STUDIES ON ACTINOBACILLUS SEMINIS INFECTION IN LAMBS

by

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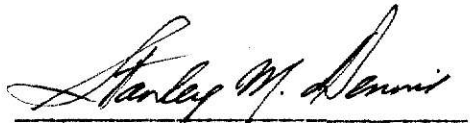

Major Professor

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INTRODUCTION

Arthritis is inflammation of intraarticular structures; in animals is commonly due to infection by a number of organisms. It is economically important because of losses or unthriftiness. In draught animals joint conditions are significant as they tend to be crippling in nature; in chronic cases, animals may become permanently incapacitated. In food animals, in addition, it is responsible for considerable economic loss because affected animals are either wholly or partly condemned. Young animals surviving the septicemic phase of certain infections frequently develop chronic arthritis that results in joint deformity and stunted growth.

Certain forms of arthritis are of public health significance because the causative organisms are transmissible to man. Most of these infections are occupationally acquired and usually take the form of a cutaneous condition (for example erysipelas), and may be either mild and localized or severe and widespread; fatal septicemic forms have also been reported.

In recent years, certain organisms not previously associated with arthritis are now playing a major role in arthritic conditions of farm animals. Studies are being carried out in many parts of the world to determine their importance and better methods of diagnosis.

Compared to Brucella ovis infection, there is little knowledge of the transmission and pathological changes of Actinobacillus seminis in sheep. As experimental studies of A. seminis infection in lambs have not been reported, this project was undertaken to study aspects of A. seminis infection in lambs. The objectives of the study were to determine the:

1. Localization of A. seminis and associated pathological changes following intravenous inoculation.

2. Sequential changes in the carpal joint following intraarticular inoculation of A. seminis.

3. Pathological changes associated with intratracheal inoculation of A. seminis.

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I. REVIEW OF LITERATURE

Introduction

Arthritis is inflammation of the synovial membrane and articular surfaces usually as a result of infection. It is characterized by lameness, swelling of the joint, heat and pain. It is one of the diseases of the musculoskeletal system that, as a group, are clinically characterized by reduced activity, difficulty in rising and mobility and adoption of unusual postures (Blood et al 1979).

Prior to further discussion, the type of joints and the anatomy of a typical joint will be reviewed.

Types of Joints

Joints connect two or more bones together and are classified according to the type of tissue and the degree of movement in a particular joint (Dellmann and Brown 1976).

Synarthroses are slightly movable or nearly immovable joints classified by the type of tissue connecting the bones. Syndesmoses, held by dense connective tissue comprising collagen and elastic fibers are usually found in skull sutures. Synchondroses, held by cartilage, are found in epiphyseal plates and sternabrae. Synostosis, held by bone, occurs as a result of aging in which the dense connective tissue and cartilage in the aforementioned are replaced by bone. Symphysis, held by hyaline cartilage caps connected by thick fibrous tissue with a transition zone of fibrocartilage are

usually found in the pubic symphysis and intervertebral disc. In the latter, the collagen tissue forms a ring around the periphery called annulus fibrosus that surrounds a space filled with semifluid material known as nucleus pulposus, a remnant of the embryonic notochord. In this location, the annulus fibrosus and the nucleus pulposus confer resiliency to the spinal column.

Diathroses are movable or synovial joints characterized by articular cartilage on opposing bony surfaces, fluid within a closed cavity and a fibrous capsule enclosing the entire joint. The articular surfaces are covered with typical hyaline cartilage composed of four layers namely: the superficial layer with fibers and flattened cells arranged parallel to the surface; middle zone in which cells are larger, spherical and arranged at right angles to the surface with anastomosing small fiber bundles arranged perpendicularly to the surface; the deep zone is composed of large cells in which the fiber bundles are larger, coarse and perpendicular to the surface. The fibers are coalescent and continuous with the perpendicularly-oriented fibers in the calcified zone.

Morphology of a Joint

The joint capsule encloses the entire joint and is composed of an outer layer with thick collagen fibers that are continuous with the periosteum and an inner layer or synovial

membrane that lines the joint cavity except at the articular surface. The synovial membrane is cellular and it secretes a viscid liquid containing blood dialysate and polymerized hyaluronic acid called synovial fluid that lubricates the closed joint. The surface of the synovial membrane is covered with undifferentiated mesenchymal cells within fine connective tissue fibers that form the inner lining. The membrane has folds or villi that project into the joint cavity with blood vessels, lymphatics and adipose tissue that vary with location within the joint. In pressure-free areas, it rests on loose connective tissue and the surface consists of fibroblasts held together by fine collagen fibers with macrophages and lymphocytes interspersed. In pressure areas the membrane is fibrous with dense underlying connective tissue and high cellularity in the surface layer. In some areas, the membrane is made up of adipose tissue with single layer of cells resting on thin connective tissue. Some diarthroses have intraarticular fibrocartilage called menisci anchored on one side of the fibrous layer of joint capsule (Dellmann and Brown 1976).

The carpal joint, bounded proximally by ulna and radius, distally by metacarpal bones, consists of six bones and it is covered by a joint capsule. Four carpal bones are located proximally while two are located distally. In the proximal row, the radial and intermediate carpal bones articulate with

the ulna and the accessory carpal bone articulates with the ulnar carpal bone. The distal row consists of carpal bones 2 and 3 that are fused and the fourth carpal bone, both of which articulate with fused third and fourth metacarpal bone distally (Getty 1975).

Arthritis in Lambs and Calves

Cause

Specific infections in farm animals in which localization occurs in joints from bacteremia or septicaemia particularly include infections of neonates arising from intrauterine infection or umbilical contamination during or immediately after birth.

Escherichia coli and Streptococcus spp. cause arthritis in all species (Platt 1977). Erysipelothrix insidiosa may cause arthritis in newborn and recently docked lambs (Tontis et al 1977) but occurs most commonly at other times in pigs and rarely in calves. Calves with hypogammaglobulinemia are particularly susceptible to bacteremia, meningitis, ophthalmitis and arthritis. Salmonella dublin and Salmonella typhimurium have been found to cause arthritis occasionally in calves. Sporadic outbreaks and individual cases of arthritis in neonates may also occur from umbilical infection with Corynebacterium pyogenes, Fusobacterium necrophorum and

Staphylococcus aureus (Blood et al 1979). C. pseudotuberculosis, Haemophilus agni and Pasteurella hemolytica have also been observed as causes of arthritis in lambs (Jensen 1974). Polyarthritis due to Chlamydia spp. is now recognized in foals and lambs (Hopkins et al 1973; McChesney et al 1974). Arthritis occurs in tick pyemia of lambs associated with Staph aureus but is also involved with extensive suppurative lesions elsewhere. Actinobacillus seminis was isolated from natural outbreaks of polyarthritis in lambs (Watt et al 1970). Occasional cases of Mycoplasma arthritis occur in calves vaccinated against contagious pleuropneumonia with cultures of Mycoplasma mycoides (Piercy and Bingley 1972).

Sporadic cases of traumatic arthritis with perforations of the joint capsule or by extension of infection from surrounding soft tissues such as footrot in cattle and pigs (Penny et al 1963), interdigital abscess of sheep or by hematogenous spread from suppurative lesions, infected umbilicus, tail docking or castration wounds.

Pathogenesis

In infectious arthritis of hematogenous origin, there is an initial synovitis, followed by changes in the articular cartilage and sometimes bone. Without almost any systemic infection there may be localization of the infectious agent in the synovial membrane and joint cavity. The synovial

membrane is inflamed, edematous and associated with varying degrees of villous hypertrophy and deposition of fibrin. Synovitis causes distention of the joint capsule with fluid and the joint becomes painful and warm. A progressive infectious synovitis commonly results in pannus formation between articular surfaces with erosion of articular cartilage, infection of subchondral bone and osteomyelitis. In the chronic stage there is extensive granulation tissue formation. Chronic synovitis and degenerative joint disease with osteophyte formation and ankylosis can occur. Depending on the organism, the arthritis may be suppurative or serofibrinous. Suppurative arthritis is particularly destructive of cartilage and bone and commonly, there is rupture of the joint capsule (Blood et al 1979).

Infectious arthritis may occur following traumatic injury to a joint but the pathogenesis is obscure. Traumatic injury of the joint capsule resulting in edema and inflammation may allow latent organisms to localize, proliferate and initiate arthritis.

SPECIFIC ARTHRITIC CONDITIONS IN LAMBS

Colibacillosis

Early workers identified colibacillosis among newborn lambs in the United States (Marsh and Tunnicliff 1938), Russia (Volkova 1938), Australia (Roberts 1957; 1958),

Argentine (Giovanelli et al 1959), Britain (Hughes et al 1962; Sutton and Gee 1963), New Zealand (Kater et al 1963), and South Africa (Botes 1966).

From clinical studies colibacillosis has been classified into two forms: enteric and septicemic infection (Sojka 1971). Investigators of enteric infections, that usually occurred in lambs 2-8 days of age, demonstrated transmission by feeding intestinal contents of sick lambs to healthy lambs and also by placing intestinal contents of sick lambs on the unsanitary floors of pens housing healthy lambs (Marsh and Tunnicliff 1938). Many strains of E. coli were isolated from infected small and large intestines. Some strains especially antigenic group 08.K, were pathogenic, while others were innocuous. In addition to pathogenicity of each strain, other supplementary factors such as weather, temperature changes and poor management contributed to the development of colibacillosis.

In the septicemic form, workers observed arthritis and meningoencephalitis in affected lambs. Although the septicemic form occasionally occurred in lambs two to three days of age (Terlecki and Shaw 1959; Hughes et al 1962; Botes 1966), the majority of outbreaks were found in lambs two to six weeks of age (Roberts 1957, 1958; Sojka 1971). E. coli was isolated in pure culture from joints, brain, liver and spleen of affected lambs. Strains from both forms of colibacillosis belonged to serotypes 078.K80, 024.K and RCD 3033

(Rees, 1958, Roberts 1958, Kater et al 1963, Botes 1966). Subcutaneous and intravenous inoculation of viable organisms of serotype 078.K80 resulted in arthritis and meningoencephalitis in neonatal lambs two to three days of age as well as older lambs four to eight weeks of age (Kater et al 1963, Terlecki and Sojka 1965, Botes 1966). Oral administration of viable organisms of serotype 078.K80 to four normal lambs resulted in arthritis and encephalitis in one lamb (Terlecki and Sojka 1965).

Gross pathological findings in the septicemic form of colibacillosis include edema and enlargement of mesenteric lymph nodes, fibrinous exudate in pericardial, thoracic and abdominal cavities, in the bursa of right biceps brachii tendon and in both elbow joints, the fetlock joint, the left hock and hind fetlock joints and the right stifle and hock joints; meninges were also affected (Roberts 1958).

Histopathologically, the affected peritoneal surfaces, joints and meninges had hyperemia, hemorrhages, fibrinous or purulent exudate with bacilli and polymorphs (Roberts 1958; Jensen 1974). Purulent synovitis is a consistent finding in E. coli arthritis.

Erysipelothritic Polyarthritis

This is a chronic infection of the lamb joints of sheep characterized by prolonged lameness and stunted growth caused by Erysipelothrix insidiosa (Jensen 1974).

Erysipelothritic arthritis was reported in Holland (Poels 1913), Germany (Reinhardt 1923), Britain (Cornell and Glober 1925), the United States (Ray 1930; Marsh 1931), New Zealand (Marsh 1933), Australia (Murnane 1938), Israel (Bar Moshe and Shimshoni 1969), Norway (Mohn and Utklev 1970), France (Jubert et al 1971; Shirrer 1971) and Germany (Tontis et al 1977).

American workers compared ovine strains of E. insidiosa from United States, New Zealand and United States swine and found them to be serologically identical (Marsh 1933). They also produced the disease in experimental sheep by intravenous inoculation of E. insidiosa, by applying contaminated soil to fresh umbilical stumps, docking wounds, castrating incisions and by maintaining lambs on pen soil seeded with E. insidiosa (Marsh 1931, 1933; Howarth 1933).

New Zealand workers reproduced the disease by dipping sheep with contused limbs in dipping solutions such as rotenone and/or benzene hydrochloride, that have been contaminated with organic material and bacteria, including E. insidiosa. Addition of copper sulfate to the dip in low concentrations prevented growth of E. insidiosa and development of the disease (McLean 1948; Whitten et al 1948). The findings were confirmed in Australia (Gill 1948).

Erysipelothritic polyarthritis has been reported in almost all breeds of sheep from one week to three months

of age, but the incidence is higher in castrated and docked lambs than in other lambs.

Variation in pathological changes found at necropsy depends on extent and duration of the disease. The carcass is usually emaciated and lesions are most marked in the knee, shoulder, hock and stifle joints. Lesions are seldom found below the knee or hock, or in vertebrae, and very rarely in the acetabulum or in the articular head of the femur. Incision of an inflamed joint reveals a chronic proliferative inflammation of the synovial membrane. The articular cartilages of the joints may have degenerative changes ranging from small pinpoint erosions to complete dissolution of the cartilage. Depending on the extent of inflammation, a thinning and erosion of cartilage and rarefaction of the bone--possibly it partial destruction--may result. Exposed proliferating, subchondral blood vessels and marrow spaces may be observed. Erosions on the opposing cartilages do not always correspond. Irregularities in the shapes of the joints are often observed and also exostosis and ankylosis (Howarth 1933).

Detailed histopathological changes have been described following experimental intraarticular inoculation of lambs with killed E. rhusiopathiae. Twenty-four hours after inoculation E. rhusiopathiae induced severe synovitis. The villi were covered by small plaques of fibrin. There were

also focal deposits of fibrin in the synovium, scattered petechial hemorrhages in both the synovium and stratum fibrosum. Isolated venules in the stratum fibrosum were thrombosed. The joint capsule was variably infiltrated with neutrophils that were most numerous in and adjacent to fibrin deposits. There were large areas of edema in the stratum fibrosum, with moderate numbers of vacuolated macrophages, monocytes, neutrophils and extravasated erythrocytes scattered throughout the transudate. There was focal mesothelial hyperplasia on the villi and a proportion of synovial cells were enlarged (Piercy 1971).

Forty-eight hours after inoculation synovitis in joints had increased in severity. There was more extensive fibrinous exudation that extended into the stratum fibrosum. A large proportion of neutrophils were pyknotic. Areas of edema in the stratum fibrosum contained increased numbers of macrophages and fibroblasts and connective tissue disrupted by the transudate had degenerative changes. Mesothelium beneath the surface plaques of fibrin was disrupted, while intact portions had moderate hyperplasia. Fibroblast proliferation and a light lymphocytic infiltration were observed in the connective tissue layer of the synovium (Piercy 1971).

Seven days post inoculation, scattered deposits of fibrin of variable size were present in and overlying the synovium. Fibrin deposits adherent to or lying within the synovium were

surrounded by a broad zone of granulation tissue. Connective tissue adjacent to the granulation tissue was edematous and contained numerous macrophages, fibroblasts and lymphocytes. A light lymphocytic infiltration was present throughout the joint capsule. The mesothelium had more intense and more widespread hyperplasia than that seen in more acute cases. An increased number of synovial cells were enlarged and some contained clear vacuoles. The synovium had moderate fibroblastic thickening. In this study, inflammatory reaction resulting from intraarticular inoculation of heat-killed E. rhusiopathiae was more severe in passively immunized lambs than in nonimmunized lambs and was followed by more extensive proliferative changes in the synovium (Piercy 1971). The histopathological changes described above in passively immunized lambs were similar to those described in the early stages of E. rhusiopathiae arthritis in lambs (Marsh 1933; Jubb and Kennedy 1963), pigs (Collins and Goldie 1940), and rats (Ajmal 1970).

Chlamydial Polyarthritis

Chlamydial polyarthritis of lambs, an acute contagious but nonfatal disease of feedlot and nursing lambs, is characterized by fever, lameness, arthritis, serositis, conjunctivitis, and emaciation, and is caused by a chlamydial organism (Jensen 1974).

The chlamydial agent causing this disease was discovered in Wisconsin in the United States. The signs and lesions were described (Mendlowski and Segre 1960; Mendlowski et al 1960). The disease was diagnosed in both feedlot and nursing lambs in Utah, Idaho and Wyoming (Storz et al 1963), Texas (Livingston et al 1965), Colorado (Pierson 1967), Iowa (Page and Cutlip 1968), and Spain (Blanco Loizelier 1969). The same disease was found in calves (Storz et al 1964), and goats (Blanco Loizelier 1969), and a similar disease, Reiter's syndrome, in man (Schachter et al 1966).

In chlamydial polyarthritis, the major location of infection is in the limb joints. From joint lesions in feedlot lambs, Wisconsin researchers first isolated the chlamydial organism in yolk sacs of embryonating chicken eggs and reproduced the disease with the isolate (Mendlowski and Segre 1960; Mendlowski et al 1960). Workers in the western states confirmed the findings in both feedlot and nursing lambs (Storz et al 1963). With higher incidence of follicular conjunctivitis and gastroenteritis in lambs with chlamydial polyarthritis, researchers isolated the organism from ocular lesions (Storz et al 1967) and from mucosal scrapings from small and large intestines (Storz and Thornley 1966).

Besides the chlamydial polyarthritis agent, similar chlamydial organisms were isolated from placentas of ewes

with enzootic abortion of ewes (Stamp et al 1950), from feces of normal lambs (Kawakami et al 1958), from placentas of cows with epizootic bovine abortion (Storz and McKercher 1962), from calves with polyarthritis (Storz et al 1964), and from feces of lambs affected with chlamydial polyarthritis (Storz and Thornley 1966).

At necropsy, gross lesions are found in and around joints, tendon sheaths, eyes and lungs. The large limb joints and the attanto-occipital articulation commonly have distention of the articular capsule with increased amounts of amber colored fluid. The synovium contained flakes and plaques of loose or attached fibrin. Edema, hyperemia and petechial hemorrhages extend, in varying degrees, through the fibrous layer into adjacent muscles. Usually the articular cartilage appeared normal. In chronic infection, the synovial layer may be roughened from villous proliferations. Tendon sheaths had changes similar to those of joints, but with lesser amounts of fibrin. Bilateral follicular conjunctivitis, and areas of atelectasis and consolidation were found in the lungs (Jensen 1974).

Histopathologically, smears of synovial fluid and impression smears of synovial membranes stained with Giemsa or Macchiavello's stain revealed, in most cases, large numbers of inflammatory cells and abnormally large rounded synovial cells with intracytoplasmic masses of chlamydiae which

stained purplish-red with the Giemsa stain and bright red with the Macchiavello technique (Mendlowski and Segre 1960). Sections of arthritic joints revealed hyperplasia of the synovial membrane and enlarged synovial cells. In advanced stages villous proliferations projected into the cavity. The synovial layer contained granulomas and the fibrous layer perivascular accumulations of mononuclear leukocytes. Affected lungs had some atelectasis, thickening of alveolar walls, and accumulations of leukocytes. Mononuclear cells may contain cytoplasmic inclusion bodies (Jensen 1974).

Mycoplasma Arthritides

Three species of Mycoplasma have been associated with arthritis in sheep, goats and calves. Mycoplasma agalactiae produced typical arthritis and mastitis in sheep following subcutaneous inoculation (Watson et al 1968). Mycoplasma capricolum was isolated from stifle joints of a sheep that was lame for months and from the knees and stifles of a moribund two-month-old lamb following natural exposure (Swanepoel et al 1977). Mycoplasma alkalescens was isolated from six of seven synovial fluid samples taken by arthrocentesis from three-week to four-month-old Holstein-Fresian calves with severe arthritis (Bennett and Jasper 1978).

In M. agalactiae arthritis, changes in infected joints occur in both the cavity and connective tissue around the

joint capsule. In the joint cavity, the synovium may be increased and clouded and may contain flakes of fibrin. After a prolonged course, articular surfaces often erode and ankylose. Some joint capsules ruptured and discharged infected exudate to the surface. The periarticular connective tissue becomes edematous and swollen and these changes cause joint enlargement and lameness (Jensen 1974).

In M. capricolum arthritis in sheep and goats, bilateral inflammation of the stifle joints with marked periarticular and subcutaneous edema are common findings. There is irregular proliferation of the synovial membrane with moderate infiltration of mononuclear cells and polymorphs and copious fibrinous exudate (Swanepoel et al 1977).

In M. alkalecens arthritis in calves, the affected joints are usually distended with masses of fibrin with little synovial fluid. Erosion of the articular surface is a common finding. The synovial membrane reveals evidence of massive surface-oriented necrosis and exudation of fibrin and neutrophils. Granulation tissue formation in the underlying tissue is heavily infiltrated by mononuclear leukocytes, including plasma cells (Bennett and Jasper 1978).

Failure to culture mycoplasma from several of the naturally infected joints appears to be a common feature of mycoplasma arthritides and isolation of mycoplasmas from arthritic joints is most likely during the early febrile phase of the disease (Taylor-Robinson and Taylor 1976).

Neonatal Polyarthritis

One of the most common forms of infectious arthritis in farm animals occurs in neonates as a result of umbilical infection or as a result of intrauterine infection (Blood et al 1979; Jubb and Kennedy 1970).

Alpha hemolytic Streptococcus sp. is commonly associated with acute to subacute, tertiary infectious arthritis. E. coli is frequently isolated from acute, tertiary infectious arthritis and a mixed infection due to C. pyogenes and Streptococcus fecalis is also classified as subacute tertiary infectious arthritis. Infection due to C. pyogenes alone is classified as primary infectious arthritis and is usually chronic (Van Pelt et al 1966).

Grossly, affected joints are enlarged with excessive turbid yellow synovial effusion and considerable amounts of flocculent debris. The joint capsules are thickened with marked evidence of periarticular fibrosis. The synovial membranes appear hyperemic and in many areas are covered with fibrinonecrotic exudate; this is usually more pronounced in calves with joint infections due to C. pyogenes or S. fecalis or both than in calves with joint infection due to Streptococcus sp. or E. coli. The articular cartilages in calves with subacute infectious arthritis undergo early erosion or dissolution; whereas in calves with chronic infectious arthritis, particularly in those due to C. pyogenes,

the articular cartilages are often eroded, with exposure of the denuded subchondral bone. Fibrinopurulent casts with little synovial effusion and exuberant granulation tissue are also found in chronic arthritis (Van Pelt et al 1966).

Microscopically, desquamation of synovial intimal cells in the majority of the villi,and in few areas where synovial intimal cells remain, the free borders of the eosinophilic cytoplasm are frayed or fimbriated. Proliferating granulation tissue infiltrated by large numbers of neutrophils fewer eosinophils, lymphocytes, mast cells, macrophages and numerous fibroblasts. Amount of fibrous tissue increases with chronicity (Van Pelt et al 1966).

Secondary infectious arthritis is primarily monarticular in nature and generally encountered in mature animals (Van Pelt et al 1966).

Other Arthritides

Arthritis associated with a septicemic disease of lambs caused by Haemophilus agni was reported. Lambs which survive 24 hours or more develop fibrinopurulent arthritis. Although all joints of the appendicular skeleton sometimes are affected, the changes are more evident in the stifle joints. In the earliest stages there is only a slight increase of joint fluid that contains small bits of fibrin. In severe cases the joint capsule and tendon sheaths are distended by

a thick gelatinous exudate. In addition to the joints of the appendicular skeleton, the atlanto-occipital articulations were involved regularly (Kennedy et al 1958).

A severe and persistent polyarthritis and synovitis was reported in young cattle associated with bovine virus diarrhea-mucosal disease complex-like infection. Affected calves had fever, aggression, diarrhea and serous ocular and nasal discharge and lameness. There was coronitis and swelling of the carpal, metacarpophalangeal, tarsal and metatarso-phalangeal joints. The swollen joints persisted for two to six months and as much as 65 ml of fluid was obtained from single samplings of a tarsal joint cavity. The joint fluid was usually opaque; some samples were clear or yellow or sanguineous (Hanly and Mossman 1977).

Histophilus ovis

This organism was first isolated in Western Australia from the udder of a ewe with mastitis (Roberts 1956). A syndrome of suppurative synovitis and pyemia in New Zealand lambs associated with the isolation of an organism which was considered indistinguishable from Histophilus ovis was reported (Kater et al 1962). The affected lambs described by Kater et al (1962) were one to six weeks old and the infection was characterized by severely swollen joints in one or more of the atlanto-occipital, atlanto-axial, costochondral,

costosternal, costovertebral or intervertebral articulations and limb joints. The articular surfaces of the joints were never involved but the joint capsules were often distended with greenish-yellow inflammatory exudate. In some lambs there was a teno-synovitis while about one-half of affected lambs had small multiple abscesses in the liver and kidneys. Suppurative meningitis and ependymitis was found in one lamb. The organism was isolated from the affected joints and abscesses. Following intravenous and intranasal inoculation of lambs with pure cultures of H. ovis in Australia (Rahaley 1978a) the organism was isolated from the joints of only one lamb. Another lamb had a swollen left carpal joint with cloudy synovial fluid. The synovial membrane was slightly congested but culture of the synovial fluid was negative for H. ovis. Abscesses were found in the subcutaneous tissues, myocardium and fascia of gracilis muscle of the legs in some other lambs. Microscopically, acute vasculitis and fibrin thrombi with leukocytes, predominantly neutrophils infiltrating the vessel walls and perivascular tissue were found in the liver, kidneys and lungs.

Actinobacillus seminis

This organism was first described and named in Queensland (Baynes and Simmons 1960) following isolation from cases of ovine epididymitis. A. seminis epididymitis has also been

diagnosed in rams in the United States (Livingstone and Hardy 1964) and South Africa (Worthington and Bosman 1968; Van Tonder 1973).

Although naturally occurring infection of pregnant ewes has not been described, experimental inoculation of pregnant ewes with viable cultures of the organism resulted in abortion or premature birth of small and weak or stillborn lambs (Baynes et al 1966; Smith and Hughes 1974). The only lesion described in these experimental ewes was necrotic placentitis (Smith and Hughes 1974).

Epidemiological studies of A. seminis infection in a Border Liecester flock suggested that the disease was primarily a genital infection of rams. The disease was not controlled, however, by removal of infected rams or by isolation of mated and unmated animals (Baynes et al 1966).

In Western Australia A. seminis was isolated from cases of polyarthritis in three separate flocks of young lambs and from a case of severe posthitis in a Merino ram (Watt et al 1970). In one flock 25 of 280 six-week-old lambs became lame within four days of dipping. The lambs had been castrated two weeks previously. Apart from involvement of the carpal joints, one lamb with posterior paresis had arthritis of the lumbar vertebrae. Four uncastrated lambs from a second Merino flock were found dead and on necropsy revealed extensive purulent polyarthritis. In the third flock a

Dorset Horn lamb had polyarthritis from which A. seminis was recovered in pure culture.

An overnight culture of A. seminis from a case of polyarthritis in the above reported lambs was injected into the teat canal of the right mammary gland of a lactating ewe. Within three days a gangrenous mastitis developed, the ewe was pyrexic, moribund, and death quickly ensued. A. seminis was recovered from milk taken from the right udder but not from the heart blood. A second lactating ewe was injected in a similar manner with an overnight culture of A. seminis, developed severe mastitis within three days and A. seminis was recovered from the udder. Treatment with penicillin and streptomycin effected a recovery (Watt et al 1970).

A. seminis is a pleomorphic gram-negative, nonmotile bacillus, 1-4um long and 1um in width. Growth occurs aerobically on primary isolation but becomes more luxuriant in a micro-aerophilic atmosphere of 10% CO_2. On primary isolation, media enriched with serum or blood support growth, but without serum or blood, growth is slight and irregular. The organism can be adapted to give reasonable growth on unenriched media. Optimum temperature for growth is 37^{o} C. A. seminis is relatively biochemically inert; sugars are not fermented, indol is not produced, urea or sodium citrate is not split, nitrate is not reduced and coagulated blood serum is not lysed. Catalase is produced. After incubation for 28 days, slight

acid production may be observed in arabinose, fructose, trehalose and mannitol. The organism is resistant to bacitracin, partially resistant to neomycin and sensitive to penicillin-streptomycin (Baynes and Simmons 1960).

In rams A. seminis produces chronic epididymitis with fibrous adhesions in the epididymis. Microscopic examination of thick, creamy semen reveals dead spermatozoa with detached head and tail and cellular debris. Microscopic findings include glandular atrophy of the testes, chronic interstitial fibrosis in the epididymis and chronic inflammatory changes with marked fibrosis and invasion of ductus deferens by neutrophils, lymphocytes and macrophages. The tunica vaginalis may be thickened and adherent to the testis and epididymis. Spermatic granuloma are seen in some cases (Baynes and Simmons 1960).

In lambs extensive purulent polyarthritis is produced, the carpal joint being almost always affected although other joints, especially the vertebral joints, have been found to be affected. Severe myocarditis with abscesses has also been reported. Gangrenous mastitis was produced in ewes following experimental inoculation of the udder with viable cultures of A. seminis (Watt et al 1970).

Mastitis, polyarthritis, epididymitis and pyemia are current disease problems with multiple causes in all sheep raising areas of the world. The extent and significance of A. seminis in these conditions is presently unknown.

Ovine epididymitis due to A. seminis was clinically and pathologically indistinguishable from that due to Brucella ovis, the major reported cause of ovine epididymitis (Baynes and Simmons 1968; Jensen 1974).

An unidentified, gram-negative pleomorphic organism was incriminated as a cause of perinatal lamb mortality in Eastern (Hughes et al 1971) and Western Australia (Dennis 1974) and as a cause of placentitis, abortion and premature birth (Dennis 1974; Smith and Hughes 1974; Rahaley and White 1977). Histophilus ovis and the unidentified, gram-negative bacillus appear to be related to A. seminis (Watt et al 1970); Hughes et al 1971; Dennis 1974; Rahaley 1978b).

Because of the similarity in pathogenicity and cultural characteristics of H. ovis and A. seminis, a serological comparison was made between H. ovis, A. seminis and B. ovis using a cross-absorption complement-fixation technique. Four strains of H. ovis were found to be serologically homologous and that an incomplete relationship existed between these organisms and A. seminis. Antiserums prepared against one strain of H. ovis and the strain of A. seminis gave a weak, apparently nonspecific cross-reaction with B. ovis antigen (Rahaley 1978b). Recently, immunofluorescent techniques have been found to be accurate and reliable for distinguishing from B. ovis and A. seminis (Ajai 1980).

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II. ACTINOBACILLUS SEMINIS INFECTION OF LAMBS

INTRODUCTION

Septicemia in lambs resulting from neonatal and post-marking wound infections have been reported. The causative organisms incriminated were Pasteurella hemolytica (Hughes 1972), Fusobacterium necrophorum (Hughes et al 1971; Hughes 1972; Dennis 1974), Clostridium sp (Moule 1954: McHugh and Edwards 1958; Dennis and Nairn 1970; Hughes 1972; Dennis 1974), Corynebacterium sp (Dennis and Bamford 1966; Hughes et al 1971; Hughes 1972; Dennis 1974), E. coli (Kater et al 1963, Hughes 1972, Dennis 1974), and other sporadic pathogens such as Streptococcus faecalis, miscellaneous streptococci, Pasteurella multocida, Proteus sp, Actinobacillus lignieresi, Dermatophilus congolense, Erysipelothrix rhusiopathiae, Actinomyces bovis, Pseudomonas aeruginosa, Haemophilus agni, Actinobacillus seminis, and Histophilus ovis (Moule 1954; McHugh and Edwards 1958; Kennedy et al 1958; Kater et al 1962; Dennis and Nairn 1970; Watt et al 1970; Hughes et al 1971; Hughes 1972). Septicemia due to these organisms was characterized by one or more of the following: pneumonia, polysynovitis, polyarthritis, peritonitis, omphalitis, pyemia and disseminated abscesses in other visceral organs.

This paper reports A. seminis infection in lambs.

MATERIALS AND METHODS

Experimental Animals

Twelve lambs, eight 6 to 8 weeks old suffolks and four 9-11 weeks old Rambouillet crosses, were divided into three groups: 6 Suffolks (Group A), 4 Rambouillet crosses (Group B), and 2 Suffolks served as controls (Group C). The lambs were dewormed with Levasole[a] two weeks before being tansferred to an isolation room with a controlled environment of 21 C. The control lambs were housed in a separate room. Each lamb was examined clinically and the heart rate, respiratory rate and rectal temperature were monitored morning and evening for two days prior to inoculation.

Preparation of Inoculum

A culture of A. seminis[b] was transferred to brain heart infusion broth (BHI)[c] and incubated at 37 C in an atmosphere of 10% CO_2 for 24 to 48 hours. For storage, the organism was grown in test tubes containing 5 ml BHI broth, 5 ml citrated bovine blood and approximately 100 sterile glass

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[a]Pitman-Moore, Inc., Washington Crossing, New Jersey 08560.

[b]American Type Culture Collection, #15768.

[c]Difco Laboratories, Detroit, Michigan.

beads, for 48 hours in an atmosphere of 10% CO_2. The cultures were checked by Gram stain, most of the supernatant was decanted, and the tubes frozen at 70 C until required.

Frozen beads of A. seminis stock culture were placed in BHI broth with 10% bovine serum and incubated in an atmosphere of 10% CO_2, relative humidity 55%, for 48 hours at 37 C. The culture was checked for purity by Gram stain and by plating on blood agar plates (BBA). Colonies of A. seminis were transferred to small 120 ml bottles containing 20 ml of BHI with 10% bovine serum. The bottles were incubated in a horizontal position in an atmosphere of 10% CO_2 for 24 hours. The purity of the cultures were checked by Gram staining, plating on BBA, and by specific immunofluorescence (Ajai 1980). Before inoculating the test lambs, the cell concentration was determined spectrophotometrically[d] to be 2×10^9 cells per ml.

Test Procedures

The inoculation site in the left jugular furrow was clipped, cleansed with alcoholic detergent solution, and disinfected with 70% ethanol. Each lamb in Groups A and B was injected intravenously with 3 ml of 24 hour culture of A. seminis via the jugular vein. The control lambs were injected with 3 ml of sterile BHI broth.

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[d]Spectronic 20, Bausch and Lomb.

Each lamb was observed for depression, anorexia and other clinical signs. Rectal temperature, heart rate and respiratory rate were recorded twice daily.

Group A lambs were euthanatized with T-61 solution[e] intravenously on day 21 postinoculation (PI) and the Group B lambs on day 42 PI. The control lambs were euthanatized on day 43 PI.

Necropsy Examination

Each lamb was subjected to an immediate standardized necropsy after euthanasia. Specimens collected for bacteriological culture included synovial fluid and/or swab from all limb joints, atlanto-occipital joint and costochondral junction, lung, liver, spleen, kidney and heart blood. The heart blood was collected by a sterile syringe after searing the heart with a red hot spatula. The surface of each tissue cultured was seared by heat, opened by a sterile scapel and the contents plated on BBA plates. The plates were incubated at 37 C in an atmosphere of 10% CO_2 and 55% relative humidity for 48 hours. The cultures were examined by Gram stain and colonies suspected to be A. seminis were tested by macroscopic slide agglutination (Kabat et al 1964) or immunofluorescence (Ajai 1980).

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[e]National Laboratories Corporation, Somerville, NJ 08876.

Thin smears of synovial fluid from the carpal joints were stained by Leishman's method (Coles 1980) and examined for cell types.

The following tissue specimens were collected and fixed in 10% buffered neutral formalin for histopathological examination: liver, lung, spleen, myocardium, kidney, lymph nodes and brain. The tissues were trimmed, processed in an autotechnicon[f], embedded in paraffin, cut at 6u, and stained by hematoxylin and eosin (H & E). Sections with evidence of bacterial infection were recut and stained by the Giemsa method.

RESULTS

Clinical Observations

Lamb #50 died between 9-12 hours post-inoculation. At 24 hours post-inoculation (PI) increased body temperature, heart rate and respiratory rate were recorded in the remaining lambs. Dullness, depression and shallow respiration involving the abdominal muscles were observed. Feed and water intake were markedly reduced. By 96 hours PI,

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[f]Technicon Instruments Corporation, 511 Benedict Ave., Tarrytown, New York 10591.

a decrease in temperature was recorded, apetite improved but depression and abdominal respiration persisted. Normal temperatures and heart rates were recorded on day 6 PI, lambs fed normally and respiratory movements involving the abdominal muscles were less obvious although the respiratory rates were still high. By day 8 PI lambs appeared clinically normal but increased respiratory rates persisted throughout the experiment. Control lambs were clinically normal during the whole experimental period.

Necropsy Findings

The findings are tabulated in Table 1 and Appendix A. At 21 days PI no significant gross findings were found in lambs 33, 34, 39, 47 and 48. At 42 days PI areas of red hepatization were present in all lobes of the lungs of lambs 52 and 58. The right apical lobe was severely affected in lamb 58. The lungs of both lambs had an irregular mottled appearance.

In addition to the wrinkled appearance of lungs, lambs 51 and 54 (Fig 1) had fibrous pleural adhesions of the left and right diaphragmatic lobes, respectively. Areas of red hepatization, especially pronounced in the right apical lobes, were present in all lobes. In lamb 54, a large encapsulated abscess was present in the left apical and cardiac lobes

(Fig 2) and adhered to the adjacent pleura. The abscess contained light, yellow inspissated material.

Microscopic Findings

The findings are summarized in Table 2 and Appendix A. At 21 days PI, the lungs were congested with areas of emphysema and atelectasis. The interalveolar septa were thickened and infiltrated by lymphocytes and occasional plasma cells (Fig 3). Numerous mononuclear cells were present in the peribronchiolar areas. In the liver, lymphocytes were observed around the bile ducts in the periportal areas (Fig 4). The cytoplasm of hepatocytes tended to be more eosinophilic than normal.

Tubular nephrosis was observed in the renal cortex. The cytoplasm of tubular epithelial cells was granular and eosinophilic and the nuclei were pyknotic. The Bowman's space contained protein reflux and proteinaceous casts were present in some tubules.

In addition to the above findings lambs 34, 39, 47 and 48 had hypercellular glomeruli (Fig 5). Only lamb 47 had depletion of some of the germinal centers in the spleen.

No significant changes were observed in tissues from the two control lambs (36 and 45).

At 42 days PI, the lung of lamb 58 had areas of carnification characterized by atelectasis and alveolar macrophages,

mononuclear cells, neutrophils and fibroblasts. The bronchial epithelium was moderately hyperplastic and was surrounded by granulomatous-like nodules of mononuclear cells and macrophages and peripheral fibroblasts (Fig 6). Bridging adjacent bronchioles by these nodules was present throughout the sections. The lamina propria of the bronchiole was hypercellular and the peribronchiolar area was infiltrated by mononuclear cells. Mononuclear cells were present within the lumen of bronchi and bronchioles. In other areas, the alveolar lining cells were fusiform with an increase in fibrillar elements of the alveolar septa and infiltration of mononuclear cells. The lymphoid nodules of the bronchial lymph nodes were hyperplastic. In the lung of lamb no. 54, a mixed purulent bronchopneumonia with mononuclear cells, neutrophils and eosinophils was present (Fig 7). Hemorrhage was observed and the border of the right apical lobe of the lung was indistinguishable because of proliferation of fibrous tissue involving the lung and pleura. The lymphod nodules of the bronchial lymph nodes were hyperplastic and in the medulla, neutrophils and eosinophils were present.

In the kidneys, the glomeruli were hypercellular and eosinophilic with protein reflux present in some Bowman's spaces (Fig 8).

In the liver of lambs no. 51 and 52, foci of mononuclear cells were present in the periportal areas (Fig 9).

In the myocardium of lambs no. 51 and 54, foci of mononuclear cells were observed (Fig 10). In addition, the spleen of lamb 51 had hyperplastic lymphoid nodules.

Bacteriological Findings

A. seminis was cultured from the lung of lamb 54 necropsied 42 days PI. No bacterial growth was obtained from the tissues and joints of the other test lambs or control lambs.

DISCUSSION

Bacteremia resulting from intravenous inoculation of lambs with A. seminis was, with one exception, non-fatal during the test period and was characterized by interstitial pneumonia and mild glomerulonephritis. The acute death of lamb 50 probably resulted from endotoxemia. Additional lesions in some lambs were focal hepatitis, myocarditis and splenitis. The renal lesions were mild. These findings are in contract with a previous report of natural acute outbreaks (Watt et al 1970) that recorded extensive purulent polyarthritis and myocardial abscesses from which A. seminis was cultured.

The presence of interstitial pneumonia supports the suggestion by Simmons (Baynes et al 1966) that Actinobacillus sp

have an affinity for respiratory tissues. It is suggested that A. seminis may become latent in the lung of some lambs until sexual maturity provides the conditions for the infection to become active resulting in epididymitis in rams and mastitis in ewes.

Inability to reproduce purulent polyarthritis and necrotizing lesions in the parenchymatous organs may be explained in part by one or more of the following: absence of stress factors that can increase severity of the disease, lower virulence of the strain of A. seminis inoculated, and immunological status of the lambs used in the experiment.

The differential diagnosis of A. seminis infection in lambs includes infection by Haemophilus agni (Kennedy et al 1958), Histophilus ovis (Kater et al 1962) and Pasteurella spp (Jensen 1974).

The muscle hemorrhages noted with Haemophilus agni with subcutaneous and subserosal hemorrhages also found in infection with Pasteurella spp (Kennedy et al 1958; Jensen 1974) were not observed with Histophilus ovis (Rahaley 1977) and A. seminis in this study. Hepatic necrosis, septic thrombi and vasculitis in parenchymatous organs associated with H. ovis infection in lambs (Rahaley 1978) differentiates it from A. seminis infection. It was suggested by Rahaley (1977) that separation of infection by H. agni, Pasteurella spp H. ovis or A. seminis, be based on bacteriological

results since primary isolation of these organisms is readily achieved on sheep blood agar and microaerophilic incubation.

It is concluded from this study that A. seminis tends to localize in the lungs of lambs following intravenous inoculation.

SUMMARY

Following intravenous inoculation of lambs with a 24 hour broth culture of A. seminis, consistent findings were interstitial pneumonia and mild glomerulonephritis. A well-encapsulated abscess was present in the left apical lobe of the lung in one lamb. Other findings included multifocal hepatitis and pericholangitis, nodular hyperplasia or depletion in the spleen, and focal, non-suppurative myocarditis.

The tendency of A. seminis to localize in the lungs of lambs suggested that it is a predilection site.

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TABLE 1--Distribution of Lesions in Lambs Infected With *Actinobacillus Seminis*.

Lamb #	Lung	Liver	Kidney	Spleen	Heart
9-12 hrs.					
50	+	-	+	+	-
21 days					
33	+	+	+	-	-
34	+	-	+	-	-
47	+	-	+	-	-
48	+	-	+	-	-
42 days					
52	+	+	+	+	-
58	+	-	+	-	-
51	+	+	+	+	+
54	+	-	+	-	+
Controls					
36	-	-	-	-	-
45	-	-	-	-	-

+ - Presence or absence of lesions.

Table 2 --Microscopic Findings in Lambs Inoculated Intravenously With A. seminis

	21 Days *					42 Days				Controls	
	33	34	39	47	48	52	58	51	54	36	45
Lung											
Pleura											
Edema	0	0	0	0	0	0	0	0	0	0	0
Fibrin	0	0	0	0	0	0	0	0	0	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	0	0	0	0	0	1	0	1	2	0	0
Plasma Cell	0	0	0	0	0	0	0	1	2	0	0
Macrophages	0	0	0	0	0	0	0	0	1	0	0
Fibrous Tissue	0	0	0	0	0	1	1	1	3	0	0
Bronchus											
Lumen											
Debris	0	0	0	0	0	0	0	0	1	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocyte	0	0	0	0	0	0	0	0	0	0	0
Plasma Cell	0	0	0	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	0	0	0	0	0	0	0
Epithelium											
Desquamated	0	0	0	0	0	0	0	0	0	0	0
Vacuolated	0	0	0	0	0	0	0	0	1	0	0
Hypertrophic	0	0	0	0	0	0	0	0	1	0	0
Hyperplasia	1	1	0	1	1	1	1	2	3	0	0
Lamina Propria											
Neutrophils	0	0	0	0	0	0	0	0	1	0	0
Lymphocytes	0	0	0	0	0	1	0	1	3	0	0
Plasma Cells	0	0	0	0	0	0	1	0	3	0	0
Macrophages	0	0	0	0	0	1	1	1	3	0	0
Eosinophils	0	0	0	0	0	0	0	0	1	0	0
Erythrocytes	0	0	0	0	0	0	0	0	1	0	0

*Post inoculation

Table 2--continued

	21 Days					42 Days				Controls	
	33	34	39	47	48	52	58	51	54	36	45
Periphery											
Neutrophils	0	0	0	0	0	0	0	0	1	0	0
Lymphocytes	2	2	2	2	2	2	2	2	3	0	0
Plasma Cells	1	1	1	1	1	0	1	1	1	0	0
Macrophages	0	0	0	0	0	0	1	1	2	0	0
Eosinophils	0	0	0	0	0	0	0	0	1	0	0
Erythrocytes	0	0	0	0	0	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	1	1	1	2	0	0
Glands											
Prominent	1	0	1	0	1	1	1	1	1	0	0
Dilated	0	0	0	0	0	0	0	0	0	0	0
Bronchiole											
Lumen											
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	0	0	0	0	0	0	0	0	1	0	0
Plasma Cells	0	0	0	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	0	0	0	0	0	0	0
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Erythrocytes	0	0	0	0	0	0	0	0	0	0	0
Epithelium											
Desquamated	0	0	0	0	0	0	0	0	0	0	0
Fetalized	1	1	1	1	1	2	1	1	2	0	0
Hyperplasia	0	0	0	0	0	0	0	0	0	0	0
Hypertrophy	0	0	0	0	0	0	0	0	0	0	0
Periphery											
Neutrophils	0	0	0	0	0	0	0	0	2	0	0
Lymphocytes	1	1	0	1	1	1	1	1	2	0	0
Plasma Cells	0	1	1	0	1	1	1	1	2	0	0
Macrophages	0	0	0	0	0	0	0	0	2	0	0
Eosinophils	0	0	0	0	0	0	0	0	0	0	0

Table 2--continued

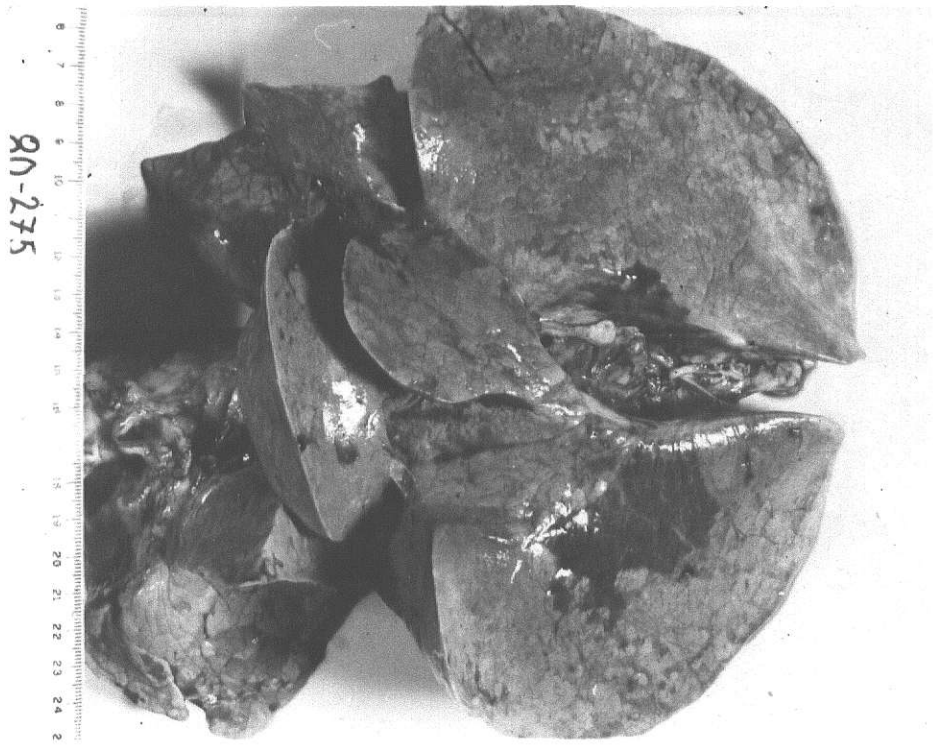
	21 Days					42 Days				Controls	
	33	34	39	47	48	52	58	51	54	36	45
Alveoli											
Epithelium											
Fetalized Cells	1	1	1	1	1	1	1	2	2	0	0
Rounded Cells	0	0	0	0	0	0	0	0	0	0	0
Wall											
Edema	0	0	0	0	0	0	0	0	0	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	2	2	2	2	2	3	3	3	3	0	0
Plasma Cells	2	1	2	1	2	1	1	1	2	0	0
Macrophages	1	2	2	1	2	1	1	1	3	0	0
Fibroblasts	2	2	2	2	2	2	2	2	3	0	0
Collagen	1	2	2	1	2	2	2	2	3	0	0
Lumen											
Dilated	3	2	3	2	2	3	3	3	3	0	0
Constricted	2	2	3	3	3	2	3	3	3	1	1
Neutrophils	0	0	0	0	0	0	0	0	2	0	0
Lymphocytes	1	1	2	1	1	2	1	1	3	0	0
Plasma Cells	0	0	1	1	1	0	0	0	2	0	0
Macrophages	0	0	0	0	0	0	2	1	3	0	0
Eosinophils	0	0	0	0	0	0	0	0	1	0	0
Erythrocytes	0	0	0	0	0	0	0	0	1	0	0
Fibroblasts	0	0	0	0	0	2	2	2	2	0	0
Fibrin	0	0	0	0	0	0	0	0	0	0	0
Bronchial L. Node											
Nodular Hyperplasia	3	3	3	3	3	3	3	3	3	0	0
Neutrophils	0	0	0	0	0	0	0	0	1	0	0
Erythrocytes	0	0	0	0	0	0	0	0	0	0	0
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0

Table 2--continued

	21 Days					42 Days				Controls	
	33	34	39	47	48	52	58	51	54	36	45
Kidney											
Glomeruli											
Proliferation	0	0	0	0	0	0	2	3	0	0	0
Membraneous	0	0	0	0	0	0	0	0	0	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	0	1	1	2	0	2	2	2	3	0	0
Tubules											
Epithelium											
Desquamated	0	0	0	0	0	0	0	0	0	0	0
Nephrosis	1	1	0	1	1	0	0	0	0	0	0
Vacuolated Cells	0	0	0	0	0	0	0	0	0	0	0
Cast	0	1	2	0	1	0	1	1	1	0	0
Spleen											
Nodular Hyperplasia	0	0	0	0	0	0	0	1	2	0	0
Depleted	0	0	0	2	0	0	0	0	0	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Eosinophils	0	0	0	0	0	0	0	0	0	0	0
Liver											
Parenchyma											
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	0	0	0	0	0	2	0	0	0	0	0
Macrophages	0	0	0	0	0	2	0	0	0	0	0
Erythrocytes	0	0	0	0	0	0	0	0	0	0	0
Portal Area											
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	2	0	0	0	0	0	0	3	0	0	0
Macrophages	1	0	0	0	0	0	0	1	0	0	0
Plasma Cell	0	0	0	0	0	0	0	0	0	0	0
Eosinophil	0	0	0	0	0	0	0	0	0	0	0
Fibroblasts	1	0	0	0	0	0	0	1	0	0	0
Myocardium											
Lymphocytes	0	0	0	0	0	0	0	2	3	0	0
Neutrophils	0	0	0	0	0	0	0	0	0	0	0
Macrophages	0	0	0	0	0	0	0	1	2	0	0

Fig 1. Lungs with irregular surface.

Fig 2. Lungs with encapsulated abscess (A) in the left apical and cardiac lobes.


80-275

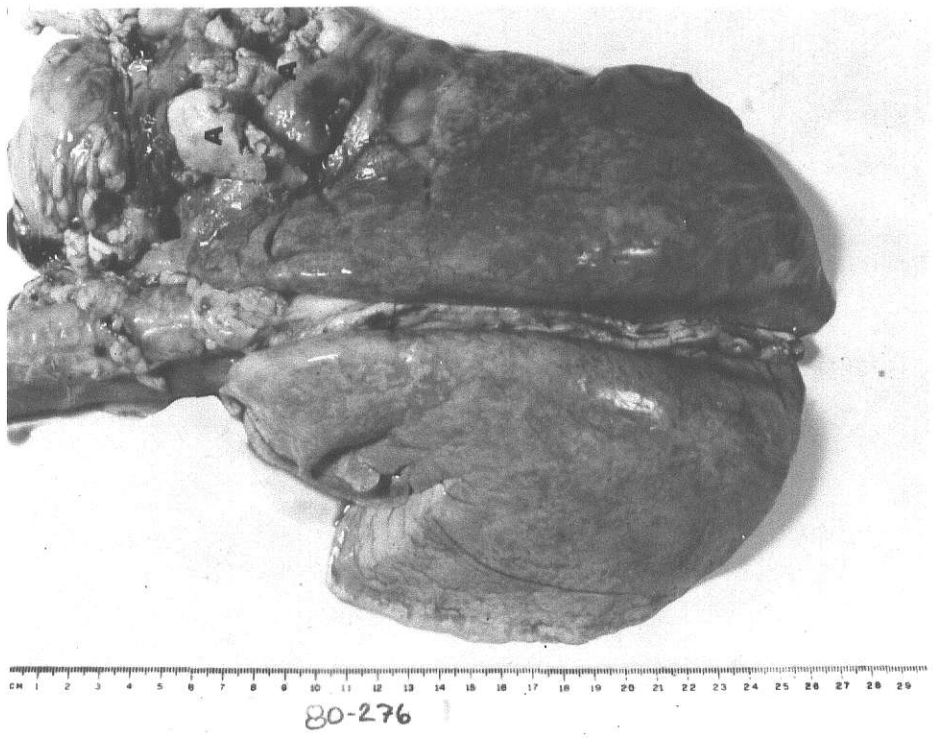

80-276

Fig 3. Section of lung with thickened interalveolar septa (B) infiltrated by lymphocytes and some plasma cells.

Fig 4. Liver section with periportal infiltration of lymphocytes (C).

Fig 5. Photomicrograph of kidney illustrating hypercellularity of the glomeruli (D).

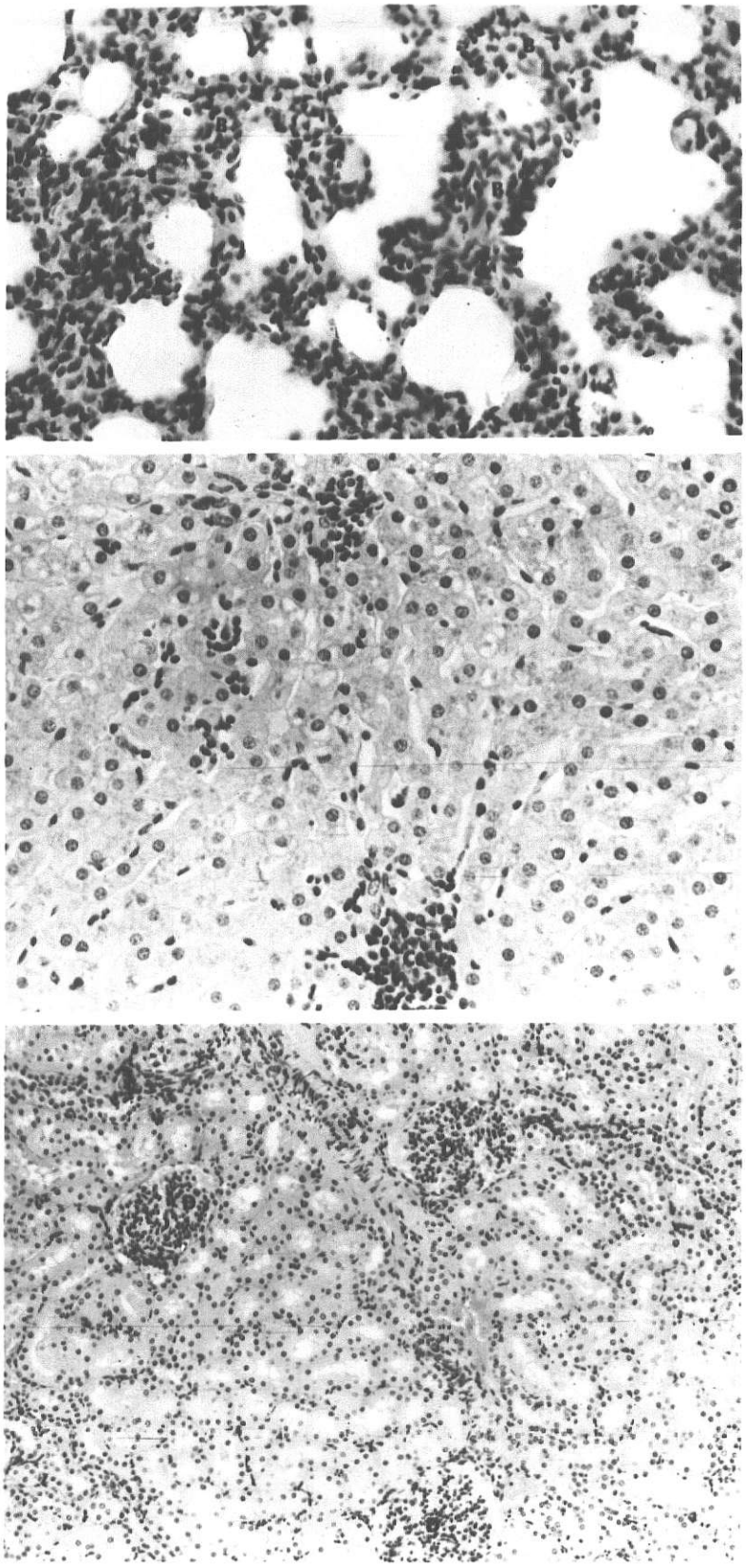
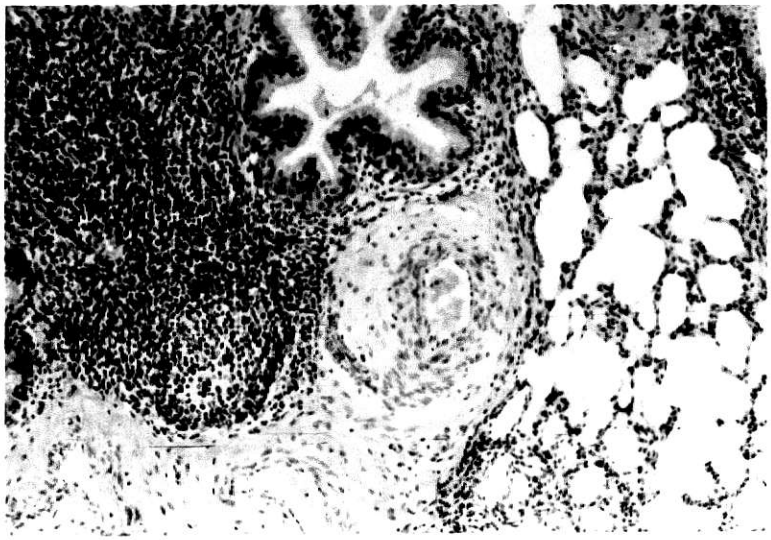
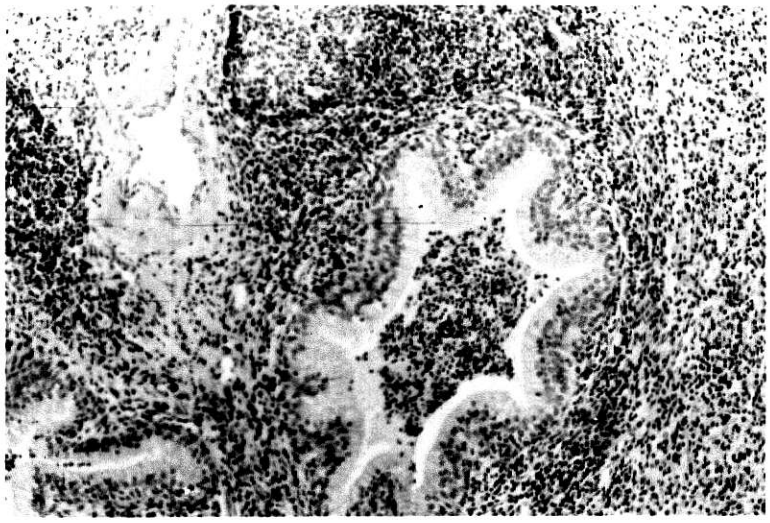


Fig 6. Photomicrograph of lung. Note hyperplasia of the bronchial epithelium (E), peribronchial lymphoid nodule (F) and general hyperplasia of the tunica media of the bronchial artery.

Fig 7. Photomicrograph of lung. Note inflammatory cells in the lumen of the bronchus (G).

Fig 8. Section of kidney with hypercellularity of the glomeruli (H).





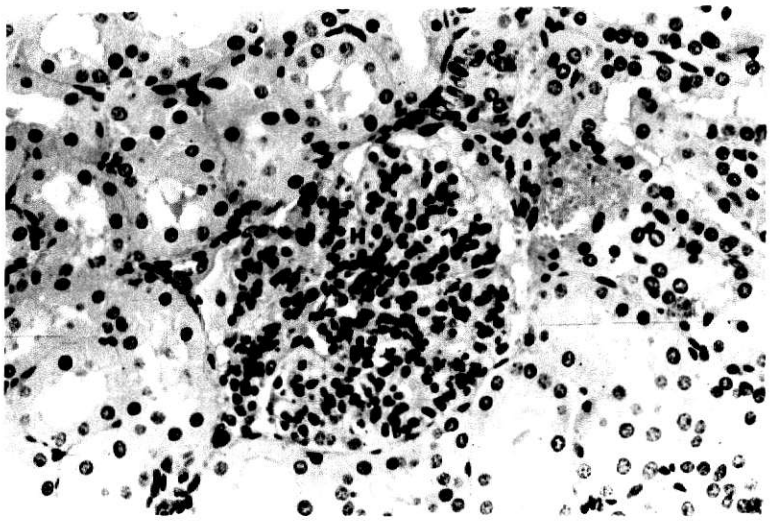
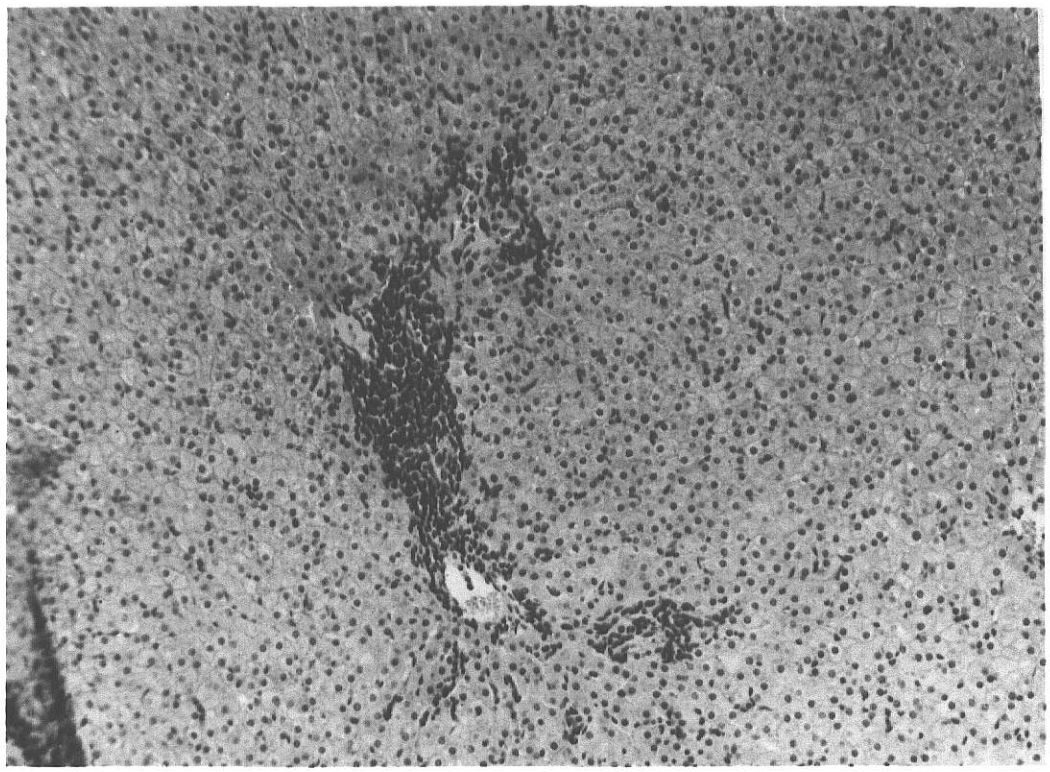
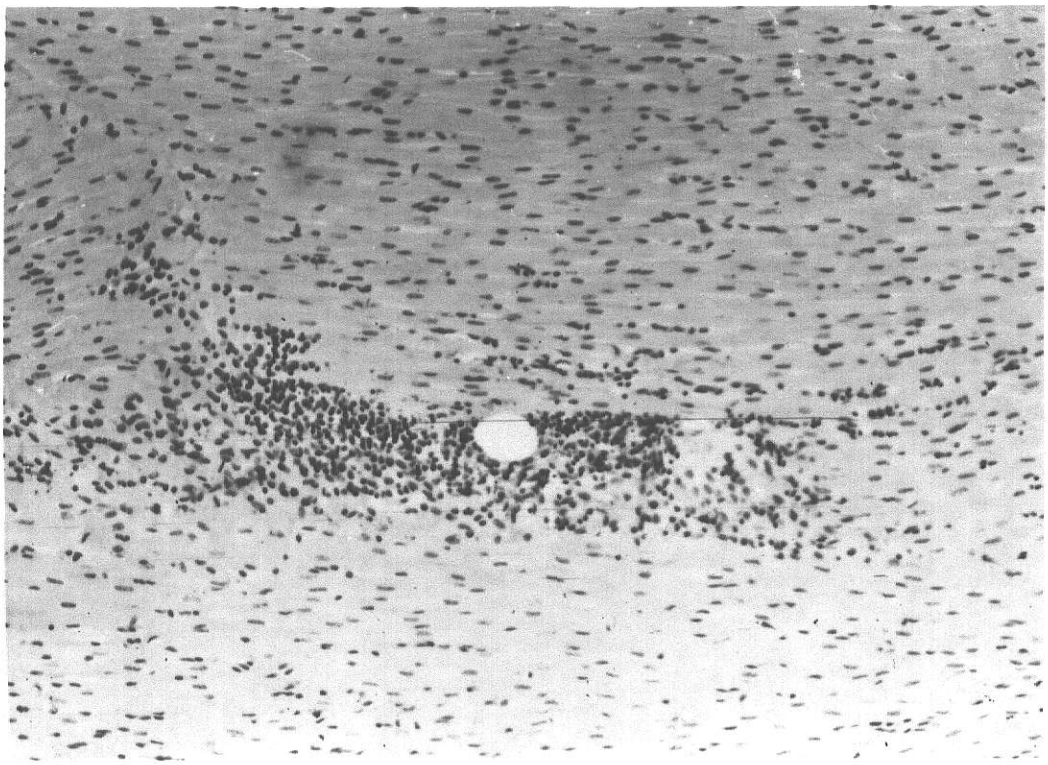


Fig 9. Section of liver with periportal infiltration by mononuclear cells.

Fig 10. Section of the myocardium with mononuclear aggregations.





III. SEQUENTIAL CHANGES IN THE CARPAL JOINT OF LAMBS DUE TO *A. SEMINIS* INFECTION

INTRODUCTION

Arthritis characterized by limited motion, reluctance to bear weight on the affected joint and lameness in lambs is due to a variety of causes acquired from neonatal infections and infected post marking wounds. Causes include Erysipelothrix insidiosa (Poels 1913), chlamydia (Mendlowski and Segre 1960; Mendlowski et al 1960), Escherichia coli (Terlecki and Shaw 1959; Hughes et al 1962; Botes 1966), Streptococcus sp. and Corynebacterium pyogenes (Van Pelt et al 1966), Mycoplasma sp (Watson et al 1968; Swanepoel et al 1977; Bennett and Jasper 1978), Hemophilus agni (Kennedy et al 1958), Histophilus ovis (Kater et al 1962) and Actinobacillus seminis (Watt et al 1970).

This paper reports the sequential articular changes following intracarpal inoculation of lambs with A. seminis.

MATERIALS AND METHODS

Experimental Animals

Fourteen Suffolk lambs, 6 to 8 weeks of age, were randomly divided into 12 test and 2 controls. Pretreatment of the test and controls lambs was the same as described in Part II.

Test Procedures

Housing of the lambs and preparation of the A. seminis inoculum was described previously (Part II). The control lambs were housed separately. The anterior surface of the left carpal joint of each lamb was carefully cleansed with an alcoholic detergent solution, dried with absorbent cotton, and disinfected with 70% ethanol. The left carpal joint of each test lamb was injected intra-articularly with 1.0 ml of a 24 hour broth culture of A. seminis. The control lambs were injected intraarticularly with 1.5 ml of sterile BHI broth. The right carpal joint of each lamb served as additional controls.

After injection, all lambs were observed closely for clinical signs, especially lameness. The temperature, heart rate and respiratory rate were recorded twice daily. Two lambs were randomly euthanatized with T-61 solution[a] administered intravenously at the following time intervals post injection (PI): 24, 48, 72, 96, 120 hours and 14 days. The control lambs were euthanatized on day 15 PI.

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[a]National Laboratories Corporation, Somerville, NJ 08876.

Necropsy Examination

Each lamb was subjected to a standardized necropsy and bacteriological examination as described in Part II. Synovial fluid and swabs from the carpal joints were also cultured.

The following tissues were collected and fixed in 10% buffered neutral formalin (BNF): left and right carpal joints, left and right prescapular lymph nodes, lung, myocardium, liver, kidney and spleen. After fixation in BNF, the carpal joints were sliced vertically into 0.5 to 1 cm thick sections and placed in RDO decalcifying agent[b] until they were flexible. The decalcified bone sections were washed under running tap water for at least 8 hours before being placed in BNF. The tissues were trimmed, processed in autotechnicon[c], embedded in paraffin, cut at 6u, and stained by hematoxylin and eosin (H & E). Sections with evidence of bacterial infection were recut and stained by the Giemsa method.

Thin smears of synovial fluid from the carpal joints were stained by Wright's-Leishman's method and examined for cell types (Coles, 1980).

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[b]Du-Page Kinetic Laboratories, Inc., Naperville, Illinois.

[c]Technicon Instruments Corporation, 511 Benedict Ave., Tarrytown, New York 10591.

RESULTS

Clinical Observations

The left carpus of test lambs became swollen, hot and painful on palpation during the first 72 hours post inoculation (PI). Slightly increased temperatures were recorded 48 hours PI that returned to normal by 72 hours PI. Lameness characterized by lifting the left forelimb off the ground, refusal to step with the left forelimb and dragging while walking was observed until 120 hours PI when the swelling, heat and pain had subsided considerably. By 144 hours PI, the remaining lambs were able to use the left forelimb but lameness was still present as the gait and posture were abnormal. Although lameness persisted throughout the experimental period, some improvement was observed as time progressed. In the control lambs, swollen left carpal joints and pain on palpation were observed during the first 48 hours PI only; after that the lambs were clinically normal.

Gross Pathological Findings

The findings are given in Appendix B. At 24 hours PI, the left carpal joint (Lamb 41 and 49) was swollen. On incision, severe congestion of the periarticular tissue was evident and the joint cavity contained creamy, light reddish-yellow contents with fibrin flakes. The left prescapular

lymph node was swollen, edematous and had a number of petechiae.

At 48 hours PI, the left carpus was still swollen and the periarticular tissue congested (lamb 46 and 35). The joint contents were thicker, grayish and of creamy consistency (Fig 1). The prescapular lymph nodes were still edematous and had petechiae.

At 72 hours PI, swelling of the left carpal joint was reduced and the periarticular tissues less congested (lamb 30 and 44). The contents of the joint was light yellow and jelly-like in consistency. The prescapular lymph nodes were less edematous and no petechiae were visible.

At 96 hours PI, the left carpal joints were slightly enlarged (Fig 2) and the periarticular congestion was subsiding (lamb 32 and 38). The joint contents were light yellow and pasty-like in consistency. The prescapular lymph node was enlarged and congested.

At 120 hours PI, the left carpal joints were slightly swollen and the capsule thickened (lamb 37 and 40). The joint contents were light yellow and paste-like (Fig 3) and the prescapular lymph nodes were still enlarged.

At 14 days PI, the capsule of the left carpal joints was thickened and fibrotic (lamb 42 and 43). The joint contents were light yellow and inspissated. The prescapular lymph node enlargement persisted.

No significant gross lesions were observed in the left carpus of the control lambs (29 and 31) and synovial fluid was clear, colorless and tenacious. The prescapular lymph nodes were enlarged.

Microscopic Findings

The microscopic findings are summarized in Table 1 and Appendix B.

At 24 hours PI, the synovium was edematous and hyperemic with fibrinous tags on the membrane. Numerous macrophages and neutrophils with eosinophilic cytoplasm were found throughout the synovial layer. The stratus fibrosum and the muscular layers of the joint capsule were also infiltrated by numerous neutrophils and few macrophages. No lesions were found on the periarticular surfaces.

The right prescapular lymph node was normal while the left prescapular lymph node was enlarged and edematous. The subcapsular and medullary sinuses were filled with neutrophils while the lymphoid nodules were hyperplastic.

Splenic nodules were surrounded by neutrophils and the sinuses were filled with neutrophils (Fig 4).

In the liver, Kupffer cells were prominent and the portal areas were infiltrated by numerous fibroblasts and few mononuclear cells.

No lesions were found in the lungs, kidneys, myocardium and brain.

At 48 hours PI, the synovium was hyperemic and more edematous, fewer macrophages were present and the nuclei of neutrophils were pyknotic (Fig 5). Fibroblasts were present in the synovial layer with dead and dying neutrophils scattered throughout the muscular and fibrous layers of the joint capsule. No changes were observed in the articular cartilage.

The left prescapular lymph node was enlarged with hyperplastic lymphoid follicles. The subcapsular sinuses contained immature lymphocytes while the medullary sinuses were stuffed with a mixture of neutrophils, immature lymphocytes and plasma cells.

The splenic nodules were hyperplastic and few neutrophils were present in the red pulp.

Activation of Kupffer cells and periportal infiltration of fibroblasts and macrophages were the main findings in the liver.

No lesions were found in the lung, kidney, myocardium and brain.

At 72 hours PI, the synovium was hyperemic and hypertrophic but less edematous. Fluid filled cysts were present (Fig 6). Neutrophils and macrophages were still present with fibroblasts laying down collagen.

Lymphoid nodules in the left prescapular lymph node were hyperplastic and occasional neutrophils were still present in the medullary sinuses. Nodular hyperplasia was present in the spleen.

In the liver, Kupffer cell activation with periportal infiltration of fibroblasts and mononuclear cells was seen.

Thickening of the interalveolar septa especially pronounced in the peribronchiolar areas of the lungs was observed. Mononuclear cells were present in the interalveolar septa and the peribronchiolar lymphoid nodules were hyperplastic.

In the kidney, the glomeruli were infiltrated by mononuclear cells and protein reflux in Bowman's spaces was evident. In addition, lamb 44 had foci of mononuclear aggregations in the interstitial tissue.

No lesions were found in the articular surfaces, myocardium and brain.

At 96 hours PI, in addition to hypertrophy, hyperemia with neutrophil and mononuclear infiltration, fibroplasia was evident within the damaged synovial membrane (Fig 7). The articular surfaces were not involved.

The lymphoid nodules in the left prescapular lymph node were hyperplastic while the spleen was normal.

Activation of Kupffer cells, thrombosis of blood vessels and mononuclear cell infiltration of the portal areas were observed in the liver.

In the lungs, thrombosis of the blood vessels, peribronchiolar infiltration of mononuclear cells and foci of interalveolar septa thickening were found.

The glomeruli of the kidneys were hypercellular with the presence of mononuclear cells and protein reflux in some of the Bowman's spaces.

No lesions were found in the myocardium, right prescapular lymph nodes and brain.

At 120 hours PI, the synovium was shrunken and contained more collagenous connective tissue with mature fibroblasts (Fig 8). Neutrophils with pyknotic nuclei and macrophages were present within the collagenous connective tissue being laid down. Edema and hyperemia of the synovium had subsided and no lesions were found on the articular surfaces.

Nodular hyperplasia persisted in the left prescapular lymph node.

Less cellular infiltrations were present in the portal areas of the liver and in the lungs, mononuclear cells were still present in the peribronchiolar areas but thickening of interalveolar septa had reduced considerably.

In the kidney, the glomeruli were hypercellular with more mononuclear cells.

At 14 days PI, the synovium had become fibrotic with completely organized mature collagenous connective tissue (Fig 9). The epithelium of the synovium was hyperplastic.

The synovial layer contained macrophages, lymphocytes and numerous plasma cells. The articular surfaces were not affected.

The lymphoid nodules of the left prescapular lymph node were hyperplastic. In the liver periportal infiltration and Kupffer cell activation were present.

Peribronchiolitis with mononuclear infiltration and thickened alveolar septa were present. The glomeruli of the kidneys were hypercellular with lymphocytic infiltration.

The synoval membrane of the left carpal joint of the two control lambs had increased amount of collagen with numerous spindle-shaped cells similar to fibroblasts while the epithelial cells of the synovium in the right carpal joint had more rounded cells with little collagen or spindle-shaped cells. The articular surfaces of both carpal joints were not affected.

The germinal centers of the left prescapular lymph node were active.

Other organs examined microscopically were normal.

Bacteriological Findings

A. seminis was cultured from the contents of the joint cavities of lambs 41 and 49 necropsied 24 hours PI. No bacterial growth was obtained from the tissues and aspirated joint contents of the other test and control lambs.

Cytology--neutrophils were predominant in thin smears of left carpal joint fluid examined (Fig 10).

DISCUSSION

Arthritis due to A. seminis in lambs is clinically indistinguishable from other arthritic conditions. Pathologically it is characterized by fibrino-purulent synovitis and capsulitis that becomes chronic with time. Villous projections of synovium are moderate and the mesenchymal connective tissue is gradually replaced by fibrous connective tissue. Nuclei of infiltrating neutrophils were pyknotic at 48 hours PI. Mononuclear infiltration was evident by 96 hours PI. Fibrosis of the synovial membrane occurred by the 14th day PI with the vascular channels arranged parallel to the surface and in the direction of which the fibrous connective tissue is oriented.

In contrast to other purulent arthritides due to C. pyogenes, streptococci and staphylococci (Roberts et al 1968), destruction of the articular cartilage was not a feature of A. seminis arthritis in lambs. Although proliferative changes were present in the synovial membrane, lymphoid follicular hyperplasia associated with E. rhusiopathiae arthritis in lambs (Piercy 1971) was not observed. The frank

suppurative changes in erysipelas arthritis (Ward 1922), chlamydial arthritis (Shupe and Storz 1964) was not present. The periarticular tissue involvement in A. seminis arthritis was not observed with mycoplasma arthritis in lambs (Leece 1960; Heinze et al 1963).

Microscopic changes in arthritis due to Haemophilus agni (Kennedy et al 1958) and Histophilus ovis (Kater et al 1962) have not been reported hence no inferences could be drawn as to the differential features of these conditions with A. seminis arthritis.

Macrophages noted in the synovium as early as 24 hour PI were due to the phagocytic capacity of mononuclear cells deep in the synovial tissue which were in excess of synovial epithelial cells (Bauer et al 1940). Because there is a rapid turnover of fluids, solutes and particulate matter between the joint cavity and synovial blood vessels and lymphatics, excessive fluid, as well as bacteria, extravasated erythrocytes and other particulate matter are drained off by the lymphatics (Sokoloff 1960); this probably accounted for synovial edema, vasculitis and regional lymphadenitis in the acute stage of A. seminis arthritis in lambs.

Neutrophils with pyknotic nuclei present at 48 hours post-inoculation can be explained as sacrificial phagocytosis representing a first line of defense to restrict the spread of invading organisms (Tizard 1977).

A number of factors might have been responsible for inability to culture A. seminis from the inoculated joints after 24 hours post-inoculation. These factors could include: complete phagocytosis and drainage of all inoculated organisms by 48 hours, the presence of antiglobulin in the synovial fluid (Wright-George et al 1976), and inability of bacteria to multiply in synovial effusions because of inherent bactericidal property of pathologic synovial effusions following localization of bacteria in the the synovium (De Gara 1943).

The pathogenesis of A. seminis arthritis in lambs is still unknown although it occurred in conjunction with septicemia in natural outbreaks (Watt et al 1970). None of the test lambs inoculated intravenously developed arthritis, but the vasculitis present in the synovium, lymphadenitis and/or nodular hyperplasia in the left prescapular lymph node intracarpal group suggests vascular and lymphatic dissemination of A. seminis in lambs.

Foci of intralobular, interalveolar septal thickening present in the lungs of lambs necropsied after 72 hours post-inoculation strongly supports the suggestion that A. seminis has a predilection for the respiratory tissues in lambs (Simmons 1966).

No gross or microscopic findings are considered to be pathognomonic for arthritis due to A. seminis in lambs. It

would be interesting to compare arthritis due to H. ovis and H. agni in lambs with the findings of this project because of their close relationship with A. seminis and reported causes of purulent polyarthritis associated with septicemia in lambs.

SUMMARY

Experimental arthritis in lambs following intracarpal inoculation of A. seminis resulted in fibrinopurulent synovitis and capsulitis. Villous hypertrophy of the synovium was moderate with gradual replacement of the mesenchymal connective tissue. In the acute stage, vasculitis in the synovium and lymphadenitis in the left prescapular lymph nodes were present. Chronic stages were characterized by synovial fibrosis and nodular hyperplasia of the left prescapular lymph node. The articular surfaces were not affected at any stage of A. seminis infection.

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TABLE 1 -- Microscopic Findings in Lambs Inoculated Intracarpally With *A. seminis*

	24 Hrs.*		48 Hrs.		72 Hrs.		96 Hrs.		120 Hrs.		14 Days		Controls	
Lamb #	41	49	35	40	30	44	32	38	37	40	42	43	29	
Joint														
Synovium														
Hypertrophy	0	0	0	0	1	1	2	2	2	2	2	2	0	(
Edema	3	3	2	2	1	1	1	1	0	0	0	0	1	1
Neutrophils	3	2	2	2	2	2	2	2	2	2	0	0	0	(
Lymphocytes	0	0	0	0	1	0	3	3	2	2	1	1	0	(
Plasma Cells	0	0	0	0	0	0	0	0	1	1	1	1	0	(
Macrophages	3	2	1	1	0	0	2	2	1	2	0	0	0	(
Eosinophils	1	1	1	0	0	0	0	1	0	0	0	0	0	(
Erythrocytes	1	1	1	1	0	0	0	0	0	0	0	0	0	(
Fibroblasts	0	0	0	0	0	0	1	1	2	1	3	3	0	(
Collagen	0	0	0	0	0	0	1	1	2	1	3	3	1	1
Fibrin	3	3	3	3	2	2	1	1	1	0	0	0	0	(
Epithelium														
Desquamated	0	0	0	0	0	0	0	0	0	0	0	0	0	(
Hyperplasia	0	0	0	0	0	0	1	1	1	1	0	0	1	1
Blood Vessels														
Congested	2	3	2	2	1	1	1	1	0	0	0	0	0	(
Neutrophils	2	2	1	1	0	0	0	0	0	0	0	0	0	(
Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	(
Eosinophils	1	0	0	0	0	0	0	0	0	0	0	0	0	(
Capsule														
Neutrophils	3	3	2	2	1	1	1	0	0	0	0	0	0	(
Lymphocytes	1	1	2	1	2	2	2	2	2	2	0	1	0	(
Macrophages	0	0	1	1	1	1	1	0	1	1	0	0	0	(
Eosinophils	1	1	1	0	0	1	0	0	0	0	0	0	0	(
Erythrocytes	2	2	1	1	1	1	0	1	0	0	0	0	0	(
Articular Surfaces														
Erosion	0	0	0	0	0	0	0	0	0	0	0	0	0	(

*Post inoculation

Table 1--continued

	24 Hrs.		48 Hrs.		72 Hrs.		96 Hrs.		120 Hrs.		14 Days		Controls	
Lamb #	41	49	35	40	30	44	32	38	37	40	42	43	29	31
Prescapular Lymph Node														
Hyperplasia	3	3	3	3	3	3	3	3	3	3	3	3	1	1
Neutrophils	2	2	1	1	0	0	0	0	0	0	0	0	0	0
Eosinophils	2	2	1	1	0	0	0	0	0	0	0	0	0	0
Lung														
Alveolar Wall														
Thickening	0	0	0	0	0	0	1	1	1	1	2	2	0	0

Key: 0 - None

1 - Slight

2 - Moderate

3 - Marked

Fig 1. Carpal joints. 48 hours PI. Note congested periarticular tissue (a) and creamy joint contents (b) in the left carpus.

Fig 2. Carpal joints. 96 hours PI. Note subsiding periarticular congestion (a) in the left carpus.

Fig 3. Carpal joints. 120 hours PI. Note thickening (a) of periarticular tissue.

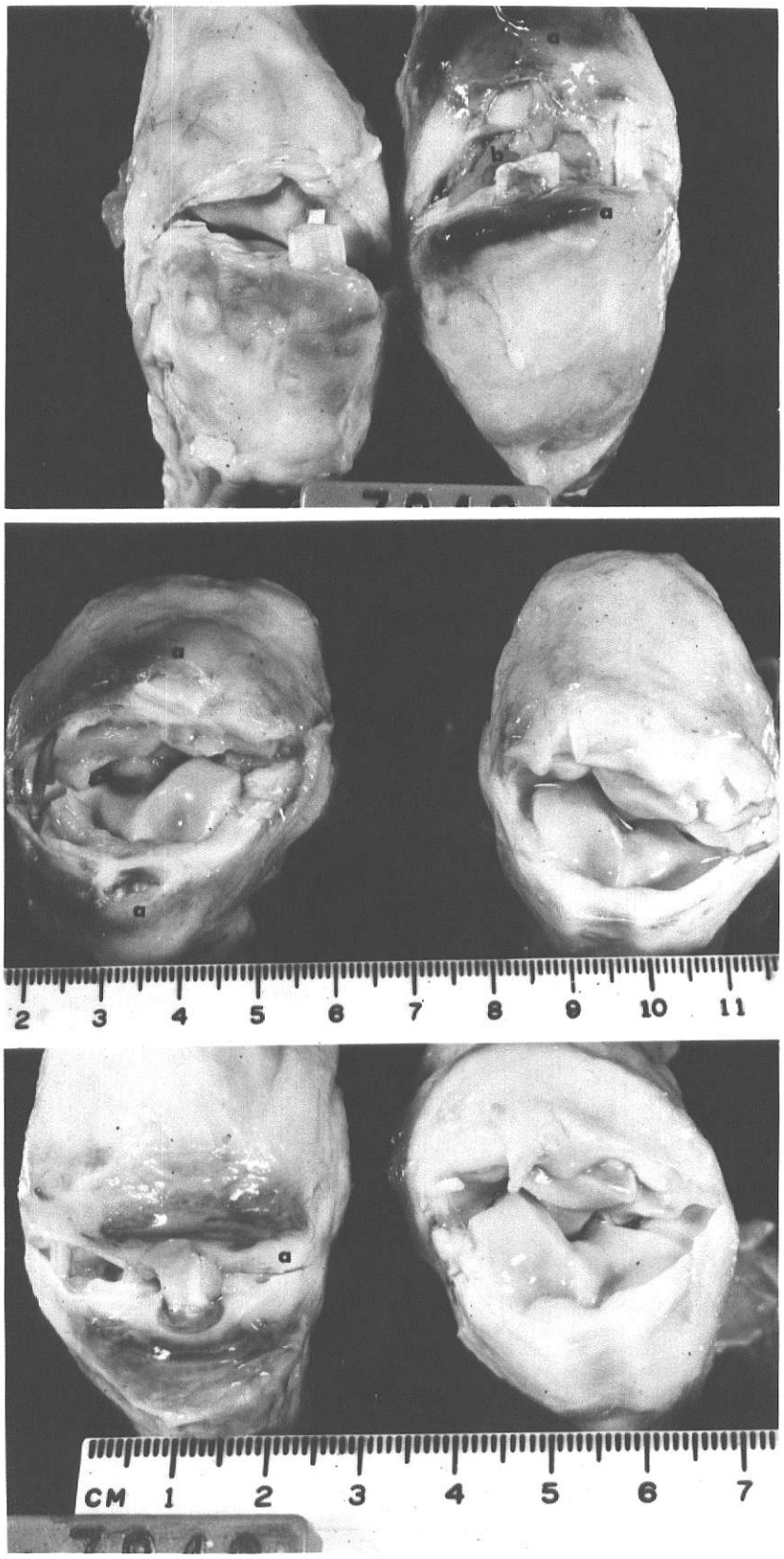
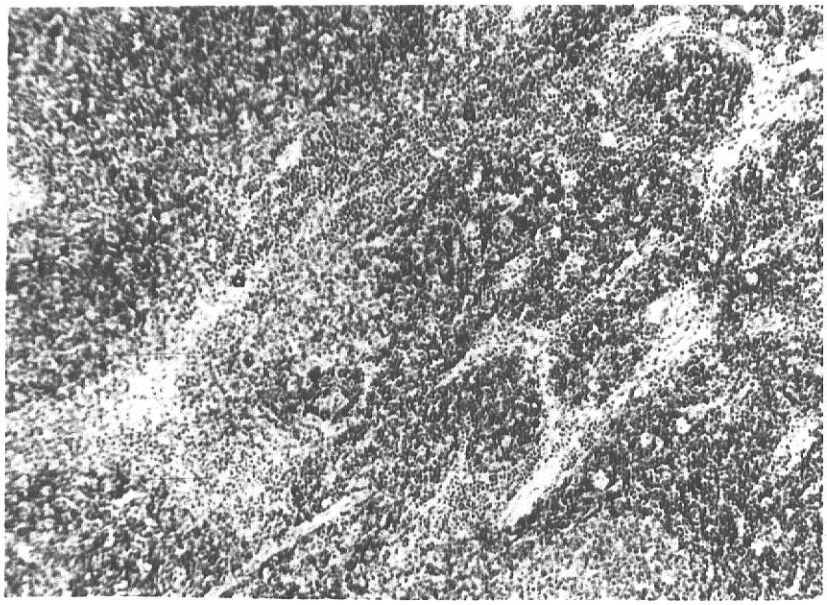
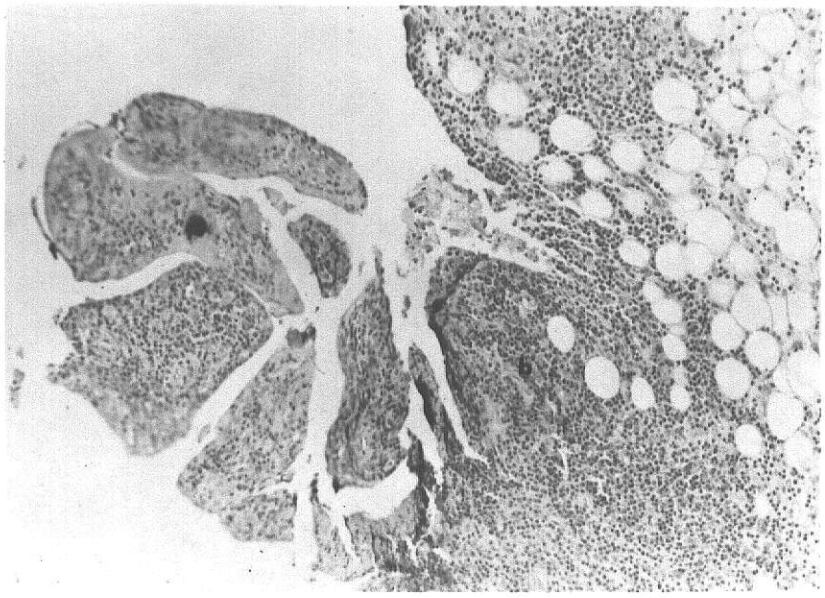

a
b
a
a
a
2 3 4 5 6 7 8 9 10 11
a
CM 1 2 3 4 5 6 7

Fig 4. Photomicrograph of spleen. 24 hours PI. Note neutrophils (a) in paracortical areas.

Fig 5. Photomicrograph of synovium 48 hours PI infiltrated by neutrophils (b) with pyknotic nuclei.

Fig 6. 72 hours PI. Fluid filled cysts (c) in the synovium.





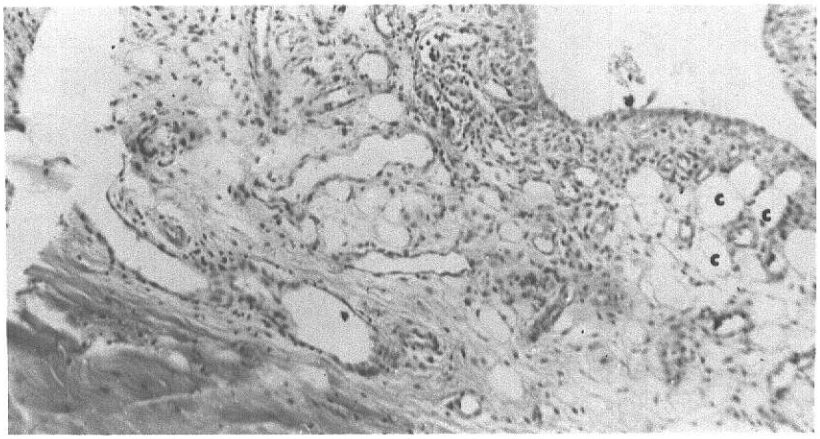

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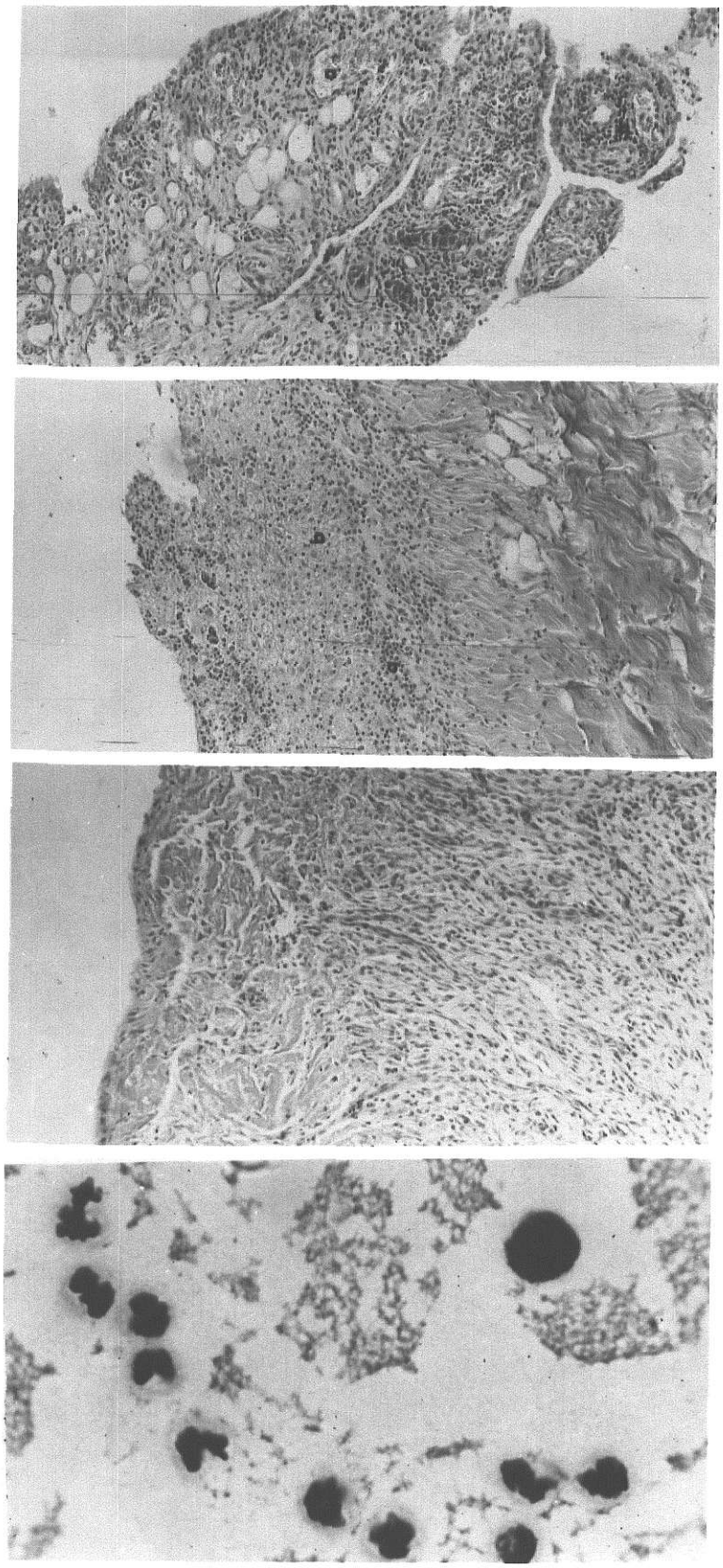
Fig 7. Photomicrograph of synovium. 96 hours PI. Note congested blood vessels (a), cellular infiltration (b) and increase in amount of collagen (c).

Fig 8. Photomicrograph of synovium. 120 hours PI. Note cellular infiltration (a) and immature fibroblasts (b).

Fig 9. Fibrosis of the synovium at 14 days PI.

Fig 10. 48 hours PI. Neutrophils in smear of contents from the left carpal joint (Wright-Leishman stain).



IV. PULMONARY CHANGES IN LAMBS FOLLOWING INTRATRACHEAL INJECTION OF *A. SEMINIS*

INTRODUCTION

Although *Pasteurella sp* are more frequently encountered in pneumonic conditions in lambs, environmental factors interacting with microorganisms and multiple infections are important in ovine pneumonia complex.

Reported causes of ovine pneumonia are *Pasteurella sp* (Newsom and Cross 1923; Montgomery *et al* 1938; Marsh 1953; McGowan *et al* 1957), *chlamydia sp* (Newsom and Cross 1923; Montgomery *et al* 1938; McKercher 1952; McGowan *et al* 1957), *Mycoplasma sp* (Greig 1955) and multiple infections involving chlamydiae, *Mycoplasma sp* and *Pasteurella sp* (Boidin *et al* 1958; Hamdy *et al* 1959b; Palov 1967; Stevenson and Robinson 1970). Pneumonia was produced experimentally following intravenous and intratracheal inoculation of lambs with the above organisms (Boidin *et al* 1958; Dungworth and Cordy 1962a).

Pneumonia associated with bacteremia due to *Fusobacterium necrophorum* (Hughes *et al* 1971; Hughes 1972; Dennis 1974) *Corynebacterium sp* (Dennis and Bamford 1966; Hughes *et al* 1971; Hughes 1972; Dennis 1974) and *Hemophilus agni* (Kennedy *et al* 1958).

Actinobacillus sp were reported to have a predilection for respiratory tissues especially in ram lambs (Baynes *et al* 1966). That plus the findings in Part II of this thesis led to this study.

Pulmonary changes due to A. seminis infection are presented in here.

MATERIALS AND METHODS

Experimental Lambs

Four Rambouillet cross lambs, 9-11 weeks old, were randomly divided into pairs, one test and one control.

Test Procedures

The housing and preparation of the A. seminis was described in Part II. The wool over the trachea in the ventral cervical area was clipped and the skin cleansed and disinfected as described previously. The two test lambs were injected intratracheally with 3 ml of a 24 hour BHI broth culture of A. seminis. The control lambs were injected with 3 ml of sterile BHI broth.

After injection, the lambs were closely observed for respiratory and other clinical signs. Respiratory rates and rectal temperatures were recorded twice daily. The lambs were euthanatized as described previously (Part II): One test lamb on day 7 post-inoculation (PI) and one on day 14 PI, and one control lamb on day 8 and one on day 15 PI.

Necropsy Examination

Standardized necropsy was performed with special emphasis on the respiratory tract and associated lymph nodes. A section of each lobe of the lungs was taken for bacteriological examination. The lungs were then profused with 10% buffered neutral formalin, and sections from each lobe were collected and fixed in BNF. Other tissues collected and fixed included bronchial and pulmonary lymph nodes, myocardium, liver, kidney, spleen and brain.

Bacteriological and histopathological procedures were performed as described in Part II.

For scanning electron microscopy, tissue from each lobe of the lung was fixed in phosphate buffered 4% formaldehyde--1% glutaraldehyde at 4° C for 24 hours. Sections were critical-point dried using CO_2 medium in a Ladd Critical Point Dryer[a]. Dried specimens were mounted on stubs and coated with gold using an Edwards Sputter Coater[b]. Coated specimens were photographed using a Hitachi H-300 TEM-SEM[c].

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[a]Ladd Research Industries, Inc., P.O. Box 901, Burlington, Vt. 05401.

[b]Edwards High Vacuum Inc., 3279 Grand Island Blvd., Grand Island, NY 14072.

[c]Hitachi, Ltd. 5-1, 1-chome, Marunouchi, Chiyoda, Tokyo.

RESULTS

Clinical Observations

Following inoculation with A. seminis broth, all lambs coughed for up to ten minutes. By 24 hours PI, intermittent coughing was more pronounced in the test lambs with labored respiration. On auscultation, moist rales were obvious; respiratory rates increased. At 96 hours PI, coughing was produced by exercising the test lambs while the control lambs did not cough. Dry rales were heard on auscultation, abdominal respiratory movements reduced although increased respiratory rates were recorded throughout the experimental period. No significant temperature changes were observed at any time but increased heart rates were recorded between 24 and 72 hours PI.

Gross Findings

The findings are tabulated in Appendix C.

At 7 days PI, fibrous pleural adhesions of the right apical and cardiac lobes was present. The right apical lobe was mottled and fibrosed. The ventral aspect of the right cardiac lobe was darker than the diaphragmatic lobe that appeared grossly normal. The left apical lobe was slightly fibrosed and mottled. The left cardiac and

diaphragmatic lobes were firm and on section increased connective tissue was present around the air passages. Slimy, straw-colored synovial fluid was present in the hock joints.

By 15 days PI, the bronchial epithelium was hyperemic and all lobes of the lungs appeared wrinkled (Fig 1). On section, the air passages were more prominent.

No significant gross lesions were observed in the control lambs. The ventral borders of all lobes of the lungs of the 16 day PI control were depressed and darkened.

Bronchial lymph nodes in all test and control lambs were enlarged.

Microscopic Findings

The findings are summarized in Table 1 and Appendix C.

At 7 days PI, the trachea was congested with hypercellularity of the lamina propria. The pulmonary blood vessels were thrombosed. Bronchial and bronchiolar lumina were filled with neutrophils, lymphocytes and macrophages (Fig 2). Severe reaction in the peribronchial, peribronchiolar and perivascular areas was characterized by massive infiltration of macrophages arranged in nests, the periphery of which were surrounded by fibroblasts (Fig 3). Adjacent bronchioles were bridged by this cellular reaction (Fig 4). Focal areas of consolidation contained a mixture of neutrophils, macrophages, mononuclear cells and alveolar macrophages.

In the myocardium, some smaller blood vessels were thrombosed. Focal and perivascular aggregations of mononuclear and/or neutrophils were present (Fig 5).

The liver and kidney were congested. There were no significant lesions present in other organs.

At 15 days PI, there was increased connective tissue in the peribronchial, peribronchiolar and perivascular areas. The epithelium of the bronchi and bronchioles was hyperplastic with perivascular cuffing by mononuclear cells (Fig 6). The interalveolar septa were thickened and the alveolar lining cells were cuboidal (Fig 7). Areas of carnification (Fig 8) adjacent to areas of emphysema were present. Peribronchial glands were prominent.

Multifocal areas of fatty change and eosinophilic cytoplasm were present in hepatocytes.

In the kidney, the glomeruli were moderately hypercellular and due to infiltration of lymphocytes (Fig 9). Some Bowman's spaces contained protein reflux.

In the 8 day PI control, there was areas of atelectasis and emphysema. Occasional nest of cells containing macrophages and mononuclear cells were located at one pole of some bronchioles. The peribronchiolar glands were prominent.

In the 16 day PI control, the epithelium of bronchi and bronchioles was hyperplastic. Mononuclear cells were

observed in some peribronchiolar areas. Many alveoli were plugged with fibrinous material while other areas were carnified.

Bronchial lymph nodes were hyperplastic in all test and control lambs.

Bacteriological Findings

Cultures of lung, liver, kidney, spleen, heart blood and synovial fluid were negative in both test and control lambs.

DISCUSSION

Pulmonary lesions following intratracheal inoculation of A. seminis in lambs are manifested by pleuritis, severe nodular peribronchial lymphoid hyperplasia, purulent bronchopneumonia with areas of hepatization in acute cases; peribronchial and parenchymal fibrosis in chronic cases.

The most striking lesion at 7 days PI was marked hyperplasia of the bronchial associated lymphoid tissue bridging adjacent bronchi. This reaction occurs as an immune response of the lung to the inoculum. The lung responds to aerogenous infection trapping organisms in the bronchial associated lymphoid tissue (Tizard 1977); antigens have been demonstrated

in the bloodstream up to 25 hours after a single intratracheal injection (Nash et al 1973). With antigens in the bloodstream, the thymus dependent lymphocytes are sensitized and when these encounter the antigens, vasoactive and chemotactic lymphokines are released that induce a severe local inflammatory reaction around the bronchi in the acute stage of infection.

By the 15th day PI, fibrosis of the connective tissue in the bronchi, alveolar walls and hepatized lobules of the lungs had occurred while the peribronchial lymphoid reaction had subsided considerably.

The interstitial reaction recorded in Parts II and III of this thesis and lesions observed following intratracheal inoculation suggest that pneumonia associated with A. seminis infection in lambs follows a chronic rather than an acute course that clearly differentiates it from pneumonia due to Pasteurella sp (Jensen 1974) in which the stage of resolution is characterized by replacement of fully or partially lysed fibrin by macrophages.

Until the present study, pneumonia due to A. seminis has not been reported. The results suggest A. seminis could be a cause of pneumonia in lambs under natural conditions.

SUMMARY

Intratracheal inoculation of A. seminis in lambs produced severe peribronchial lymphoid nodular hyperplasia with mixed purulent bronchopneumonia on day 7 post-inoculation; peribronchial and parenchymal fibrosis with hyperplastic bronchial epithelium on day 15 post-inoculation. Areas of fibrosis characterized by atelectasis with mononuclear cells and fibrous connective tissue were adjacent to areas of emphysema. Nodular hyperplasia was present in bronchial and pulmonary lymph nodes of test lambs, with nonsuppurative myocarditis in one lamb.

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TABLE 1--Microscopic Findings in Lambs Following Intratracheal Injection With A. seminis.

	#53	#58	#51	#54
Lung				
Pleura				
Edema	0	0	0	0
Fibrin	0	0	0	0
Neutrophils	0	0	0	0
Lymphocytes	1	0	0	0
Plasma Cell	1	0	0	0
Macrophages	0	0	0	0
Bronchus				
Lumen				
Debris	1	1	2	0
Neutrophils	2	0	0	0
Lymphocytes	2	0	1	0
Plasma Cell	1	0	0	0
Macrophages	2	0	0	0
Epithelium				
Desquamated	0	0	2	0
Vacuolated	0	0	0	0
Hypertrophic	2	1	1	0
Hyperplasia	1	2	0	1

Table 1--continued

	#53	#58	#51	#54
Lamina Propria				
Neutrophils	0	0	0	0
Lymphocytes	0	0	2	1
Plasma Cells	0	0	0	0
Macrophages	0	0	0	0
Eosinophils	0	0	0	0
Erythrocytes	0	0	0	1
Periphery				
Neutrophils	0	0	0	0
Lymphocytes	3	2	1	1
Plasma Cells	1	0	0	0
Macrophages	3	1	0	0
Eosinophils	0	0	0	0
Erythrocytes	0	0	0	0
Fibrosis	0	0	3	1
Glands				
Prominent	0	0	3	1
Dilated	0	0	3	1
Bronchiole				
Lumen				
Neutrophils	1	0	0	0
Lymphocytes	1	0	1	0
Plasma Cells	0	0	0	0
Macrophages	1	0	0	0

Table 1--continued

	#53	#58	#51	#54
Eosinophils	0	0	0	0
Erythrocytes	0	0	0	0
Epithelium				
Desquamated	0	0	0	0
Fetalized	2	0	2	1
Hyperplasia	0	0	0	0
Hypertrophy	0	0	0	0
Periphery				
Neutrophils	0	0	0	0
Lymphocytes	2	1	1	0
Plasma Cells	0	0	0	0
Macrophages	1	0	0	0
Eosinophils	0	0	0	0
Alveoli				
Epithelium				
Fetalized Cells	2	0	2	1
Rounded Cells	0	0	1	0
Wall				
Edema	2	0	0	0
Neutrophils	0	0	0	0
Lymphocytes	1	0	1	0
Plasma Cells	0	0	1	0
Macrophages	0	0	0	0
Fibroblasts	0	0	0	0
Collagen	1	1	2	1

Table 1--continued

	#53	#58	#51	#54
Lumen				
Dilated	3	3	3	2
Constricted	0	3	1	0
Neutrophils	2	0	0	0
Lymphocytes	2	1	1	1
Plasma Cells	1	0	1	0
Macrophages	3	0	1	1
Eosinophils	0	0	0	0
Erythrocytes	0	0	0	0
Fibroblasts	0	0	1	0
Bronchial L. Node				
Nodular Hyperplasia	3	0	3	1
Neutrophils	0	0	0	0
Erythrocytes	0	0	0	0
Eosinophils	0	0	0	0
Kidney				
Glomeruli				
Proliferation	0	0	0	0
Membraneous	0	0	0	0
Neutrophils	0	0	0	0
Lymphocytes	0	0	2	0

Table 1--continued

	#53	#58	#51	#54
Tubules				
Epithelium	0	0	0	0
Desquamated	0	0	0	0
Nephrosis	0	0	0	0
Vacuolated Cells	0	0	0	0
Cast	0	0	0	0
Spleen				
Nodular Hyperplasia	0	0	0	0
Depleted	0	0	0	0
Neutrophils	0	0	0	0
Eosinophils	0	0	0	0
Liver				
Parenchyma				
Neutrophils	0	0	0	0
Lymphocytes	0	0	0	0
Macrophages	0	0	0	0
Erythrocytes	0	0	0	0
Portal Area				
Neutrophils	0	0	0	0
Lymphocytes	0	0	2	0
Macrophages	0	0	1	0
Plasma Cell	0	0	0	0
Eosinophils	0	0	0	0
Fibroblasts	0	0	0	0

Table 1--continued

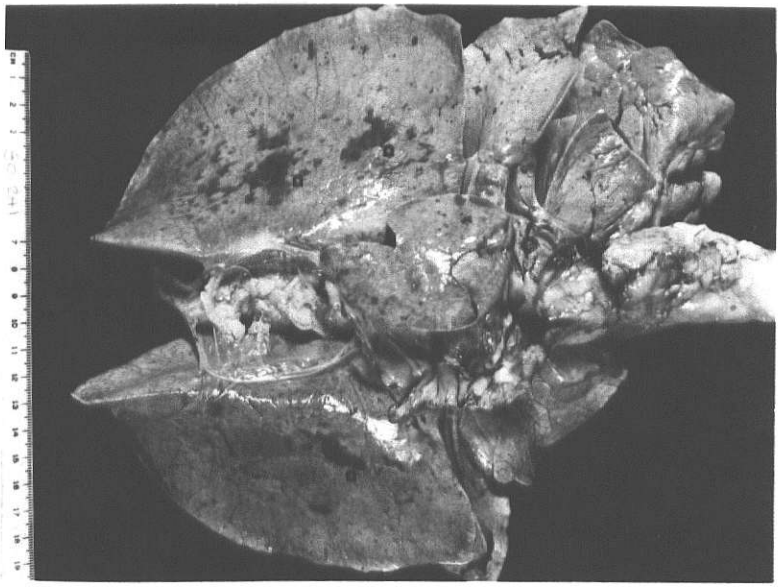
	#53	#58	#51	#54
Myocardium				
Lymphocytes	2	0	0	0
Neutrophils	0	0	0	0
Macrophages	2	0	0	0

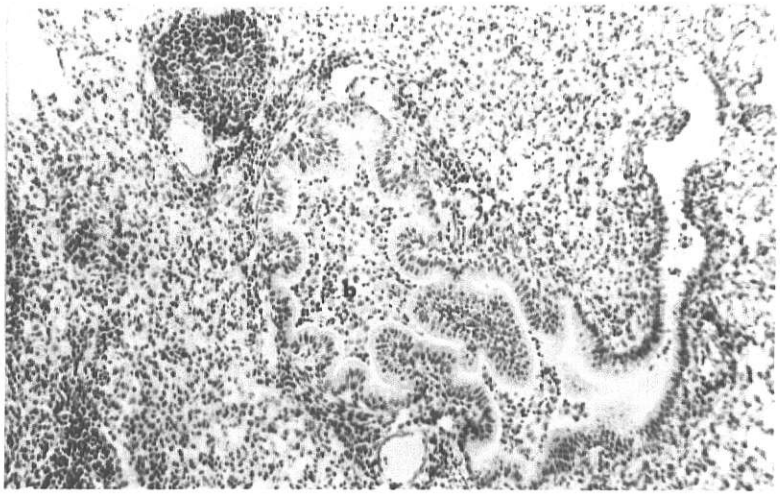
IV. PULMONARY CHANGES IN LAMBS FOLLOWING INTRATRACHEAL INJECTION OF *A. SEMINIS*

Fig 1. Lungs with wrinkled appearance. Note darkened areas (a) on the diaphragmatic lobes.

Fig 2. Photomicrograph of lung with inflammatory cells (b) in the bronchus.

Fig 3. Day 7 PI. Peribronchial hyperplastic lymphoid nodules (c) in lungs.





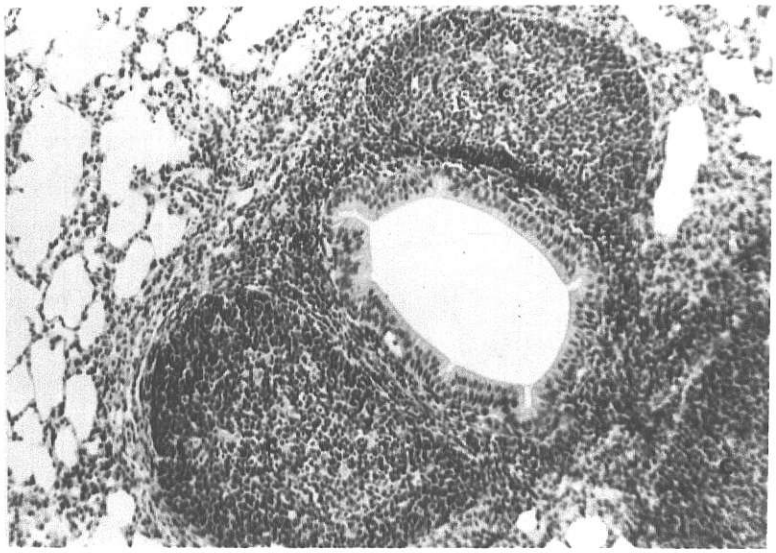
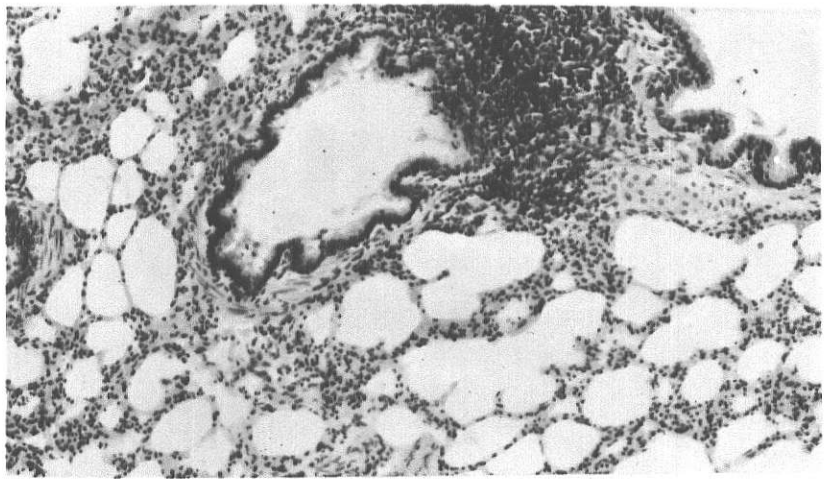
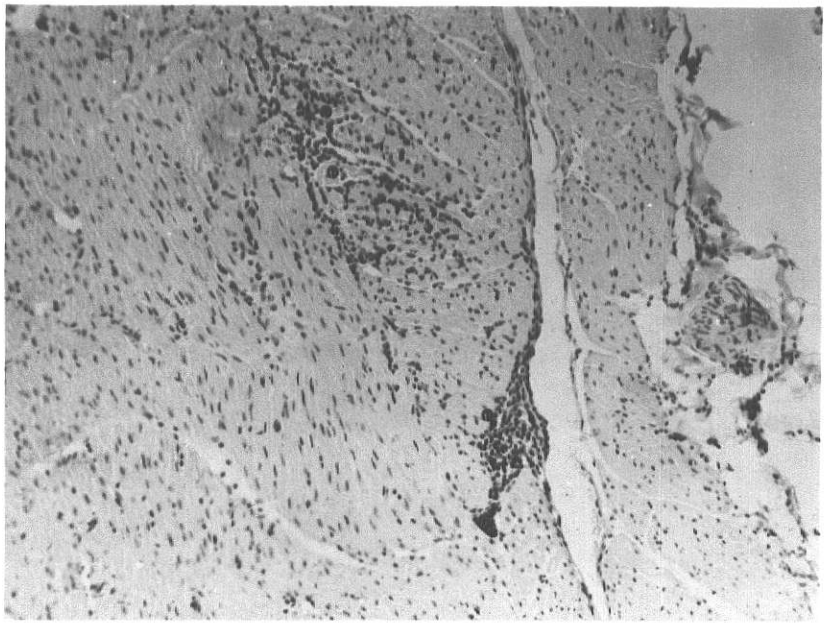


Fig 4. Day 7 PI. Note cellular reaction (d) bridging adjacent bronchioles.

Fig 5. Foci of mononuclear aggregations (e) in the myocardium.

Fig 6. Day 15 PI. Lung. Note peribronchial fibrosis (f).





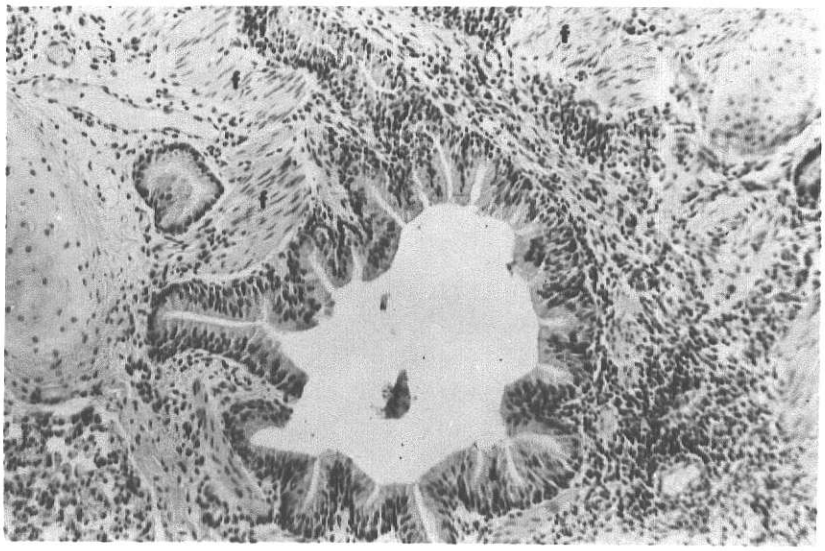
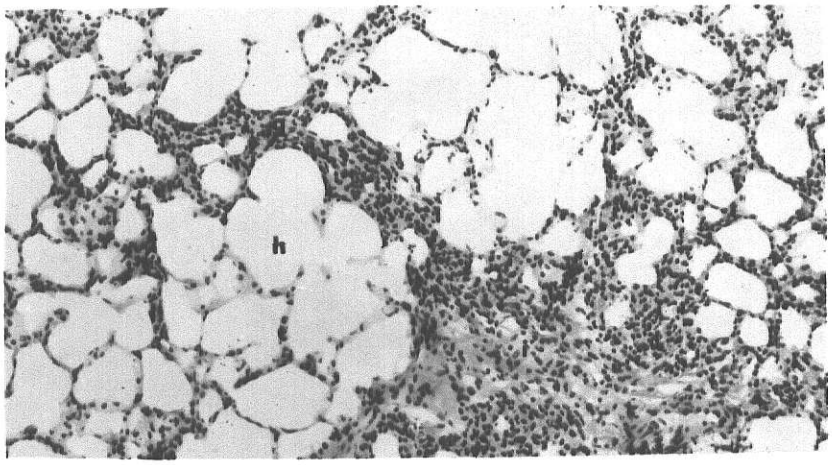


Fig 7. Day 15 PI. Lung. Note septal thickening (g), emphysema (h) and atelectasis (i).

Fig 8. Day 15 PI. Carnified area in the lungs.

Fig 9. Kidney with hypercellular glomeruli.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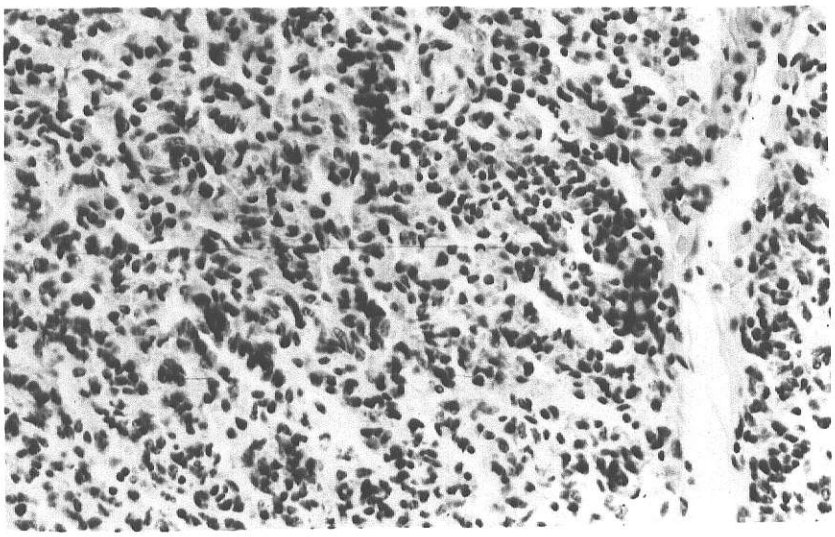

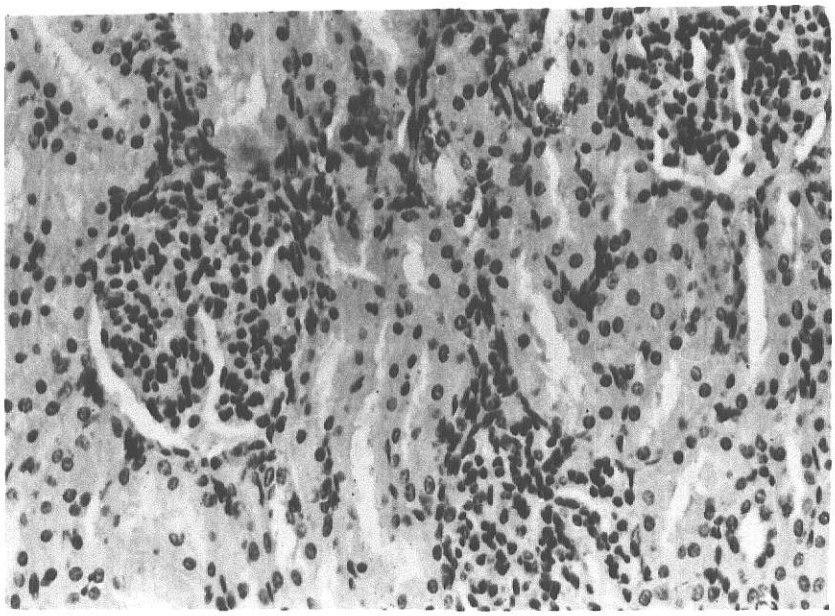

APPENDICES

A. Intravenous Inoculation With *A. seminis*

Table [1] --Pathological Findings in Lambs Inoculated Intravenously With A. seminis

PI Period*	Lamb #	Gross Findings	Microscopic Findings
9-12 hrs.	50	Hydrothorax, 470 ml straw-colored. Fibrinous pleuritis. Pulmonary edema. Ecchymoses and petechiae in thymus, lungs, liver and sternal muscles. Friable liver.	Serofibrinous pneumonia, peribronchitis and peribronchiolitis with hyperplastic peribronchial lymphoid nodules. Glomerulonephritis. Depleted nodules, spleen. All organs congested.
21 days	33	None	Interstitial pneumonia. Pulmonary emphysema and atelectasis with hyperplastic peribronchial lymphoid nodules. Pericholangitis. Cortical tubular nephrosis.
42 days	34	None	Interstitial pneumonia. Pulmonary emphysema and atelectasis with hyperplastic peribronchial lymphoid nodules. Cortical tubular nephrosis and glomerulonephritis.
	39	None	Interstitial pneumonia. Pulmonary emphysema and atelectasis with hyperplastic peribronchial lymphoid nodules. Glomerulonephritis.
	47	None	Interstitial pneumonia. Hyperplasia of peribronchial lymphoid nodules. Cortical tubular nephrosis and glomerulonephritis. Nodular depletion spleen.
	48	None	Interstitial pneumonia. Pulmonary emphysema and atelectasis with hyperplastic lymphoid nodules. Cortical tubular nephrosis and glomerulonephritis.
42 days	52	Areas of grey hepatization and congestion scattered throughout lungs.	Chronic pneumonia, interstitial pneumonia and fibrosis with hyperplastic peribronchial lymphoid nodules. Multifocal hepatitis. Glomerulonephritis.

*Post inoculation

Table 1--continued

PI Period*	Lamb #	Gross Findings	Microscopic Findings
	58	Wrinkled appearance with areas of grey hepatization present in all lobes of the lungs.	Chronic pneumonia, interstitial pneumonia and fibrosis. Hyperplasia bronchial epithelium and peribronchial lymphoid nodules. Bronchiolitis. Nodular hyperplasia bronchial lymph node. Proliferative glomerulonephritis.
	51	Wrinkled appearance with areas of grey hepatization in right apical and left diaphragmatic lobes of lungs. Fibrous pleural adhesion of left diaphragmatic lobe.	Chronic pneumonia, interstitial pneumonia and fibrosis with hyperplastic peribronchial lymphoid nodules. Bronchitis and peribronchiolitis. Pericholangitis. Nodular hyperplasia spleen. Glomerulonephritis. Myocarditis.
	54	Wrinkled appearance with a large encapsulated abscess involving right apical and cardiac lobes of the lungs. Areas of hepatization in all lobes of lungs. Fibrous adhesion of right diaphragmatic lobes. Enlarged bronchial and mediastinal lymph nodes.	Chronic pleuritis. Mixed purulent chronic bronchopneumonia with fibrosis. Bronchitis, peribronchitis and peribronchiolitis. Hyperplasia bronchiolar epithelium. Nodular hyperplasia in bronchial and mediastinal lymph nodes. Myocarditis. Glomerulonephritis.

*Post inoculation

B. Intracarpal Inoculation With *A. seminis*

TABLE 1--Pathological Findings in Lambs Inoculated Intracarpally With A. seminis

PI Period*	Lamb #	Gross Findings	Microscopic Findings
24 hours	41 and 49	Swollen left carpus with thickened periarticular sheath. Thick creamy yellow exudate aspirated from joint cavity. Left prescapular lymph node enlarged.	Edema of synovial membrane with vasculitis and neutrophils. Capsulitis. Lymphadenitis left prescapular lymph node.
48 hours	35 and 46	Swollen left carpus with thickened capsule and thick creamy yellow pus in the joint cavity. Enlarged left prescapular lymph node.	Edema of synovial membrane with vasculitis and neutrophils. Capsulitis. Nodular hyperplasia left prescapular lymph node with neutrophils.
72 hours	30 and 44	Reduced swelling left carpus with thickened periarticular sheath and thick creamy yellow exudate in the joint cavity. Enlarged left prescapular lymph node.	Villous projections of synovial membrane with vasculitis, neutrophils, macrophages and increased collagen. Capsulitis. Nodular hyperplasia left prescapular lymph node. Peribronchiolitis and thickened interalveolar septa.
96 hours	32 and 38	Reduced thickening of periarticular sheath with grey pasty pus in the joint cavity. Enlarged left prescapular lymph node.	Villous projections of synovial membrane with increased collagen, immature fibroblasts, mononuclear cells, neutrophils and macrophages. Capsulitis. Nodular hyperplasia left prescapular lymph node.
120 hours	37 and 40	Swollen left carpus with thickened periarticular sheath and pasty light yellow pus in the joint cavity. Enlarged left prescapular lymph node.	Villous projections of synovial epithelium with increased collagen, fibroblasts, macrophages, mononuclear cells and neutrophils. Nodular hyperplasia left prescapular lymph node. Thickening of interalveolar septa of lung.
14 days	42 and 43	Fibrous thickening of the periarticular sheath of left carpus with light yellow inspissated pus. Enlarged left prescapular lymph node.	Flattened synovial epithelial cells with increased collagen, mature fibroblasts, lymphocytes and plasma cells. Nodular hyperplasia left prescapular lymph node. Thickened interalveolar septa of lung.
15 days	29 and 31	None	Synovial membrane acellular but the amount of collagen increased.

*Post inoculation

C. Intratracheal Inoculation With *A. seminis*

TABLE 1--Pathological Findings in Lambs Inoculated Intratracheally With A. seminis

PI Period*	Lamb #	Gross Findings	Microscopic Findings
7 days	53	Fibrous pleural adhesions of right apical and cardiac lobes. Left apical lobe wrinkled with a large area of grey hepatization. Foci of grey hepatization present in both diaphragmatic lobes. Foci of red hepatization present in all lobes of the lungs.	Thrombosis of pulmonary blood vessels. Bronchitis and bronchiolitis. Hypercellular lamina propria of the bronchi with peribronchial lymphoid hyperplasia bridging adjacent bronchi. Pneumonic areas with mononuclear cells, alveolar macrophages, macrophages and neutrophils. Myocarditis.
8 days	56	Foci of atelectasis present in all lobes of the lungs.	Foci of atelectasis with occasional peribronchial hyperplastic lymphoid nodule. Prominent peribronchial glands.
15 days	57	Foci of grey hepatization present in all lobes of the lungs. Air passages prominent on incission. Enlarged bronchial and mediastinal lymph nodes.	Hyperplasia of bronchial and bronchiolar epithelium. Peribronchial and peribronchiolar fibrosis with prominent peribronchial glands. Focal pulmonary fibrosis and emphysema present in the parenchyma. Thrombosis of hepatic blood vessels with perivasculitis and pericholangitis. Glomerulonephritis. Nodular hyperplasia bronchial lymph node.
16 days	55	Atelectasis present in ventral borders of all lobes of the lungs.	Hyperplasia of bronchial and bronchiolar epithelium with peribronchial mononuclear aggregation. Foci of atelectasis present.

*Post inoculation

STUDIES ON ACTINOBACILLUS SEMINIS INFECTION IN LAMBS

by

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AN ABSTRACT OF A THESIS

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Actinobacillus seminis was initially thought to be a venerally transmitted cause of epididymitis in rams. This generated considerable research to characterize and differentiate it from epididymitis due to Brucella ovis. Because of reduced fertility and subsequent economic loss associated with epididymitis, researchers have concentrated on this aspect of A. seminis infection in sheep. Since no experimental work had been reported on A. seminis infection in lambs, this project was undertaken. It was designed to determine the possible sites of localization and associated pathological changes in lambs following intravenous inoculation of A. seminis, sequential carpal changes in lambs following intra-articular inoculation of A. seminis, and the pathological changes in lungs of lambs following intratracheal inoculation of A. seminis.

In the intravenous group, the most consistent findings were interstitial pneumonia and glomerulonephritis. Occasional findings included mixed purulent bronchopneumonia, focal hepatitis or pericholangitis, nodular hyperplasia or depletion of the spleen and foci of mononuclear aggregations in the myocardium. Arthritis was not reproduced in this experiment. It was concluded that the target organ for localization of A. seminis in lambs following intravenous inoculation was the lung since the renal lesions were mild.

Pathological changes in the intra-articular group were restricted to the synovial membrane and joint capsule of the left carpal joint in all test lambs; the articular cartilage was not affected. Fibrinopurulent synovitis and capsulitis were the main findings up to 120 hours post-inoculation (PI). By day 14 PI, fibrosis of the synovium with very few inflammatory cells was present. Nodular hyperplasia was present in the left prescapular lymph node of all test lambs and at 24 hours PI numerous neutrophils were found in the subcapsular sinus, paracortical areas and in the medulla. In addition, thickening of the interalveolar septa was observed as from 96 hours PI.

Intratracheal inoculation of A. seminis was carried out as a result of the findings following intravenous challenge with A. seminis. At day 7 PI, mixed purulent bronchopneumonia and severe hyperplasia of peribronchial lymphoid nodules bridging adjacent bronchi were present. At day 15 PI, peribronchial and parenchymal fibrosis with areas of carnification were present in the lungs. It was concluded that complete resolution of hepatized areas of the lungs does not occur in experimental A. seminis induced bronchopneumonia in lambs; instead, carnification and fibrosis occurred in subacute and chronic stages.

Results of these experiments indicate that A. seminis has an affinity for respiratory tissues in lambs. It is

suggested that A. seminis may localize in the lungs and that some lambs may become carriers. Susceptible animals may be infected by carriers coughing or by contaminating food and water. Intermittent bacteremia in carrier rams, after sexual maturity, may also lead to localization in the epididymis and result in epididymitis. It is hypothesized that one or more of the above methods of spreads may lead to perpetuation of actinobacillosis in a flock.