following inoculation. This study resulted in the recovery of C. equi from the lungs of one, and the limb joints of two experimentally infected ewes. Three ewes (No. 812, 814, and 833) developed severe fibrous adhesions between the lungs and parietal pleura. The condition was that of a chronic interstitial pneumonia and a fibrinous pleuritis. These observations are similar to the findings of Roberts, D.S.,

who also isolated C. equi from a sheep affected with chronic pneumonia and pleurisy (23).

C. equi was recovered from the lungs and tarsal joint of the right rear leg of ewe (No. 814), and also from the tarsal joint of the left rear leg of ewe (No. 833). Because of similar lesions in the lungs of all three ewes, it is possible to consider C. equi responsible for the chronic interstitial pneumonia and fibrinous pleuritis in the three ewes (Nos. 812, 814, and 833) in the group.

In group four, four ewes were inoculated intravenously with a C. pyogenes strain, isolated from a case of bovine mastitis. Fifteen cc of inoculum was used because the organism is considered to be of low pathogenicity. All four ewes responded with a febrile response within five hours following inoculation. Two ewes (No. 934 and 841) developed lung abscesses. A chronic inflammatory papillitis was observed in both kidneys of ewe (No. Z). C. pyogenes was recovered from the right kidney of ewe (No. Z). Attempts to recover C. pyogenes from the abscesses in the lungs of ewes (Nos. 934 and 841) were without success.

In the controls group, four ewes were inoculated with 15 cc of broth intravenously. No febrile response was detected in these animals. Three ewes (Nos. 910, 861, and 932) developed lung abscesses and one ewe (No. 910) also had liver abscesses. C. pseudotuberculosis was isolated from the liver and lungs in two ewes (Nos. 861 and 932).

SUMMARY

Twenty ewes were divided into four test groups (16 ewes) and one control group (4 ewes) for this study. Group one and two consisted of eight ewes, four ewes held for 45 days (group 1), and four held for 90-100 days (group 2). All eight ewes were inoculated intravenously with 2 cc of C. renale. Cultures were diluted to a count of 4.9×10^8/ml. All eight ewes developed a febrile response on day 1-4 (P.I.). Lung abscesses were observed in three ewes (Nos. 800, X, and 180) in group one. Liver abscesses were found in ewe (No X). In group two, ewe (No. 815) developed multiple abscesses in the lungs, liver, and the kidneys. Microscopically, all abscesses were found to be thickly encapsulated, and contained necrotic debrises and mineralized material.

C. renale was recovered and reidentified from the tarsal joint of the left rear leg in ewe (No. 800), and carpal joint of the left front leg in ewe (No. 180) in group one. In group two, C. renale was recovered and reidentified from the lungs of ewe (No. 898) and carpal joints of the left front legs in ewes (No. 832 and 815).

Group three (3) consisted of four ewes held for 90-100 days, and each was inoculated with 2 cc of C. equi (I.V.). Cultures were diluted to a count of 4.6×10^8/ml. All four ewes had a febrile response from day 1-3 (P.I.). One ewe (No. 892) died on day 36 (P.I.) with impaction of the colon. Three

ewes (Nos. 812, 814, and 833) each developed fibrous adhesions between the parietal and visceral pleura. Microscopically, there were thickenings of the alveolar walls in the lungs, particularly in the edges close to pleural surfaces. C. equi was recovered and reidentified from the lungs and tarsal joint of the right rear leg in ewe (No. 814), and also from the tarsal joint of the left rear leg in ewe (No. 833).

In group four (4), four ewes were inoculated with 15 cc of C. pyogenes (I.V.). Cultures diluted to a count of 4.6×10^7/ml. These ewes held for 90-100 days. A febrile response was detected in all four ewes from day 1-4 (P.I.). One ewe (No. 926) that died on day 38 (P.I.) had two large ventral abdominal abscesses. Ewes (Nos. 934 and 481) developed multiple lung abscesses. In microscopic examinations, multiple focal round cell accumulations were found in the pelvic papilla in the kidneys of ewe (No. Z). C. pyogenes was recovered and reidentified from the right kidney of ewe (No. Z).

Group five (controls) consisted of four ewes held for 90-100 days; and, each was inoculated with 15 cc of B.H.I. broth (I.V.). Three ewes developed lung abscesses, and one also had liver abscesses. C. pseudotuberculosis was isolated from the abscesses in two ewes (Nos. 861 and 932). C. pseudotuberculosis was isolated from lesions in animals from each group and was cultured from abscesses in ewes (Nos. X, 815, 812, 934, 841 and controls Nos. 861 and 932).

REFERENCES

1. Benham, C. L., Seaman, A. and Woodbine, M. Corynebacterium pseudotuberculosis and its role in disease of animals. Vet. Bull., 32:645-655. 1962.

2. Blood, D. C. and Henderson, J. A. Veterinary Medicine. 3rd ed. The Williams and Wilkins Company, Baltimore, 298-305. 1968.

3. Brumer, D. W. and Gillespie, J. H. Hagan's Infectious Disease of Domestic Animals. 5th ed. Cornell University Press, Ithaca, New York, 151-166. 1966.

4. Dennis, S. M. and Bamford, V. W. The role of Corynebacterium in perinatal lamb mortality. Vet. Rec., 79: 105-108. 1966.

5. Hard, G. C. Electron microscopic examination of Corynebacterium ovis. J. Bact., 97:1480-1485. 1969.

6. Jubb, K. V. F. and Kennedy, P. C. Pathology of Domestic Animals. Vols. 1 and 2, New York Academic Press. 1970.

7. Merchant, I. A. and Packer, R. A. Veterinary Bacteriology and Virology. 6th ed. The Iowa State University Press, Ames, Iowa, 527-546. 1969.

8. Roberts, D. S. Corynebacterium equi infection in a sheep. Aust. Vet. J., 33:21. 1957.

9. Smith, R. E., Reynolds, I. M., Clark, G. W., and Milbery, J. A. Fetoplacental effects of C. pyogenes in sheep. Cornell Vet., 61:573-590. 1971.

10. Stableforth, A. W. and Gallaway, I. A. Infectious Diseases of Domestic Animals. Vol. 1, New York Academic Press, 239-254. 1959.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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Table 1. A summary of clinical observations on four groups of sheep inoculated with *Corynebacterium species*, and the control group.

Group	Sheep No.	(Clinical Observations) Maxim P.I. Temp. (F^{o})	Other Signs of Illness
(1)	800	105.0	depression, anorexia, coughs, nasal discharge
inoculated	X	104.0	depression, anorexia
with a *C.*	825	104.1	depression, anorexia
renale	180	105.5	depression, anorexia, coughs, nasal discharge
strain			
(2)	898	104.0	-----
inoculated	832	104.0	-----
with a *C.*	796	103.0	depression, anorexia, hypersensitivity died on day 46 P.I.
renale			
strain	815	104.2	depression, anorexia, coughs, weakness
(3)	892	105.5	hypersensitivity, yellowish-brown adhesive feces, died on day 36 P.I.
inoculated			
with a *C.*	812	106.5	-----
equi	814	104.5	-----
strain	833	105.8	depression, coughs
(4)	926	103.5	Found death on day 38 P.I. without any prior signs
inoculated			
with a *C.*	Z	106.5	-----
pyogenes	934	103.5	-----
strain	841	103.8	-----
(5)	910	102.6	-----
controls	861	102.5	Found death on day 33 P.I., showed no clinical signs of illness
inoculated			
with broth	932	102.5	-----
	Y	102.6	-----

Table 2. A summary of macroscopic and microscopic findings on four groups of sheep inoculated with Corynebacterium species, and the control group.

Group	Sheep No.	Macroscopic Findings	Microscopic Findings
(1) inoculated with C. renale	800	Two large (5x5x3) abscesses localized in the subcutaneous tissue in abdominal area. Purulent adhesions between liver, lungs and diaphragm.	Lungs--encapsulated abscesses suppurations, necrosis, mineralization. Liver--fatty changes, fibrosis, infiltration of non-suppurative inflammatory cells around bile ducts.
	X	Enlarged, caseated bronchial, lymph nodes. A large abscess localized between lungs, liver, and diaphragm.	Lungs--abscessation, suppuration necrosis, mineralization. Liver--multiple abscesses, necrosis, mineralization, infiltration of non-suppurative inflammatory cells around bile ducts.
	825	N.S.L.	Lungs--congestion. Liver--mild fatty changes. Kidney--medullary congestion.
	180	Large consulidated areas on both lungs. Enlarged, caseated bronchial lymph nodes.	Lungs--multiple granulomas neutrophils in bronchial lumen and alveolar spaces. Liver--congestion.
(2) inoculated with C. renale	898	N.S.L.	Lungs--thickened alveolar walls specially about pleural surfaces. Liver--mild fatty changes. Kidney--medullary congestion.
	832	N.S.L.	Lungs--slight thickened alveolar walls about pleural surfaces. Liver--mild fatty changes.

Table 2. (continued)

Group	Sheep No.	Macroscopic Findings	Microscopic Findings
	796	Congested lungs, greasy pale liver.	Lungs--congestion. Liver--severe fatty changes. Kidney--fat in tubules, crystals in the tubules.
	815	Adhesion between lungs and parietal pleura. Lung included abscesses. Liver--abscesses. Kidney--abscesses in cortex and medulla.	Lungs--abscessations, suppurations necrosis, mineralizations. Liver--abscesses, granulomas, fibrosis, fatty change. Kidney--abscesses, necrosis, mineralizations.
(3) inoculated with *C. equi*	892	Congested lungs, pale liver. A large mass of wool bundles and dry feces localized in the colon.	Lungs--N.S.L. Liver--non-suppurative inflammatory cells accumulated around bile ducts, and fatty changes. Kidney--crystals in the tubules. Congestion and mineralizations.
	812	Severe fibrous adhesions between lungs and parietal pleura.	Lungs--thickened alveolar walls close to the pleural surfaces and infiltration of mononuclear cells. Liver--mild fatty changes. Kidney--N.S.L.
	814	Severe fibrous adhesions between lungs and parietal pleura. A caseated nodule was found in the lungs.	Lungs--lesions were the same of those in ewe (No. 812). Liver--N.S.L. Kidney--N.S.L.

Table 2. (continued)

Group	Sheep No.	Macroscopic Findings	Microscopic Findings
	833	Severe fibrous adhesions between lungs and parietal pleura.	Lungs--lesions were the same of those in ewes (Nos. 814 and 812). Liver--mild fatty changes.
(4) inoculated with C. pyogenes	926	Two large abscesses (3x3x5 cm) were found in the subcutaneous tissue in the abdominal areas. No other gross changes.	Lungs--N.S.L. Liver--mild fatty changes. Kidney--N.S.L.
	Z	Lungs contained consuliated and some emphysematous areas.	Lungs--emphysema and atlectasis. Liver--N.S.L. Kidney--multiple circumscribed round cell nodules found in the renal pelvis.
	934	Lungs - included a few small abscesses. Excessive amount of tenacious yellowish-brown fluid in the carpal joint of the right front leg.	Lungs--abscesses, necrosis, mineralizations. Liver--infiltration of mononuclear cells around the intrahepatic bile ducts. Kidney--N.S.L.
	841	Lungs had multiple abscesses. Bronchial lymph nodes were enlarged and caseated.	Lungs--areas of abscessation and necrosis and mineralization. Kidney--accumulation of mononuclear cells in the medulla.
(5) (Controls) inoculated with broth	910	A few caseated nodules found in the lungs and liver.	Encapsulated abscesses were seen in the lungs and liver containing necrotic debrises and mineralized material.

Table 2. (continued)

Group	Sheep No.	Macroscopic Findings	Microscopic Findings
	861	Enlarged, caseated bronchial lymph nodes. Multiple abscesses in the lungs.	Abscess in the lungs, with areas of necrosis and mineralization. Liver--mild fatty changes. Kidney--fat in tubules, congestion.
	932	Lungs contained a few caseated nodules.	Lungs--included abscesses, necrosis, mineralization.
	Y	N.S.L.	N.S.L.

Table 3. A summary of microbiologic findings on four groups of sheep inoculated with *Corynebacterium* species, and the control group.

Group	Sheep No.	Maxim. P.I. Temp. (°F)	Lung	Liver	Kidney	Urinary Bladder	Joint	Lymph Nodes
(1) inoculated with a *C. renale* strain	800	105.0	---	---	---	---	*C. renale* L.R. leg	---
	X	104.0	*	*	---	---	---	---
	825	104.1	---	---	---	---	---	---
	180	105.5	---	---	---	---	*C. renale* L.F. leg	---
(2) inoculated with a *C. renale* strain	898	104.0	*C. renale*	---	---	---	---	---
	832	104.0	---	---	---	---	*C. renale* L.F. leg	---
	796	103.0	---	---	---	---	---	---
	815	104.2	---	*	*	*	*C. renale* L.F. leg	*
(3) inoculated with a *C. equi* strain	892	105.5	---	---	---	---	---	---
	812	106.5	---	---	---	---	---	*
	814	104.5	*C. equi*	---	---	---	*C. equi* R.R. leg	---
	833	105.8	---	---	---	---	*C. equi* L.R. leg	---
(4) inoculated with a *C. pyogenes* strain	926	103.5	---	---	---	---	---	---
	Z	106.5	---	---	*C. pyogenes*	---	---	---
	934	103.5	*	---	---	---	---	---
	841	103.8	*	---	---	---	---	---
(5) Control group	910	102.6	---	---	---	---	---	---
	861	102.5	---	*	---	---	*	---
	932	102.5	*	---	---	---	---	---
	Y	102.6	---	---	---	---	---	---

*C. pseudotuberculosis was isolated from this tissue.

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Fig. 1. Left kidney of ewe (No. 815) inoculated with C. renale. Multiple large and small abscesses in the medulla and cortex.

Fig. 2. Right kidney of ewe (No. 815) inoculated with C. renale. Two medium sized abscesses, one at each pole.

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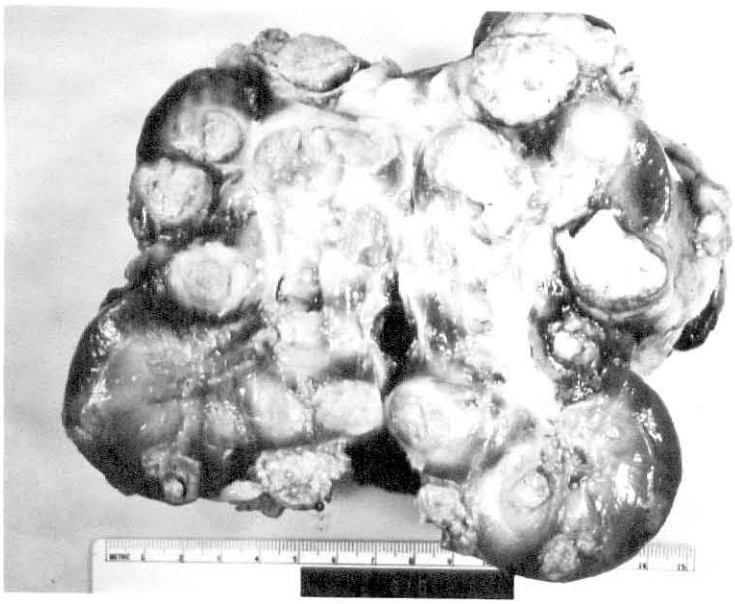

Fig. 1

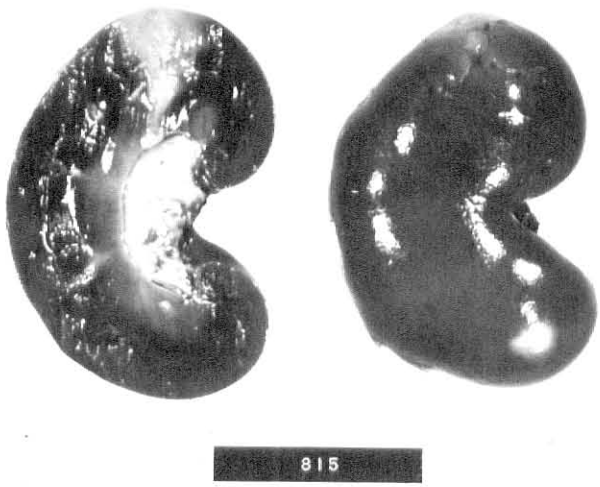

Fig. 2

Fig. 3. Lungs of ewe (No. 815) inoculated with C. renale. Abscesses in the left apical and diaphragmatic lobes. Focal adhesions between lungs and parietal pleura.

Fig. 4. Liver of ewe (No. 815) inoculated with C. renale. A few abscesses in the ventral surface of the liver and bile ducts.

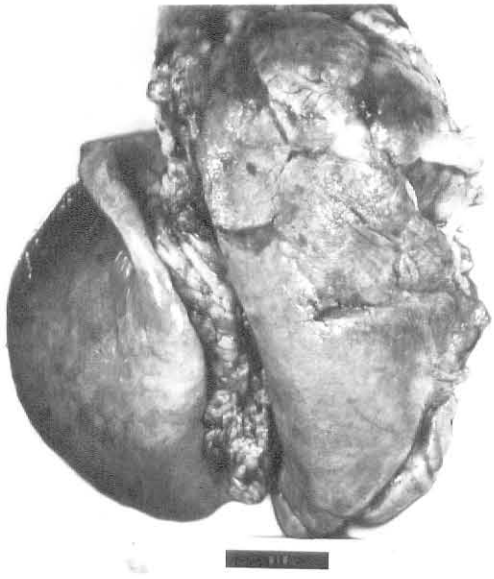

Fig. 3

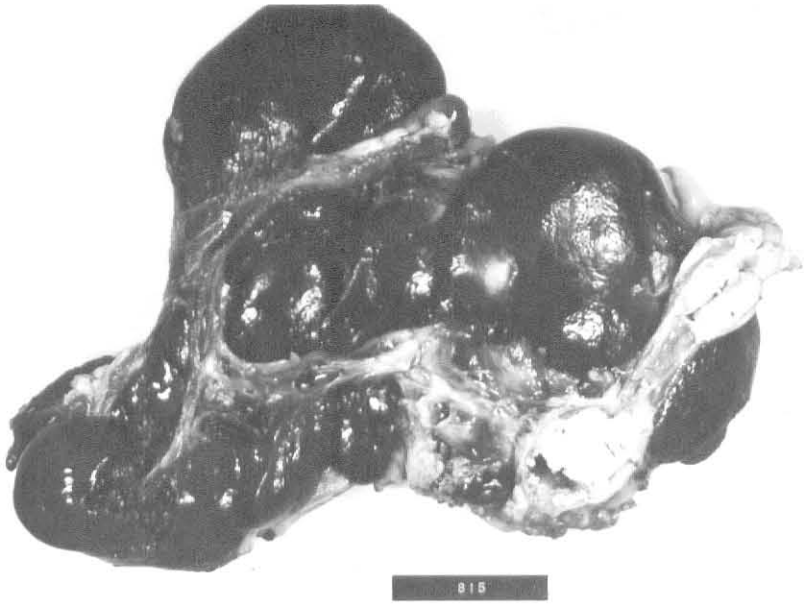

Fig. 4

Fig. 5. Lungs of ewe (No. 841) inoculated with C. pyogenes. Numerous abscesses in the lungs, and enlarged bronchial lymph nodes.

Fig. 6. Lungs of ewe (No. 841) inoculated with C. pyogenes. Inspissated granular material in the bronchial lymph nodes and lungs.

Fig. 5

Fig. 6

Fig. 7. Lungs of ewe (No. 814) inoculated with C. equi. Severe fibrous adhesions between lungs and parietal pleura.

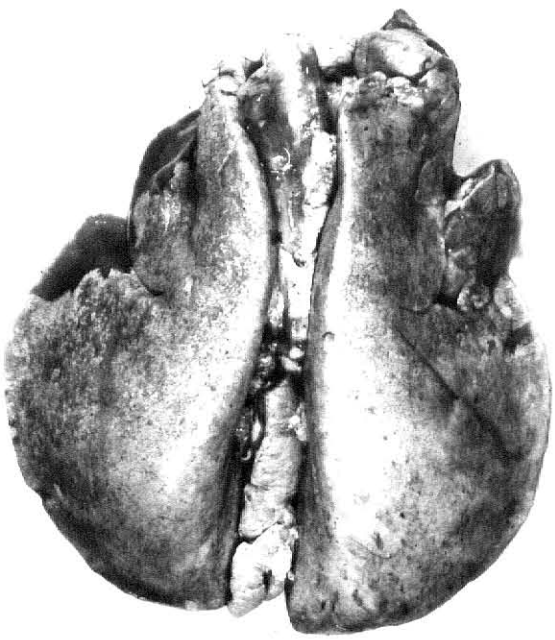

Fig. 7

Fig. 8. Photomicrograph of a chronic interstitial reaction in the kidney of ewe (No. 815) inoculated with C. renale. Infiltration and accumulation of mononuclear cells around tubules (X 60).

Fig. 9. Photomicrograph of a area of suppuration and necrosis in the liver of ewe (No. 815) inoculated with C. renale. Extensive fibrosis and replacement of hepatic cord cells by fibrous tissues (X 60).

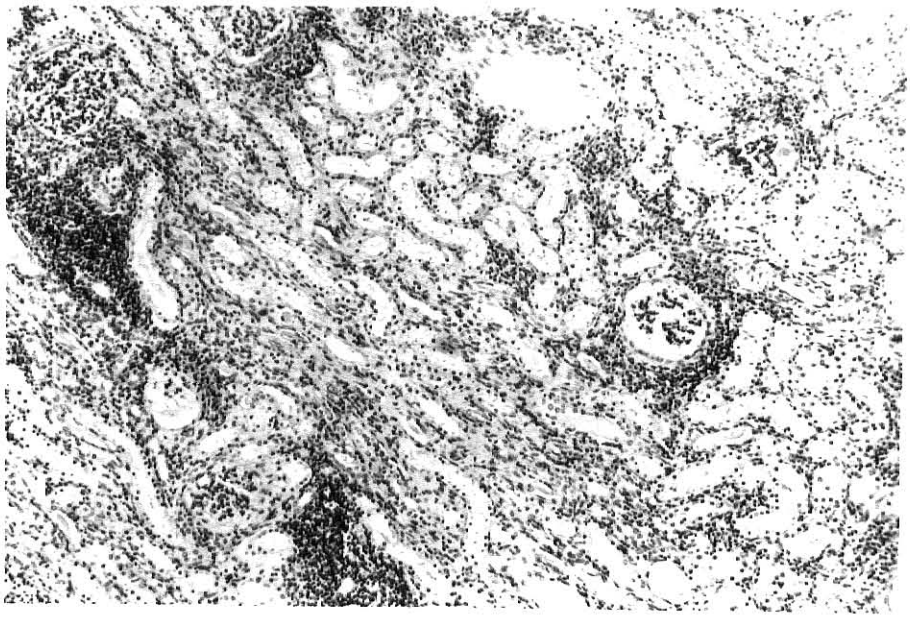

Fig. 8

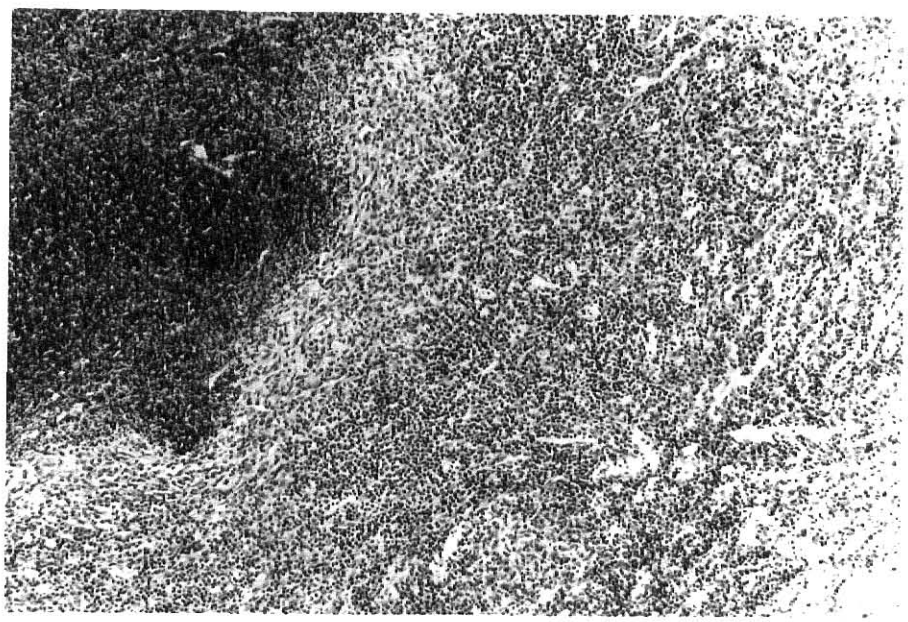

Fig. 9

Fig. 10. Photomicrograph of a large abscess in the lungs of ewe (No. 815) inoculated with C. renale. The abscess is surrounded by a thick fibrous capsule, and included central necrosis (X 60).

Fig. 11. Photomicrograph of a large encapsulated abscess in the liver of ewe (No. 815) inoculated with C. renale. Most hepatic cord cells are replaced by fibrous tissue (X 60).

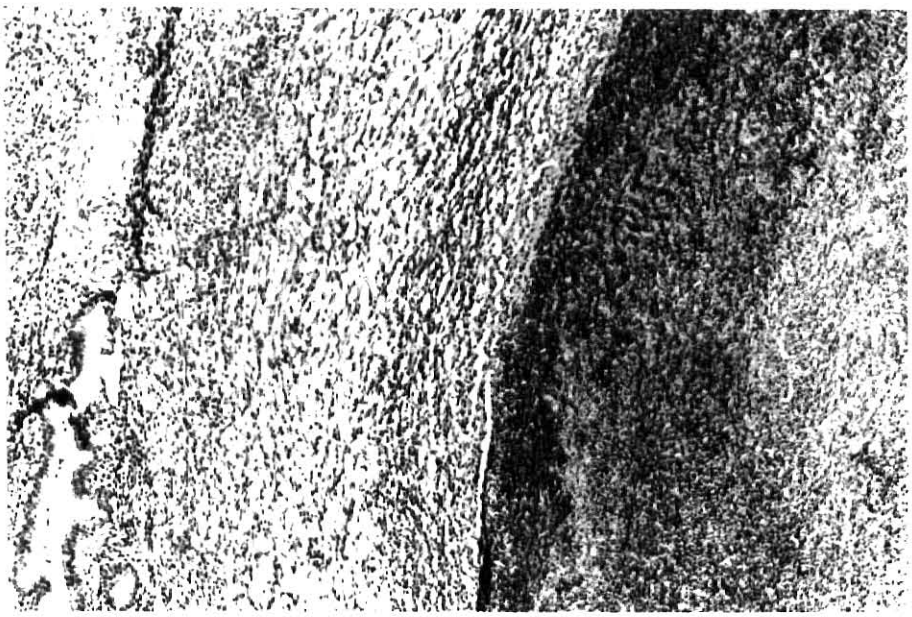

Fig. 10

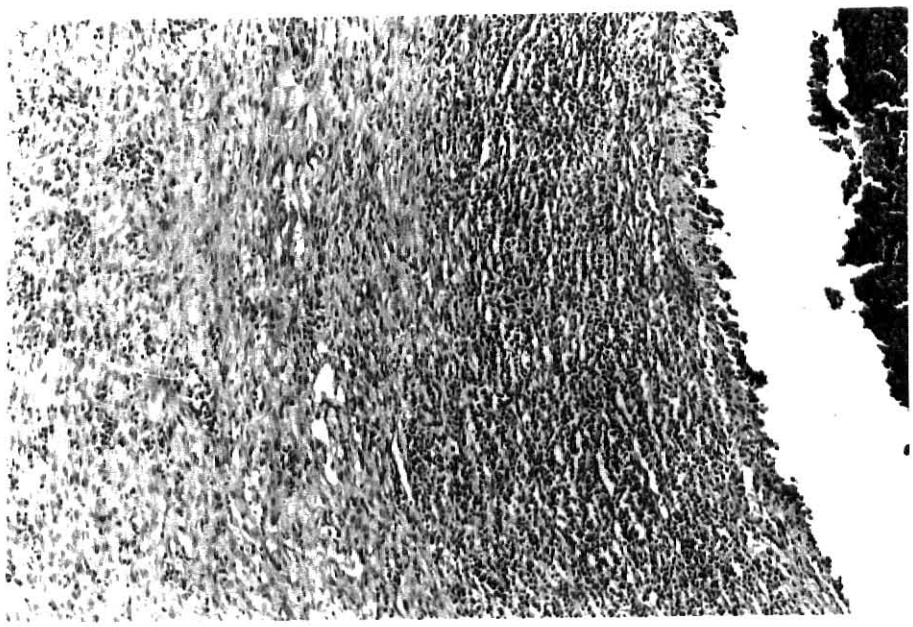

Fig. 11

Fig. 12. Photomicrograph of a lung tissue from ewe (No. 898) inoculated with C. renale. Proliferation of fibrous tissue and thickened alveolar walls close to the pleural surface (X 60).

Fig. 13. Photomicrograph of a lung tissue from ewe (No. 898) inoculated with C. renale. Proliferation of fibrous tissues and thickened alveolar walls and pleural surfaces (X 60).

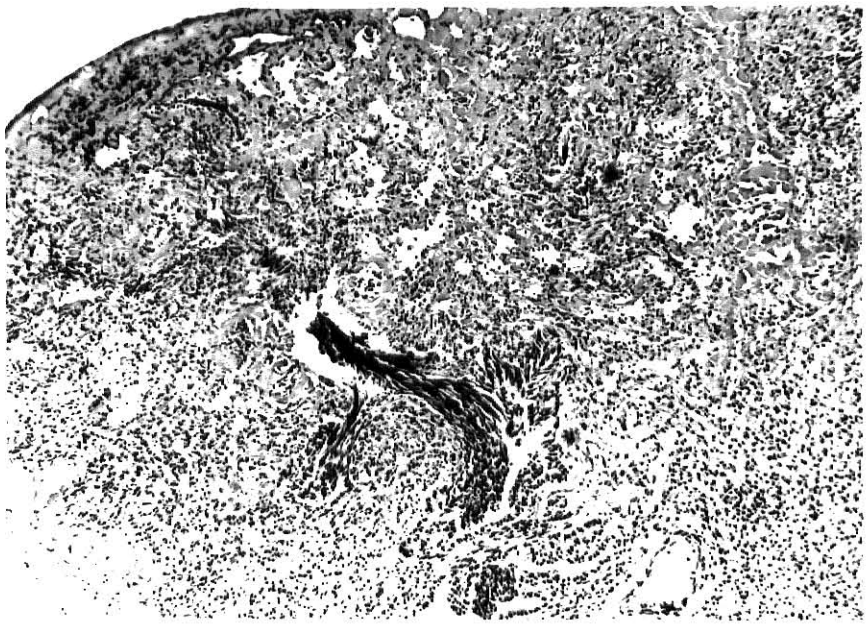

Fig. 12

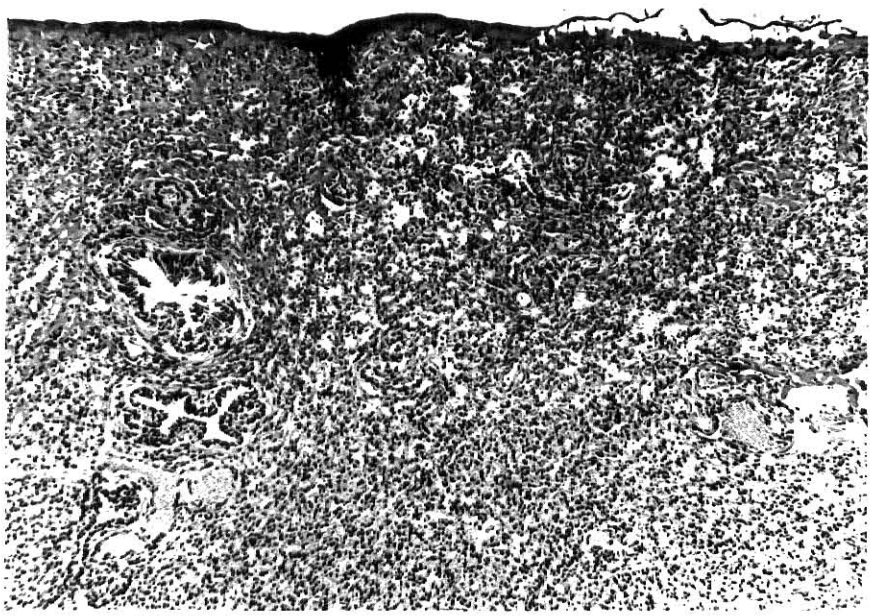

Fig. 13

Fig. 14. Photomicrograph of a lung tissue from ewe (No. 180) inoculated with C. renale. Note severe fibrosis, and multiple focal and diffuse granulomas (X 60).

Fig. 15. Photomicrograph of a large focal granuloma in the lungs of ewe (No. 180) inoculated with C. renale (X 60).

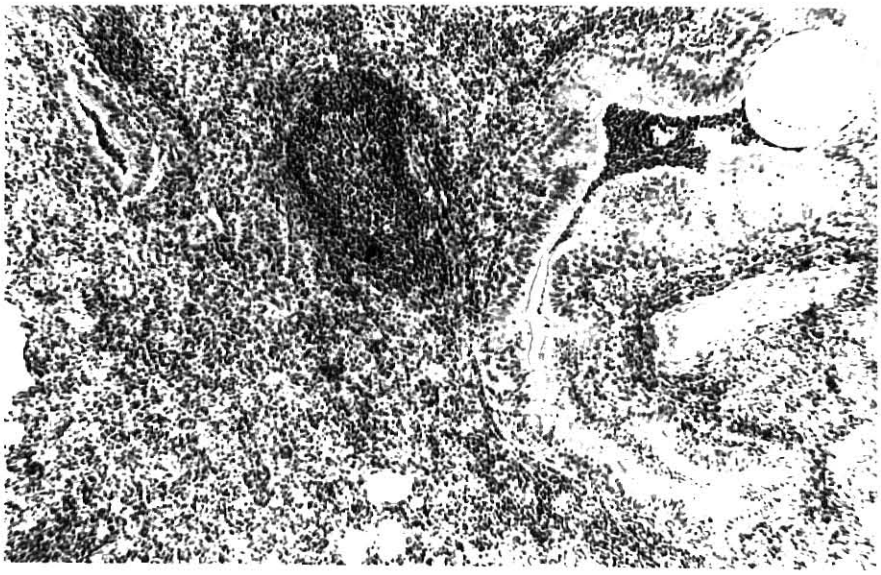

Fig. 14

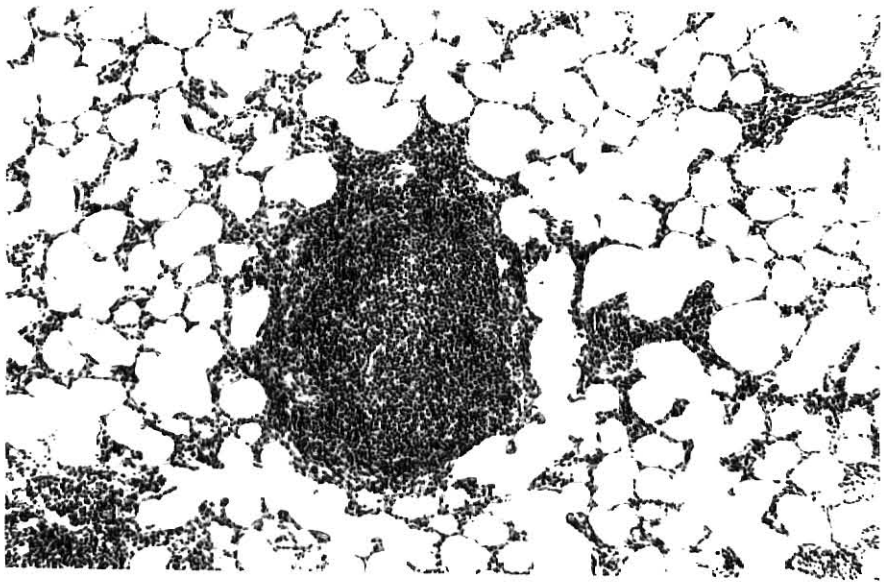

Fig. 15

Fig. 16. Photomicrograph of a section of the lungs from ewe (No. 180). Severe fibrosis and multiple granulomas (X 60).

Fig. 17. Photomicrograph of a granuloma in the lungs of ewe (No. 180). Infiltrated cells are mostly mononuclear phagocytes (X 250).

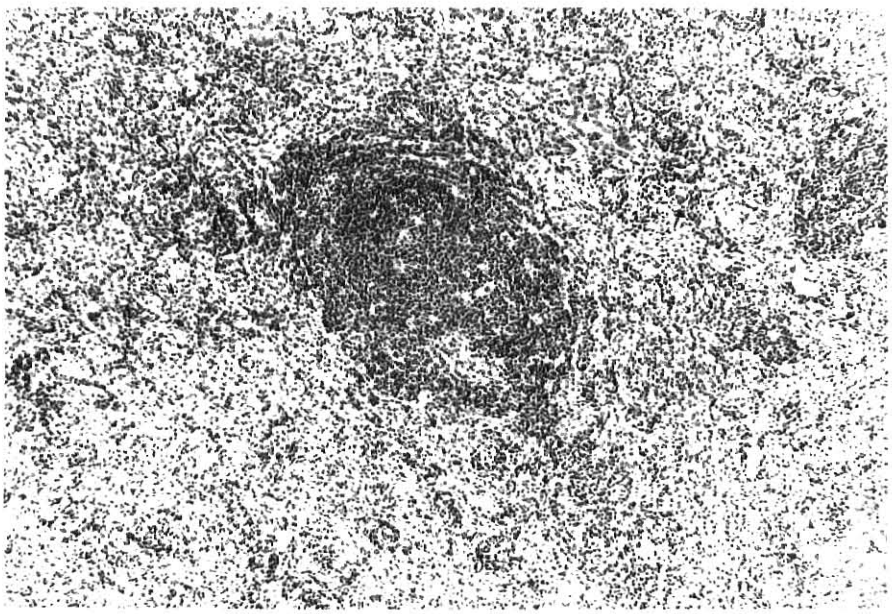

Fig. 16

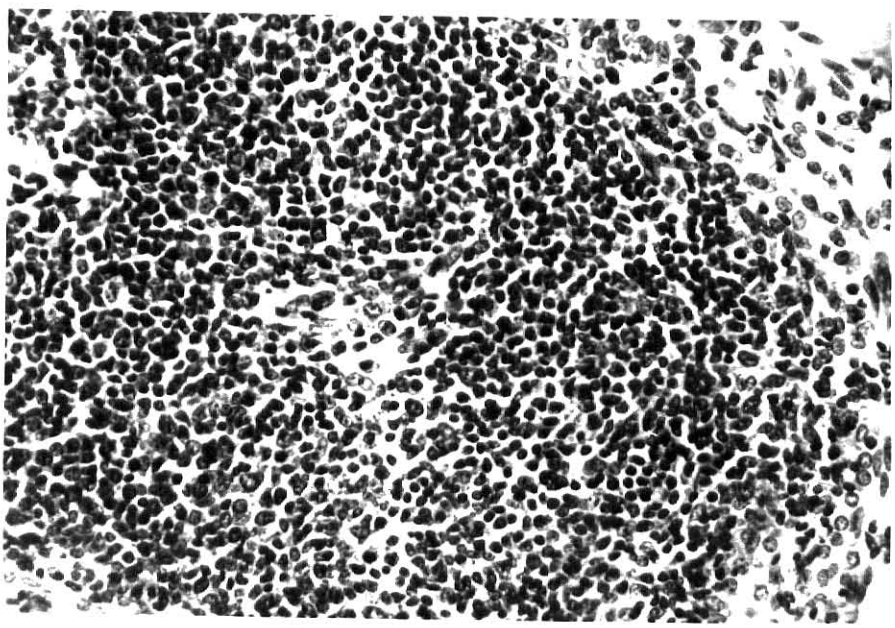

Fig. 17

Fig. 18. Photomicrograph of the liver from ewe (No. 800) inoculated with C. renale. Widespread fatty changes in the hepatic cord cells, and infiltration and accumulation of the non-suppurative inflammatory cells around the intrahepatic bile ducts (X 60).

Fig. 19. Photomicrograph of a severe fibrous tissue proliferation in the lungs of ewe (No. 800) (X 250).

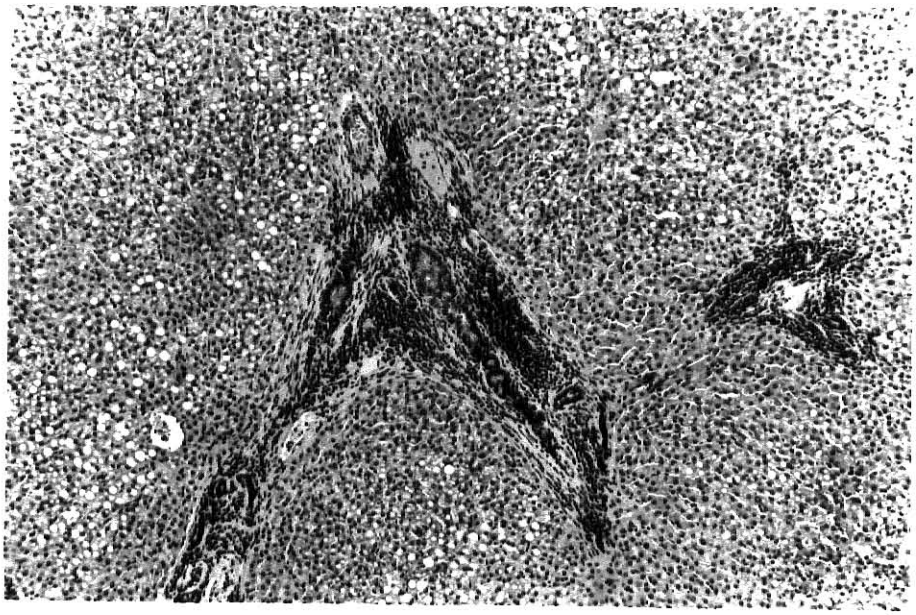

Fig. 18

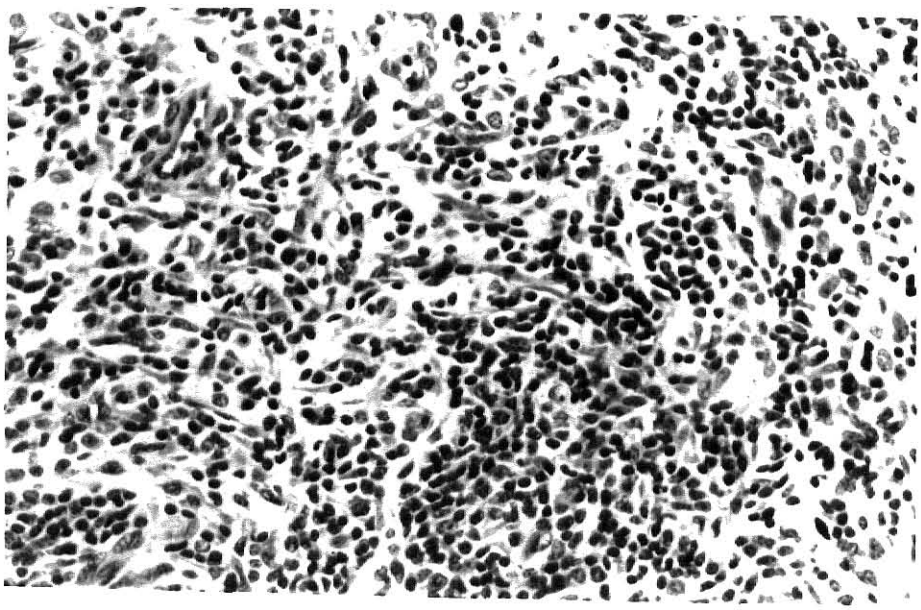

Fig. 19

Fig. 20. Photomicrograph of a chronic inflammatory papillitis in the kidneys of ewe (No. Z) inoculated with C. pyogenes (X 60).

Fig. 21. Photomicrograph of a lung tissue from ewe (No. 814) inoculated with C. equi. Thickened alveolar walls and infiltration of non-suppurative inflammatory cells into the thickened areas (X 60).

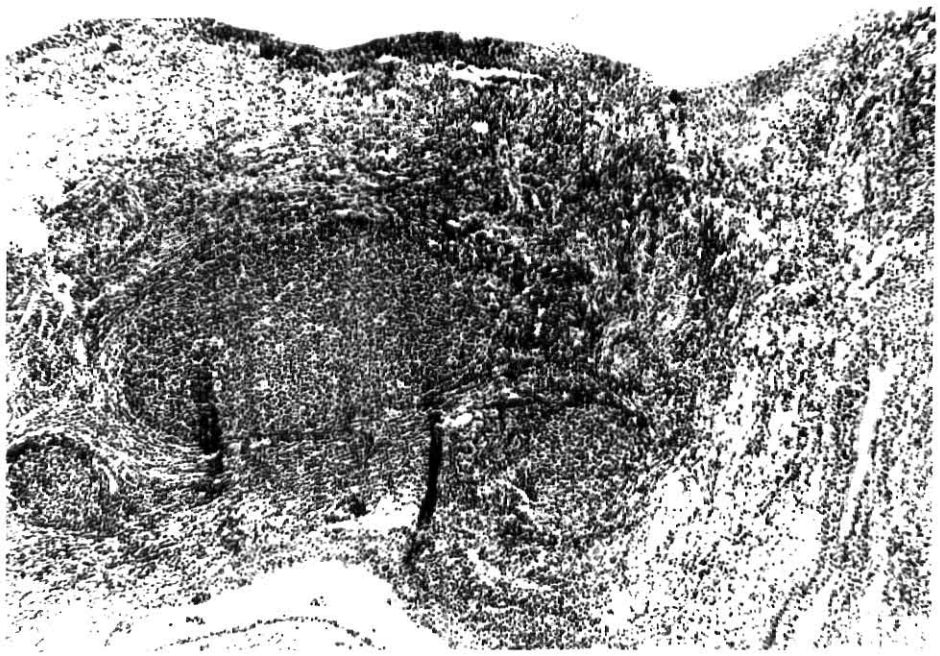

Fig. 20

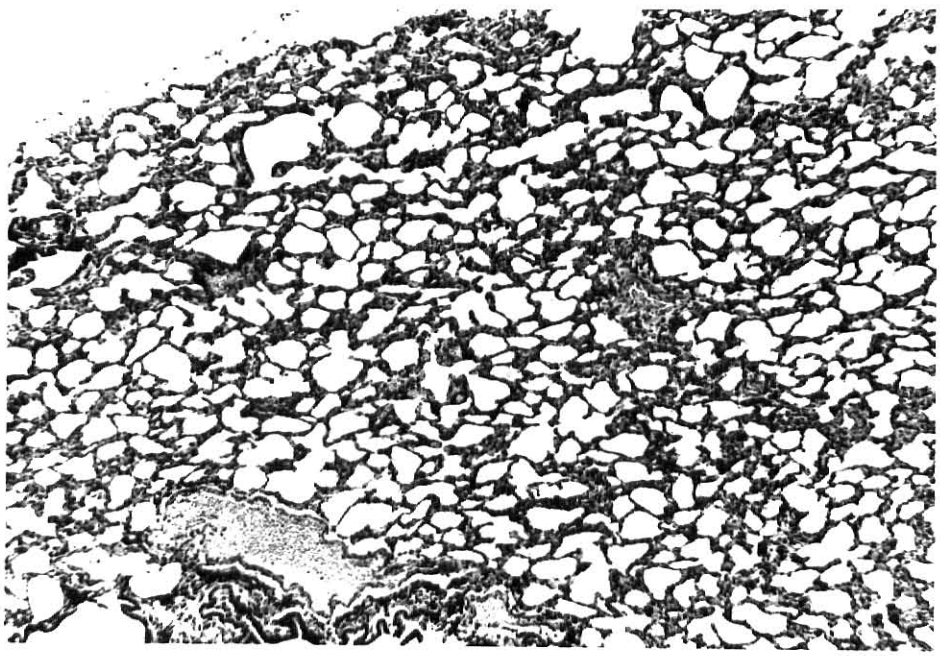

Fig. 21

Fig. 22. Photomicrograph of the lung from ewe (No. 841) inoculated with C. pyogenes. Multiple focal areas of abscessations, and slight fibrosis (X 60).

Fig. 23. Photomicrograph of a large abscessed area in the lungs of ewe (No. 841). Note infiltrated polymorphonuclear leukocytes in the periphery and necrotic tissues and mineralized material in the center of the abscess (X 250).

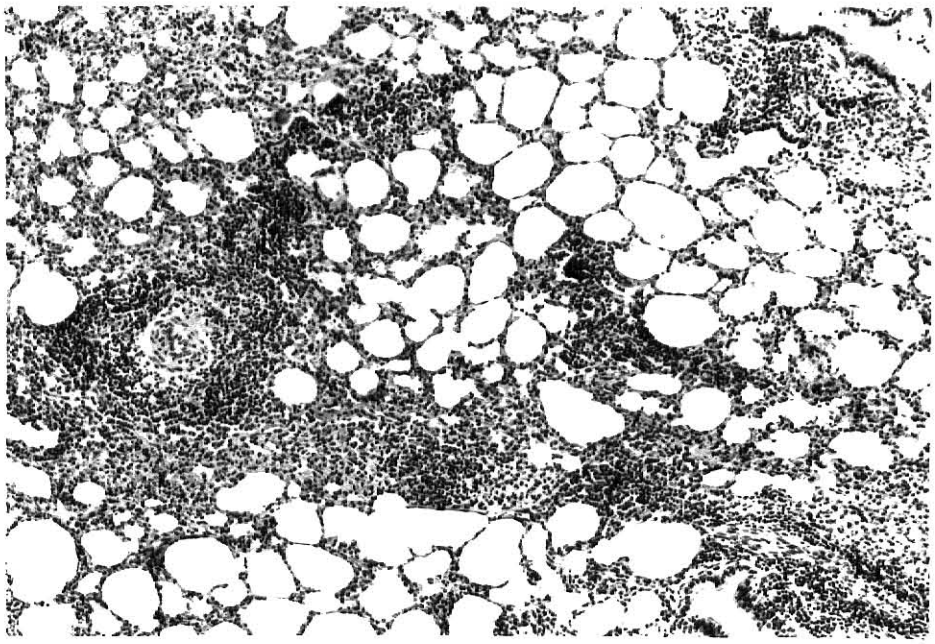

Fig. 22

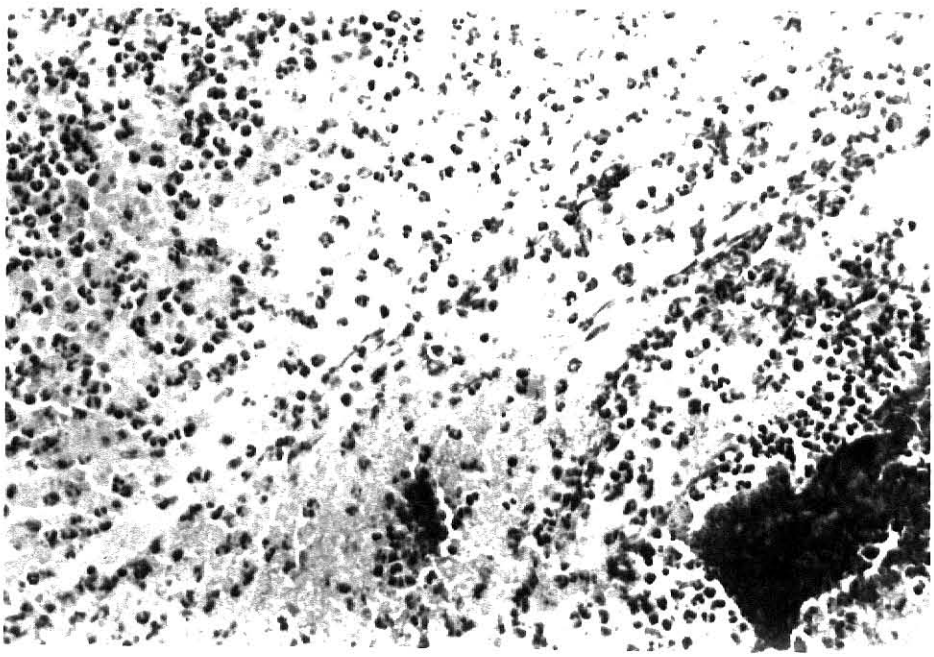

Fig. 23

CORYNEBACTERIA STUDIES IN SHEEP

Paper II: Diseases Caused by Corynebacteria (A Literature Review).

INTRODUCTION

The diphtheroid bacilli are organisms that have a resemblance to Corynebacterium diphtheria, the cause of human diphtheria, and are classified in the same group (4, 20, 27). Pathogenic members of the genus Corynebacteria are non-motile, gram positive rods, frequently banded or beaded with metachromatic granules and arranged in pairs of palisades with club-shaped swellings at the poles (2, 20). The diphtheroid organisms are members of a large group of bacteria which are common on the mucous membranes and skin of man and animals (20, 27). In animals, four species have been found of major importance. These are C. pyogenes, C. pseudotuberculosis, C. renale, and C. equi (3, 4, 6). Corynebacteria have also been isolated from pulmonary abscesses in mice, from throat lesions in chickens and other birds, from the conjunctiva of dogs and horses, and from abscesses in numerous other animals (2, 20). This review is concerned primarily with the four pathogenic species listed above as having major importance in animal disease.

Corynebacterium pyogenes synonyms are Bacillus pyogenes, Bacterium pyogenes, and Hemophilus pyogenes. The organism was first observed in the pig from a case of caseous pneumonia in 1890 by Kitt (20, 27). It was recovered from the cow in 1893 by Lucet (20). In 1897, it was isolated from a case of arthritis in a calf and named Bacillus pyelonephritis by Poles (20). This

organism was first recovered from a form of swine pneumonia by Grip in 1902 (3, 16, 20). In 1903, Kunneman observed C. pyogenes in a case of suppuration in the cow (20). This organism was first included in the diphtheroid group and named C. pyogenes by Eberson in 1918 (20).

C. pyogenes is the most common pus-forming organism reported in domestic ruminants, and appears to be frequently involved in sporadic infections of the reproductive system (25). The bacterium has been associated with abortion, pyosalpinx, purulent endometritis, and mastitis in cows, and with seminal vesiculitis in bulls (3, 25).

C. pyogenes has been recovered from cattle with "summer mastitis", endometritis and pyometra, liver abscesses, polyarthritis, and some granulomatous lesions (3, 4, 16). Pyometra and endometritis may follow calving even though parturation and cleansing may have occurred naturally (27). It is also responsible for a chronic abscess forming mastitis, suppurative pneumonia, actinomycosis-like lesions, and abscesses forming in the peritoneal and thoracic cavities secondary to trauma and arthritis (20). It has been reported to be associated with abortion in cows by Maxwell, Boyed, and Kelly (20).

C. pyogenes is frequently found in purulent metritis in cattle, usually in association with staphylococci (4). In adult cattle, it causes embolic suppurative nephritis secondary to endometritis, and it is also the cause of septic embolism and cerebral abscess in the central nervous system and vertebral

osteomyelitis (20). C. pyogenes can cause sporadic cases of suppurative mastitis, but the specific cause of "summer mastitis" is not necessarily this organism alone (3). Streptococci, staphylococci and other bacteria are commonly present and play some part in the development of the disease (3, 16).

Acute mastitis caused by C. pyogenes in cattle is usually a complication of injury and, as a enzoatic disease, is known as "summer mastitis" in United Kingdom and "Holstein udder plague" in Europe (27). Calf pneumonia is sometimes caused by C. pyogenes, also umbilical infections and destructive arthritis in calves can be caused by this organism (4, 16).

C. pyogenes has been isolated from ovine and caprine mastitis, chronic purulent pneumonia, bronchopneumonia, and from joint infection resulting in acute lameness (20, 25).

The location and character of the lesions produced by C. pyogenes in swine are similar to those in cattle (22). It may cause suppurative pneumonia, and formation of focal, encapsulated abscesses followed by extensive causeous pneumonia, polyarthritis, stiffness of gait, lameness, and progressive weight loss (4, 6, 20, 27).

Horses are rarely infected, but it has been reported from one case of sinusitis (33). Zaki and Farrage have reported C. pyogenes to be a rare cause of metritis in horses and pyometra in the dog (33).

Fowls are considered resistant, but the organism has been isolated from suppurative lesions in birds (33).

It is commonly associated with pneumonia in the camel and has been isolated from traumatic reticulitis in buffalos (4).

C. pyogenes produces subcutaneous abscesses in the rabbit, and localizes in the joints causing deforming arthritis (20).

Guinea pigs and rats are resistant to the infection (20). In mice following intraperitoneal inoculation, abscesses are formed in the liver and on the omentum (20).

C. pyogenes is an ubiquitous organism and occurs in many animal diseases both as a primary or secondary invader (3, 16). Abscesses caused by this organism usually form slowly and develop thick fibrotic capsules (27). The pus may be typically thick, greenish-white, and nonodorous; or it may be thin and fetid, especially where other organisms are also presented (4). C. pyogenes may also act along with other infectious agents and complicate diseases such as foot and mouth disease, swine plague, and contagious agalactia of sheep and goats (16).

It is strongly supported that C. pyogenes infection in cattle, sheep, goats, and pigs is endogenous and is the result of the stimulation of a latent infection (4, 16). In some cases, trauma alone may be sufficient, and in others viruses, or other living agents may play a part (4, 16, 20).

C. bovis is a common inhabitant of the bovine udder and is usually considered to be non pathogenic, but has been suggested as a cause of mastitis in some herds (10). The spread

of the infection is uncertain in sporatic cases, but flies have been reported to play an important role in outbreaks of the disease (7).

Corynebacterium pseudotuberculosis has been known under a variety of names: C. pseudotuberculosis nocard (1885); pseudotuberkulose-Bacterien preisz (1891); Bacillus pseudotuberculosis ovis, Lehmann and Neumann (1896); Bacillus pseudotuberculosis, Buchanan (1911); C. ovis, Bergey et al. (1923); C. pseudotuberculosis ovis, Hauduroy et al. (1937); C. Preisz-nocard, Hauduroy et al. (1937); and, Bacillus of Preisz-Guinard, Bacillus Preisz-nocard (cited by Bergey, 1948) (2). Preisz and Guinard (1891) first described an organism found in a renal abscess of a sheep (2). This was named Bacillus pseudotuberculosis ovis by Preisz (1894) (2). Lehmann and Neumann (1896) introduced the generic name Corynebacterium for the diphtheria bacillus and diphtheroid organism (2). As they had not studied Preisz's organism, they did not alter the generic name, but grouped it with the Corynebacteria (2). After the adoption of the generic name Corynebacterium in the classification of the American Society of Bacteriologist (1923), the organism was named Corynebacterium ovis (2).

In 1969, C. pseudotuberdulosis was examined by electron microscopy after being subjected to various methods of fixation (12). The organism exhibited a fine structure similar to other corynebacterial species in the appearance of its cell wall, plasma membrane, nuclear apparatus, cytoplasmic matrix, wealth

and complexity of intracytoplasmic membrane system, and polyphosphate granules (12). An outstanding structural feature was the existence of an electron-dense, floccular layer external to the cell wall which both ligroin and acetonemetanol extractions demonstrated to be the previously postulated surface lipid of this organism (12). The only variation in structure evident between virulent and attenuted strains was a quantitative difference in the thickness and appearance of the surface lipid (12, 15).

C. pseudotuberculosis is the cause of caseous lymphadenitis in sheep and ulcerative lymphangitis in horses and cattle (27). Caseous lymphadenitis is a suppurative infection of the lymph nodes of sheep, and is usually the result of shearing wounds becoming infected, occasionally the infection may occur by injection (3, 16). Infection is usually transmitted by contamination of wounds with pus from a discharging lesion (16, 20). The pathogenecity of C. pseudotuberculosis is related to production of a heat-labile exotoxin, heat stable pyogenic factors associated with the bacterial bodies, and a surface lipid leukotoxin (16, 25). The role of the surface lipid is unclear and it may be indirectly responsible of the persistence of the caseous lesions, and their failure to soften (16). The sequence of events in progressive disease is infection of a superficial wound, spread of infection to the local lymph nodes which suppurate, and then lymphagenous and hematogenous extension to produce abscesses in the internal organs (14, 16). The

typical lesion of caseous lymphadenitis is the encapsulated abscess with a distinct greenish exudate of a caseous or caseo-purulent consistency (4, 16, 20).

When the organisms reach the lymph nodes, multiple microscopic foci form in the pulp, with a prominant eosinophilic reaction (16). These foci rapidly coalesce, and the central area becomes a structureless caseated mass (2, 16). Abscesses are rapidly encapsulated, but continue to enlarge by migration of phagocytes through the capsule (16). With progressive enlargement, there is also progressive necrosis and a reformation of the capsule, which gives to the lesion a very characteristic structure of concentric lamellation (16). Metastatic lesions may occur in the lungs, and occasionally in the renal pelvis, liver, and spleen. The mortality in a flock of adult sheep may be as high as 70 percent (3, 16, 27). The disease has also been observed in goats (8).

C. pseudotuberculosis (C. ovis) has also been found to cause a nonsuppurative arthritis and bursitis in lambs; the joints were slightly enlarged (18). C. pseudotuberculosis may also be presented in outbreaks of foot abscess in sheep (3). Suppurative orchitis in rams caused by C. pseudotuberculosis has been recorded in South Africa, New Zealand, and the U.S.A. (9, 19, 24). This orchitis may be confused with epididymitis caused by Brucella ovis (3, 19). C. pseudotuberculosis (C. ovis) infection has been reported in mares (13). Encapsulated abscesses were found in the abdominal cavity, perirenal tissues, and kidneys.

Ulcerative lymphangitis of horses is a chronic progressive inflammation of the subcutaneous lymphatics of horses with C. pseudotuberculosis as the primary pathogen (16). It typically begins about fetlocks or the rear limbs (16, 27). As a result of the lymphangitis, there is diffuse swelling in the legs with abscesses forming in the dermal nodes. The nodes ulcerate, and discharge a thick creamy exudate which may be blood stained (16, 27). The ulcers heal and leave small areas of depilated and depigmented skin (16). Milliary abscesses occur as a result of internal dissemination and may form in kidneys (3, 16, 27). Ulcerative lymphangitis also occurs in cattle, within the dermis or subcutis of the neck and trunk (16, 27).

Corynebacteria species have been reported as a cause of ovine abortion (28). Southcott (1965) reported ovine posthitis due to corynebacteria (26). Dennis and Bamford (1966) reported the role of corynebacteria in perinatal lamb mortality (6). In deer, C. pseudotuberculosis (C. ovis) causes a disease similar to that in sheep, with the exception of subcutaneous abscesses of the extremities in deer (20). It is also reported in goats (8).

Intravenous injection of C. pseudotuberculosis (C. ovis) cultures into the guinea pig causes death in four to ten days, with abscess formations in the lungs and livers (20). Intraperitoneal injections into male guinea pigs produces orchitis (20). C. pseudotuberculosis (C. ovis) is reported to produce suppurative osteomyelitis and arthritis in white mice (32).

Synonyms of Corynebacterium renale are Bacillus pyelonephritis boum, Bacillus renalis, C. renalis and Bacillus renalis bovis. Dammann (1877) observed small bacteria in lesions of typical pyelonephritis of the cow, and in 1890, Hess observed a curved bacillus in the kidneys and urine of cows suffering with pyelonephritis (20, 27). Bollinger (1890) reported an organism under the name of Bacillus renalis bovis. Enderlen (1891) was the first to cultivate the organism on artificial media and describe it in detail (10, 27). The most exact early description of the organism and of the pyelonephritis was made by Ernest in 1905 and 1906 (20).

Contagious bovine pyelonephritis is the specific infection of the urinary tract of cattle caused by C. renale, and is characterized by chronic purulent inflammation in the bladder, urethers, and kidneys (3, 16). C. renale may be isolated from the urine of clinically affected or clinically silent carrier animals (3). In natural infections, the urinary bladder is always involved. The walls of affected bladders are thickened and the mucosa is superficially ulcerated (4). Typically, the kidney pelvis is found to be enlarged. The papillae are necrotic and abscesses occur throughout the kidney (4, 16, 27). The vulva is thought to be the portal entry in the cow (3).

C. renale is found principally in cattle, but has been observed in horses and sheep, and one case has been described in a dog (4).

Intravenous injection of C. renale has been shown to produce renal lesion in mice (3). Cystitis and pyelonephritis in mice have been produced by Morse and Margon (20).

The pathogenecity of C. renale for rabbits was first reported by Enderlen, but typical renal pathology produced in rabbits was described in 1949 by Feenstra, Thorp, and Gray (20). They were able experimentally to produce papillitis and pyelitis, characterized necrosis (20). The organism has not been found to be pathogenic for guinea pigs (20).

In 1923, Magnusson isolated and named the organism, Corynebacterium equi from a case of suppurative pneumonia in a foal (4, 20). Holth and Amundsen (1936) reported a small coccobacillus in tuberculosis-like lesions of the cervical lymph nodes of swine, and in 1938 Bendixen and Jespen reported that the Holth bacilli were C. equi (4, 20, 27).

C. equi is capable of producing a suppurative broncho-pneumonia in foals, and the pneumonia is accompanied by the formation of small abscesses throughout the lung tissue (16, 20). There is an age incidence and infected foals are typically two to three months of age or older (1, 27). The disease can be produced experimentally in foals by either oral or intranasal inoculation of the organism (3).

The method of natural transmission of C. equi is unknown; presumably, it gains entrance through the respiratory tract in foals, although hematogenous origin of the pulmonary infection is reportedly possible (20). A genital mode has also been

proposed with infection occurring either prenatally or by invasion via the navel soon after birth (29). Other experience points to migrating parasitic larvae as carriers of infection through the body (1). Magnusson reports that it is possible to produce infection by intratracheal inoculation, and Flatla has found that C. equi infection can be produced experimentally by feeding cultures of the organism to foals (20).

This organism has been isolated from the uterine discharge of a number of aborting mares and is suspected of being a causative factor of this condition in some cases (4).

C. equi affects horses principally, but may also be found in lymph node abscesses in the pig, and in ulcerative lymphangitis in cattle (3, 4, 16). C. equi has also been isolated from a sheep affected with pneumonia and pleurisy (23).

In the pig, C. equi is associated with small, soft, encapsulated abscesses found commonly in the submaxillary lymph glands; also, it has been found in pneumonia of the pig by Thal and Rutquist (20, 27). C. equi has been determined as a cause of infectious arthritis in horses, to cause pneumonia in calves, and to be associated with pyeometra in cattle (16). Pathogenecity of C. equi for experimental animals is low (4). Rabbits and rats are not susceptible, but some strains of the organism are able to kill mice and guinea pigs (20).

DISCUSSION

A variety of diphtheroid organisms have been isolated from various tissues in animals. They have been isolated from

obviously pathogenic conditions and on occasion, the tissues from which the organism were isolated appeared normal.

In domestic animals, four corynebacterium species have been found to be of great importance. These are C. pyogenes, C. pseudotuberculosis, C. renale, and C. equi.

Reports of C. pyogenes have been contributed from all over the world where animals have been domesticated. C. pyogenes is the most frequently recovered pus forming bacterium in domestic animals.

In cattle, the organisms are found in abscesses, in necrotic and suppurative pneumonia, suppurative arthritis, and purulent metritis and mastitis. In calves, the organism has been isolated from umbilical infections. In swine, the organism produces a disease resembling those in cattle. In sheep and goats, purulent pneumonia and abscesses in the upper respiratory tract have been reported due to this organism.

C. pseudotuberculosis causes ulcerative lymphangitis in horses and caseous lymphadenitis of sheep and goats.

C. renale is commonly associated with bacillary pyelonephritis of cattle, in which chronic purulent cystitis and urethritis accompany the inflammatory changes in the ureters and renal pelvis. Horses and sheep may become infected but dogs rarely.

C. equi is involved in a specific pneumonia of foals and has been isolated from arthritic joints in lambs, and granulomatous lesions in swine.

Diseases caused by corynebacterium species are very important economic problems to the food animal industry, but in most cases, they have not been studied in sufficient detail.

SUMMARY

A number of species of corynebacteria are pathogen for domestic animals.

C. pyogenes is one of the most common causes of purulent infections in cattle, sheep, pigs, and goats. It is the cause of a specific mastitis, a few cases of abortion, arthritis, and granulomatous lesions in bovine. It has been observed in endometritis, pyometra, liver abscess, suppurative pneumonia, and embolic suppurative nephritis in cattle, and is associated with calf pneumonia.

C. pseudotuberculosis is also a common pathogen of domestic animals, it produces a caseous lymphadenitis in sheep and ulcerative lymphangitis in horses. It is found to cause nonsuppurative arthritis and bursitis in lambs, orchitis in rams, and foot abscess in sheep. The organism is also observed from ulcerative lesions in cattle, mares, deer, and goats.

C. renale produces purulent infections of the urninary tract in cattle, but has also been reported in horses, sheep, and dogs.

C. equi is the cause of spontaneous pneumonia in foals and other infection in horses. It is reported to cause abscesses in the pig and cattle, and has been isolated from a case of pneumonia and pleurisy in sheep.

REFERENCES

1. Bain, A. M. Corynebacteria infection in the equine. Aust. vet. J., 39:116-121. 1963.

2. Benham, C. L., Seaman, A. and Woodbine, M. Corynebacterium pseudotuberculosis and its role in disease of animals. Vet. Bull., 32:645-655. 1962.

3. Blood, D. C. and Henderson, J. A. Veterinary Medicine. 3rd ed., The Williams and Wilkins Company, Baltimore, 298-305. 1968.

4. Bruner, D. W. and Gillespie, J. H. Hagan's Infectious Disease of Domestic Animals. 5th ed., Cornell University Press, Ithaca, New York, 151-166. 1966.

5. Cameron, C. M. and Swart, C. F. A new liquid medium for cultivation of Corynebacterium ovis. J. S. Afr. Vet. Med. Ass., 36:185-188. 1965.

6. Dennis, S. M. and Bamford, V. W. The role of Corynebacterium in perinatal lamb mortality. Vet. Rec., 79:105-108. 1966.

7. Derbyshire, J. B. Immunity in bovine mastitis. Vet. Bull., 32:1-10. 1962.

8. Dhanda, M. R. and Singh, M. M. Corynebacterium ovis infection in goats. Indian vet. J., 32:43-47. 1955.

9. Dood, D. C. and Hartly, W. J. A specific suppurative epididymitis of rams. N. Z. vet. J., 3:105-111. 1951.

10. Duckitt, S. M., Seaman, A., and Woodbine, M. The bacteriology of Corynebacterium bovis. Vet. Bull., 33:67-73. 1963.

11. Fraser, G. Haemolytic activity of Corynebacterium ovis. Nature. London, 189:246. 1961.

12. Hard, G. C. Electron microscopic examination of Corynebacterium ovis. J. Bact., 97:1480-1485. 1969.

13. Hughes, J. B. Biberstein, E. L. and Richards, W. P. C. Generalized C. pseudotuberculosis infection in mares. Cornell Vet., 52:51-62. 1962.

14. Jolly, R. D. The pathogenic action of the exotoxin of Corynebacterium ovis. J. Comp. Path., 75:417-431. 1965.

15. Jolly, R. D. Some observation on surface lipids of virulent and attenuated strains of C. ovis. J. Appl. Bact., 29:189-196. 1966.

16. Jubb, K. V. F. and Kennedy, P. C. Pathology of Domestic Animals. Vols. 1 and 2, New York Academic Press. 1970.

17. Lovell, R. and Zaki, M. M. Studies on growth products of Corynebacterium ovis. Res. Vet. Sci., 7:302-311. 1966.

18. March, H. C. ovis associated with an arthritis in lambs. Amer. J. Vet. Res., 8:294-298. 1947.

19. McGown, B., and Shultz, G. Epididymitis of rams, clinical and descriptions and field aspects. Cornell Vet., 46:277-281. 1956.

20. Merchant, I. A. and Packer, R. A. Veterinary Bacteriology and Virology. 6th ed., The Iowa State University Press, Ames, Iowa, 527-546. 1969.

21. Nadim, M. A., Mahmoud, A. H. and Soliman, M. K. The significance of the blood picture in the diagnosis and prognosis of caseous lymphadenitis in sheep. Zentbl. Vet. Med., 13A:715-718. 1966.

22. Roberts, R. J. Isolation of Corynebacterium hemolyticum from a case of ovine pneumonia. Vet. Res., 84:490. 1969.

23. Roberts, D. S. Corynebacterium equi infection in a sheep. Aust. Vet. J., 33:21. 1957.

24. Roberts, D. S., Graham, N. P. H., Egerton, J. R. and Parsonson, I. M. Infective bullbar necrosis (hell abscess) of sheep, a mixed infection with F. necrophorus and C. pyogenes. J. Comp. Path., 78:1-8. 1968.

25. Smith, R. E., Reynolds, I. M., Clark, G. W. and Milbury, J. A. Fetoplacental effects of C. pyogenes in sheep. Cornell Vet., 61:573-590. 1971.

26. Southcott, W. H. Etiology of ovine postitis. Aust. Vet. J., 41:193-200. 1965.

27. Stableforth, A. W. and Galloway, I. A. Infectious Diseases of Animals. Vol. I, New York Academic Press, 239-254. 1959.

28. Szemardi, G. and Temesi, Z. Abortion in ewes due to Corynebacterium infection. Mag. allator. Iapja, 14:276-279. 1959.

29. Wilson, M. M. A study of Corynebacterium equi infection in a stud of thorough bred horses in Victoria. Aust. Vet. J., 31:175-182. 1955.

30. Zaki, M. M. Production of soluble substances by C. ovis. Nature, London, 205:928-929. 1965.

31. Zaki, M. M. The application of a new technique for diagnosis of C. ovis infection. Res. Vet. Sci., 9:489-493. 1968.

32. Zaki, M. M. The ability of Corynebacterium ovis to produce suppurative osteomyelitis and arthritis in white mice. J. Comp. Path., 76:121-126. 1966.

33. Zaki, O. A., and Farrag, H. Incidence of C. pyogenes among animals and birds in Egypt. Vet. Med., 50:219-220. 1955.

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CORYNEBACTERIA STUDIES IN SHEEP

by

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AN ABSTRACT OF A THESIS

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The genus corynebacterium is composed of species of bacteria which are found in man and animals, and are a very important economic problem to the food animal industry.

Among the domestic animal species, four have been found to be of the greatest importance. These are Corynebacterium pyogenes, C. pseudotuberculosis (ovis), C. renale, and C. equi.

The purpose of this study was to induce corynebacterial infection in sheep with C. renale, C. equi, and C. pyogenes to gain information regarding the specific lesions produced by these three organisms in sheep.

A total of 20 western crossbred ewes were divided into four test groups (16 ewes) and one control group (four ewes), and kept in isolation quarters and fed alfalfa pellets. The body temperature of each ewe was recorded twice daily for two days prior and each day post inoculum until death.

Group one and two consisted of eight ewes each inoculated with 2 cc of C. renale (IV). Four ewes held for 45 days (group one), and four held for 90-100 days (group two). All eight ewes developed a febrile response on day 1-4 (PI). Lung abscesses were observed in three ewes in group one. Liver abscesses were found only in one ewe in group one. In group two, one ewe developed multiple abscesses in the lungs, liver, and the kidneys. These abscesses were thickly encapsulated and contained

necrotic debrises and mineralized material. C. renale was recovered from the limb joints of two ewes in group one. In group two, C. renale was recovered from the lungs of one ewe and limb joints of two ewes.

In group three, four ewes were inoculated with 2 cc of C. equi (IV), and held for 90-100 days. All four ewes had a febrile response six hours following inoculation. Three ewes developed fibrous adhesions between the lung and pareital pleura. The conditions were those of a chronic interstitial pneumonia and pleurisy. C. equi was recovered from the lungs of one ewe, and limb joints of two ewes.

Group four consisted of four ewes each inoculated with 15 cc of C. pyogenes strain. All four ewes showed a febrile response within five hours post inoculum. Two ewes developed lung abscesses. One ewe had a granulomatous papilitis in both kidneys. C. pyogenes was recovered from the right kidney of the ewe with granulomatous papilitis. Attempt to culture C. pyogenes from the abscesses in the lungs were without success.

In group five (controls), four ewes were inoculated with 15 cc of B.H.I. broth (IV) and held for 90-100 days. One ewe in this group had a few caseated abscesses in the lungs, and C. pseudotuberculosis was isolated in this ewe.

A literature review concerning diseases caused by corynebacteria was done. Four species have been reported of being of greatest importance in animals. These are: C.

pyogenes, *C. pseudotuberculosis* (*C. ovis*), *C. renale*, and *C. equi*. It seems difficult from the published reports to determine whether these organisms have or have not played a significant pathogenic role. In many cases and in most instances, they have not been studied in sufficient detail.