STUDIES ON ACTINOBACILLUS SEMINIS INFECTION IN LAMBS

by

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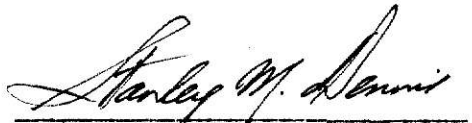

Major Professor

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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